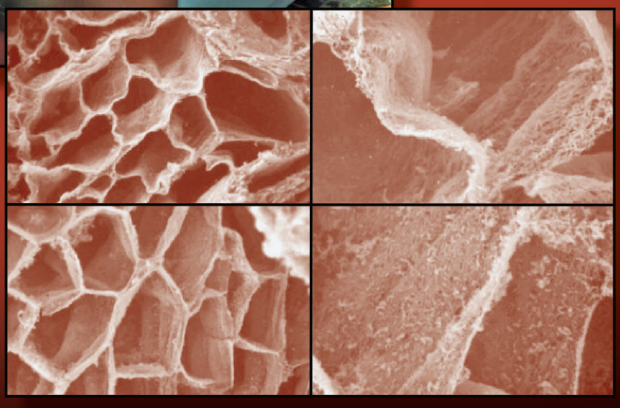
SECOND EDITION

Advanced Technologies for Meat Processing





Edited by

Fidel Toldrá • Leo M. L. Nollet

CRC Press
Taylor & Francis Group

Advanced Technologies for Meat Processing

Downloaded From Vet-eBooks.com



Taylor & Francis
Taylor & Francis Group
http://taylorandfrancis.com

Downloaded From Vet-eBooks.com

Advanced Technologies for Meat Processing
Second Edition

Edited by
Fidel Toldrá and Leo M. L. Nollet


CRC Press
Taylor & Francis Group
Boca Raton London New York

CRC Press is an imprint of the
Taylor & Francis Group, an **informa** business

Downloaded From Vet-eBooks.com

CRC Press
Taylor & Francis Group
6000 Broken Sound Parkway NW, Suite 300
Boca Raton, FL 33487-2742

© 2018 by Taylor & Francis Group, LLC
CRC Press is an imprint of Taylor & Francis Group, an Informa business

No claim to original U.S. Government works

Printed on acid-free paper

International Standard Book Number-13: 978-1-4987-5459-0 (Hardback)

This book contains information obtained from authentic and highly regarded sources. Reasonable efforts have been made to publish reliable data and information, but the author and publisher cannot assume responsibility for the validity of all materials or the consequences of their use. The authors and publishers have attempted to trace the copyright holders of all material reproduced in this publication and apologize to copyright holders if permission to publish in this form has not been obtained. If any copyright material has not been acknowledged please write and let us know so we may rectify in any future reprint.

Except as permitted under U.S. Copyright Law, no part of this book may be reprinted, reproduced, transmitted, or utilized in any form by any electronic, mechanical, or other means, now known or hereafter invented, including photocopying, microfilming, and recording, or in any information storage or retrieval system, without written permission from the publishers.

For permission to photocopy or use material electronically from this work, please access www.copyright.com (http://www.copyright.com/) or contact the Copyright Clearance Center, Inc. (CCC), 222 Rosewood Drive, Danvers, MA 01923, 978-750-8400. CCC is a not-for-profit organization that provides licenses and registration for a variety of users. For organizations that have been granted a photocopy license by the CCC, a separate system of payment has been arranged.

Trademark Notice: Product or corporate names may be trademarks or registered trademarks, and are used only for identification and explanation without intent to infringe.

Visit the Taylor & Francis Web site at
http://www.taylorandfrancis.com

and the CRC Press Web site at
http://www.crcpress.com

Downloaded From Vet-eBooks.com

Contents

Preface vii
About the Editors ix
Contributors xiii

1. **Genetically Modified Farm Animals: Control and Traceability** 1
   Antoon Lievens and Mauro Petrillo

2. **Hyperspectral Imaging Technique for Online Monitoring of Meat Quality and Safety** 17
   Jun-Li Xu and Da-Wen Sun

3. **Raman Spectroscopy for Predicting Meat Quality Traits** 83
   Stephanie M. Fowler, Heinar Schmidt, Rico Scheier, and David L. Hopkins

4. **Real-Time PCR for the Detection of Pathogens in Meat and Meat Products** 113
   Alicia Rodríguez, María J. Andrade, Mar Rodríguez, and Juan J. Córdoba

5. **Sensors and Biosensors for Meat Safety: Recent Advances in Nanotechnology Integration** 153
   Rosa Pilolli, Nicoletta Ditaranto, and Linda Monaci

6. **Meat Decontamination by Irradiation** 197
   Dong U. Ahn, Eun Joo Lee, and Aubrey Mendonca

7. **Advances in High Hydrostatic Pressure for Meat and Meat Processing** 227
   Sencer Buzrul

8. **Hydrodynamic Pressure Processing to Improve Meat Quality and Safety** 259
   Tomás Bolumar and Stefan Toepfl

9. **Emerging Technologies for the Meat Processing Industry** 297
   Kumari Shikha Ojha, Joseph P. Kerry, and Brijesh K. Tiwari

10. **Reduction of Contaminant' Content in Processed Meats** 319
    Peter Šimko

Downloaded From Vet-eBooks.com

11. Nutrigenomics in Food-Producing Animals 355
Werner G. Bergen

12. Bioactive Properties of Peptides Generated from Meat Proteins 371
Keizo Arihara and Yusuke Komiya

13. New Approaches for the Development of Functional Meat Products 403
Francisco Jiménez-Colmenero, Milagro Reig, and Fidel Toldrá

14. Salt Reduction in Processed Meats 443
Fidel Toldrá and José M. Barat

15. Fat Reduction in Processed Meats 461
Marise A. Rodrigues Pollonio

16. Processing of Nitrite-Free Cured Meats 513
Fereidoon Shahidi and Ronald B. Pegg

17. Proteomic Tools for Improved Processing of Dry-Cured Meats 535
Leticia Mora and Fidel Toldrá

18. The Use of Bacteriocins against Meat-borne Pathogens 559
Carmen A. Campos, Marcela P. Castro, María E. Cayre, and Franco P. Rivas

19. Functionalities of Meat Bacterial Starters 597
Régine Talon and Sabine Leroy

20. Modified Atmosphere Packaging 615
Joseph G. Sebranek and Terry A. Houser

21. Nanotechnology-Based Packaging Materials for Fresh and Processed Meats 647
Brandon Guild, Hans Bernard Tee, and Loong-Tak Lim

Index 689

Downloaded From Vet-eBooks.com

Preface

Meat and meat products constitute some of the most important foods in Western societies and their presence is also increasing very rapidly in the Asian countries. The processing technology has been developing very fast over the last decade, as can be observed in the number of publications, which is growing exponentially year by year. The meat industry is progressively incorporating such technological advances and it is evident that an updated compilation of these recent developments is really needed by meat scientists and technologists.

The first edition of this book dates back to 2006 and the contents were spread throughout 18 chapters. The second edition brings 21 chapters with new approaches to a dynamic field, combining updated and revised versions of several chapters with new chapters dealing with new online monitoring techniques such as hyperspectral imaging and Raman spectroscopy, the use of nanotechnology for sensor devices or new packaging materials, and the application of omics technologies such as nutrigenomics and proteomics for meat quality and nutrition.

The main goal of this book is to provide the reader with recent developments in new advanced technologies for the full meat processing chain. The book starts with the control and traceability of genetically modified farm animals, followed by four chapters reporting the use of online nondestructive monitoring techniques such as hyperspectral imaging and Raman spectroscopy, real-time polymerase chain reaction for pathogen detection, and nanotechnology-based sensors. Then, the following five chapters describe different advanced technologies for meat decontamination, such as irradiation, hydrostatic and hydrodynamic pressure processing, other nonthermal technologies, and reduction in contaminant generation. One chapter covers nutrigenomics in animal nutrition and production, and this is followed by six chapters dealing with nutrition-related issues such as bioactive peptides, functional meats, fat and salt reduction, processing of nitrite-free products, and the use of proteomics for the improved processing of dry-cured meats. The last four chapters report the latest developments in bacteriocins against meat-borne pathogens, the functionality of bacterial starters, modified atmosphere packaging, and the use of new nanotechnology-based materials for intelligent and edible packaging.

This book is written by distinguished international contributors with recognized expertise and strong reputations, and brings together all the advances in varied and different technologies such as biotechnology, hyperspectral imaging and Raman spectroscopy, nanotechnology, decontamination technologies, omics technologies, and packaging for application in different stages of meat processing.

Downloaded From Vet-eBooks.com

We would like to thank very cordially all the authors of this book for all their efforts and for sharing their knowledge on these different topics, as well as the production team at CRC Press for their dedication to the publication of this new edition.

Downloaded From Vet-eBooks.com

About the Editors

Fidel Toldrá obtained his BSc in chemistry (1980) and PhD in chemistry (1984) from the University of Valencia. Since 2001, he has been a research professor and head of the Laboratory of Biochemistry, Innovation, and Technology of Meat and Meat Products at the Instituto de Agroquímica y Tecnología de Alimentos, in Paterna, Valencia, Spain.

He has filed 11 patents and directed 24 PhD theses and is currently directing one PhD theses. He has published over 270 manuscripts in worldwide recognized scientific journals and 120 book chapters. His work is quite cited (>700 citations in 2016) and he holds an h-index of 47.

Prof. Toldrá has received numerous awards such as the 1992 Iber Award on food and cardiovascular diseases; the 2001 Danone Institute Award to the best scientific trajectory within the last 10 years in food, nutrition, and health; the 2002 International Prize for meat science and technology from the International Meat Secretariat, Paris, France; the 2002 GEA Award on R&D activity in agro-food in the Valencia community; the 2010 Distinguished Research Award and the 2014 Meat Processing Award, both from the American Meat Science Association; and the 2015 Dupont Science Award. In 2008, he was elected as a fellow of the International Academy of Food Science and Technology in Shanghai, China; in 2009, he was elected as a fellow of the Institute of Food Technologists.

Prof. Toldrá is the European editor of *Trends in Food Science and Technology* (Elsevier, since 2005) and an associate editor of *Meat Science* (Elsevier, since 2014); he was editor-in-chief of *Current Nutrition and Food Science* (Bentham, 2005–2012) and section editor of the *Journal of Muscle Foods* (Wiley-Blackwell, 2009–2010). He is a member of the Editorial Boards of *Food Chemistry* (Elsevier, since 1999), *Journal of Food Engineering* (Elsevier, since 2011), *Journal of Muscle Foods* (Wiley-Blackwell, 2001–2010), *Food Analytical Methods* (Springer, since 2008), *Journal of Food and Nutrition Research* (VUP, since 2008), *The Open Nutrition Journal* (Bentham, since 2008), *The Open Enzyme Inhibition Journal* (Bentham, since 2008), *Recent Patents in Agriculture, Food and Nutrition* (Bentham, since 2009), *Food Science and Nutrition* (Wiley, since 2012), and *Current Opinion in Food Science* (Elsevier, since 2014).

In 2002, he authored a book entitled *Dry-Cured Meat Products* (Wiley-Blackwell) and has worked as an editor or associate editor of numerous books for major publishers, such as Springer, CRC Press, Wiley-Blackwell, and Elsevier. He was an associate editor of the *Handbook of Food and Beverage Fermentation Technology* and the *Handbook of Food Science, Technology and Engineering,* both published by CRC Press (2004 and 2006, respectively), *Handbook of Food Product Manufacturing* (John Wiley & Sons, 2007), and the third edition of *Food Biochemistry and Food Processing* (Wiley-Blackwell, 2012).

Downloaded From Vet-eBooks.com

In 2006, he coedited two books with Dr. Nollet: *Advanced Technologies for Meat Processing* (CRC Press) and *Advances in Food Diagnostics* (Blackwell Publishing). He was the editor of *Meat Biotechnology* (Springer, 2008) and *Safety of Meat and Processed Meat* (2009, Springer), the editor-in-chief of first and second editions of the *Handbook of Fermented Meat and Poultry* (Wiley-Blackwell, 2007 and 2015), and editor of the *Handbook of Meat Processing* (Wiley-Blackwell, 2010). With Leo M. L. Nollet, he has published several books on food analysis published by CRC Press: *Handbook of Muscle Foods Analysis* (2009), *Handbook of Processed Meats and Poultry Analysis* (2009), *Handbook of Seafood and Seafood Products Analysis* (2010), *Handbook of Dairy Foods Analysis* (2010), *Handbook of Analysis of Edible Animal By-Products* (2011), *Safety Analysis of Foods of Animal Origin* (2011), *Sensory Analysis of Foods of Animal Origin* (2011), *Handbook of Analysis of Active Ingredients in Functional Foods* (2012), *Food Analysis by HPLC* (2013), and the third edition of the *Handbook of Food Analysis* (2015). He also coedited *Proteomics in Foods: Principles and Applications* for Springer (2013). He is one of the three editors-in-chief of the *Encyclopedia of Food and Health* with five volumes published by Academic Press/Elsevier (2016). He is the editor of the eighth edition of the famous book *Lawrie´s Meat Science* published by Woodhead/Elsevier (2017). Since 2016, he has been the editor of the prestigious series of books entitled *Advances in Food and Nutrition Research* (Academic Press/Elsevier).

Prof. Toldrá has been a member, by strict selection within the European Union, for two mandates on the scientific panel on food additives (2003–2008) and three mandates on the panel on flavorings, enzymes, processing aids, and materials in contact with foods (2008–2015) of the European Food Safety Authority where he was chairman of the Working Groups on Irradiation (2009–2010), Processing Aids (2011–2014), and Enzymes (2010–2015). In 2008–2009, he joined a group of Food and Agriculture Organization/World Health Organization experts to evaluate chlorine-based disinfectants in the processing of foods.

Leo M. L. Nollet obtained his MS (1973) and PhD (1978) in biology from the Katholieke Universiteit Leuven, Belgium. He is an editor and associate editor of numerous books. He edited for M. Dekker, New York—now CRC Press of Taylor & Francis—the first, second, and third editions of the books *Food Analysis by HPLC* and *Handbook of Food Analysis*. The last edition is a two-volume book. He also edited the *Handbook of Water Analysis* (first, second, and third editions) and *Chromatographic Analysis of the Environment,* third edition (CRC Press).

With Fidel Toldrá, he coedited two books published in 2006 and 2007: *Advanced Technologies for Meat Processing* (CRC Press) and *Advances in Food Diagnostics* (Blackwell Publishing—now Wiley). With M. Poschl, he coedited the book *Radionuclide Concentrations in Foods and the Environment,* also published in 2006 (CRC Press). Dr. Nollet has also coedited several books with Y. H. Hui and other colleagues: *Handbook of Food Product Manufacturing*

Downloaded From Vet-eBooks.com

(Wiley, 2007), *Handbook of Food Science, Technology and Engineering* (CRC Press, 2005), *Food Biochemistry and Food Processing* (first and second editions; Blackwell Publishing—now Wiley—2006 and 2012), and the *Handbook of Fruits and Vegetable Flavors* (Wiley, 2010). In addition, he edited the *Handbook of Meat, Poultry and Seafood Quality*, first and second editions (Blackwell Publishing—now Wiley—2007 and 2012).

From 2008 to 2011, he published five volumes on animal product–related books with Toldrá, namely, the *Handbook of Muscle Foods Analysis*, *Handbook of Processed Meats and Poultry Analysis*, *Handbook of Seafood and Seafood Products Analysis*, *Handbook of Dairy Foods Analysis*, and *Handbook of Analysis of Edible Animal By-Products*. In 2011, also with Toldrá, he coedited two volumes for CRC Press: *Safety Analysis of Foods of Animal Origin* and *Sensory Analysis of Foods of Animal Origin*. In 2012, they both published the *Handbook of Analysis of Active Compounds in Functional Foods*.

Dr. Nollet coedited, with Hamir Rathore, the books *Handbook of Pesticides: Methods of Pesticides Residues Analysis* in 2009, *Pesticides: Evaluation of Environmental Pollution* in 2012, and *Biopesticides Handbook* in 2015. Other finished book projects include *Food Allergens: Analysis, Instrumentation, and Methods* (with A. van Hengel, CRC Press, 2011) and *Analysis of Endocrine Compounds in Food* (Wiley-Blackwell, 2011).

Dr. Nollet's recent projects include *Proteomics in Foods* with Toldrá (Springer, 2013) and *Transformation Products of Emerging Contaminants in the Environment: Analysis, Processes, Occurrence, Effects and Risks* with D. Lambropoulou (Wiley, 2014). In the series *Food Analysis and Properties*, he edited *Flow Injection Analysis of Food Additives* with C. Ruiz-Capillas (CRC Press, 2015) and *Marine Microorganisms: Extraction and Analysis of Bioactive Compounds* (CRC Press, 2016).

Downloaded From Vet-eBooks.com



Taylor & Francis
Taylor & Francis Group
http://taylorandfrancis.com

Downloaded From Vet-eBooks.com

Contributors

Dong U. Ahn
Department of Animal Science
Iowa State University
Ames, Iowa

María J. Andrade
Department of Veterinary Science, Food Hygiene and Safety, Meat and Meat Products Research Institute
University of Extremadura
Cáceres, Spain

Keizo Arihara
Laboratory of Food Science
Kitasato University
Towada-shi, Japan

José M. Barat
Departamento de Tecnología de Alimentos
Universitat Politècnica de València
Valencia, Spain

Werner G. Bergen
Department of Animal Sciences
Auburn University
Auburn, Alabama

Tomás Bolumar
Group of Food Structure, Meat Science Team
CSIRO Agriculture and Food
Queensland, Australia

Sencer Buzrul
Tütün ve Alkol Piyasası Düzenleme Kurumu (TAPDK) Auditing Department
Ankara, Turkey

Carmen A. Campos
Universidad de Buenos Aires
Facultad de Ciencias Exactas y Naturales
Departamento de Industrias
Buenos Aires, Argentina

and

CONICET
Buenos Aires, Argentina

Marcela P. Castro
Departamento de Ciencias Básicas y Aplicadas
Laboratorio de Microbiología de Alimentos
Universidad Nacional del Chaco Austral
Presidencia Roque Sáenz Peña, Chaco, Argentina
Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET)
Buenos Aires, Argentina

María E. Cayre
Departamento de Ciencias Básicas y Aplicadas
Laboratorio de Microbiología de Alimentos
Universidad Nacional del Chaco Austral
Presidencia Roque Sáenz Peña, Argentina

Juan J. Córdoba
Food Hygiene and Safety, Meat and Meat Products Research Institute
University of Extremadura
Cáceres, Spain

Downloaded From Vet-eBooks.com

Nicoletta Ditaranto
Dipartimento di Chimica
Università degli Studi di Bari Aldo Moro
Bari, Italy

Stephanie M. Fowler
NSW Department of Primary Industries
Centre for Sheep and Red Meat Development
Cowra, Australia

Brandon Guild
Department of Food Science
University of Guelph
Guelph, Ontario, Canada

David L. Hopkins
NSW Department of Primary Industries
Centre for Sheep and Red Meat Development
Cowra, Australia

Terry A. Houser
Animal Sciences and Industry
Kansas State University
Manhattan, Kansas

Francisco Jiménez-Colmenero
Products Department
Instituto de Ciencia y Tecnología de Alimentos y Nutrición (ICTAN-CSIC)
Madrid, Spain

Joseph P. Kerry
Food Packaging Group
School of Food and Nutritional Sciences
University College Cork
Cork, Ireland

Yusuke Komiya
Laboratory of Food Science
Kitasato University
Towada-shi, Japan

Eun Joo Lee
Department of Food and Nutrition
University of Wisconsin-Stout
Menomonie, Wisconsin

Sabine Leroy
INRA
UR454 Microbiologie
Centre de Recherche
Auvergne-Rhône-Alpes, France

Antoon Lievens
European Commission
Joint Research Centre
Directorate F – Health, Consumers and Reference Materials
Unit F4 – Fraud Detection and Prevention
Geel, Belgium

Loong-Tak Lim
Department of Food Science
University of Guelph
Guelph, Ontario, Canada

Aubrey Mendonca
Department of Food Science and Human Nutrition
Iowa State University
Ames, Iowa

Linda Monaci
Istituto di Scienze delle Produzioni Alimentari
Consiglio Nazionale delle Ricerche
Bari, Italy

Downloaded From Vet-eBooks.com

Leticia Mora
Department of Food Science
Instituto de Agroquímica y Tecnología de Alimentos (CSIC)
Valencia, Spain

Kumari Shikha Ojha
Food Chemistry and Technology Department
Teagasc Food Research Centre
Dublin, Ireland

Ronald B. Pegg
Department of Food Science and Technology
University of Georgia
Athens, Georgia

Mauro Petrillo
European Commission
Joint Research Centre
Directorate F – Health, Consumers and Reference Materials
Unit F7 – Knowledge for Health & Consumer Safety
Ispra, Italy

Rosa Pilolli
Istituto di Scienze delle Produzioni Alimentari
Consiglio Nazionale delle Ricerche
Bari, Italy

Marise A. Rodrigues Pollonio
Department of Food Technology
School of Food Engineering
University of Campinas
Campinas, Brazil

Milagro Reig
Instituto de Ingeniería de Alimentos para el Desarrollo
Universidad Politécnica de Valencia
Valencia, Spain

Franco P. Rivas
Departamento de Ciencias Básicas y Aplicadas
Universidad Nacional del Chaco Austral
Presidencia Roque Sáenz Peña, Argentina and
Consejo Nacional de Investigaciones Científicas y Técnicas
Presidencia Roque Sáenz Peña, Argentina

Alicia Rodríguez
Food Hygiene and Safety, Meat and Meat Products Research Institute
University of Extremadura
Cáceres, Spain

Mar Rodríguez
Food Hygiene and Safety, Meat and Meat Products Research Institute
University of Extremadura
Cáceres, Spain

Rico Scheier
Department of Bioanalytical Science and Food Analysis
University of Bayreuth
Kulmbach, Germany

Heinar Schmidt
Department of Bioanalytical Science and Food Analysis
University of Bayreuth
Kulmbach, Germany

Joseph G. Sebranek
Animal Science and Food Science and Human Nutrition
Iowa State University
Ames, Iowa

Downloaded From Vet-eBooks.com

Fereidoon Shahidi
Department of Biochemistry
Memorial University of Newfoundland
St. John's, Newfoundland, Canada

Peter Šimko
Institute of Food Science and Nutrition
Slovak University of Technology
Bratislava, Slovakia

Da-Wen Sun
Food Refrigeration and Computerised Food Technology (FRCFT)
School of Biosystems and Food Engineering
University College Dublin
National University of Ireland
Agriculture and Food Science Centre
Dublin, Ireland

Régine Talon
INRA
UR454 Microbiologie
Centre de Recherche
Auvergne Rhône Alpes, France

Hans Bernard Tee
Department of Food Science
University of Guelph
Guelph, Ontario, Canada

Brijesh K. Tiwari
Food Chemistry and Technology Department
Teagasc Food Research Centre
Dublin, Ireland

Stefan Toepfl
Process Technologies
German Institute of Food Technologies
Quakenbrueck, Germany

Jun-Li Xu
Food Refrigeration and Computerised Food Technology (FRCFT)
School of Biosystems and Food Engineering
University College Dublin
National University of Ireland
Agriculture and Food Science Centre
Dublin, Ireland

Downloaded From Vet-eBooks.com

1

Genetically Modified Farm Animals: Control and Traceability

Antoon Lievens and Mauro Petrillo

CONTENTS

1.1 Introduction 1
1.1.1 Authorization Process 2
1.1.2 Field Use of GM Animals 3
1.1.3 Modification Strategies 4
1.1.4 Detection Options 7
1.1.4.1 Visual Inspection 7
1.1.4.2 Protein Detection 7
1.1.4.3 DNA Detection 7
1.1.5 Protein-Based Detection Strategies 7
1.1.5.1 Flow Strip 8
1.1.5.2 Enzyme-Linked Immunosorbent Assay 9
1.1.5.3 Mass Spectrometry 10
1.1.6 DNA Bases Detection Strategies 10
1.1.6.1 Polymerase Chain Reaction-Based Methods 10
1.1.6.2 Sequencing 11
1.1.7 Quantification Strategies 13
1.1.7.1 Quantitative Protein Methods 13
1.1.7.2 Quantitative PCR Methods 13
1.1.8 Outlook 14
References 14

1.1 Introduction

Traceability is one of the current key aspects of the European food safety policy. It has rapidly gained importance in the wake of the bovine spongiform encephalopathy (BSE) crisis, and currently, there is a widespread adoption of control and traceability systems in the entire agri-food industry.

Transparent and trustworthy regulatory and traceability processes are a cornerstone in addressing consumer concerns and providing a wider acceptance of animal biotechnology. In addition, proper traceability increases

Downloaded From Vet-eBooks.com

safety and quality control and allows faster identification and resolving of problems, and it also allows correct labeling, which provides consumers with information that is important for health and lifestyle choices. Traceability also forms an essential aspect of risk management and will be a major requirement for postmarketing surveillance when genetically modified (GM) animals enter the consumer market. However, before any GM product may enter the European market, it needs to be officially authorized (Frewer et al., 2013).

The main traceability legislation is stipulated in European Commission (EC) regulation 1830/2003 concerning the traceability and labeling of genetically modified organisms (GMOs) and the traceability of food and feed products produced from GMOs.* It requires member states to take measures to ensure traceability and labeling of authorized GMOs at all stages of their placing on the market. The European Union (EU) law further requires that foods and ingredients that contain, or are directly produced from, (authorized) GMOs must be clearly labeled as such. These requirements do not apply to foods containing GM material at <0.9%, provided that this presence is adventitious or technically unavoidable.

Although member states are free to choose how to implement these regulations, most have chosen a strategy of sampling and analysis throughout the food chain to check the labeling claims (note that, depending on the member state, it may or may not be allowed to label as "GMO-free"). This is carried out by member-state-run laboratories (Reference labs) that together with the European Reference Laboratory for GM Food and Feed (EURL GMFF) make up the European Network of GMO Laboratories (ENGL), a platform that develops, validates, and shares methods and information to harmonize the identification and quantification of GM samples. It is expected that the mandate of this current network will be expanded to also encompass samples derived from animal products.

In the following sections, we take a closer at the procedure for GMOs to enter the European market and discuss what methods will allow for control and verification of labeling claims at various stages of the food chain. Ideally, such methods should allow us to accurately distinguish GM from non-GM, and should preferably be fast and not require extensive sample preparation. In addition, the ideal detection method should be mobile and allow on-site control (be it on the farm, in the supermarket, or in the restaurant) (Loftus, 2005). However, as we see, depending on the case, only certain of these boxes may be checked.

* Regulation (EC) 1830/2003 of the European Parliament and of the Council of 22 September 2003 concerning the traceability and labelling of genetically modified organisms and the traceability of food and feed products produced from genetically modified organisms and amending directive 2001/18/EC.

Downloaded From Vet-eBooks.com

1.1.1 Authorization Process

Before a GMO is allowed to enter the European food or feed market, an authorization application it must be submitted to a national authority. The requirements for authorization are stipulated in regulation (EC) 1829/2003,* (EC) 641/2004,† and (EU) 503/2013.‡ Among other requirements, an application must include a monitoring plan, labeling proposal, and detection method.

The national authority will send the application to the European Food Safety Agency (EFSA) for a risk assessment. EFSA then makes the application summary available to the public.

In the risk assessment procedure, the safety of the gene product is assessed on a case-by-case basis. In the case of well-characterized proteins, a limited evaluation, such as amino acid sequence and expression rates in different tissues, may suffice. In the case of less well-documented proteins, there may be extensive toxicity testing, since, theoretically, GM animals may contain new proteins for which there is no history of safe use in the human diet.

Once EFSA has published its risk assessment, the public has 30 days to comment on the EC's website for applications, after which the commission will produce its final decision (within 3 months). The authorizations are valid for a maximum of 10 years and are renewable.

1.1.2 Field Use of GM Animals

Currently, the main European legislation consists of directive 2001/18/EC§ on the deliberate release of GMOs into the environment and directive 2015/412¶ amending the former, which allows member states to restrict or prohibit the cultivation of GMOs in their territory.

These directives explicitly incorporate the precautionary principle, and state that the introduction of GMOs into the environment should be carried out according to a "step-by-step" principle. Thus, the containment

* Regulation (EC) No 1829/2003 of the European Parliament and Council of 22 September 2003 on genetically modified food and feed.

† Regulation (EC) No 641/2004 of 6 April 2004 on detailed rules for the implementation of Regulation (EC) No 1829/2003 of the European Parliament and of the Council as regards the application for the authorization of new genetically modified food and feed, the notification of existing products and adventitious or technically unavoidable presence of genetically modified material which has benefited from a favourable risk evaluation.

‡ Commission implementing regulation on applications for authorisation of genetically modified food and feed in accordance with regulation (EC) No 1829/2003 of the European Parliament and of the council amending commission regulations (EC) No 641/2004 and (EC) No 1981/2006.

§ Directive 2001/18/EC of the European Parliament and of the concould of 12 March 2001 on the deliberate release of genetically modified organisms and repealing council directive 90/220/EEC.

¶ Directive (EU) 2015/412 of the European Parliament and of the Council of 11 March 2015 amending Directive 2001/18/EC as regards the possibility for the member states to restrict or prohibit the cultivation of genetically modified organisms in their territory.

Downloaded From Vet-eBooks.com

of a GMO should be gradually reduced and the scale of its release increased, step by step, but only after a positive evaluation of the previous step (in terms of the protection of human health and the environment) (Garcia, 2006).

A person or company applying for an authorization for "deliberate release" of a GMO must submit its application to the competent national authority of the member state where the release is to take place, and the eventual authorization will only be valid in this member state. Among other requirements, an application for deliberate release should contain a technical dossier including a full environmental risk assessment, a monitoring plan, and appropriate safety measures.

In practice, such safety measures will probably boil down to treating the GM animals as a completely different species than their non-GM counterparts. This may include separate housing and transport facilities and mandatory animal identification systems (Kok and Jones, 2003). However, member states may impose additional restrictions on the use and cultivation of GM animals.

1.1.3 Modification Strategies

To fully understand the complications that may arise in detecting GMOs, one has to have a basic understanding of the origin of their modifications and their effects on the microbiological level.

Historically, the most common methods for generating GM animals are probably *microinjection* (injecting DNA into the nucleus of fertilized egg cell) and *retrovirus-mediated gene transfer.* The success rate is rather low and the results are variable: the insertions are highly random in terms of location and number of copies inserted, making the disruption of essential genetic information a possibility (Houdebine et al., 2009; Robl et al., 2007; Kues and Niemann, 2011). Even in plants, where the existence of *Agrobacterium tumefaciens* makes for a more straightforward transformation, the random nature of the insertions complicates GMO production.

These technical limitations favored the insertion of larger sequence constructs that could operate independently from the region of insertion and carry selection markers to distinguish successfully modified cells. As a consequence, such inserts tend to come with easy targets for detection, either on the protein level or on the DNA level.

The advent of clustered regularly interspaced short palindromic repeat (CRISPR) technology (see Figure 1.1) is changing all this by allowing targeted insertion, thus taking part of the randomness out of the equation. In addition, CRISPR may allow for site-specific mutagenesis that can create variants of one specific protein (Go and Stottmann, 2016). As we see, such small changes may be problematic for detection.

Downloaded From Vet-eBooks.com

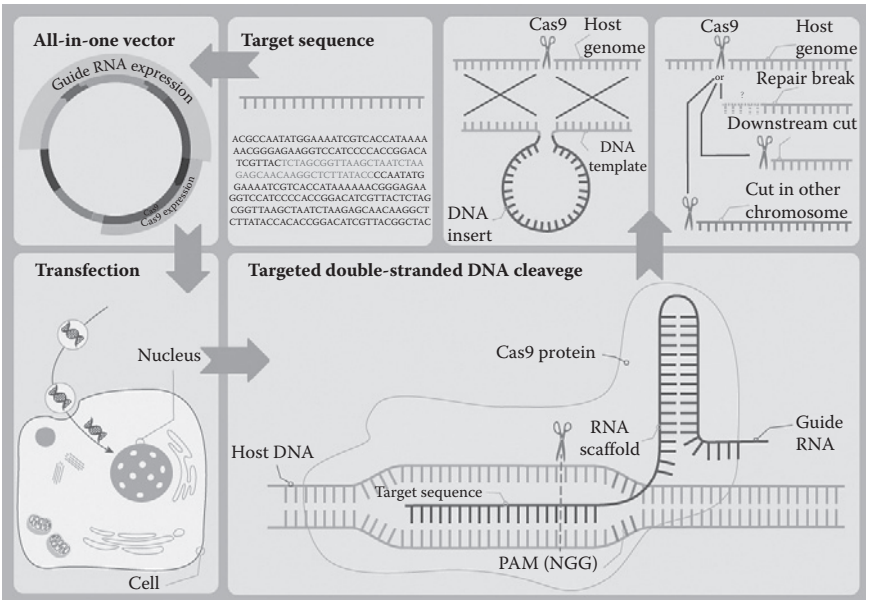


FIGURE 1.1
The clustered regularly interspaced short palindromic repeat (CRISPR) system consists initially of a single (circular) piece of DNA into which the target sequence is coned. Upon transfection into the cell, it is the cell's own which builds and assembles the protein complex. The CRISPR/Cas9 system recognizes specific DNA sequences using a guide RNA/protein complex based around a protospacer adjacent motif (PAM). The complex creates a double-stranded break in the DNA at the target site. The latter is subsequently repaired by the cell's DNA repair system, which will incorporate a template provide (homology-directed repair) or just close the break(s) (nonhomologous end joining).

Independent of the desired final effect (e.g., faster growth, virus resistance), we can divide the current types of modification into classes relevant to their detection as follows:
At the DNA level the following can occur:

- *Insertion of a sequence* into the host genome inevitably generates two unique junctions in addition to the (usually foreign) inserted gene, making this the most straightforward type of modification to detect. Even if the donor organism is the same species, the junctions at the insertion sites are unique to the GM and specific detection is possible.
- *Deletion of a sequence* yields, similar to insertions, a new, unique, junction that may be targeted for detection. Theoretically, exceptions are possible if the deletion took place in a high-repeat region; this might create an undetectable (nonunique) junction region.
- *Mutation of a sequence* consists of changing very few bases in the DNA, resulting in a highly similar variant of gene. Depending on

Downloaded From Vet-eBooks.com

the kind of change and its sequence context, the design of a routine detection method can be anything from straightforward to near impossible.

At the protein level there the following possible options:

- A *new protein is produced,* which has no equivalent in the wild-type organism. This is the most straightforward type of modification to detect. Independent of the technology used, a novel protein is an excellent detection marker.
- A protein is *no longer produced*. Essentially, the same situation as in the above, but with the GM and non-GM roles reversed. A negative test will now indicate a GMO. However, such a strategy will not work in mixed samples.
- A *modified version* of an existing protein is produced. Depending on if the modification results in unique protein-surface features, this protein may be detected using immunological methods, otherwise more complicated methods will be required.
- Changes in protein *timing or dosage*. GM animals may produce more or less of an endogenous protein, or may produce the protein in a different tissue or at a different stage of their life. Protein quantification approaches may yield a workable strategy in some stages of the food chain, but may not be a universal solution.
- *No change at all*. Not all DNA modifications yield proteomic changes and in these cases, protein testing will not be able to distinguish between GM and non-GM.

The *classes* of DNA modification are not causally linked to the *classes* of proteomic change: the insertion of DNA may not lead to the production of a protein, or may even prevent the production of a protein, and the deletion of part of the DNA may modify rather than suppress the protein production. And even more complicated constructs exist, for example, recombinase-mediated deletions where, depending on the construct, the resulting organism may or may not be mosaic for the deletion.

Theoretically, all modifications can be detected at the DNA level, but it is more difficult to develop detection methods for small changes than for large insertions. Likewise, some protein changes are more readily detected than others (e.g., a change in protein level vs. a new protein).

Overall, small changes are harder to detect than large changes. Because of the limitations of the techniques involved in producing GMOs, small changes were never much of an option . . . until recently. The advent of CRISPR has the potential to greatly accelerate the production of more subtle GMOs and threatens to throw the proverbial wrench in the current detection strategies.

Downloaded From Vet-eBooks.com

1.1.4 Detection Options

Depending on the place in the food chain, there may be different needs for detection. In the early part of the chain where animals are whole or their meat is unprocessed, qualitative detection (positive/negative) may suffice. Near the end of the chain, in processed materials or mixed samples, quantitative analysis may be required (How much of the sample is GM?) (Bonfini et al., 2002; Corbisier et al., 2007).

1.1.4.1 Visual Inspection

In live animals, visual identification of GM animals may be possible: fluorescent light can be used to recognize GloFish™ and featherless chickens are easily identified as such. However, other traits (disease resistance, meat composition) are not so straightforward to confirm, and one has to resort to other means.

1.1.4.2 Protein Detection

Changes in the proteome form an attractive target for detection. "New" or modified proteins are often suitable for immunological detection (see Figure 1.3). Such methods are highly specific, fast, and can be made in portable formats. However, not all proteins resits processing well and by the end of the food chain they may no longer be detectable.

1.1.4.3 DNA Detection

As changes to the DNA are the very definition of GMOs, the detection of these changes is a reliable way to detect and identify GMs (Holst-Jensen, 2009). As a consequence, DNA forms the current gold standard in detection and quantification of GMOs. In addition, its resistance to processing and its amplifiable nature (see Figure 1.2) makes for a low limit of detection throughout the entire production chain. As a drawback, DNA detection methods often require extensive sample preparation and highly specified equipment.

1.1.5 Protein-Based Detection Strategies

Proteins are manufactured in the cell according to the information coded by DNA. This DNA can be completely transgenic (GMO) and the protein produced can be foreign to the species (e.g., a bacterial toxin, CryIAb, in insect-resistant maize) (Magg et al., 2001) or it could be a modified version of a natural protein (e.g., a glyphosate-tolerant version of a plant enzyme, CP4-epsps, in herbicide-resistant soy) (Funke et al., 2006). The changes to the proteome can also be temporal (e.g., growth hormone at lower temperature in aquadvantage™ salmon) (Du et al., 1992) or in the amount of protein produced (e.g., sheep overexpressing insulin-like growth factor 1 in their skin to improve wool growth) (Damak et al., 1996).

Downloaded From Vet-eBooks.com

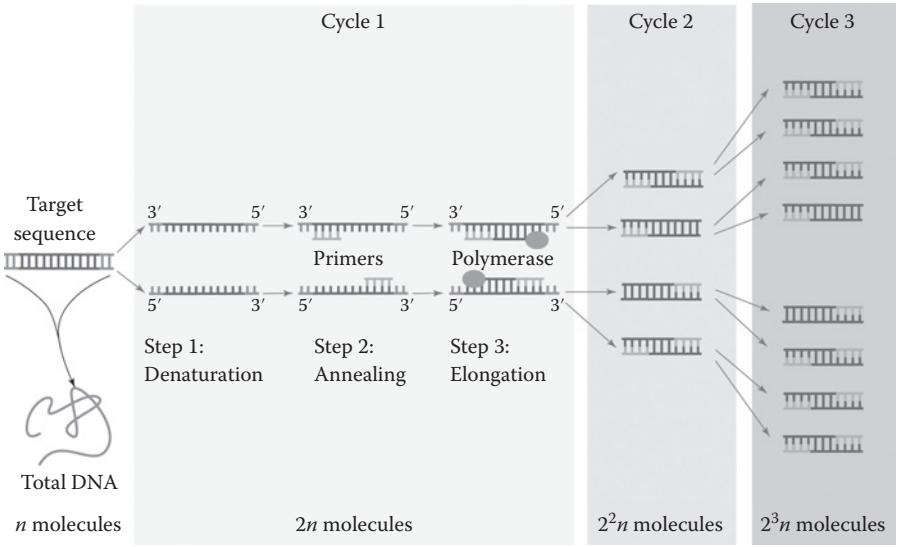


FIGURE 1.2
Enzymatic amplification of DNA (polymerase chain reaction [PCR]). An amplification cycle typically consists of three steps that allow duplication of the target DNA. The denaturation step consists of heating the reaction to a temperature of 95°, which disrupts the hydrogen bonds between complementary bases, yielding single-stranded DNA molecules. During the annealing step, the temperature is lowered to 60°, allowing binding of the primers to the single-stranded DNA template. For the elongation step, the temperature is raised to the optimum activity temperature of the polymerase enzyme (72° for Taq polymerase). At this step, the DNA polymerase synthesizes a new DNA strand complementary to the DNA template strand by adding nucleotides present in the reaction mix.

Proteins that have surface characteristics that are unique to GM animals are usually detectable by immunological methods (using antibodies that bind specifically to that protein) whereas other changes may only be detectable by more elaborate methods such as 2D gel electrophoresis and mass spectrometry (MS).

1.1.5.1 Flow Strip

The most commonly used immuno- or antibody-based test for GMO detection is the strip test (also known as a lateral flow device). The "devices" are thin strips of a nitrocellulose membrane covered by a sample pad on one end and a wicking pad on the other end (see Figure 1.3).

The business end of the strip is submersed in a solution of homogenized test sample (usually an aqueous solution). The solution wicks up the strip, passing the fluid over an area containing an excess of gold-labeled antibody specific to the GM protein being tested. If this protein is present then it will bind to the gold-labeled antibodies, and the antibody–protein complex will continue moving up the strip with the flow of fluid.

Downloaded From Vet-eBooks.com

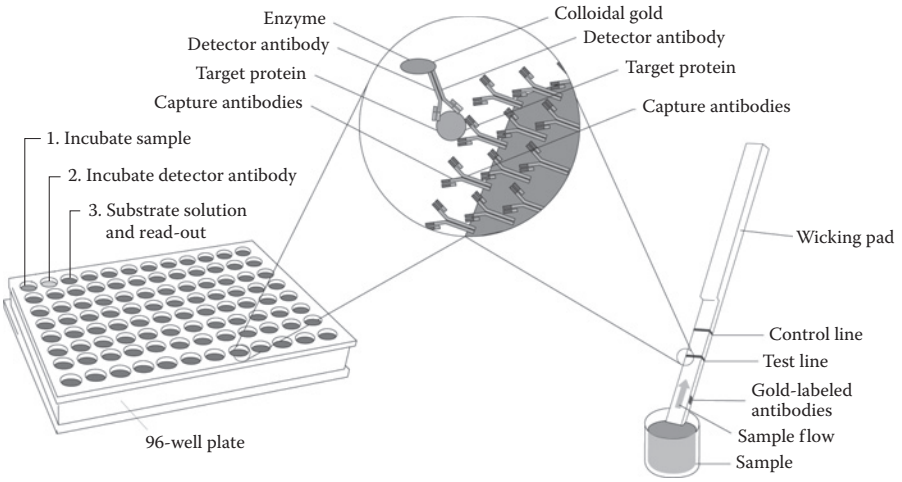


FIGURE 1.3
Immunological protein detection methods: Enzyme-linked immunosorbent assay (ELISA) (left) and flow strips (right) employ a common mechanism of capturing the protein of interest using an immobilized capture antibody and visualizing the protein using a second enzyme/gold-linked antibody.

The sample fluid then passes over two additional areas on the strip: (1) the test line, containing a second (immobilized) antibody specific for the GM protein that captures the antibody–protein complex, which results in formation of a visible line on the strip indicating the presence of the GM protein in the sample, and (2) the control line, containing a third (immobilized) antibody that will bind any other antibody (free or carrying protein) and which indicates that the strip has functioned correctly.

1.1.5.2 Enzyme-Linked Immunosorbent Assay

Enzyme-linked immunosorbent assay (ELISA) is a more sensitive antibody-based protein detection method. Here, the sample solution is added to a multiwell plate on which GM-protein-specific antibody has been immobilized (capture antibodies). If the GM protein is present in the sample, it will bind to the capture antibody. After washing, the detector antibody, also specific for the GM protein and tagged with an enzyme, is added to the well. The enzyme-linked detection antibody will bind any GM protein already immobilized to the well by the capture antibody, forming an antibody–protein–antibody sandwich. After another round of washing to remove any unbound antibody, a substrate is added, which is converted by the enzyme inducing a color change in the solution. Not only does the enzymatic reaction amplify the signal, but it also lowers the detection limit; the intensity of the color change is proportional to the amount of protein present, allowing quantification.

Downloaded From Vet-eBooks.com

1.1.5.3 Mass Spectrometry

Although antibody-based approaches are powerful and versatile, their main bottleneck is the availability of antibodies with high specificity. The development of high-quality antibody-based assays can be costly, time consuming and resource intensive. Another disadvantage of antibody-based assays is that they may not be able to discriminate between closely related proteins. MS provides a powerful alternative and complementary approach.

Essentially, a mass spectrometer measures the masses of the molecules within a sample. The technique relies on magnetic and electric fields which exert forces on charged particles (ions) in vacuum in order to determine their mass–charge ratio. Therefore, the sample must be ionized prior to be analysis. In addition, the ions must be introduced into the system as a gas so that the molecules are individualized, allowing the determination of their single masses. However, proteins are nonvolatile and thermally unstable, and require special "soft" ionization methods that do not cause extensive degradation. Beside the ionization source, a mass spectrometer also consist of a mass analyzer, a detector, and the data processing electronics.

The workflow usually consists of breaking down the protein in smaller parts (peptides), which yields a spectrum of masses (a peptide mass fingerprint) which is used as input for a search in a database that holds the predicted masses for each protein. Complex samples can hold an overwhelming number of components that yield spectrums that are very difficult to interpret. Therefore, it is usually necessary to enrich, purify, or separate the proteins prior to MS analysis (e.g., via 2D gel or high-performance liquid chromatography [HPLC]).

As MS is a much more versatile tool for protein analysis, it is able to see very small differences between proteins and is not limited to any specific protein. Unfortunately, a mass spectrometer is an expensive, very much nonportable piece of equipment that may require extended sample preparation, and relies on the availability of databases for the identification of proteins.

1.1.6 DNA Bases Detection Strategies

As mentioned above, DNA analysis forms the current gold standard in detection and quantification of GMOs within the EU. The technology has been optimized to produce a high-throughput screening and quantification system that has been harmonized between member states and has been standardized in terms of performance.

1.1.6.1 Polymerase Chain Reaction-Based Methods

At present, (real time) polymerase chain reaction (PCR)-based analysis is the method of choice for the routine analysis of food and feed samples for

Downloaded From Vet-eBooks.com

their GMO content and authenticity (Loftus, 2005; Mafra et al., 2008). They work by targeting specific DNA sequences (between 60 and 200 bp long) for enzymatic amplification (also see Figure 1.2), revealing the presence (amplification) or absence (no amplification) of the target sequence. By following the amplification process fluorometrically (in real time,) quantification of the initial amount of target sequence becomes possible.

Traditionally, three levels of specificity are distinguished within GMO-detection methods (Figure 1.4):

Screening methods target DNA sequences that are frequently inserted into GMOs, for example, promoters, terminators, sequences of genes conferring certain tolerances, or resistances. These methods allow the detection of a broad range of GMOs, but they do not allow identification with absolute certainty of which GM event(s) is (are) present in the sample.

Construct-specific methods target two or more adjacent genetic elements in a transgene construct by amplifying their junction. These methods are more informative in terms of presence or absence of specific events, but cannot distinguish between different events containing the same (or similar) constructs.

Event-specific methods target the host-transgene DNA junction. As a consequence, these methods are highly specific and amplify only a single event. They are usually applied in downstream confirmation of GM-positive samples.

The current workflow surrounding GM sample processing includes the following steps: (1) analysis of the meta information surrounding a sample in order to decide which screening methods to used (e.g., only markers related to a single crop or a full screening), (2) running of these screening methods and if all are negative, stopping the testing, (3) in case of positive results, identifying possible events and running the event-specific tests, possibly quantifying any positive result in the same run, and (4) if no event-specific tests show a positive result, declaring the sample contaminated with a nonauthorized GMO.

The downside of PCR-based detection is that, much like immunological methods, you can only find what you are looking for: each reaction tests the absence/presence of a specific sequence. Any GM sequence that is present but not tested for cannot be detected. In addition, PCR-based tests are difficult to design and optimize for small sequence changes.

1.1.6.2 Sequencing

Where PCR cannot distinguish between GM and non-GM, or when a complete characterization of a sample is required, direct DNA sequencing may offer a solution (Lievens et al., 2015). Sequencing has come a long way in

Downloaded From Vet-eBooks.com

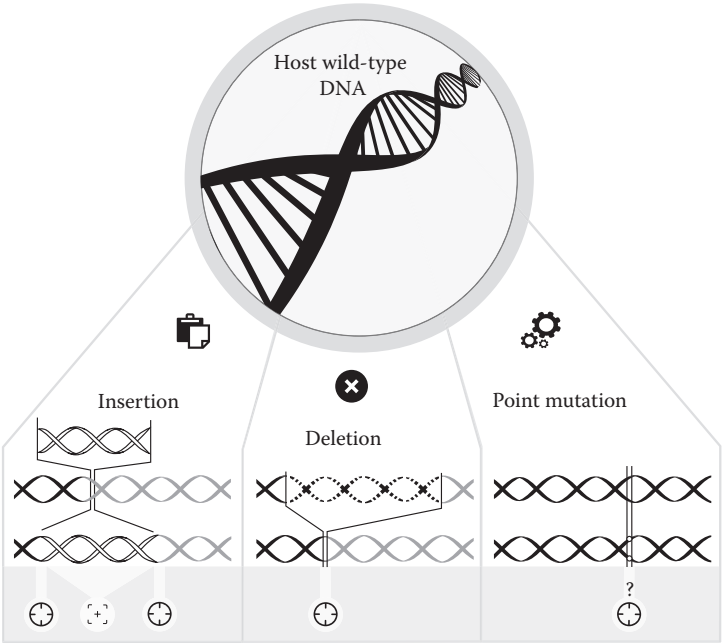


FIGURE 1.4
Schematic overview of detection targets. DNA is represented in different ways for explanatory purpose. Insertions are represented as outlined stretches of DNA, deletions as the omission of dashed stretches of DNA, grey is used to help indicate the new junction after modification. Depending on the type of modification (insertion, deletion, and point mutation), there are different options for event-specific identification (circular crosshairs) or construct-specific detection (square crosshairs).

recent decades, and the current state of the art allows the massive parallel sequencing of entire genomes (so-called "next generation sequencing" [NGS]).

Several approaches exist (semiconductor sequencing, pyro-sequencing, nanopore sequencing, etc.), but the common strategy entails "reading" many short sequences (usually 100–400 bp depending on the technology). From the overlap between the millions of "reads" generated the original sequence is rebuilt. The technology allows either concentrating on a specific sequence (captured or preamplified) or addressing the entire sequence diversity of the sample.

Much like MS, the equipment is expensive compared to standard PCR (although prices are steadily declining) and may require extended sample preparation. In addition, there is a bioinformatics bottleneck in the processing of the "reads," which may be challenging without dedicated software and computing cluster. On the plus side, most sequencing platforms have the

Downloaded From Vet-eBooks.com

ability to "tag" DNA with specific short sequences (barcoding), which allows pooling of samples since the software can use the barcode to distinguish which sequence came from which sample.

1.1.7 Quantification Strategies

The current European labeling requirement applies to food and feed when their GM content is higher than 0.9% (weight/weight) on a per ingredient base. Other thresholds exist (e.g., 5% in Japan and 1% in Brazil) but irrespective of their exact value, there is a need for accurate quantification (Zel et al., 2012; Trapmann et al., 2010).

Most quantification strategies are relative in nature and require standards or reference materials to which a sample is compared and, generally speaking, a quantification result is often only as good as the standard that has been used. Materials of high purity and well-defined GM content are key in the process of efficiently controlling for a threshold.

1.1.7.1 Quantitative Protein Methods

The current GM quantification strategies are almost exclusively nuclear DNA based, which has the advantage that each (somatic) cell carries the exact same amount of target copies. Although not straightforward, the relation between the number of DNA targets and sample weight is more direct than for proteins. Protein levels can change over time and may differ between tissues. Compared to DNA, a lot less work has been done in the establishment of a protein-based GM quantification approach. Nevertheless, ELISA, MS, and even strip tests can be made to function quantitatively and combined with the right sampling strategies may provide a useful tool in quality assurance.

1.1.7.2 Quantitative PCR Methods

Real time PCR-based GM quantification involves the parallel detection of two targets: a wild-type endogene and an event-specific transgene. Calibration curves for both genes are constructed using reference materials. The complete result then allows accurate quantification of the GM content of the sample over a wide dynamic range. Digital PCR, on the other hand, allows the direct absolute quantification of the target and could function without the need for reference materials.

The current quantification approach is based on the assumption of equivalence of the percentage of GM target in DNA and the weight/weight percentage of GM in the ingredient of the original sample. Even though there are many factors that may complicate this assumption (cell size varies between plant tissues, fruits and seeds may be (partially) haploid, etc.) a large body of

Downloaded From Vet-eBooks.com

practical experience currently justifies its use and its existence as an official recommendation from the EC (i.e., EC 2004/787).*

In animals, there are again several biological facts that complicate the assumption of equivalence between DNA ratio and mass percentage. The most straightforward relating to the nature of muscle tissue is that muscle consists of multinucleated myofibers and their growth mainly happens by increases in length and width of the muscle fibers rather than by an increase of the number of fibers (and therefore, in the number of cell nuclei). However, there is significant postnatal DNA accumulation in muscle tissue insofar that there is a quite linear relation between the number of nuclei and the muscle. Transferability of the equivalence of DNA and weight percentages to animals thus seems likely but will have to be confirmed and validated before it can be put to practice at the regulatory level.

1.1.8 Outlook

Traceability is currently well established as a key risk management tool and component of the agri-food quality system. Animal identification systems are already mandatory in several regions worldwide and GMO testing is commonplace where labeling requirements have been installed. These developments have largely paved the way for the advent of biotech-derived animals, as most of the building blocks are already in place. The further adoption of molecular-based systems in the food production process has the potential to answer most traceability challenges GM animals will pose. It is likely that the biggest bottleneck in the introduction of biotech animals will not be of a technical nature, but rather regulatory.

References

Bonfini, L., Kay, S., Heinze, P. and Van den Eede, G. (2002). Report on GMO detection identification and quantification methods submitted to collaborative studies. *European Communities, EUR 20383 EN*, 1–29.

Corbisier, P., Broothaerts, W., Gioria, S. et al. (2007). Toward metrological traceability for DNA fragment ratios in GM quantification. 1. Effect of DNA extraction methods on the quantitative determination of Bt176 corn by real-time PCR. *Journal of Agricultural and Food Chemistry,* **55**, 3249–3257.

Damak, S., Sul, H.-Y., Jay, N. P. and Bullock, D. W. (1996). Improved wool production in transgenic sheep expressing insulin-like growth factor 1. *Biotechnology,* **14**(2), 185–188.

* Commission recommendation 2004/787/EC of 4 October 2004 on technical guidance for sampling and detection of genetically modified organisms and material produced from genetically modified organisms as or in products in the context of Regulation (EC) No 1830/2003.

Downloaded From Vet-eBooks.com

Du, S. J., Gong, Z., Fletcher, G. L., Shears, M. A., King, M. J., Idler, D. R. and Hew, C. L. (1992). Growth enhancement in transgenic Atlantic salmon by the use of an "all fish" chimeric growth hormone gene construct. *Nature Biotechnology,* **10**(2), 176–181.

Frewer, L., Kleter, G., Brennan, M. et al. (2013). Genetically modified animals from life-science, socio-economic and ethical perspectives: Examining issues in an EU policy context. *New Biotechnology,* **30**, 447–460.

Funke, T., Han, H., Healy-Fried, M. L., Fischer, M. and Schönbrunn, E. (2006). Molecular basis for the herbicide resistance of roundup ready crops. *Proceedings of the National Academy of Sciences,* **103**(35), 13010–13015.

Garcia, P. R. (2006). Directive 2201/18/EC on the deliberate release into the environment of GMOs: An overview and the main provisions for placing on the market. *Journal for European Environmental and Planning Law,* **3**, 3–12.

Go, D. E. and Stottmann, R.W. (2016). The impact of CRISPR/Cas9-based genomic engineering on biomedical research and medicine. *Current Molecular Medicine,* **16**(4), 343–352.

Holst-Jensen, A. (2009). Testing for genetically modified organisms (GMOs): Past, present and future perspectives *Biotechnology Advances,* **27**, 1071–1082.

Houdebine, L.-M. (2009). Methods to generate transgenic animals. *In:* Engelhard, M., Hagen, K. and Boysen, M. (eds.) *Genetic Engineering in Livestock* (Vol. 34). Berlin: Springer-Verlag, 31–48.

Kok, E. J. and Jones, W. (2003). The food safety risk assessment of GM animals. Topic Paper 4. FAO/WHO Expert Consultation on Safety Assessments of Foods Derived From Genetically Modified Animals, Including Fish. FAO, Rome.

Kues, W. and Niemann, H. (2011). Advances in farm animal transgenesis. *Preventive Veterinary Medicine,* **102**, 146–156.

Lievens, A., Petrillo, M., Querci, M. and Patak, A. (2015). Genetically modified animals: Options and issues for traceability and enforcement. *Trends in Food Science and Technology*, **44**, 159–176.

Loftus, R. (2005). Traceability of biotech-derived animals: Application of DNA technology. *Revue Scientifique et Technique, Office International des Epizooties,* **24**, 231–242.

Mafra, I., Ferreira, I. and Oliveira, M. (2008). Food authentication by PCR-based methods. *European Food Research and Technology,* **227**, 649–665.

Magg, T., Melchinger, A. E., Klein, D. and Bohn, M. (2001). Comparison of Bt maize hybrids with their non-transgenic counterparts and commercial varieties for resistance to European corn borer and for agronomic traits. *Plant Breeding,* **120**, 397–403.

Robl, J., Wang, Z., Kasinathan, P. and Kuroiwa, Y. (2007). Transgenic animal production and animal biotechnology. *Theriogenology,* **67**, 127–133.

Trapmann, S., Corbisier, P., Schimmel, H. and Emons, H. (2010). Towards future reference systems for GM analysis. *Analytical and Bioanalytical Chemistry,* **396**, 1969–1975.

Zel, J., Milavec, M., Morisset, D., Plan, D., Van den Eede, G. and Gruden, K. (2012). *How to Reliably Test for GMOs.* New York, NY: Springer.

Downloaded From Vet-eBooks.com



Taylor & Francis
Taylor & Francis Group
http://taylorandfrancis.com

Downloaded From Vet-eBooks.com

2

Hyperspectral Imaging Technique for Online Monitoring of Meat Quality and Safety

Jun-Li Xu and Da-Wen Sun

CONTENTS

2.1 Introduction 18
2.2 Fundamentals of Hyperspectral Imaging 20
2.2.1 Basic Principles 20
2.2.2 Data Structure 22
2.2.3 Image Sensing Modes 23
2.2.4 Acquisition of Hyperspectral Images 24
2.3 Hyperspectral Imaging Instruments 26
2.3.1 Illumination Unit 27
2.3.2 Wavelength Dispersion Devices 28
2.3.2.1 Imaging Spectrographs 28
2.3.2.2 Filter Wheels 29
2.3.2.3 Tunable Filters 29
2.3.3 Area Detectors 30
2.3.4 Hyperspectral Imaging System Calibration 30
2.3.4.1 Spatial Calibration 31
2.3.4.2 Spectral Calibration 31
2.3.4.3 Flat-Field Correction 32
2.4 Hyperspectral Imaging Data Processing and Multivariate Analysis 33
2.4.1 Image Segmentation 33
2.4.1.1 Thresholding and Morphological Processing 34
2.4.1.2 Spectral Image Segmentation 36
2.4.2 Spectral Preprocessing 37
2.4.3 Multivariate Analysis 39
2.4.3.1 Multivariate Classification 39
2.4.3.2 Multivariate Regression 41
2.4.3.3 Model Evaluation 43
2.4.4 Selection of Important Wavelengths 44
2.5 Online Applications for Meat Quality and Safety Assessment 45
2.5.1 Poultry Carcass 48

Downloaded From Vet-eBooks.com

2.5.1.1 Contamination Detection....49
2.5.1.2 Tumor and Bone Detection....52
2.5.1.3 Microbiological Spoilage Detection....55
2.5.2 Fish....56
2.5.2.1 Freshness....57
2.5.2.2 Chemical Compositions....58
2.5.2.3 Parasites and Defects....60
2.5.2.4 Microbial Spoilage....61
2.5.2.5 Differentiation and Classification/Sorting....62
2.5.3 Red Meats....62
2.5.3.1 Sensory Attributes....64
2.5.3.2 Chemical Compositions....67
2.5.3.3 Microbiological Properties....69
2.5.3.4 Technological Attributes....70
2.6 Conclusions....72
References....73

2.1 Introduction

Accurate and rapid assessment of meat quality parameters has always been a big concern because consumers are always demanding and expecting safe meat and meat products with superior quality. To meat consumers' needs, it is significant for the meat industry to ensure the safe production of meat during processing operations and the correct labeling of products with regard to safety, quality, authenticity, and compliance. Quality and safety of meat are normally defined by physical attributes (e.g., color, texture, tenderness, and marbling), chemical attributes (e.g., moisture, protein, and fat content), and microbiological attributes (e.g., total viable count [TVC]). Traditionally, assessment of meat quality and safety involves human visual inspection which, however, is subjective, laborious, and inconsistent. Currently, different techniques such as sensory analysis, chemical procedures, instrumental methods, and screening methods, such as mass spectrometry (MS) and high-performance liquid chromatography (HPLC), are commonly used to provide information about meat quality. These methods play a pivotal role in current industrial meat quality and safety inspection, and some of them have been applied as old standards and regulation methods in scientific research because of their relative accuracy and validity. However, they have some unavoidable limitations, such as being destructive, tedious, time consuming, sometimes environmentally unfriendly, and unable to handle a large number of samples. These obvious disadvantages of conventional methodologies necessitate the need for rapid, accurate, fast, noninvasive, and real-time detection techniques, so as to optimize quality and assure safety of meat.

In order to surmount the aforementioned disadvantages, the meat industry has experienced dramatic changes by adopting the most advanced

Downloaded From Vet-eBooks.com

technological innovations. A great number of methods, which are based on different principles, procedures, and/or instruments, are available now to measure meat traits. One of these methods is a spectroscopic technique that depends on the interaction between incident light and molecules in matters. Recently, near-infrared spectroscopy (NIRS) has received considerable attention as a fast and noncontact analytical tool in food quality assessment. NIRS is a useful and reliable technique and is relatively easy to implement for both offline and online applications. Meanwhile, NIRS has the potential for the measurement of multiple attributes at the same time. In other words, NIRS is capable of simultaneously collecting the physical, chemical, and microbiological information within the tested products by scrutinizing the changes of spectra. However, this spectroscopic technique is not able to provide important spatial information because it analyzes the sample in bulk and evaluates an average composition across the whole sample. In order to obtain spatial information, a smart technology, that is, computer vision (Sun, 2007) has been developed for automated visual inspection to replace the traditional inspection by human inspectors. Computer vision imitates the principle of human vision, using three bands (red, green, and blue) to acquire the characteristics of the tested object, such as color, shape, size, and texture. Unfortunately, computer vision has some limitations that make certain industrial applications unavailable. External attributes such as shape, size, texture, color, and external defects can be easily detected by ordinary computer vision, yet internal attributes are difficult to evaluate with the traditional imaging means. Working in visible range, it is generally not efficient to predict chemical and biological parameters and detect invisible defects. Both spectroscopy and computer vision techniques have found a wide range of applications in the food industry even though they have their own inherent drawbacks (Brosnan and Sun, 2004). As an integrated alternative, hyperspectral imaging was developed to obtain both spectral and spatial information from the target.

In recent years, the hyperspectral imaging technique, also known as imaging spectroscopy or imaging spectrometry, has been considered a promising and versatile analytical tool for the food research and industry. Hyperspectral imaging was originally developed in the early 1970s for mainly remote sensing applications (Adam et al., 2010). The invention of the first charge-coupled device (CCD) detector by George Smith and Willard Boyle played a crucial role in moving hyperspectral imaging technology forward. It started blooming in many other different fields, such as pharmaceutical research, food science, forensic science, and agriculture (ElMasry and Sun, 2010). Indeed, the feasibility of measuring a whole spectrum over the ultraviolet (UV), visible, and near-infrared (NIR) spectral regions (300–2600 nm) for every single pixel within the sample has made it very attractive for many different applications.

Hyperspectral imaging goes beyond conventional imaging and spectroscopy through obtaining a spatial map of spectral variation (Gowen et al., 2015). Therefore, the merits in spectroscopy and computer vision are both

Downloaded From Vet-eBooks.com

reflected in hyperspectral imaging. In addition, more benefits are obtained besides these aggregated advantages from both methods. For example, hyperspectral imaging can generate distribution maps to visualize different biochemical constituents presented in a tested sample based on their special spectral signatures. The huge spectral information stored in the hyperspectral images allows different objects to be identified and distinguished even if they have similar morphological features, colors, and overlapping spectra. Compared to conventional methods for evaluation of meat quality parameters, hyperspectral imaging is a fast, sensitive, noninvasive, and chemical-free analytical technique with minimal sample preparation, providing both qualitative and quantitative measurements for numerous meat properties simultaneously. For example, it can be used as an automatic inspection protocol to identify contaminants with the capability of causing illness in humans in meat and meat products. The pioneering research of applying hyperspectral and multispectral imaging techniques to determine various contaminants on poultry carcasses was carried out by the United States Department of Agriculture's (USDA) Agricultural Research Service, and some of them have already been set up in an online monitoring line (ElMasry and Sun, 2010). Additionally, research on meat safety inspection using hyperspectral imaging has been extended to reach the area of microbial spoilage detection. Some other fundamental applications, such as bone fragments detection, overall quality inspection, grade classification, and fraud authentication have also been investigated in many published works and good results were collected (Wu and Sun, 2013b). Therefore, hyperspectral imaging is a powerful analytical technique in the meat industry with great potential for inspection, monitoring, control, quantification, classification, and identification purposes. In this chapter, a comprehensive overview of the recent developments in using hyperspectral imaging systems for the online applications of monitoring meat quality and safety parameters is presented. Basics and theoretical aspects of this technique, the main features of the instrumentation, and the commonly used chemometrics algorithms to handle the hyperspectral data are also provided and briefly discussed. In particular, the latest research and online applications using hyperspectral imaging on white meat (poultry and fish) and red meat (pork, lamb, and beef) are described and highlighted in detail.

2.2 Fundamentals of Hyperspectral Imaging

2.2.1 Basic Principles

At the molecular level, all sample materials continuously absorb and emit energy by reducing or raising their molecular energy levels. Thus, spectroscopic tools provide detailed fingerprints by using physical characteristics

Downloaded From Vet-eBooks.com

of the interaction between electromagnetic radiation and physicochemical materials of foods, such as absorbance, reflectance, transmittance, phosphorescence, fluorescence, and radioactive decay. Electromagnetic waves usually consist of UV radiation, visible light (VIS), NIR, mid-infrared, and far-infrared (FIR) (Huang et al., 2014). NIRS is one of the most popular strategies among these spectroscopic techniques within the food industry. As with any biological material, food tissues are the combination of many various pure compounds with different molecular bonds and forces. Water, fats, and carbohydrates are rich in C–H or O–H bonds, while organic compounds and petroleum derivatives contain many C–H or N–H bonds. The absorption bands in the NIR range arise from overtones and combination bands of C–H, O–H, N–H, and S–H stretching and bending vibrations, enabling NIRS to provide characteristic and detailed fingerprints of food samples by using these observed changes in molecular energy levels. Since all the biological substances consist of thousands of molecular bonds, the exposure of a sample to electromagnetic waves accounts for a complex spectrum that provides the detailed qualitative and quantitative information about the physical and chemical changes of that sample. At the macro level, the electromagnetic wave is observed as light, and the change of the incident electromagnetic wave is presented as the scattering, reflection, and transmission of light. After the light penetrates into the tissue of samples, the emerging light obtained is converted to a spectrum, which could indicate the chemical constituents and physical properties of the food samples.

On the other hand, measurement of the external features, such as color, texture, shape, and size parameters, of food products can be achieved by a conventional imaging system or more specifically computer vision. Computer vision involves the capturing, processing, and analyzing of images, accounting for the rapid, objective, and nondestructive assessment of visual quality characteristics (Timmermans, 1995). Images are first acquired with a sensor and dedicated computing hardware and software are implemented to analyze the images with the aim of performing a predefined visual task. This technology attempts to duplicate the effect of human vision by electronically perceiving and understanding an image (Brosnan and Sun, 2004). It has been tested successfully for objective measurement of various agricultural and food products (Wang and Sun, 2001). Nevertheless, a computer vision system is incapable of inspecting targets with similar color, predicting chemical components, classifying complex objectives, and detecting invisible defects because it operates at visible wavelengths in the forms of monochromatic or color images.

Hyperspectral imaging is defined as a system that is able to simultaneously acquire spatial images in a very large number of spectrally discrete or contiguous bands measured from a remotely operated platform (Schaepman, 2007). By integration of the main advantages of spectroscopy and imaging, hyperspectral imaging enables us to simultaneously provide external physical and geometrical features as well as the inside chemical properties of the product. In other words, if conventional spectroscopy answers the question

Downloaded From Vet-eBooks.com

of what and conventional imaging answers the question of where, hyperspectral imaging can provide the answer to the question of where is what.

2.2.2 Data Structure

A hyperspectral image is a three-dimensional (3D) hyperspectral cube (also called a hypercube, spectral cube, data cube, or spectral volume), which comprises two spatial dimensions (of x rows and y columns) and one spectral dimension (of λ wavelengths). A hypercube provides rich information about the objective food product, including physical and geometric observations of size, shape, color, and texture, together with chemical/molecular information such as fat, water, protein and other hydrogen-bond constituents (Sun, 2010). The 3D dataset contains a stack of images of the same object, where each one of them is measured at a different wavelength. Since pixels are digitalized intensities or gray values at a certain wavelength, they are interpreted as integers. The intensity value of each pixel might have 8-bit gray values meaning that 0 is the black and 255 is the white.

Figure 2.1 displays one example of the hypercube acquired from a piece of salmon fillet. The raw hyperspectral image comprises a series of continuous subimages and each of them presents the intensity and spatial distribution of the tested sample at a certain waveband. It should be noted that the collective images for all wavelengths are intrinsically the same as a conventional digital image: they both provide optical perception in a spatial domain. The only difference exists in the number of wavelengths involved and thereby the amount of characteristic information captured. Particularly, for a hypercube, the object of interest in a certain subimage may either be apparent or merged in the background and noise, based on the wavelength concerned. Therefore, effective image analysis approaches should be carefully established and employed to sift through useful bands, which will be discussed later.

As shown in Figure 2.1, the hyperspectral image described as $I(x, y, \lambda)$ can be viewed either as a separate spatial image $I(x, y)$ at each individual wavelength λ, or as a spectrum $I(\lambda)$ at each individual pixel (x, y). Full spatial features of the tested object could be viewed at a different single waveband (pseudocolor image) as depicted in Figure 2.1 to show how the sample gives different reflectance values at different wavelengths based on its chemical composition. Meanwhile, spectral features can be viewed in the form of a spectrum, which acts like a fingerprint to characterize the composition of that particular pixel. For a given tested object, when the reflectance value (also absorbance or transmittance) is plotted against wavelengths, the resulting curve is considered the spectral signature or spectral fingerprint for that object. In essence, the spectral signature can be utilized to uniquely identify, characterize, and discriminate between classes/types of any given material(s) in each pixel of the image (Elmasry et al., 2012b). For instance, salmon is a fat-rich fish with a large proportion of lipids congregated in white stripes of connective tissue, segmenting the red-colored muscle tissue

Downloaded From Vet-eBooks.com

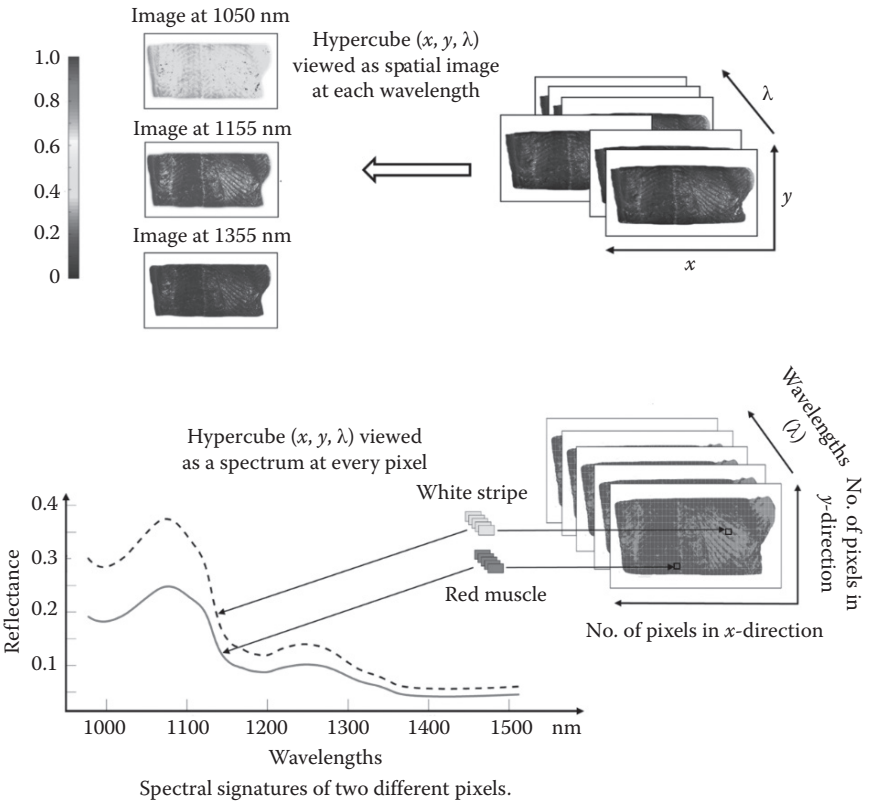


FIGURE 2.1
The conceptual view of a hypercube for a salmon fillet.

in vertical blocks and presenting a zebra-like appearance. Figure 2.1 shows the difference in spectral signatures between white stripe pixel and red muscle pixel of the tested salmon fillet. According to Xu et al. (2016), though these two spectra shared a similar spectral pattern, the white stripe demonstrated higher reflectance (lower absorbance) values over the whole wavelength range owing to different concentrations of major chemical compositions. Therefore, it is possible to identify either white stripes or red muscles from captured spectral signatures.

2.2.3 Image Sensing Modes

There are three common sensing modes for hyperspectral imaging, namely reflectance, transmittance and interactance, based on the relative arrangement between the light source and the optical detector (spectrograph, camera, and lens). In reflectance mode, the detector collects the reflected light from the illuminated sample in a specific conformation to avoid specular

Downloaded From Vet-eBooks.com

reflection. External quality features such as shape, size, surface texture, color, and external defects are typically detected using reflectance mode. In transmittance mode, the detector is located in the opposite side of the light source and captures the transmitted light penetrated through the substance. The amount of light through the sample is normally very small but carries more valuable information. Transmittance mode is mostly used to determine internal ingredient concentration and detect internal defects of relatively transparent materials such as vegetables, fruit, and fish (Wu and Sun, 2013a). However, transmittance mode has a low signal level from light attenuation and is affected by the thickness of the sample. Therefore, a more powerful light source and a sensitive detector are required in this mode. Interactance mode is a combination of transmittance and reflectance where both the light source and the detector are located on the same side and parallel to each other (ElMasry and Wold, 2008). Compared to reflectance mode, the interactance method can detect information deeper into the sample and has less surface effects. In addition, the interactance mode can reduce the influence of thickness, which is a practical advantage when compared to transmission mode. However, a special setup with a light seal is required in interactance mode to avoid interference from the environment, which may affect the received light.

2.2.4 Acquisition of Hyperspectral Images

Technically, there are four approaches to acquire hyperspectral images, which are point scanning, line scanning, area scanning, and the single-shot method, as illustrated in Figure 2.2. The point scanning method, known as the whiskbroom approach, is completed by acquiring a complete spectrum of a single point (pixel) at a time and then moving either the detector or the sample along two spatial dimensions (x and y), after which and another spectrum is taken. The great advantage of point scanning is that the light for every spatial element passes through the same path of the optical system. However, it is very time consuming for positioning the sample and needs advanced repositioning hardware to ensure repeatability. The hypercube obtained from this configuration is stored in the band-interleaved-by-pixel (BIP) format. This format is optimal for accessing the spectral information of each pixel and is commonly used for microscopic imaging where the acquisition time is usually not a problem.

The line scanning method (also known as the pushbroom method) can be regarded as an extension of the whiskbroom method. It simultaneously records a whole line of an image rather than a single pixel, together with spectral information corresponding to each pixel in the line at a time. A slit of the object is imaged onto a row of pixels on one dimension of the sensor chip and the spectrograph yields a spectrum for each point on the second dimension of the chip. As illustrated in Figure 2.2, a special image (y, λ) with one spatial dimension (y) and one spectral dimension (λ) is acquired at a

Downloaded From Vet-eBooks.com

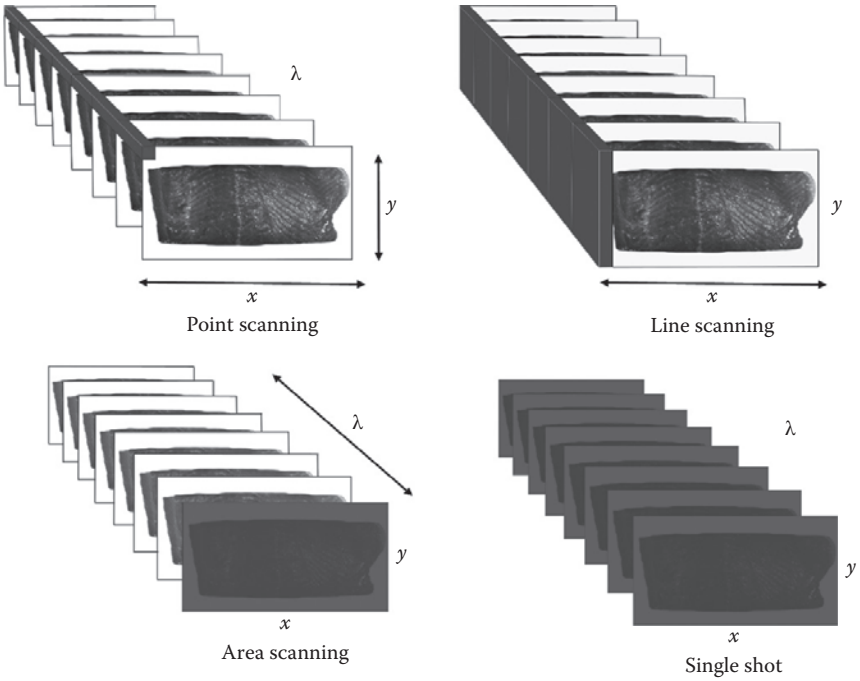


FIGURE 2.2
Four approaches to acquire hypercube $I(x, y, \lambda)$.

time and the complete hypercube $I(x, y, \lambda)$ is obtained as the line is scanned along the direction of x dimension. This configuration stores a hypercube in the format of band-interleaved-by-line (BIL). Because of its characteristics of continuous scanning in one direction, line scanning is particularly well suited for conveyor belt systems that are commonly used in food process lines. Therefore, line scanning is the most popular method of acquiring hyperspectral images for food industry applications.

Both point scanning and line scanning are spatial scanning methods, while area scanning (also known as plane scanning) is a spectral scanning method. This method keeps the image field of view fixed and there is no movement at all either for the imaging unit or for the tested object itself. This configuration acquires a 2D grayscale image (x, y) with full spatial information at a single spectral band at a time and it repeats over the whole wavelength range, accounting for a stack of single-band images stored in the band sequential (BSQ) format. As the detector is exposed to only a single wavelength each time, a suitable exposure time can be set for each spectral band. Particularly, area scanning is suitable for applications where the object should be stationary for a while, such as excitation-emission in florescence imaging. However, the disadvantage is that it is not suitable for a moving sample or the inspection of real-time delivery. Finally, the single

Downloaded From Vet-eBooks.com

shot method was developed to record both spatial and spectral information using an area detector with one exposure, making it attractive when fast hyperspectral imaging is anticipated. Nevertheless, this method is not fully developed and has limited resolutions for the spatial dimension and narrow ranges for the spectral dimension. To conclude, each approach has its own advantages, and the choice among these methods depends on the specific application. Point scanning is useful if a small surface has to be analyzed to find out minor compounds (i.e., impurity detection), while area scanning is more appropriate in situations where an overview of the sample is required and a lot of samples must be analyzed. As for the line scanning approach, it is a typical choice for online applications where the sample is moving.

2.3 Hyperspectral Imaging Instruments

The instrumentation of hyperspectral imaging is basic and it is very desirable to acquire reliable hyperspectral images with high quality in the food industry. Therefore, the main components of a hyperspectral imaging system will be outlined in this section and more efforts will be focused on the system employing the line scanning method due to the fact that line scanning is more consistent and useful for food online applications. The schematic representation of the main configuration of a line-scan hyperspectral imaging system used for nondestructive meat quality assessment at University College Dublin (UCD), Ireland is illustrated in Figure 2.3. A typical pushbroom hyperspectral imaging system consists of an illumination unit, a spectrograph coupled with C-mount lens, CCD, or complementary metal oxide semiconductor (CMOS) cameras, and a computer equipped with image acquisition software.

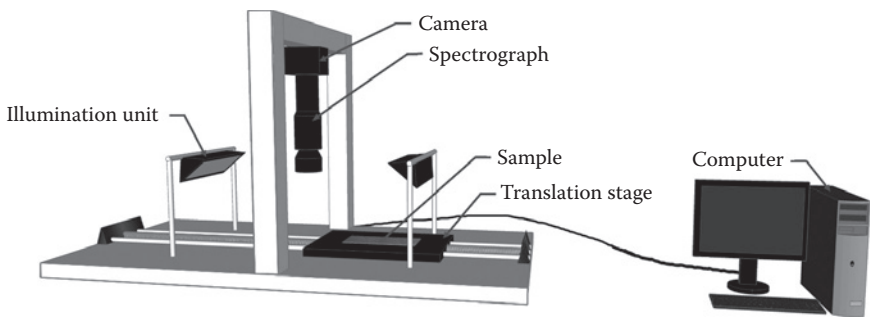


FIGURE 2.3
Schematic diagram of the main components of a pushbroom hyperspectral imaging system.

Downloaded From Vet-eBooks.com

2.3.1 Illumination Unit

Light serves as an information carrier to excite or illuminate the target sample. The illumination unit is an essential part of optical inspection systems to generate light. Choice of light sources and development of the lighting setup are paramount to guarantee the excellent performance and reliability of any imaging system. Typical light sources used in hyperspectral imaging systems include halogen lamps, light-emitting diodes (LEDs), lasers, and tunable sources.

Halogen lamps are usually used for illumination in the visible and NIR spectral regions. These broadband sources cover a wide spectral range, from 400 to 2500 nm, and are low cost (Gowen et al., 2015). Typically, a lamp filament made of tungsten wire is housed in a quartz glass bulb filled with halogen gas (Sun, 2010). The output light of quartz–tungsten–halogen (QTH) lamps is generated from incandescent emission when the filament has a high temperature. The QTH lamps are bright and work with low voltage, and thereby they are considered all-purpose illumination sources. However, the heat generated by a tungsten–halogen bulb is significant and may alter or even burn the sample. In order to overcome this issue, fiber optic line lights are usually applied to provide distance between the tungsten–halogen bulb and sample (Gaston et al., 2010).

LED technology has developed rapidly owning to its advantages of small size, low cost, long lifetime, fast response, low heat generation, low frequency of bulb replacement, low energy consumption, robustness, power, coolness to the touch without burning concerns, and insensitivity to vibration (Wu and Sun, 2013a). Instead of having a filament for incandescent emission, LEDs are solid-state sources that emit light when a semiconductor is electrified. LEDs can be assembled in different arrangements (e.g., spot, line, and ring lights) to meet different illumination requirements. With the different benefits mentioned above, LED lights have found application in hyperspectral imaging systems in food inspection (Park et al., 2011). The growth of LED technology is still ongoing with the development of new materials and electronics, and it has great potential to become a mainstream light source.

Unlike broadband illumination sources, lasers are directional monochromatic light sources. Lasers have three unique properties, namely monochromaticity, directionality, and coherence. Lasers are widely used as excitation sources in the application of fluorescence and Raman measurements. When some biological materials are excited by a monochromatic beam of light with a high energy, they will emit light of a lower energy in a broad wavelength range, accounting for fluorescence emission or Raman scattering. In recent years, lasers have been used as excitation sources in hyperspectral Raman imaging (Qin et al., 2011) and fluorescence imaging (Cho et al., 2009) applications.

Tunable light sources are mostly used for area scanning hyperspectral imaging systems. Tunable light sources put the wavelength dispersion device in the illumination light path instead of the imaging light path, allow-

Downloaded From Vet-eBooks.com

ing the detector directly performing area scanning to obtain both spatial and spectral information from the sample. Tunable light sources are still in the early stage of development and different wavelengths dispersion methods are explored. Currently, hyperspectral imaging with tunable light sources have been used for identification of fraudulent documents (Klein et al., 2008).

2.3.2 Wavelength Dispersion Devices

Wavelength dispersion devices are recognized as the core component for any hyperspectral imaging system using broadband illuminating light sources. Different optical and electro-optical instruments can be used to disperse broadband light into different wavelengths so that detectors can record light changes in specific wavelengths with associated spatial information after light-matter interaction. Typical devices include imaging spectrographs, bandpass filter wheels, and tunable filters.

2.3.2.1 Imaging Spectrographs

The imaging spectrograph normally operates in a hyperspectral imaging system with line scanning mode. This optical device is capable of dispersing incident broadband light into different wavelengths while preserving its spatial information and generating a spectrum for each point on the scanned line. The dispersed light is mapped onto the detector array and then a two-dimensional (2D) image is constructed with one dimension representing the spectral axis and the other containing spatial information for the scanning line. A complete 3D hyperspectral image cube is generated after scanning the entire surface of the sample. Most contemporary imaging spectrographs are established based on diffraction grating, which is a collection of equally spaced reflecting or transmitting elements separated by a distance that is on the order of the wavelength of the light under investigation. The physical characteristic of the diffraction grating is based on the spatial modulation of the refractive index, by which the incident electromagnetic wave on a grating will have its electric field amplitude, or phase, or both, modified in a predictable manner (Palmer et al., 2005).

There are two main methods for building imaging spectrographs, namely reflection gratings (i.e., a grating laid on a reflective surface) and transmission gratings (i.e., a grating laid on a transparent surface). The imaging spectrograph utilizing a transmission grating is based on the prism-grating-prism (PGP) principle and no moving mechanical components are included. Specifically, the light source, such as a halogen lamp, illuminates the object and entrance optics, for example, a lens, captures the radiation coming from the tested object. After passing through the entrance slit of the spectrograph, the incoming beam is then collimated and dispersed at the PGP component into different directions according to wavelength. At last, the dispersed light is projected onto an area detector resulting in a 2D image; one dimension

Downloaded From Vet-eBooks.com

represents the spectral axis (λ), and the other contains one spatial dimension (y) of the scanned line. The second spatial dimension (x) is generated by moving the sample perpendicularly to the scanning direction. As a result, hypercube $I(x, y, \lambda)$ is created after the surface of the sample is completely scanned. As another main form of imaging spectrographs, a typical reflection grating generally consists of an entrance slit, a pair of concentric spherical mirrors coupled with an aberration-corrected convex reflection grating, and a detector. After passing through the entrance slit, the incoming light is reflected by the lower mirror to the reflection grating, where the incident beam is dispersed into different wavelengths in a reflection manner. The upper mirror then reflects the dispersed light to the detector, resulting in a continuous spectrum at different pixels. Reflection grating is believed to have several advantages, such as lack of higher order aberrations, high image quality, low distortion, low f-number, and large field size (Bannon and Thomas, 2005). Additionally, the efficiency of the reflective optical components (e.g., mirror) is usually higher than that of the transmission components (e.g., prism). Hence, reflection grating-based imaging spectrographs can provide high signal-to-noise ratio (SNR) and are more suitable for low-light measuring conditions, such as fluorescence imaging and Raman imaging, which make the reflection grating approach increasingly accepted in line-scan hyperspectral imaging systems.

2.3.2.2 Filter Wheels

A rotatable disk called a filter wheel is the most basic implementation of spectral imaging for wavelength dispersion. A filter wheel carries a set of discrete bandpass filters that transmit the light at a particular wavelength while rejecting light at other wavelengths out of the passband. Filter wheels are relatively cheap and easy to use. Nevertheless, they have some disadvantages for applications in hyperspectral imaging, such as mechanical vibration from moving parts, slow wavelength switching, low resolution, and image misregistration owing to the filter movement. There is a broad range of filters from UV and visible to NIR wavelength with different specifications commercially available to satisfy different requirements.

2.3.2.3 Tunable Filters

An acousto-optic tunable filter (AOTF) is a solid-state device that works based on acousto-optic interactions in a crystal. An AOTF can isolate a single wavelength from a broadband source through an applied acoustic field. The wavelength of the dispersed light is a function of the frequency of the acoustic waves that are applied to the crystal (Sun, 2010). The AOTF only diffracts one particular spectral band at a time, which is quite similar to a bandpass filter with a narrow bandwidth. A liquid crystal tunable filter (LCTF) is another commonly used tunable filter and it is established from a

Downloaded From Vet-eBooks.com

series of optical stacks. A LCTF has electronically controlled liquid crystal cells inserted between two parallel polarizers to transmit light at a specific wavelength while eliminating light at other wavelengths. Each optical stack comprises a combination of a birefringent retarder and a liquid crystal layer inserted between two parallel polarizers. Both AOTFs and LCTFs are electronically tunable bandpass filters. Tunable filters have moderate spectral resolution (approximately 5–20 nm) and a broad wavelength range covering 400 to 2500 nm. In addition, electronically tunable filters like AOTFs and LCTFs can be flexibly controlled for various wavelengths by differentiating the frequency of the radio frequencies using a computer, which is a major difference from fixed interference filters. Compared to the rotating filter wheels, tunable filters have no problem of speed limitation, mechanical vibration, and image misregistration because they have no moving parts.

2.3.3 Area Detectors

Area detectors are used to quantify the intensity of the acquired light by converting radiation energy to electrical signals. The quality of hyperspectral images are directly based on the performance of the detector. Hyperspectral imaging systems operating in the spectral range of 400–1000 nm typically utilize two major types of solid-state area detectors, namely, CCD and CMOS cameras. Both types of detectors are sensitive in the wavelength region of 300–1000 nm, but typically subject to a sharp decrease in quantum efficiency below 400 nm and above 900 nm (Gowen et al., 2015). Longer wavelength systems require more expensive detectors, for example, InGaAs, which is made of an alloy of indium arsenide (InAs) and gallium arsenide (GaAs). A standard InGaAs (53% InAs and 47% GaAs) sensor is generally used for detecting the spectra at 900–1700 nm (ElMasry et al., 2012c) and it has fairly flat and high quantum efficiency in the NIR region. Photodiodes made of light-sensitive materials are the basic unit of both CCD and CMOS sensors to convert incident photons into electrons.

2.3.4 Hyperspectral Imaging System Calibration

Proper calibrations for a hyperspectral imaging system are necessary to ensure the consistent performance of the instrument and reliability of the acquired data. Inconsistent spectral profiles of reference spectra may be recorded by some systems even if the environment of data measurement is under carefully controlled conditions. The variability suggests that it is essential to apply a standardized and objective calibration protocol. The aims of calibration process are to (1) standardize the spectral and spatial axes of the hyperspectral image, (2) evaluate the accuracy of the extracted spectral and spatial data and validate the acceptability and reliability, (3) determine whether the hyperspectral imaging system is in a stable running condition, and (4) diagnose instrumental errors if necessary. The commonly

Downloaded From Vet-eBooks.com

used calibration methods include spatial calibration, spectral calibration, and flat-field correction.

2.3.4.1 Spatial Calibration

Spatial calibration for hyperspectral imaging systems is intended to adjust the field of view and estimate the spatial detection limit. There are different spatial calibration methods that can be used according to different image acquisition modes for imaging systems. In area scanning mode, as mentioned before, each single-band image at different wavelengths is a regular 2D grayscale image with full spatial information. Therefore, spatial calibration is carried out on a selected image with high SNR using resolution test charts, for example, the NBS 1952 Resolution Test Chart, ISO 12233 Test Chart, and 1951 USAF resolution test chart. When the same binning is employed for the horizontal and vertical axis of the camera, the area scanning systems generally provide the same resolution for both spatial dimensions. However, the resolution for two spatial dimensions might be different in line scanning systems. The y direction is parallel to the slit of the imaging spectrograph (see Figure 2.2), and the resolution has a relationship with the working distance, lens, imaging spectrograph, and camera. The pixels along the x direction are acquired by the stepwise movement of the sample, and the resolution is determined by the step size of the movement.

2.3.4.2 Spectral Calibration

The goal of spectral calibration (also called wavelength calibration) is to identify each pixel along the spectral dimension with a specific wavelength. Wavelength calibration will convert the form of hyperspectral data from pixel intensity versus pixel index to intensity versus wavelength. The calibration results are useful to determine the range and resolution of spectra. Wavelength calibration is not necessary for area scanning systems because single-band images at a series of known wavelengths are generated in area scanning mode. However, imaging spectrograph-based line scanning systems disperse indecent light into different spectral bands, which are then charged at different locations on the detector. Thus, the wavelength of each pixel is usually unknown. Technically, standard emission lamps are used as light sources to determine the correlations between distance (in pixels) on the spectral axis and wavelength. These calibration lamps generate constant, narrow, intense, stable, and specific lines from the excitation of different rare gases and metal vapors. Different spectral calibration lamps are available for different wavelength ranges from UV to infrared for different systems. The most acceptable wavelength calibration lamps include battery-powered lamps, pencil style lamps, and high power lamps utilizing Argon (Ar), Mercury (Hg), Krypton (Kr), Neon (Ne), Mercury/Argon (Hg/Ar), Xenon (Xe), and so forth. Once the calibration lamp is scanned by the hyperspectral

Downloaded From Vet-eBooks.com

imaging system, the spectral peaks with known wavelengths as well as their corresponding pixel indices along the spectral dimension can be identified. Then, the relationship between wavelength and the pixel indices can be established by a quantitative regression equation. The resulting regression equation can be used to identify the wavelengths of all pixels along the spectral dimension.

2.3.4.3 Flat-Field Correction

Due to the imperfections of each component (e.g., source, lens, spectrograph, and camera) and different measurement environments, the acquired raw hyperspectral images generally contain noises and artifacts. Many factors, such as nonuniform illumination, pixel-to-pixel sensitivity variations of the detector, and dust on the lens, will contribute to different image artifacts, leading to the raw images not suitable for quantitative analysis. Flat-field correction (also known as reflectance calibration) can be performed to calibrate the raw intensity image into a relative (or percent) reflectance or absorbance image and remove the effects of the noise and artifacts. White diffuse reflectance panels, which have high, uniform, stable, and flat reflectance over a broad spectral range (about 99.9% reflectance), are generally employed as standards for the reflectance calibration. The dark current image (about 0% reflectance) is obtained with the light source completely off and the camera lens completely covered. These two reference images are then used to correct the original hyperspectral images using the following equation:

$$R = \frac{I_S - I_D}{I_W - I_D} \tag{2.1}$$

where R is the relative reflectance image, I_S the raw hyperspectral image, I_D the dark image, and I_W the white reference image. The original image can also be corrected into absorbance (A) by taking logarithms of Equation 2.1 as

$$R = -\log_{10}\left(\frac{I_S - I_D}{I_W - I_D}\right) \tag{2.2}$$

Figure 2.4 shows an example of flat-field correction for a hyperspectral image of a salmon sample. The plot displayed in Figure 2.4a consists of original spectra extracted from three hyperspectral images (i.e., salmon sample, white calibration tile made of Teflon [Gilden Photonics Ltd., Glasgow, UK], and dark current). The white panel is observed to have the highest intensity values and the dark current is relatively low and flat over the whole wavelengths. A relative reflectance spectrum of the sample (Figure 2.4b) is achieved by performing Equation 2.1 and this spectrum is suitable for the subsequent spectral data analysis.

Downloaded From Vet-eBooks.com

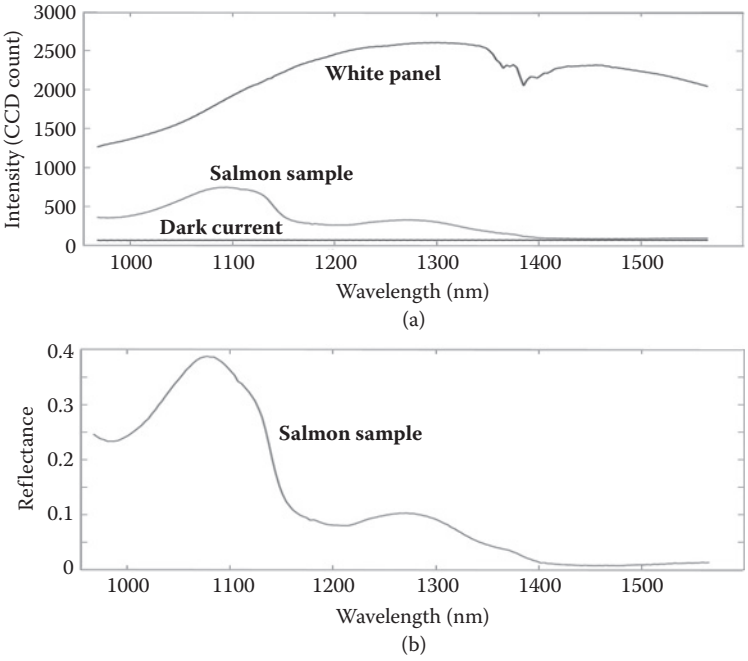


FIGURE 2.4
Flat-field correction for hyperspectral image. (a) Raw reflectance spectra. (b) Relative reflectance spectrum after flat-field correction.

2.4 Hyperspectral Imaging Data Processing and Multivariate Analysis

2.4.1 Image Segmentation

Segmentation is the process that divides an image into disjoint objects or regions and locates the regions of interest (ROIs) in a form of mask for further spectral and textural feature extraction. Technically, image segmentation can be obtained by attempting to detect either the discontinuity of object boundaries or the similarity of regions in an image. Discontinuity is defined as the abrupt changes of intensity, and the places where discontinuity occurs are named edges. On the other hand, the approach based on similarity is to identify the relatively homogeneous regions which are believed to be similar according to the predefined criteria. There are some classic techniques for image segmentation, such as thresholding, morphological processing (such as erosion, dilation, open, close, and watershed algorithms), and edge-based segmentation.

With a hyperspectral image, each pixel contains a vector of intensity values and this vector is well recognized as the spectral signature. Therefore, image

Downloaded From Vet-eBooks.com

segmentation can be achieved by classifying pixels into background and the tested object based on the spectral difference. Spectral image segmentation, which is regarded as a higher level analysis compared to traditional segmentations, actually integrates segmentation and classification into a single process.

2.4.1.1 Thresholding and Morphological Processing

Thresholding is popularly used to separate samples from the background because it is easy to use and it can provide relatively good results. Better performance can be obtained for the image containing the object against a contrasting background. When ROIs present a high gray level on a low gray level background, the thresholding image $J(x,y)$ can be defined as

$$J(x,y) = \begin{cases} 1, & \text{if } I(x,y) \geq T \\ 0, & \text{otherwise} \end{cases} \tag{2.3}$$

where $I(x,y)$ represents the original image, and T is the threshold value. Therefore, all pixels at or above the threshold are assigned value 1 corresponding to the ROI, whereas pixels corresponding to the background are assigned value 0. Global thresholding, which is known as the simplest thresholding technique, is accomplished by partitioning the image histogram with a single global threshold and it is widely acceptable in hyperspectral image processing. This approach is competent when the gray level histogram is bimodal and the threshold T is appropriately selected. Morphological processing is flexible and powerful for image segmentation and it can be utilized if the initial segmentation by thresholding is not satisfactory. Binary morphological processing consists of typical neighborhood operations by sliding a structure element containing any complement of 0s and 1s with any size over the image. Erosion and dilation are two primitive operations of morphological processing techniques. Specifically, erosion is the process to eliminate all the pixels on object boundaries in an image, while dilation is the process of incorporating pixels to the boundaries of objects.

Figure 2.5 demonstrates examples of hyperspectral image segmentation by using the thresholding technique. The spectra of three pixels belong to the salmon sample and three pixels of background were randomly selected and presented in the form of a plot (Figure 2.5a). A grayscale image at a waveband of 1400 nm (B2) was obtained due to the high contrast between the sample and the background (Figure 2.5b). Simple histogram thresholding (value of 0.3) was determined and it subsequently produced a segmented image for the whole sample. Then, morphological operations were implemented to boost the segmentation performance. This method is simple and useful; however, as we can see from the final mask (Figure 2.5b), some pixels of the sample were wrongly classified into the background. Figure 2.5c

Downloaded From Vet-eBooks.com

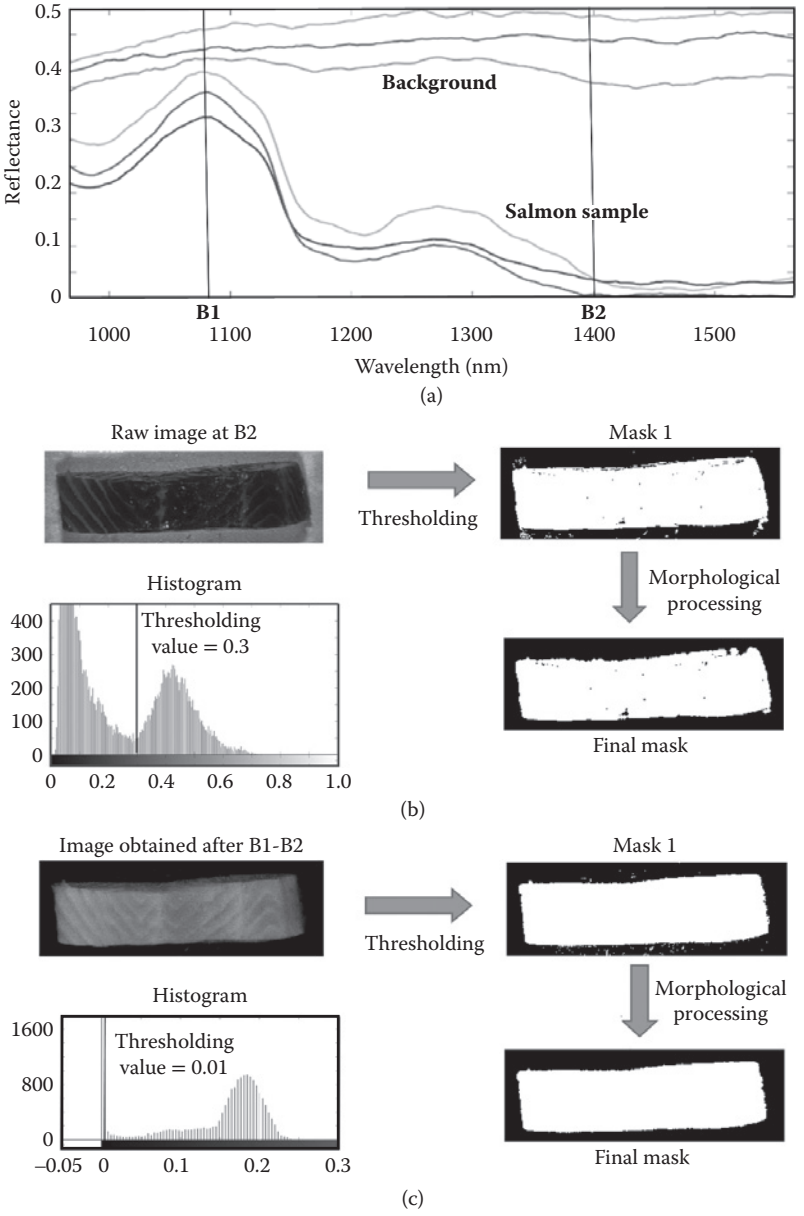


FIGURE 2.5
Hyperspectral image segmentation by thresholding.

shows another thresholding method that is widely used in hyperspectral imaging processing (Barbin et al., 2013c; Kamruzzaman et al., 2011). Two images were selected, one with high (B1 of 1077 nm) and the other with low reflectance values (B2 of 1400 nm), and subtracted from each other to obtain

Downloaded From Vet-eBooks.com

a grayscale image. This grayscale image has uniform gray level within the entire sample region and no extreme pixel can be observed. All the pixels in the background were set to approximately 0 because each spectrum of background remains constant along the whole wavelength region. As a result, a thresholding value of 0.01 was obtained and a final mask was generated. As shown, the segmentation result was greatly improved.

2.4.1.2 Spectral Image Segmentation

Classification-based techniques intend to partition pixels into several classes based on various classification methods. Generally, they can be categorized into two groups, supervised and unsupervised, where output target values of learning samples are involved in the former and missing in the latter. There are a great number of supervised techniques, such as support vector machines (SVMs), artificial neural networks (ANNs), and Bayesian classification, among which Bayesian classification is considered as the most commonly used method (Kleynen et al., 2005). Unsupervised classification does not offer guidance in the learning process because of lack of target values and the widely used methods include K-means and principal component analysis (PCA). K-means is a classical dynamic clustering algorithm and is simple in theory and fast in computation. It intends to partition the image into K clusters so that intercluster similarity is low and intracluster similarity is high. K-means has been successfully implemented in hyperspectral image segmentation (Piqueras et al., 2011).

Meanwhile, PCA is a mathematical approach used to segment a hyperspectral image by seeking the maximum variances from the original image. It decomposes the raw hyperspectral image into several score images described by a small amount of uncorrelated data (principal components [PC]) and loadings (eigenvectors). The first and the second PC score images usually interpret the highest variance. Figure 2.6 shows an example of using PCA for identification of the loin eye, fat part, and marbling pixels in a piece of pork meat (Barbin et al., 2012a). The first two PCs were used to compose the score plot for identifying the pixels with similar spectral signatures. This plot represents the most useful information of the original image due to the highest variance and the lowest correlation to avoid the colinearity of the spectral data. Since each material has a unique spectral signature based on different physicochemical properties, the loin eye reveals a distinctive spectral signature compared with fat portions and any other muscles adjoining the loin eye. Pixels with similar spectral signatures were projected together as one cluster in the 2D scatter plot, which facilitates its identification in the PC score image and consequently in the original image. The correspondent pixels recognized in the score plot were highlighted in the PC score image simultaneously, which allowed for the identification of the corresponding clusters, namely loin eye, fat, and muscles adjacent to the loin eye.

Downloaded From Vet-eBooks.com

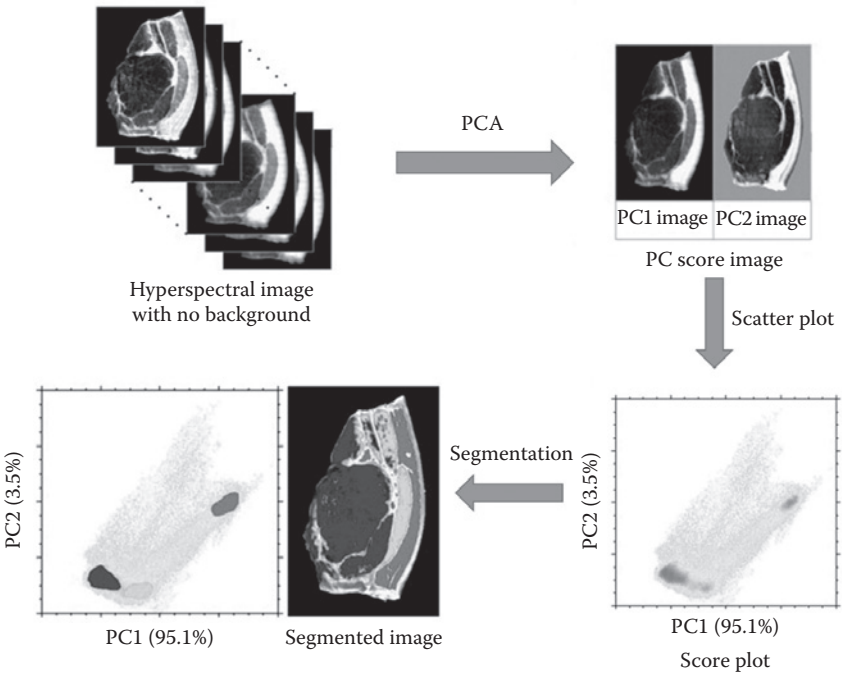


FIGURE 2.6
Principal component analysis (PCA) classification-based segmentation method for hyperspectral images. (Adapted from Barbin, D. et al., *Meat Science*, 90, 259–268, 2012a.)

2.4.2 Spectral Preprocessing

After image segmentation, spectral data can be extracted from different ROIs, which present different quality features in the calibrated image. Spectral preprocessing algorithms are mathematically used to improve the spectral data. They aim to correct undesired effects such as random noise, length variation of light path, and light scattering resulting from variable physical sample properties or instrumental effects, to produce a robust model with the best predicting ability. This step is generally performed prior to multivariate modeling so as to reduce, eliminate, or standardize the impact on the spectra and to greatly enhance the robustness of the calibration model (Reich, 2005). The most widely used preprocessing algorithms include smoothing, derivatives, standard normal variate (SNV), multiplicative scatter correction (MSC), Fourier transform (FT), and some new methods including orthogonal signal correction (OSC) and wavelet transforms (WTs) (Luypaert et al., 2004).

Smoothing, which includes moving smoothing and Savitzkye–Golay smoothing (Savitzky and Golay, 1964), can be applied to eliminate noises in the raw spectra without reducing the number of spectral variables. Generally, smoothing is combined with other preprocessing techniques to accomplish the denoising function. Derivatives (mainly first and second derivatives) are

Downloaded From Vet-eBooks.com

performed to correct baseline effects in spectra, remove background, and increase spectral resolution. The second derivative also has the function of resolving nearby peaks and sharpening spectral features. Figure 2.7a and b shows original and processed spectra by the Savizkye–Golay second derivative with a window width of 15. It can be observed that the peaks and valleys not very obvious in raw spectra became clear after preprocessing.

SNV and MSC intend to remove the multiplicative interferences of scatter, particle size, and the change of light distance (Blanco et al., 1997). In order to remove slope variations on individual spectrum basis, each object is transformed independently using the following calculation:

$$X_{i,\mathrm{SNV}} = (X_i - \bar{X}) / \sqrt{\frac{\sum_{i=1}^{m} (X_i - \bar{X})^2}{(m-1)}} \tag{2.4}$$

where $X_{i,\mathrm{SNV}}$ is the ith variable in the transformed spectrum after SNV preprocessing, X_i is the ith variable, and $\bar{X}$ is the average of all variables in the raw spectrum, respectively, $i = 1, 2, 3, \ldots, m$, where m is the number of wavelength variables in the spectrum. Figure 2.7c shows spectra processed by the SNV algorithm, and it is observed that the baseline shift was successfully

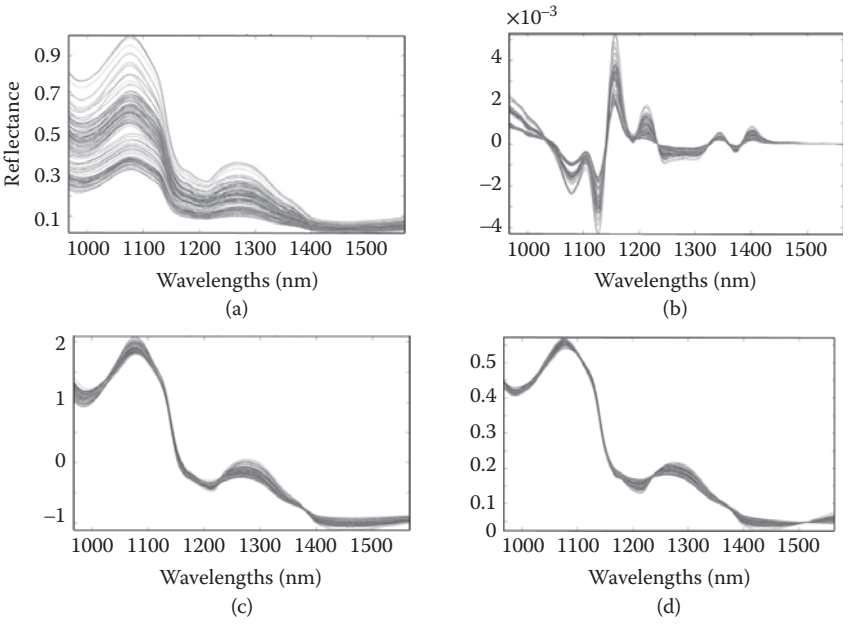


FIGURE 2.7
Near-infrared (NIR) spectra of salmon samples obtained from (a) raw data, (b) Savizkye–Golay second derivative, (c) standard normal variate, and (d) extended multiplicative signal correction.

Downloaded From Vet-eBooks.com

removed. Extended multiplicative signal correction (EMSC) is considered an extension of MSC that can be used to eliminate additional unwanted interferences in the signal (Martens and Stark, 1991). This method cannot only correct the spectra for multiplicative effects but can also correct the baseline of the spectra and suppress the effect of known interfering compounds. This method outperforms MSC in many cases (Esquerre et al., 2012). The performance of EMSC with the default setting in MATLAB® 8.6 R2015b software (The Mathworks Inc., MA, USA) is displayed in Figure 2.7d.

2.4.3 Multivariate Analysis

As mentioned previously, hyperspectral imaging contains huge amount of spectral and spatial data; therefore, a combination of multivariate chemometrics and visualization tools to appropriately extract desired information in an efficient way is undoubtedly required. Chemometric techniques are mathematical and statistical methods which decompose a massive quantity of features into simple and easier interpretable structures, which enable the establishment of easier to understand relationship between hyperspectral data and the desired attributes of tested samples (Bro et al., 2002). Multivariate techniques commonly applied in spectral data can be classified into multivariate classification for qualitative analysis and multivariate regression for quantitative analysis.

2.4.3.1 Multivariate Classification

Qualitative classification, also called pattern recognition, can be further divided into unsupervised and supervised classification. Unsupervised classification is accomplished based on the natural characteristics, which can be correlation, distance, or some combination of both, requiring no prior knowledge about the class information of the data. Generally, it is quite useful for preliminary evaluation of the information contents in the spectral dataset. Typical unsupervised classification algorithms for the analysis of hyperspectral data include hierarchical clustering, K-means clustering, and PCA.

PCA is a dimension-reduction tool, which can reduce a large set of variables to a small set but retain most of the information in the original dataset. It is a mathematical procedure that transforms a number of (possibly) correlated variables into a (smaller) number of uncorrelated variables called PCs. The first PC interprets as much of the variability in the data as possible, and each succeeding component accounts for as much of the remaining variability as possible. PCA decomposes the data matrix X (m samples and n variables) as the sum of the outer product of vectors t_i and p_i plus a residual variance (Penha and Hines, 2001):

$$X = t_1 p_1^{\mathrm{T}} + t_2 p_2^{\mathrm{T}} + t_3 p_3^{\mathrm{T}} + \ldots + t_k p_k^{\mathrm{T}} + E = T_k P_k^{\mathrm{T}} + E \tag{2.5}$$

Downloaded From Vet-eBooks.com

where t_i vectors refer to principle component scores and provide information on how the samples are correlated with each other, and p_i vectors are the eigenvectors of the covariance of data matrix X. These eigenvectors are known as the PC loadings, which reveal how variables are related to each other. In other words, the PCA model aims to split the matrix X in two parts: one accounts for the system variation (the process model $T_k P_k^T$) and the other captures the noise or unmodeled information (residual variance E). This division is able to reduce the dimensionality and eliminate the redundant information existent in highly correlated variables of the original dataset.

Figure 2.8 demonstrates an example of PCA model used to evaluate spectral variations in chicken meat, lamb, and salmon. Each data point in the score plot represents a spectrum of the sample. This plot was established for exploration and identification of any special features (clusters or groupings) within the data set. As shown, all sample points were generally clustered into three groups based on their first three PCs, which account for 91.26% of the total variance. Loading plots in Figure 2.8 can be used to identify important variables that are responsible for the specific features appearing in the corresponding scores. Generally, several wavelengths with high (local maxima) and low (local minimum) values from the PC loadings should be selected as the dominant wavelengths which contribute most to the classification performance. As the most frequently used unsupervised technique for qualitative classification, PCA has proved useful in initial investigation of many meat species to visualize the spectral data and

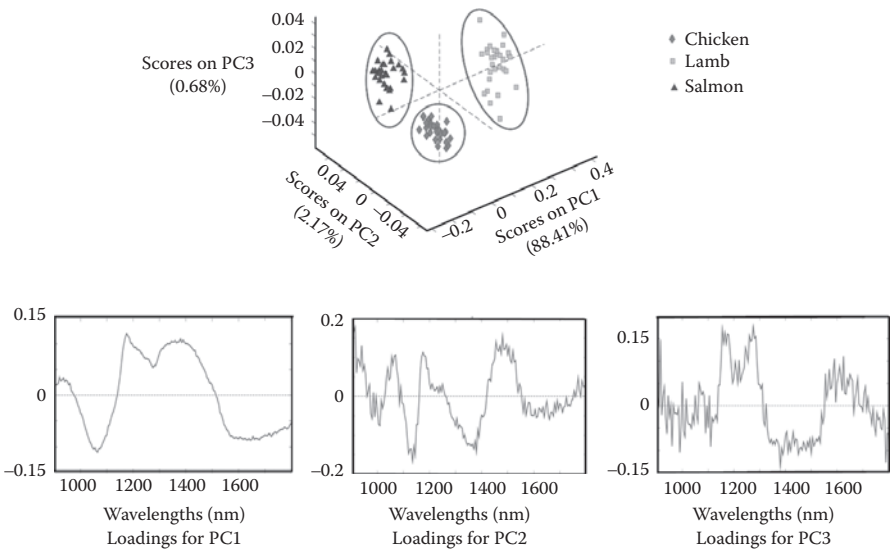


FIGURE 2.8
Example of PCA model performance to analyze chicken meat, lamb, and salmon using NIR hyperspectral imaging.

Downloaded From Vet-eBooks.com

to examine any possible grouping of samples according to spectral features (Kamruzzaman et al., 2012a; Wu et al., 2012).

Supervised pattern recognition intends to establish a classification model to group new unknown samples into predefined known classes on the basis of their measured features. Linear discrimination analysis (LDA) and partial least squares-discriminant analysis (PLS-DA) are typical chemometric techniques for supervised classification of the spectra and they are increasingly used in hyperspectral data analysis for classification and discrimination problems (Chevallier et al., 2006). LDA aims to find optimum boundaries among classes by maximizing the ratio of between-groups variance and minimizing the ratio of within-groups variance. PLS-DA is based on the partial least squares regression (PLSR) approach and seeks the optimum separation of classes by encoding each dependent variable of PLSR with dummy variables interpreting the classes. When performed using the PLS2 algorithm, the dependent Y matrix in the PLS-DA model has as many columns as classes, and as many rows as samples used for the calibration. This is known as the dummy matrix and it contains only 1's and 0's, indicating that the spectrum belongs or not belongs to a specific class, respectively. Technically, a threshold must be set in the predictions because the results will not be either a 1 or a 0 perfectly.

Machine learning methods, including SVM, artificial neural networks (ANN), and random forests (RF), have also been applied in the supervised multivariate analysis. Among them, RF is a powerful statistical classifier, which is well established in many disciplines but is relatively unknown in the food research area. It has been shown effective when applied for classification of hyperspectral data (Lawrence et al., 2006). As the name suggests, the RF classifier first generates many classification trees and then combines the predictions to provide a more accurate classification result (Cutler et al., 2007). The trees are fully grown and each can be used to predict the out-of-bag (OOB) observations. The predicted class is calculated by majority vote of the OOB predictions, with ties split randomly. Accuracies and error rates are calculated for each observation, and then averaged over all observations. Meanwhile, SVM has also been successfully used for NIR classification tasks, such as material identification (Langeron et al., 2007) and food discrimination (Chen et al., 2007). This method aims at finding the optimal separation surface between classes on the basis of the identification of support vectors which are the most representative training samples of the side of the class (Mercier and Lennon, 2003). If the training data are not linearly separable, a kernel method is applied to simulate a nonlinear projection of the data in a higher dimension space, where the classes are linearly separable.

2.4.3.2 Multivariate Regression

The goal of multivariate regression is to establish a quantitative relationship between the desired physical, chemical, or biological attributes

Downloaded From Vet-eBooks.com

of the tested sample and its spectrum response. The most widely used multivariate linear regression methods are multiple linear regression (MLR), principle component regression (PCR), and PLSR. MLR establishes a relationship between the spectrum and its target features with explanatory or predictive purposes in the form of a linear equation. It is simple and easy to interpret. The regression coefficients (RCs) of the equation are estimated by the process of minimizing the error between reference and predicted values in a least squares sense. MLR typically does not perform well when the number of variables is more than that of samples and is easily affected by high collinearity between the variables. PCR is a two-step multivariate method that consists of PCA and MLR. The PCs are first computed by the PCA model and then used as independent variables in MLR to replace the original variables. Compared to MLR, the PC calculation in PCR makes the independent variables uncorrelated and less noisy. However, PC calculation does not take the reference values of dependent variables into consideration; therefore, the achieved PCs may be not informative with respect to the response variables. On the other hand, PLSR decomposes both the spectra (independent variables) and properties of objects (dependent variables) simultaneously. During the decomposition process, dependent variables are actively considered to extract a set of orthogonal factors called latent variables (LVs) to ensure that the first several LVs are the most related for predicting dependent variables. The number of LVs to be extracted relies on the prediction error, typically determined by cross-validation.

When the spectra of the tested sample and its quality are not linearly related, nonlinear methods, such as ANN and support vector regression (SVR), are very suitable for analysis. ANN is inspired by the behavior of biological neural networks and is used for learning and prediction purposes. The most widely used ANN technique is the multilayer feed forward neural network where the artificial neurons are arranged into three layers of input, hidden, and output layer. The intensity or reflectance value at each spectral wavelength is fed to the input layer, while the output layer exports the prediction of the target attribute. A feed forward neural network usually involves more than one hidden layer, which makes the network capable of processing nonlinear and complex correlation. SVR employs a nonlinear map function and the input is first mapped to a high-dimensional feature space, then a linear regression is established in this space. There are two methods to build an SVR model, epsilon-SVR and nu-SVR, and the difference lies in the fact that nu-SVR introduces and uses parameter nu to control the number of support vectors while epsilon-SVR uses parameter epsilon. The radial basis function (RBF) kernel is normally used as the kernel function of epsilon-SVR to deal with the linear and nonlinear relationships between the spectral information and target attributes. Both ANN and SVM are able to deliver promising solutions in different classification and regression tasks.

Downloaded From Vet-eBooks.com

2.4.3.3 Model Evaluation

To perform either qualitative classification or quantitative regression using hyperspectral imaging, data must be trained or calibrated to develop a model to relate spectral data with the real properties of these samples. This is generally carried out by first selecting representative calibration samples (which span the values of attributes under investigation) and then building a multivariate model. The established model is subsequently validated by cross-validation or/and the prediction process with a new set of samples. Cross-validation is a method to measure generalization error by using hold-out data. Leave-one-out cross-validation and venetian blinds cross-validation methods are widely used to validate hyperspectral data.

Within the processes of quantitative regression, the performance of a calibration model is usually evaluated by the following criteria: standard error of calibration (SEC) and standard error of prediction (SEP), root mean square error of calibration (RMSEC), root mean square error of cross-validation (RMSECV) and root mean square error of prediction (RMSEP), coefficients of determination of calibration (r^2_C), coefficients of determination of cross-validation (r^2_{CV}) and coefficients of determination of prediction (r^2_P), and residual predictive deviation (RPD). Generally, a good model would present higher values of r^2_C, r^2_{CV}, r^2_P, and RPD, lower values of SEC, SEP, RMSEC, RMSECV, and RMSEP, with a small difference between SEC and SEP (Wu and Sun, 2013a).

When it comes to qualitative classification, the performance of each established model can be evaluated by calculating the correct classification rate (CCR) in calibration, validation, and cross-validation, which is expressed according to the following equation:

$$\text{CCR} = \frac{N_1}{N_2} \times 100\% \tag{2.6}$$

where N_1 is the number of correctly classified samples and N_2 is the total number of samples. Meanwhile, model performance can also be statistically evaluated by using the sensitivity, specificity, and class error:

$$\text{Sensitivity} = \frac{\text{TP}}{\text{TP} + \text{FN}} \tag{2.7}$$

$$\text{Specificity} = \frac{\text{TN}}{\text{FP} + \text{TN}} \tag{2.8}$$

$$\text{Class error} = 1 - \frac{\text{Sensitivity} + \text{Specificity}}{2} \tag{2.9}$$

Downloaded From Vet-eBooks.com

In Equations 2.7 through 2.9, TP and TN refer to true positive and true negative, respectively, resulting in the samples that have been correctly assigned as belonging (TP), or not belonging (TN), to a specific class. FP and FN denote false positive and false negative, respectively, accounting for the samples that have been wrongly assigned as belonging (FP), or not belonging (FN), to a specific class (Amigo et al., 2015). Another important statistic evaluation often used in classification is the receiver operator characteristics (ROC) curves, which are graphical plots that present the performance of the classification as its discrimination threshold (relationship between the specificity and sensitivity) is varied. The closest point in the ROC curve to the top left corner of the chart usually results from an optimal threshold. The best prediction model would generate a point in the upper left corner or coordinate (0, 1) of the ROC space, representing 100% sensitivity (no false negatives) and 100% specificity (no false positives). The area under the ROC curve (AUC) can also be applied to compare general classification performances due to its statistical property: the AUC of a classifier is equivalent to the probability that the classifier will rank a randomly chosen positive instance higher than a randomly chosen negative instance.

2.4.4 Selection of Important Wavelengths

It is very critical to select the most important wavelengths in hyperspectral imaging in order to optimize data analysis and save computation time. Irrelevant wavelength elimination and spectral dimension reduction are necessary in spectral data analysis. According to Wold et al. (1996), effective wavelengths may be equally or more efficient than full wavelengths in many situations. The elimination of irrelevant wavelengths can predigest calibration modeling and enhance the results in terms of accuracy and robustness. Besides, contiguous wavelengths are likely to have a problem of multicollinearity, which refers to strong correlations among the independent variables (wavelengths). The selection of spectral variables can minimize the collinearity among contiguous wavelengths. Once a few optimal wavelengths with characteristic information are collected, a multispectral imaging system with the advantages of simple structure and low cost can be developed and it will be incomparable for process monitoring and real-time inspection.

There are numerous mathematical selection algorithms developed for choosing optimum wavelengths. Some classical methods include correlation coefficients, analysis of spectral differences (ASD), spectrum derivatives, and stepwise regression. Simulated annealing (SA) and genetic algorithms (GAs) are two elaborate search-based strategies. Interval-based algorithms usually include interval partial least squares (iPLS), windows PLS, and backward interval partial least squares (biPLS). The choice of a particular method should be determined by the nature of the problem, ease of implementation, the size of the data set, and economic feasibility (ElMasry and Wold, 2008).

Downloaded From Vet-eBooks.com

2.5 Online Applications for Meat Quality and Safety Assessment

From its manifestation as a means of remote sensing exploration, to its current success in several distinct applications, hyperspectral imaging has established itself as a key analytical tool for nondestructive quality and safety evaluation of meat and meat products. When implemented in processing lines, hyperspectral imaging systems enable further increasing throughput by providing precise inspection control over key steps in the production and packaging process. Stimulated by rapid advances in instrumentation, software, and algorithmic developments as well as chemometrics, hyperspectral imaging has found use in many important applications in the meat industry such as classification or sorting of meat into different groups based on quality traits, such as color, fat, protein, and moisture, either to screen or eliminate lower quality products before processing or packaging or to sort a single meat source into a quality stack. Meanwhile, it is applied for uniformity control and inspection purposes to identify meat adulteration and exclude contaminated or substandard meat sources from the food chain with minimal additional cost. There are a number of areas for which hyperspectral imaging holds great potential in online quality and safety evaluation of meat and meat products, as summarized in Table 2.1.

TABLE 2.1

Applications of Hyperspectral Imaging for Quality and Safety Evaluation of Meat

Products	Imaging Mode	Spectral Range (nm)	Applications	Data Analysis	References
Poultry carcass	Reflectance	385–735	Differentiation of wholesome and unwholesome chicken	Fuzzy algorithm	Chao et al. (2007)
	Reflectance	400–1000	Fecal detection and wholesomeness inspection	Image processing algorithm	Park et al. (2010)
	Fluorescence	425–711	Skin tumor detection	SVM, PCA	Du et al. (2007)
	Reflectance	447–733	Skin tumor detection	FS-MBB, KNN	Nakariyakul and Casasent (2007)
	Reflectance/ transmittance	400–1000	Bone fragment detection	PCA	Yoon et al. (2008)
	Reflectance	900–1700	TVC prediction	PLSR	Feng and Sun (2013a)
	Reflectance	900–1700	*Pseudomonas* loads	PLSR	Feng and Sun (2013b)
	Reflectance	900–1700	Enterobacteriaceae loads	PLSR	Feng and Sun (2013)

(*Continued*)

Downloaded From Vet-eBooks.com

TABLE 2.1 (*Continued*)

Applications of Hyperspectral Imaging for Quality and Safety Evaluation of Meat

Products	Imaging Mode	Spectral Range (nm)	Applications	Data Analysis	References
	Reflectance	400–1000	Hydroxyproline content in chicken meat	PLSR	Xiong et al. (2015b)
	Reflectance	400–1000	Springiness of fresh chicken meat	PLSR, ANN	Xiong et al. (2015a)
Fish	Interactance	400–1000	Freshness assessment of cod	PCA, PLSR, KNN	Sivertsen et al. (2011)
	Reflectance	400–1000	Sensory QIS in grass carp fillet	LS-SVM	Cheng and Sun (2015a)
	Interactance	760–1040	Distribution of fat and salt contents	PLSR	Segtnan et al. (2009)
	Reflectance	899–1694	Fat content in Atlantic salmon fillets	PLSR	Zhu et al. (2014)
	Interactance	448–752	Nematodes detection in cod fillets	GML classifier	Sivertsen et al. (2012)
	Reflectance	400–1700	TVC of salmon fillets	PLSR, LS-SVM	Wu and Sun (2013d)
	Reflectance	400–1000	Enterobacteriaceae loads in grass carp	PLSR	Cheng and Sun (2015b)
	Reflectance	380–1030	Discrimination of fresh and frozen-thawed halibut fillets	LS-SVM	Zhu et al. (2013)
	Reflectance	400–1000	Categorization of salmon fillets stored under different atmospheres	PCA, KNN	Sone et al. (2012)
	Reflectance	400–1000	Distinguish expired and nonexpired vacuum-packed salmon	PLS-DA	Ivorra et al. (2013)
Red meat	Reflectance	900–1700	Color, pH, and tenderness of fresh beef	PLSR	ElMasry et al. (2012c)
	Reflectance	900–1700	Color, pH, and drip loss of pork	PLSR	Barbin et al. (2012b)

(*Continued*)

Downloaded From Vet-eBooks.com

TABLE 2.1 (*Continued*)

Applications of Hyperspectral Imaging for Quality and Safety Evaluation of Meat

Products	Imaging Mode	Spectral Range (nm)	Applications	Data Analysis	References
	Reflectance	400–1000	Beef marbling grade	MLR	Li et al. (2011)
	Reflectance	400–1000	Pork tenderness	MLR	Tao et al. (2012)
	Reflectance	900–1700	Lamb tenderness	PLSR	Kamruzzaman et al. (2013)
	Reflectance	400–1000	Beef tenderness	FLD, SVM, DT	Konda Naganathan et al. (2016)
	Reflectance	380–1700	Moisture content in beef	MLR	Wu et al. (2013)
	Reflectance	900–1700	Water, fat, and protein contents of beef	PLSR	ElMasry et al. (2013)
	Reflectance	400–1000	Fat content in beef	SAM, EDM	Lohumi et al. (2016)
	Reflectance	1000–2500	Moisture content of porcine meat	PLSR	Liu et al. (2014b)
	Reflectance	400–1000	Moisture content in pork	PLSR	Ma et al. (2016)
	Reflectance	900–1700	Moisture, protein, and fat of intact and minced pork	PLSR	Barbin et al. (2013a)
	Reflectance	900–1700	Moisture, protein, and fat content in lamb	PLSR	Kamruzzaman et al. (2012b)
	Reflectance	400–1000	Moisture content in beef, lamb, and pork	MLR	Kamruzzaman et al. (2016b)
	Reflectance	900–1700	TVC and PPC in pork	PLSR	Barbin et al. (2013b)
	Reflectance	472–1000	TVC in pork	SMLR	Tao and Peng (2015)
	Reflectance	400–1000	pH of salted pork meat	PLSR	Liu et al. (2014a)
	Reflectance	400–1000, 900–2500	Drip loss of frozen pork	PLSR	Xie et al. (2015)
	Reflectance	900–1700	Drip loss of lamb	PLSR	Kamruzzaman et al. (2012c)

(*Continued*)

Downloaded From Vet-eBooks.com

TABLE 2.1 (*Continued*)

Applications of Hyperspectral Imaging for Quality and Safety Evaluation of Meat

Products	Imaging Mode	Spectral Range (nm)	Applications	Data Analysis	References
	Reflectance	900–1700	WHC in fresh beef	PCA ,PLSR	ElMasry et al. (2011)
	Reflectance	400–1000	WHC in beef, lamb, and pork	PLSR, LS-SVM	Kamruzzaman et al. (2016a)

ANN, artificial neural network; DT, decision tree; EDM, Euclidian distance measure; FLD, Fisher's linear discriminant; FS-MBB, forward selection-modified branch and bound; GML, Gaussian maximum likelihood; KNN, K-nearest neighbor; LS-SVM, least squares-support vector machine; MLR, multiple linear regression; PCA, principal component analysis; PLS-DA, partial least squares-discriminant analysis; PPC, psychrotrophic plate count; PLSR, partial least squares regression; QIS, quality index scores; SAM, spectral angle measure; SVM, support vector machines; TVC, total viable counts; WHC, water holding capability.

2.5.1 Poultry Carcass

The Poultry Products Inspection Act of 1957 requires the United States Department of Agriculture's Food Safety and Inspection Service (FSIS) to inspect all domesticated birds when slaughtered and processed into products by a commercial facility. Since then, on-site organoleptic inspection is carried out on each chicken carcass processed at U.S. poultry plants for indications of diseases or defects and it is usually accomplished by sight and by touch for the body, the inner body cavity surfaces, and the internal organs during processing operations. Each inspector employed in FSIS is limited to a maximum of 35 birds per minute and an inspector working more than eight hours per day in these conditions tends to develop repetitive motion injuries and fatigue problems. As a result, fast and automatic methods for precise inspection are necessary in the poultry industry for efficient management. In recent years, the hyperspectral imaging technique has been extensively implemented for safety and quality evaluation and monitoring of chicken and poultry carcasses in offline and online applications through many research endeavors (Feng and Sun, 2014; Park et al., 2007). The widely conducted research of hyperspectral imaging systems in poultry quality and safety evaluation is mostly focused on the differentiation between unwholesome and wholesome freshly slaughtered chickens or identification of different contaminants or tumors on the surface of the poultry carcasses. In fact, some online hyperspectral imaging detection systems for inspection of poultry carcasses have been successfully installed and implemented (Chao et al., 2008). Generally, the spectral image is first captured for each poultry carcass along the processing line, and then the image is processed by computer software and the result to determine whether or not the chicken has a contaminant, disease, or systemic defects will be obtained in seconds (Chao et al., 2014).

Downloaded From Vet-eBooks.com

2.5.1.1 Contamination Detection

Undoubtedly, consumers require some assurance that the poultry product available for purchase is wholesome and without any food safety hazards. Contamination of poultry carcasses with bacterial food-borne pathogens potentially results from exposure of the animal carcass to fecal materials during or after slaughter. Microbial pathogens are likely to be transmitted to humans after consumption of contaminated undercooked or mishandled poultry meat. In the United States, about 9000 deaths and 6.5–33 million illnesses each year are food related (Park et al., 2002). As a result, fecal and ingesta contaminants on poultry carcasses are prohibited in order to reduce the risk of an adverse health effect. For safety purposes, the successful identification of poultry carcasses contaminated by feces and/or ingesta is critical to protect the public health from a potential source of food-borne illnesses. Generally, the color of fecal material ranges from different shades of yellow to green, brown, and white and the consistency of feces is normally semisolid to paste. These guidelines are used by inspectors to prevent visibly fecal-contaminated carcasses from entering the chilling tanks. In addition to being very tedious, this manual inspection method is not only labor-intensive but also prone to both human error and inspector-to-inspector variation.

Hyperspectral imaging systems with different designs have been developed for the identification of fecal matter and ingesta on the surfaces of poultry carcasses. Intensive research has been conducted by the USDA Agricultural Research Service (ARS) for calibrating hyperspectral imaging systems, identifying spectral signatures of different contaminants, building models for fecal detection, and exploiting the system in online multispectral applications (Chao et al., 2008; Park et al., 2007). Park et al. (2006) attempted to find out the optimal supervised classification algorithms for the identification of fecal and ingesta contaminants on the surfaces of poultry carcasses. In this research, a pushbroom hyperspectral imaging system was used to acquire hyperspectral images, with 512 narrow bands ranging from 400–900 nm. Three different feces from digestive tracts (duodenum, ceca, and colon) and ingesta were regarded as contaminants. Figure 2.9 shows typical hyperspectral images of an uncontaminated carcass, surface contaminants with regions of interest (ROIs), and the corresponding spectra from each ROI. As shown, 25 pixels were recognized as duodenum, 27 pixels as ceca, 78 pixels as colon, 93 pixels as ingesta, and 195 pixels as skin of different locations of thigh, breast, and wing. The reflectance spectra of skin were observed to be much higher than those of the contaminants. After spectra were extracted from different ROIs, six different supervised classification methods were investigated and the performances were compared. Those supervised classification algorithms were parallelepiped, minimum distance, maximum likelihood, Mahalanobis distance, spectral angle mapper, and binary encoding classifier. The classification accuracies varied with the algorithms as well as with the type of feed used to grow the broilers. The highest classification

Downloaded From Vet-eBooks.com

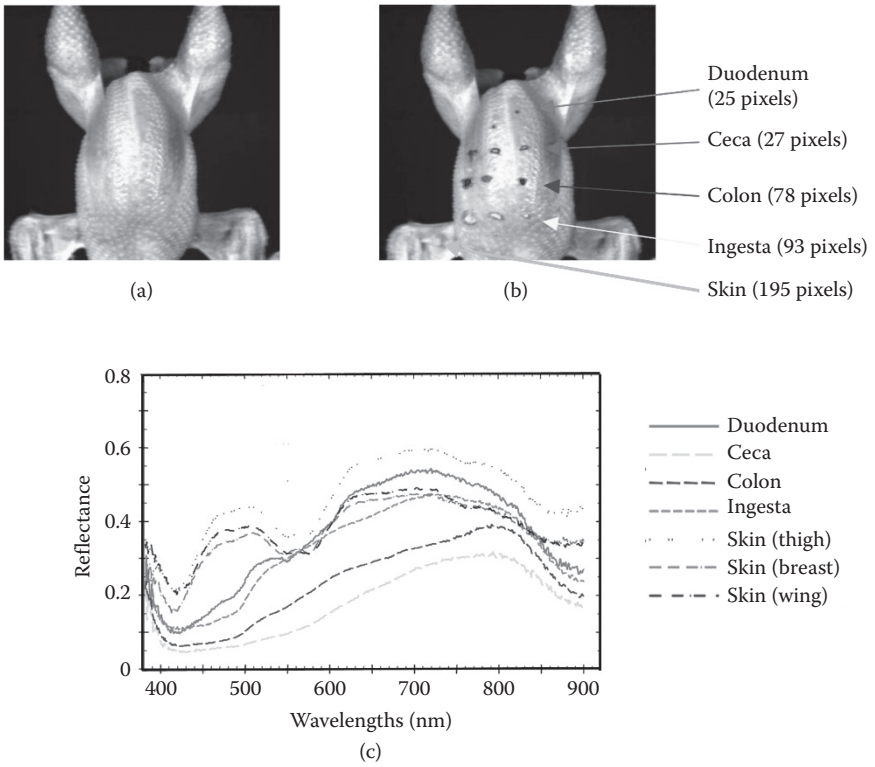


FIGURE 2.9
Regions of interests (ROIs) of a corn-fed poultry carcass and the corresponding mean spectra. (a) Uncontaminated carcass. (b) Fecal contaminant ROIs. (c) Mean spectra of ROIs. (Reproduced from Park, B. et al., *Transactions of the ASABE,* 49, 2017–2024, 2006.)

accuracy for identifying contaminants from corn-fed carcasses was observed to be 92.3% with a spectral angle mapper classifier.

A multispectral imaging system to detect fecal and ingesta contaminants on the surface of poultry carcasses was developed for a real-time, online poultry inspection application (Park et al., 2004). Three optimum wavelengths of 515.4, 566.4, and 631 nm was selected according to the results of previous research (Park et al., 2002; Windham et al., 2003) and these three-band spectral images were collected using a common aperture camera with three selected optical filters that were preinstalled into the camera assembly. The detailed process for the real-time image processing is described in Figure 2.10. It was found that the ratio of 566.4-nm image/515.4-nm image could identify feces (duodenum, ceca, and colon) and ingesta contaminants. Meanwhile, a masking process was implemented to remove the noisy background by using a 631-nm image. After a threshold process, three white spots of each row from the top

Downloaded From Vet-eBooks.com

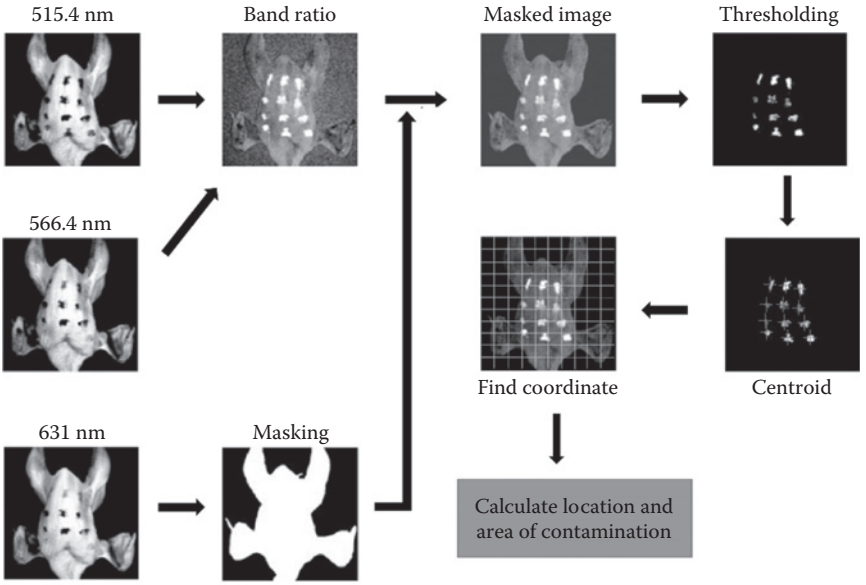


FIGURE 2.10
(a) The process of a real-time multispectral imaging method for automated fecal and ingesta detection on poultry carcasses (b) and the corresponding flowchart. (Reproduced from Park, B. et al., *Journal of Food Process Engineering*, 27, 311–327, 2004.)

Downloaded From Vet-eBooks.com

to the bottom were obtained to represent contaminants from duodenum, ceca, colon, and ingesta from the crop or gizzard, respectively. The crossline-markers denoted the centroid of each contaminant spot. Finally, blob analysis was used to determine the location of each contaminant spot. The total processing time to analyze one bird was 251 ms or 3.99 frames per second, which means processing capability would exceed 239 birds per minute. The overall accuracy to detect fecal and ingesta contaminants was 96.8% and the prediction accuracy of identifying each contaminant was 92.4% for duodenum and 98% for ceca, colon, and ingesta with moderate false positives. The algorithm developed in this research confirmed that a multispectral imaging system with batch image-processing algorithm can be effectively used for the real-time, online poultry processing speed of at least 140 birds per minute.

In addition, Chao et al. (2007) have developed a multispectral imaging platform using a line-scan hyperspectral camera with a spectrograph for differentiation of wholesome and systemically diseased poultry carcasses at a commercial line speed. Using a threshold of 0.4 for fuzzy output decision values, multispectral classification was able to obtain 97.6% accuracy for wholesome birds and 96.0% accuracy for systemically diseased birds in the testing dataset. More recently, Park et al. (2010) attempted to merge the fecal detection and systemic disease detection systems onto a common platform using a line-scan hyperspectral imaging system in real-time mode during poultry processing. Figure 2.11 presents a line-scan hyperspectral imaging system setup developed in this research to collect image data for contaminate detection on poultry carcasses at a commercial line speed at the Beltsville imaging laboratory. The results testified to the feasibility of a line-scan hyperspectral imaging system in terms of processing speed and detection accuracy for real-time, online fecal detection and wholesomeness inspection in a commercial poultry plant.

2.5.1.2 Tumor and Bone Detection

The presence of tumors and bones in chicken carcasses poses serious problems to the producers because they are not acceptable for consumers. Chicken skin tumors refer to round ulcerous lesions that are surrounded by a rim of thickened skin and dermis. Skin cancer makes skin cells unable to divide and grow normally, and induces abnormal cells to grow out of control to form tumors. Tumors are not as visually obvious as other pathological diseases because their spatial signature appears as shape distortion rather than a discoloration. Moreover, tumors vary in size and shape and sometimes they can be found in both sides of the chicken. Therefore, identification is a big challenge because some blemishes are rather difficult to discern by using traditional manual inspection and conventional vision-based inspection systems operating in the visual spectrum may also suffer limitations (Du et al., 2007). Additionally, different tumors present different spectral responses and some parts of normal chicken skin even have similar spectral response

Downloaded From Vet-eBooks.com

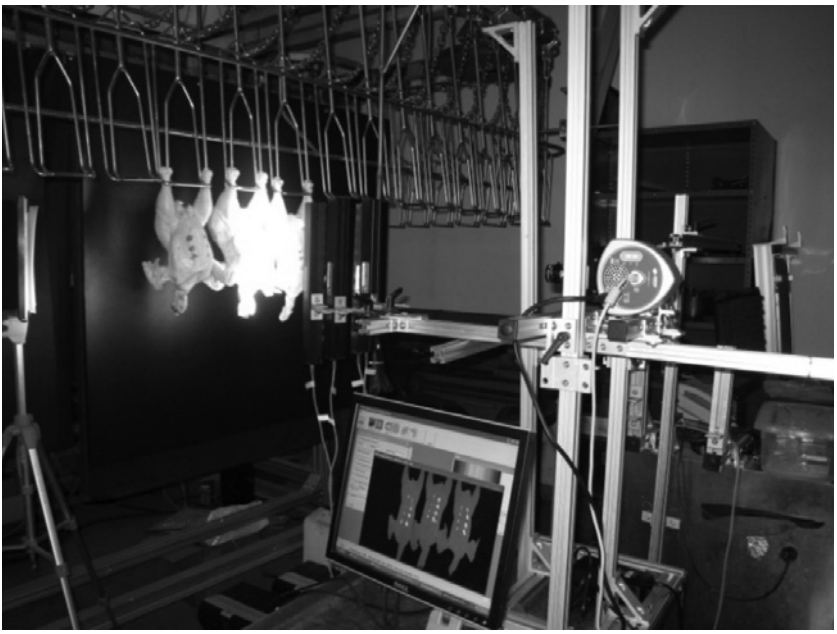


FIGURE 2.11
Line scanning real-time hyperspectral imaging system setup. (Reproduced from Park, B. et al., Line-scan hyperspectral imaging for real-time poultry fecal detection. *SPIE Defense, Security, and Sensing*. International Society for Optics and Photonics, Orlando, Florida, 76760I, 2010.)

as that of tumors, which makes detection a more difficult task. In this aspect, hyperspectral imaging shows potential for identification and classification of biomedical abnormalities due to its ability of simultaneously providing spatial and spectral features of the objects of interest in the image.

Currently, two methods, namely hyperspectral fluorescence imaging and hyperspectral reflectance imaging, are widely used to detect tumors on poultry carcasses. Prior work on detection of chicken skin tumors used PCA on hyperspectral reflectance images to select three effective wavelengths (465, 575, and 705 nm) and developed a multispectral imaging system (Chao et al., 2002). In this study, they achieved classification accuracy of 91% and 86% for normal and tumorous skin tissue regions, respectively. Kim et al. (2004) investigated the use of a hyperspectral fluorescence imaging system for identification of chicken skin tumors. A spectral classifier generated a spectral map that locates candidate regions for skin tumors. Then, a spatial classifier was employed to select the real tumor spots from the potential tumorous regions, which yielded final results showing the location of tumors. This method was believed to be useful to detect chicken carcasses with tumors, but it failed to detect some tumors smaller than 3 mm in diameter. The detection rate, false positive rate, and miss rate of this proposed method were 76%, 28%, and 24%, respectively. Nakariyakul

Downloaded From Vet-eBooks.com

and Casasent (2007) developed a novel feature selection method to detect skin tumors on chicken carcasses using hyperspectral reflectance data. Figure 2.12 demonstrates the whole process to obtain the final classification image (Figure 2.12d) by using a fusion system. As mentioned earlier, a chicken skin tumor consists of an ulcerous lesion region, which is surrounded by a region of thickened skin; the spectral responses of the lesion and the thickened-skin regions of tumors are remarkably different. In this research, the feature selection algorithm was applied to choose different sets of spectral bands to separately detect the lesion and thickened-skin regions of the tumors. A pair of binary images were produced to locate a candidate lesion and thickened-skin pixels and then morphologically processed and finally fused to reduce false alarms. As shown in Figure 2.12d, the initial test results were promising with 11 of 14 skin tumors detected without any false alarms. Two of the three missed tumors were very small and were expected to be missed. Meanwhile, the feature selection algorithm only included five bands, therefore making a fast and inexpensive multispectral sensor system possible.

Fragments of bone in boneless poultry meat also pose significant hazards to consumers. Bone fragments may result from bones that are already broken before or during slaughter or a misaligned cutting blades shaving pieces from the skeleton. Bone fragments, including the clavicle (wishbone), which is a long, sharp, and completely calcified bone, have the potential to cause injury. Yoon et al. (2008) evaluated how reflectance spectra can be exploited for detection of bone fragments in chicken fillets. Specifically, a fusion of hyperspectral transmittance and reflectance imaging, which is a nonionized and nondestructive imaging modality, was developed in this study as an

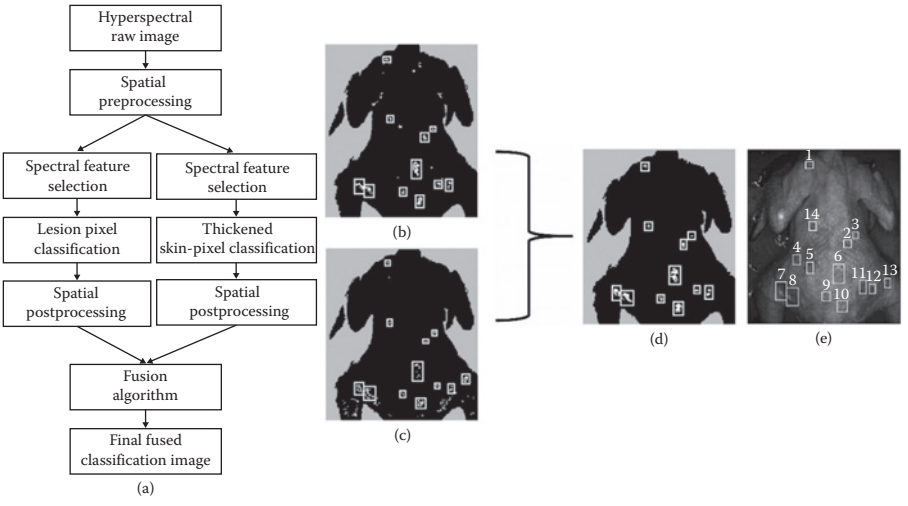


FIGURE 2.12
Chicken skin tumor detection by using hyperspectral imaging aided with a fusion algorithm.

Downloaded From Vet-eBooks.com

alternative method to the conventional transmittance x-ray imaging technique. An enhancement method using an illumination-transmittance model was proposed to correct nonuniform illumination effects so that embedded bones were more easily identified by a simple segmentation method with a single threshold value. Predicted bones were classified by the nearest neighbor classifier which was trained by the library of mean spectral reflectance of chicken tissues such as fat, meat, and embedded bones. Their study observed that bones embedded in compressed 1 cm thick samples can be successfully detected when both sides of the sample were examined. Their results were promising, indicating that a hyperspectral imaging system was effective for detecting bones on poultry carcasses when appropriate image processing algorithms were applied.

2.5.1.3 Microbiological Spoilage Detection

All chicken meat provided to the markets must undergo quality control in order to guarantee consumer safety. Without proper storage conditions, undesirable microorganisms may grow to cause sensory changes, discoloration, and changes in taste. Therefore, fast and automatic methods for precise prediction of bacterial loads in chicken meat are required in the poultry industry for efficient management. A laboratory-based push-broom hyperspectral imaging system in the NIR range was investigated to evaluate TVCs in raw chicken fillets (Feng and Sun, 2013a). To simplify the models, important wavelengths were selected by stepwise regression. Meanwhile, the resulting reflectance images were transformed into hypercubes in absorbance and Kubelka–Munck (K–M) units. Three PLSR models were built based on reflectance spectra, absorbance data, and K–M parameters, named RS-PLSR, AS-PLSR, and KMS-PLSR, respectively. More robustness was found in the resulting simplified KMS-PLSR model with high correlation coefficients (R) of 0.96 and 0.94 as well as low root mean squared errors (RMSEs) of 0.40 and 0.50 $\log_{10}$ CFU/g, for calibration and cross-validation, respectively. With respect to detecting specific bacteria, the hyperspectral imaging technique as a nondestructive tool has also been employed for quantitative and direct determination of *Pseudomonas* loads (Feng and Sun, 2013b) and Enterobacteriaceae loads (Feng et al., 2013) on chicken fillet. The best model for *Pseudomonas* loads prediction produced R and RMSEs of 0.91 and 0.55 $\log_{10}$ CFU/g, 0.87 and 0.65 $\log_{10}$ CFU/g as well as 0.88 and 0.64 $\log_{10}$ CFU/g for calibration, cross-validation, and prediction, respectively. Three optimal wavelengths (930, 1121, and 1345 nm) were selected and competent for predicting Enterobacteriaceae loads with R^2 of 0.89, 0.86, and 0.87 and RMSEs of 0.33, 0.40, and 0.45 $\log_{10}$ CFU/g for calibration, cross-validation, and prediction, respectively. All the mentioned studies had the advantage of building a prediction map to visualize the distribution of bacteria on chicken fillet, which is not available by conventional methods.

Downloaded From Vet-eBooks.com

In addition to the abovementioned applications, there have been an increasing amount of research about predicting quality parameters of poultry meat using hyperspectral imaging systems. For instance, Xiong et al. (2015b) used a hyperspectral imaging system (400–1000 nm) to predict hydroxyproline content in chicken meat and good results were achieved from the simplified regression model with R_p of 0.854 and low RMSEP of 0.049. Springiness, as one of the most important quality characteristics of chicken meat, was also measured by a hyperspectral imaging technique with satisfying results presented (Xiong et al., 2015a). However, future work should be carried out to investigate the feasibility of multispectral imaging systems for online and at-site inspections in terms of predicting these parameters in chicken meat.

2.5.2 Fish

Fish and fish products play a significant part in our daily diet; in addition to an appetizing taste, they are regarded as the best sources of fats, proteins, and essential micronutrients to promote human health. Globally, fish and fish products are consumed by nearly two-thirds of the world's population, providing roughly 40% of the protein intake (Cheng et al., 2013). Nevertheless, there are some concerns and challenges related with the evaluation of fish quality and safety at an industrial level. Fish quality and safety is a scientific discipline referring to handling, preparation, processing, transportation, and storage in ways that prevent food-borne illness and supply the consumer with premium quality products. However, fish is one of the most perishable and heterogeneous aquatic products, and it serves as a growth medium for microorganisms that may cause fish spoilage or even cause disease in the consumer. In addition, chemical composition and sensory attributes of fish are highly affected by preharvest (species, sex, age, feeding, and environmental habits) and postharvest factors (storage methods, time, and temperature). Subtle changes in color, juiciness, texture, and biochemical properties of fish are likely to affect consumers' sensory evaluation of fish quality as well as their decisions in making a second purchase. Therefore, appropriate tools for inspection are required to maintain good quality and safety in production and trade of fish and fish products.

During the past few decades, a great number of different techniques have been explored as possible instrumental methods for quality and safety evaluation of fish, including sensory evaluation based on the quality index method (QIM) (Larsen et al., 1992), biochemical methods with HPLC (Mendes et al., 2009), and microbial inspection based on TVCs (Song et al., 2012). These techniques play a critical role in the current fish industry; some of them have always been used as gold standards and regulation methods serving scientific research because of their relative validity and accuracy. However, these methods are normally expensive, time consuming, laborious, and require highly

Downloaded From Vet-eBooks.com

skilled operators, and are not suitable for online and real-time monitoring. Applications of hyperspectral imaging for quality and safety assessment of fish are mainly concentrated on overall fish freshness, chemical composition determination, and microbial inspection. Compared with NIR spectroscopy, applications of hyperspectral imaging in freshness grading and quality evaluation of fish in both research and industry have a limited number of publications, although there is a prevailing tendency of promising success. The reason probably lies in the fact that hyperspectral imaging is still a relatively new technique, and its full potential has yet to be exploited so far. The hyperspectral imaging technique is able to optimize processing of the raw fish, determing the correct pricing, labeling, and documentation, and identify quality authentication when appropriately applied in production and packaging plants.

2.5.2.1 Freshness

Fresh fish normally refers to the fish being caught/harvested and then chilled and stored for a short period before use. Freshness is an ambiguous term summing up many factors, mostly associated with a sensory impression or understanding. The freshness is reduced during storage time until spoilage occurs due to physical, chemical, biochemical, and microbiological processes, which are mainly affected by time and temperature. Traditionally, fish freshness has been measured by sensory inspection (Anastasio et al., 1999), which is carried out through the sense of smell, sight, taste, and touch. Descriptive analysis like QIM for sensory analysis of marine products is currently increasingly used. These methods are relatively simple, but inefficient, time consuming, costly, and very subjective because the assessment is based on individual preferences, although the bias could be reduced if proper sensory training is implemented. Instrumental methods are also developed, such as an electric nose that simulates the human sense of smell for analyzing the odor profile of fish samples. These methods enable objective and reliable results to a certain degree, but they are still not efficient when a large number of samples need to be measured.

In recent years, hyperspectral imaging has been employed as an advanced method for automatic freshness assessment of different kinds of fish during production. Chau et al. (2009) employed the hyperspectral imaging system in the region of 892–2495 nm to determine overall freshness of fish. Their research revealed that hyperspectral imaging was able to evaluate the freshness in all spots (pixels) of the fish surface instead of evaluating the fish freshness at specific regions on the fish surface. It also showed that there were differences observed among the mean spectra of a whole fish, the fillet flesh, and belly flap regions of a fillet. These dissimilarities between the spectra are basically due to the significant differences between chemical compositions of these parts. Meanwhile, hyperspectral interactance imaging in the region 400–1000 nm was investigated for differentiating between fresh

Downloaded From Vet-eBooks.com

and frozen-thawed cod fillets and for measuring the freshness at industrial speed (40 cm/s or 1 fillet/s) (Sivertsen et al., 2011). In this study, PCA, K-nearest neighbor (KNN) classifier, and PLSR were applied as the multivariate analysis. Their results showed that the freshness, defined as storage days on ice, can be well predicted for each of the unfrozen samples with a RMSECV of 1.64 days for the simplified PLSR model calculated from only four wavelengths. This result was comparable, or better, than that obtained from sensory evaluation by the QIM with a group of trained panelists.

More recently, Cheng and Sun (2015a) explored the feasibility of using hyperspectral imaging (400–1000 nm) in tandem with a data fusion technique for rapid and nondestructive determination of sensory quality index scores (QIS) of grass carp fish fillet during cold storage. In the QIM scheme, QIS is assigned for each quality parameter in a numeric scale using a demerit score system, ranging from 0 to 3. Score 0 normally refers to the very fresh fish, with scores increasing with storage time. In their study (Cheng and Sun, 2015a), a total of 135 fresh fish subsamples were involved and the reflectance hyperspectral images were acquired in a line scanning mode. Five important wavelengths (441, 560, 598, 639, and 684 nm) were collected by the successive projections algorithm (SPA) and 13 textural feature variables were extracted by the gray-level gradient co-occurrence matrix (GLGCM) method. In order to boost the robustness of models, these selected spectral variables and textural feature variables were fused by applying the feature level fusion method. Figure 2.13 shows the flowchart of data fusion for measurement of fish fillet QIS using hyperspectral imaging. The least squares-support vector machine (LS-SVM) model developed by data fusion of effective spectra and texture data presented the most excellent performance with RPD of 4.23, R^2_P of 0.944, and RMSEP of 0.703. These results verified that hyperspectral imaging is an admirable alternative to the conventional evaluation by trained panelists for assessment of fish freshness and it also indicated the potential and promise for making more efforts to facilitate this emerging technique for online applications in the fish industry.

2.5.2.2 Chemical Compositions

Another aspect related to fish quality and safety properties is intrinsic chemical attributes such as water, protein, fat, and salt. These key quality parameters vary greatly from one species or one individual fish to another and these variations can cause a severe deterioration of sensory quality, and loss of nutritional and commercial values. Water is the largest component of fish composition and is closely associated with other attributes such as fat and lipid. Furthermore, moisture plays an essential part in affecting microbial growth, leading to influencing the shelf life of fish and fish products. The first application of hyperspectral imaging for moisture content prediction was conducted by Wold et al. (2006) using a multispectral imaging NIR transflectance system in the region of 760–1040 nm for online evaluation

Downloaded From Vet-eBooks.com

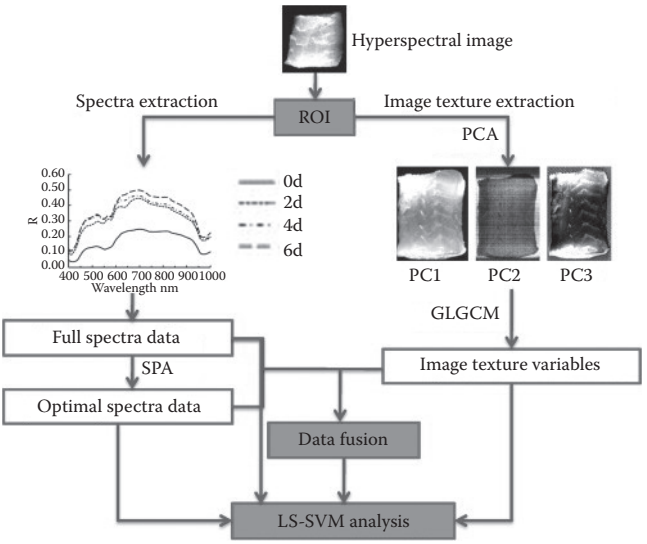


FIGURE 2.13
Flowchart of data fusion for prediction of quality index scores in grass carp fish fillet by reflectance hyperspectral imaging. GLGCM, gray-level gradient co-occurrence matrix; LS-SVM, least squares-support vector machine; PCA, principal component analysis; ROI, regions of interest; SPA, successive projections algorithm. (Reproduced from Cheng, J.-H. and Sun, D.-W., *LWT—Food Science and Technology*, 63, 892–898, 2015a.)

of dried salted coalfish (*Bacalao*). Fish was placed on the conveyor belt and moved at a speed of approximately 0.1 ms^{-1}. It was scanned line by line to collect the entire spectral image, which was acquired with a spectral resolution of approximately 20 nm at the speed of 10,000 spectra per second. Their research achieved the best prediction model with R^2 and RMSECV equal to 0.92 and 0.70%, respectively. In addition, hyperspectral imaging was also explored to determine the spatial distribution of moisture content in farmed Atlantic salmon fillets (He et al., 2013). Three spectral regions of 400–1000 nm (Spectral Range I), 900–1700 nm (Spectral Range II), and 400–1700 nm (Spectral Range III) were considered, and optimized PLSR models were built using only the important wavelengths selected from each spectral range, resulting in R^2_P of 0.893, 0.888 and 0.884, and RMSEP of 1.517%, 1.553%, and 1.578% for the three spectral regions, respectively.

Fat content is also one of the most significant quality criteria of fish, not only from a nutritional point of view, but also because of its sensory and functional properties. Nevertheless, conventional chemical analytical methods for fat content assessments are destructive and time consuming and require the use of hazardous chemicals that may be harmful to analysts and the environment (Xu et al., 2015). Therefore, online NIR hyperspectral interactance imaging (760–1040 nm) was used as an advanced and noncontact technique to determine the fat distribution in raw and salted salmon (*Salmo salar*) fillets (Segtnan et al., 2009).

Downloaded From Vet-eBooks.com

Satisfactory results were obtained with correlation R and prediction error (RMSECV) for raw fillets of 0.947 and 1.96% and for salted fillets of 0.966 and 1.95%, respectively. Likewise, Zhu et al. (2014) used a pushbroom NIR (899–1694 nm) hyperspectral imaging system to determine the spatial distribution of fat in Atlantic salmon fillets. The quantitative relationship between spectral data and the reference fat contents was successfully established based on PLSR with R_P of 0.93 and RMSEP of 1.24.

In another interesting endeavor for the application of compositional analysis in fish, an NIR hyperspectral imaging technique was employed for noninvasive distributional analysis of salt (NaCl) and fat in salmon fillets during salting, salt equilibration, and smoking, which are recognized as very critical for modern fish processing plants (Segtnan et al., 2009). In spite of the fact that NaCl molecules are not infrared active, several studies have been implemented on the use of NIR spectroscopy for prediction of NaCl contents in foods. Segtnan et al. (2009) acquired hyperspectral images of salted and smoked salmon fillets and built models with the use of PLSR. It was found that NIR interactance imaging alone can predict NaCl contents locally in salted salmon fillets with RMSECV of 0.56% and R of 0.86. In addition, a large number of other studies have also stressed the potential of hyperspectral imaging for automatic inspection and evaluation of physical properties in fish, such as the measurement of color distribution in salmon fillet (Wu et al., 2012), determination of water holding capacity (Wu and Sun, 2013c), and analysis of texture (Wu et al., 2014).

2.5.2.3 Parasites and Defects

As with other foods, safety is a key issue in the fish industry. Therefore, producers must deliver fish and fish products free from parasites and nematodes because these incidents about parasites in fish muscle to a great extent lead to an instant negative response from consumers toward the product. So far, the only reasonable solution is the manual inspection and trimming of each fillet on a candling table to avoid the occurrence of parasites in fish products and detect them online (Elmasry et al., 2012a). This is a very labor-intensive and time-consuming method because the operator has to inspect the fillet first and then manually remove the defects from the fillet. As a consequence, the hyperspectral imaging technique has been used to detect parasite contamination in fish. In an early study, Wold et al. (2001) applied a multispectral imaging technique in the visible and NIR spectral region to detect parasites in cod (*Gadus morhua*) fillets. It was observed that parasites in fish flesh presented distinctive spectral fingerprints compared to the normal fish muscles. They further confirmed that this methodology was capable of detecting parasites at a depth of 6 mm below the fillet surface, which is 2–3 mm deeper than what can be found by manual inspection of fish fillets.

Recently, interactance hyperspectral imaging was conducted under industrial conditions to automatically detect nematodes in cod fillets (Sivertsen et al.,

Downloaded From Vet-eBooks.com

2012). The test was performed at a fish processing plant in northern Norway and this system operated at a conveyor belt speed of 400 mm/s, which meets the industrial speed requirements. A detailed sketch of the main system components are illustrated in Figure 2.14a, and a photograph of the inspection machine, with the front cover removed, is presented in Figure 2.14b. A Gaussian maximum likelihood (GML) classifier was utilized to classify pixels as nematodes or not according to their corresponding spectra. Results indicated that the detection rate was 61.5% for all nematodes, and 70.8% and 60.3% for the dark and pale nematodes, respectively. To some degree, these studies reveal that the hyperspectral imaging technique has the potential to replace manual inspection in the fish processing industry.

2.5.2.4 Microbial Spoilage

A hyperspectral imaging system in the region of 400–1700 nm was developed to rapidly and noninvasively determine TVC of salmon fillets during the spoilage process (Wu and Sun, 2013d). The reference TVC values were measured using a standard plate count method and calibration models were established using PLSR and LS-SVM. The competitive adaptive reweighted sampling (CARS) algorithm was used to identify the effective wavelengths, which largely reflected the changes of microbial spoilage. A CARS-PLSR

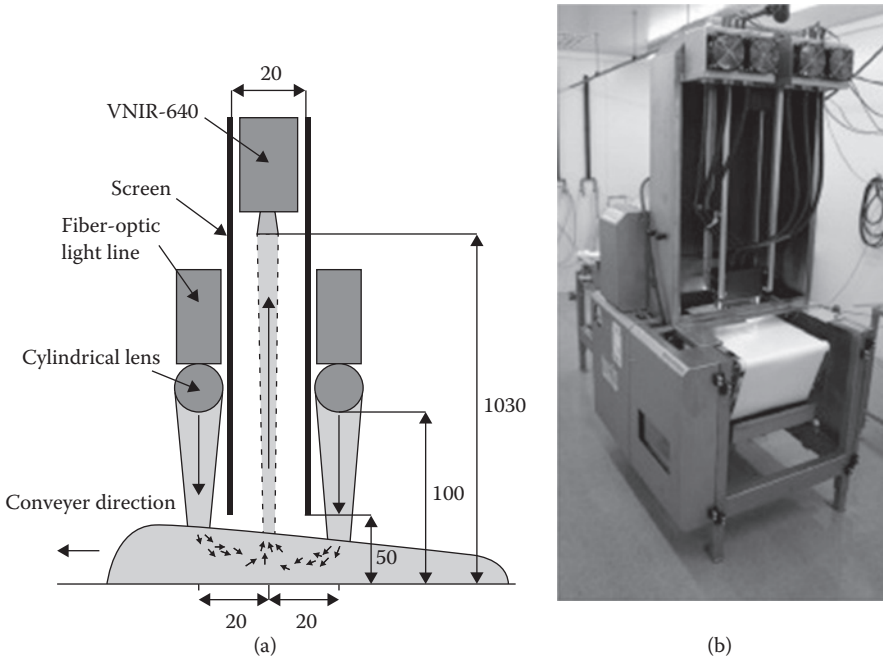


FIGURE 2.14
(a) A sketch illustrating the dimensions and position of the spectrometer and fiber lines and (b) a photo of the hyperspectral imaging machine with the front cover removed.

Downloaded From Vet-eBooks.com

model built with only eight spectral variables demonstrated the best performance with R^2_P of 0.985 and RPD of 5.127. More recently, Cheng and Sun (2015b) used hyperspectral imaging in the spectral range of 400–1000 nm to measure the *Escherichia coli* loads in grass carp fish for evaluation and visualization of microbial spoilage. A PLSR model built with full wavelengths showed good performance with RPD of 5.47 and $R^2_P = 0.880$. A simplified PLSR with six characteristic wavelengths also demonstrated good prediction capability (RPD = 5.22 and $R^2_P = 0.870$). The results presented in these studies confirmed that hyperspectral imaging systems are suitable for microbial spoilage control in view of the online requirements of fish industry.

2.5.2.5 Differentiation and Classification/Sorting

Fish product differentiation and classification is an emerging area of concern in the fish processing industry. The feasibility of Vis/NIR hyperspectral imaging (380–1030 nm) in alliance with LS-SVM classifiers was investigated to discriminate the fresh and frozen-thawed halibut fillets (Zhu et al., 2013). In this work, a classification model was established based on the textural feature variables extracted by gray-level co-occurrence matrix analysis. Satisfactory results with a classification rate of 97.22% were obtained. In another work, categorization of fresh Atlantic salmon fillets stored under different atmospheres using hyperspectral imaging was conducted by Sone et al. (2012). PCA was used to study changes occurring in the spectral characteristics of samples under different storage atmospheres and a KNN classifier was used for the classification of fillets by packaging type. Successful classification of over 88% was obtained, which was mainly associated with spectral characteristics at the wavelengths of 606 and 636 nm. Meanwhile, Ivorra et al. (2013) also applied Vis/NIR hyperspectral imaging to distinguish expired and nonexpired vacuum-packed salmon based on PLS-DA method for classification purposes. The result obtained has a classification success rate of 82.7% in cross-validation from real commercial samples, which makes this technique promising for the nondestructive detection of expired packaged smoked salmon. On the basis of the results obtained from the earlier studies, it is necessary to develop suitable classification algorithms and enhance the hardware of the hyperspectral imaging system to boost and accelerate the industrial application of a hyperspectral imaging technique with a higher correct classification rate near 100%.

2.5.3 Red Meats

Red meats, such as pork, beef, and lamb, play a significant part in people's daily diet as they are good sources of animal protein, vitamins, and minerals. For meat and meat products, it is very critical to keep them at a high quality for the modern meat industry because superior quality of these products is always demanded by consumers. Red meat is a nutritious, expensive,

Downloaded From Vet-eBooks.com

and perishable food commodity, and its quality is always associated with individual experience and preference. Generally, meat quality is defined as a measurement of attributes or properties which determine the suitability of meat as fresh or stored for a reasonable period without deterioration (Elmasry et al., 2012b). Particularly, quality attributes of red meats usually comprise sensory, chemical, microbiological, and technological attributes (Xiong et al., 2014), as demonstrated in Figure 2.15. Regrettably, there is a great variability in these attributes of raw meat, accounting for highly variable meat products being marketed without a controlled level of quality. This issue will be aggravated when the industry cannot satisfactorily characterize this level of quality and thus is unable to market products with a certified quality level (Damez and Clerjon, 2008).

Traditionally, sensory attributes (color, juiciness, firmness, marbling, tenderness, etc.) of many foods, including red meats, are evaluated by well-trained assessors. For meat color and marbling, the inspection methods are similar and usually conducted by comparing the color of the rib eye muscle (*Musculus longissimus dorsi*) or the proportion of intramuscular fat within the *Musculus longissimus dorsi* and scoring against the reference standards specific for each of the meat species (Xiong et al., 2014). Today, other methods like chemical methods and instrumental techniques have also been used in evaluation of quality attributes, which are more convenient and effective than manual inspection. For chemical methods, the universal standard for crude protein analysis in food is the Kjeldahl method (Kirk, 1950) while for moisture content the oven-drying method is the standard (Willits, 1951). However, most of the chemical and instrumental techniques are destructive and require lengthy sample preparation. Hence, these methods are not feasible for rapid analysis of quality attributes in industrial and commercial processing. In addition, from the viewpoint of quality assurance, it is desirable to inspect all meat and meat products and thereby an ideal sensing technique for the meat industry should be online and nondestructive.

In recent years, many studies have been reported on developing nondestructive techniques for detecting internal and/or external red meat quality attributes (Kamruzzaman et al., 2012b). Different methodologies based on

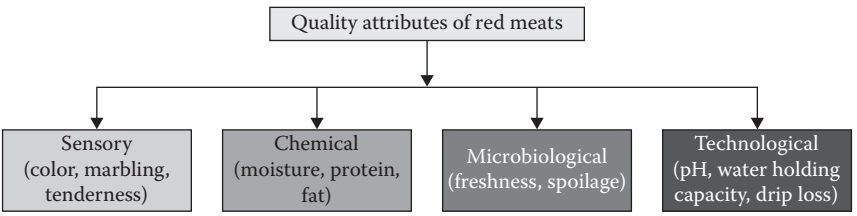


FIGURE 2.15
Common quality attributes of red meats analyzed in the meat industry.

Downloaded From Vet-eBooks.com

different principles, procedures, and instruments, such as ultrasound technology and computed tomography (CT) scanning, are currently available for measuring different meat quality attributes. Among them,the hyperspectral imaging technique is widely accepted as a new inspiring and versatile technique for rapid, reagentless, and noninvasive quantitative applications in the red meat industry. As a matter of fact, a large amount of research has been done in the application of hyperspectral imaging in red meats analysis, highlighting its capability of measuring many different quality attributes such as pH, color, tenderness, and fat content in beef, pork, and lamb (Barbin et al., 2013a; Kamruzzaman et al., 2012c).

2.5.3.1 Sensory Attributes

Today, the application of hyperspectral imaging systems to measure sensory attributes of red meats are largely focused on prediction of color, marbling, and tenderness. Sensory attributes are the main quality attributes that have a huge influence on consumers' overall evaluation of red meats. As consumers usually use color as an indicator of freshness and wholesomeness of meat, an attractive and stable color in the meat has a major influence on the buying decision made by the consumer. Marbling refers to the white flecks of fat present within the lean in the muscle, and the marbling score normally evaluates the distribution density of fat in the carcass rib eye region. As marbling enhances juiciness, retail cuts with little marbling tend to account for cooked products that lack flavor and juiciness. Ideally, it should be uniformly and finely distributed throughout the lean, as this is preferred by consumers to enhance eating satisfaction. With respect to tenderness, it is an expression of meat texture, which is regarded as the most important sensory quality attribute related to consumer satisfaction. Meat tenderness is also positively associated with juiciness and flavor, and therefore consumers accept paying more for tender meat. Sources of tenderness variation in meat may be ascribed to the animal's sex, age, breed, and antemortem stress, as well as postmortem treatments. Indeed, meat tenderness is a complex phenomenon that encompasses many characteristics such as chewiness, springiness, cohesiveness, hardness, and even juiciness (Elmasry et al., 2012a).

Color is an important feature that is used in conjunction with other characteristics to determine the grade or suitability of red meat for a particular market. Beef and lamb meat in bright red and pork in pink are desirable to consumers. Retailers have to cut prices for meat with undesired surface color, which leads to revenue losses. ElMasry et al. (2012c) developed a hyperspectral imaging system in the NIR region for noncontact measurements of surface color, pH, and tenderness of fresh beef. Hyperspectral images were acquired in a pushbroom mode and reference color values were measured in a $L^* a^* b^*$ color system with a chromometer (CR-400, Konica Minolta Corp., Japan). It was found that PLSR models for prediction of color components L^* and b^* were reasonably good; yet prediction of color component a^* was not

Downloaded From Vet-eBooks.com

satisfactory. The resulting PLSR models generated R^2_{CV} of 0.88 and 0.81 and RMSECV of 1.21 and 0.57 for lightness (L^*) and yellowness (b^*), respectively. In addition, an image processing algorithm was developed to transfer the simplified model to every pixel in the image for visualizing color distribution in all portions of the beef sample. ElMasry et al. (2012c) thereby concluded that a hyperspectral imaging technique could be effectively implemented in commercial meat processing plants for rapid and nondestructive quality analysis when more improvements in terms of speed and processing are achieved. In addition to beef, the objective determination of the pork color attribute has also been investigated using a hyperspectral imaging technique (Barbin et al., 2012b). In this research, hyperspectral images in the NIR region were acquired for pork samples from the *longissimus dorsi* muscle by a push-broom hyperspectral imaging system. Reference color (L, a, and b values) was measured using a Minolta Chromameter (CR-400, Konica Minolta Corp., Japan) calibrated against white ceramic tile. Meanwhile, chroma ($(a^2 + b^2)^{1/2}$) and hue angle (arctan b/a) were also calculated, as it is believed that these parameters can change due to differences in meat quality. The optimized PLSR models were developed to predict color features (L, a, b, chroma, and hue angle) and good performance was achieved in the prediction of lightness (L) with R^2_{CV} of 0.93, R^2_P of 0.90, RMSECV of 1.36 < and RMSEP of 1.63. Distribution maps for lightness (L) prediction in pork meats were generated by applying this reduced PLS-R model (Figure 2.16b).

As for marbling, some researchers have used hyperspectral imaging technique in marbling evaluation of red meat and obtained good results. For instance, Li et al. (2011) investigated assessment of beef-marbling grade using a hyperspectral imaging system in the spectral region of 400–1100 nm. A charactersitic band was collected at the point where the maximal ratio of gray value of fat and lean occurs and therefore the images at 530 nm were used to differentiate beef-marbling. In addition, three characteristic parameters of samples, namely the density of large-particle fat, that of medium-particle fat, and that of small-particle fat, were applied to establish a prediction model by the MLR method and the beef samples were successfully graded with R^2 of 0.92 for full cross-validation.

With respect to meat tenderness, the most common method is to measure the mechanical properties of a meat sample using the Warner–Bratzler shear force (WBSF) or slice shear force (SSF), yet both of them are not suitable for the commercial and fast-paced production environment. Recently, interest in exploiting hyperspectral imaging for the assessment of red meat tenderness is growing (ElMasry et al., 2012c). In an early study, Naganathan et al. (2008) developed and tested a Vis/NIR hyperspectral imaging system to predict meat tenderness of 14-day aged beef. On the basis of the extracted features, discriminant models were established to predict three beef tenderness categories, namely tender (SSF $\leq$ 205.80 N), intermediate (205.80 N < SSF < 254.80 N), and tough (SSF $\geq$ 254.80 N). The prediction

Downloaded From Vet-eBooks.com

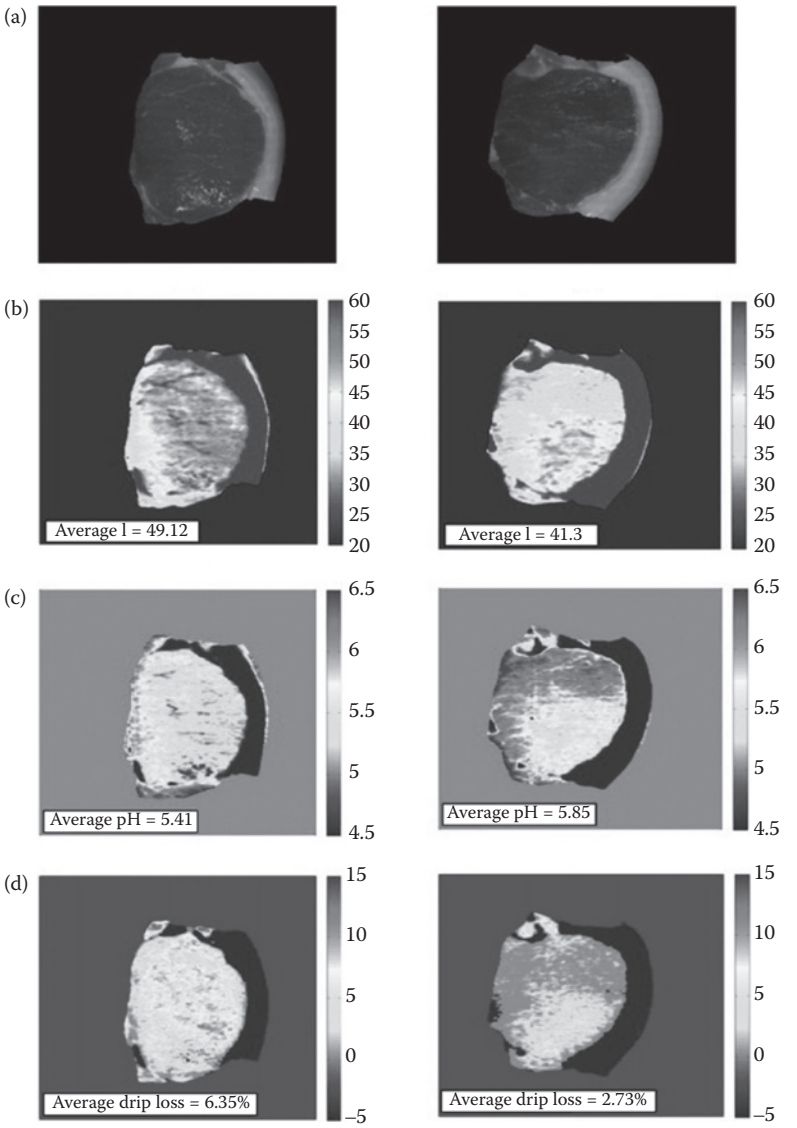


FIGURE 2.16
Distribution maps for some quality attributes in pork samples.

model achieved an overall accuracy of 96.4% in leave-one-out cross-validation and all of the tough samples were correctly classified. Apart from that, Tao et al. (2012) implemented hyperspectral imaging in 400–1000 nm to nondestructively determine pork tenderness. The overall results showed that the spatially resolved hyperspectral imaging technique coupled with MLR was capable of predicting tenderness in pork. More recently, a hyper-

Downloaded From Vet-eBooks.com

spectral imaging system (900–1700 nm) was utilized to predict instrumental and sensory tenderness of lamb meat (Kamruzzaman et al., 2013). WBSF values and sensory scores by trained panelists were recorded as the indicators of instrumental and sensory tenderness, respectively. Reasonable accuracy was obtained from PLSR models with R_{CV} of 0.84 for WBSF values and 0.69 for sensory tenderness scores. Further results demonstrated that hyperspectral imaging can quickly categorize lamb steaks into good (i.e., tender) and bad (i.e., tough) based on WBSF values and sensory scores with overall accuracy of 94.51% and 91%, respectively. More recently, a prototype online hyperspectral imaging system (400–1000 nm) was built to acquire images of exposed rib eye muscle on hanging beef carcasses at two-day postmortem in a commercial beef packing plant (Konda Naganathan et al., 2016). In this research, 3D chemometric analyses coupled with discriminant models were applied to forecast beef tenderness by predicting WBSF values. Four different dimensionality reduction approaches including sample principal component analysis (SPCA), chemometric principal component analysis (CPCA), mosaic principal component analysis (MPCA), and partial least squares analysis (PLSA), as well as three different discriminant analysis algorithms, Fisher's linear discriminant (FLD), SVM, and the decision tree (DT) model, were involved. The best prediction model was achieved by the CPCA and FLD combination with a tender certification accuracy of 86.7% and accuracy index value of 66.8%, in a true validation set (101 samples).

2.5.3.2 Chemical Compositions

Chemical constituents are intrinsic factors that affect red meat quality. It is believed that color, flavor, as well as tenderness of red meats might be changed by a series of reactions among different chemical constituents. For instance, moisture, as the major chemical constituent in red meats, plays a key part in affecting microbial growth, leading to influencing the shelf life of meat products. In addition to moisture, protein and fat are also main components in red meats. There are abundant different kinds of proteins contained in red meats and these proteins can be easily digested and absorbed by the human body for essential good health. As another major component of red meats, fat contributes greatly to the flavor when they are cooked and has a positive effect on eating satisfaction.

Attempts on using hyperspectral imaging as a nondestructive method for assessing beef chemical constituents have been fully investigated by many researchers (Elmasry et al., 2012a). The feasibility of determination of water distribution within beef during dehydration was investigated by using a time series hyperspectral imaging system (380–1700 nm) (Wu et al., 2013). Hyperspectral images of beef slices were first acquired at different periods of the dehydration process and the reference value of water content was determined based on the oven-drying method. Then,

Downloaded From Vet-eBooks.com

the selection of effective wavelengths was carried out using SPA to reduce the computational and predigest calibration modeling. Good results were achieved from the SPA-MLR model with R^2_{CV} equal to 0.953 and an RMSECV of 1.280%. Finally, the visualization of water distribution within beef slice along with dehydration was also created. Moreover, a laboratory-based pushbroom hyperspectral imaging system (900–1700 nm) in reflectance mode was tested to simultaneously predict water, fat, and protein contents of beef (ElMasry et al., 2013). PLSR models for predicting water, fat, and protein contents yielded a reasonable accuracy with R^2_P of 0.89, 0.84, and 0.86 concomitant with standard error of prediction (SEP) of 0.46%, 0.65%, and 0.29%, respectively. More recently, Lohumi et al. (2016) used a hyperspectral imaging system (400–1000 nm) for determination and visualization of intramuscular fat concentration in beef samples. An analysis of variance (ANOVA) test revealed that the ratio image based on wavelengths of 650.4 and 736.4 nm was found to be effective for the visualization of the intramuscular fat distribution, since a notable difference in the contrast between the intramuscular fat and the lean area was observed. Furthermore, spectral similarity analysis methods, which are based on the quantification of spectral similarities using a predetermined end-member spectrum vector, yielded comparable results for characterization of intramuscular fat in beef.

Besides beef, intensive research using hyperspectral imaging for prediction of chemical compositions has also been reported on pork. The suitability of using a hyperspectral imaging system (1000–2500 nm) for predicting moisture content during the salting process of porcine meat was assessed (Liu et al., 2014b). In this research, three different spectral profiles including reflectance spectra (RS), absorbance spectra (AS), and Kubelka–Munk spectra (KMS) were applied to build PLSR models and their results were compared. It was shown that the best full-wavelength PLSR model was obtained based on reflectance spectra (R^2_P = 0.941, RMSEP = 1.23%). Based on the important wavelengths selected using the RC, the optimal RS-MLR model also demonstrated good performance to predict moisture content of salted pork with R^2_P of 0.917 and RMSEP of 1.48%. More recently, Ma et al. (2016) proved that the spectral absorption index (SAI) was promising in spectral analysis to predict moisture content in pork samples using a hyperspectral imaging technique. Based on SAI features, PLSR models presented satisfactory performance with R^2_P of 0.952 and RMSEP of 1.396. An extended experiment was conducted by predicting moisture, protein, and fat of intact and minced pork (Barbin et al., 2013a). The results obtained by cross-validated PLSR models indicated that the NIR spectral range (900–1700 nm) had an extraordinary ability to determine the content of moisture (R^2_{CV} = 0.87), protein (R^2_{CV} = 0.88), and fat (R^2_{CV} = 0.95) in pork samples.

When it comes to lamb, relatively few studies have been carried out using hyperspectral imaging for chemical composition prediction.

Downloaded From Vet-eBooks.com

Kamruzzaman et al. (2012b) were the first to use hyperspectral imaging (900–1700 nm) for simultaneous prediction of the moisture, protein, and fat content in lamb meat. Multivariate calibration models were constructed by using PLSR in full spectral range and reasonable prediction abilities for these chemical constituents were presented with R^2_P of 0.88, 0.88, and 0.63 and SEP of 0.51%, 0.40%, and 0.34% for moisture, protein, and fat content, respectively. Six spectral variables were selected as the most relevant wavelengths in the spectrum for water and fat prediction and six wavelengths were identified as the effective wavelengths for protein prediction. The new PLSR models based on feature wavelengths generated R^2_P of 0.84, 0.87 and 0.82 and SEP of 0.57%, 0.35% and 0.47% for water, fat, and protein contents, respectively. Meanwhile, the resulting distribution maps have provided detailed information on the compositional gradient in the tested lamb samples. Interestingly, a hyperspectral imaging system (400–1000 nm) was investigated to develop a multispectral real-time imaging system allowing the meat industry to predict moisture content in red meat including beef, lamb, and pork (Kamruzzaman et al., 2016b). In their study, instead of respectively selecting different sets of effective wavelengths for beef, lamb, and pork, a set of 10 optimal wavelengths was selected for convenient industrial application for the prediction of moisture content in red meat. The best optimized MLR model yielded R^2_P of 0.97 and RMSEP of 2.19%, which confirmed that a fast, reliable, and accurate multispectral system for real-time monitoring of moisture content in red meat is available.

2.5.3.3 Microbiological Properties

Spoilage in red meats usually results from the growth and enzymatic activity of microorganisms; this will lead to the decomposition of nutrition compositions and the formation of metabolites. Red meats are described as spoiled if organoleptic changes make them unacceptable to the consumer. These organoleptic characteristics normally include changes in appearance (discoloration), changes in taste, the development of off odors, or any other characteristic that makes the food undesirable for consumption (Doyle and Buchanan, 2012). Excessive bacterial spoilage in red meats would cause harm to human health, thus it is crucial to guarantee the safety of red meats supplied to the markets. However, present methods of detecting bacterial spoilage in meats, such as enumeration methods based on microscopy, the plate count method, ATP bioluminescene, and the measurement of electrical phenomena, are not able to rapidly and nondestructively detect bacterially contaminated meat.

The hyperspectral imaging technique has unique advantages over the aforementioned methods and its potential for predicting microbiological properties of red meats has been intensively exploited through many research

Downloaded From Vet-eBooks.com

endeavors (Tao et al., 2012; Wang et al., 2011). For example, a NIR pushbroom hyperspectral imaging system (900–1700 nm) was explored to determine the TVC and *psychrotrophic* plate count (PPC) in chilled pork during storage (Barbin et al., 2013b). First, hyperspectral images were utilized to classify the tested samples into fresh or spoiled pork and good results were obtained with over 95% accuracy for spoilage detection. In the second stage, TVC and PPC were predicted simultaneously by correlating classical microbiological plating methods with the spectral information extracted from the pork samples. PLSR was applied and the best models demonstrated promising performance with an R^2 of 0.86 and 0.89 for log (TVC) and log (PPC), respectively. Finally, effective wavelengths were selected for regression and spatial visualization of microbial contamination. Similarly, Tao and Peng (2015) also applied hyperspectral scattering imaging in the spectral range of 472–1000 nm to predict TVC in pork meat. A stepwise multiple linear regression (SMLR) method was applied to build prediction models and models constructed by the Lorentzian parameter b and the Gompertz parameter β performed best for determining TVC of pork meat, with R_P of 0.94 and 0.93, respectively.

As for beef, Peng et al. (2011) investigated the feasibility of hyperspectral imaging technique for prediction of the beef spoilage. In this research, PLSR was carried out between the $\log_{10}$(TVC) value and three combinations of scattering parameters. The best predictions were obtained with $r^2 = 0.95$ and SEP = 0.30. Likewise, the application of a multispectral imaging system has been evaluated to monitor aerobically packaged beef filet spoilage at different storage temperatures (Panagou et al., 2014). Spectral data in the spectral range of 405–970 nm were extracted and correlated with microbiological data (log counts), for TVC, *Pseudomonas* spp., and *Brochothrix thermosphacta,* respectively. Qualitative analysis (PLS-DA) was first applied for the classification of meat samples in three microbiological quality classes based on the values of TVC, namely Class 1 (TVC < 5.5 $\log_{10}$ CFU / g), Class 2 (5.5 $\log_{10}$ CFU / g < TVC < 7.0 $\log_{10}$ CFU / g), and Class 3 (TVC > 7.0 $\log_{10}$ CFU / g). Meanwhile, PLSR models were also built to provide quantitative estimations of microbial counts during storage. Encouraging results were obtained in discrimination of meat samples with the overall CCR for cross-validation amounting to 85.9% and respective sensitivities of 73.1%, 75.0%, and 95.7% for classes 1, 2, and 3, respectively. The developed PLSR models yielded r_{CV} of 0.918, 0.898, and 0.903 for TVC, *Pseudomonas* spp., and *Brochothrix thermosphacta,* respectively. The overall results confirmed that multispectral imaging has significant potential as a rapid and nondestructive technique in assessing the microbiological quality of beef fillets.

2.5.3.4 Technological Attributes

Besides sensory attributes, chemical constituents attributes, and microbial spoilage determination, in recent years, research on red meat quality and safety

Downloaded From Vet-eBooks.com

inspection using hyperspectral imaging has been extended to predict important technological attributes such as pH, drip loss, and water holding capability (WHC), generally with satisfactory results. pH is defined as the concentration of the hydrogen ion in aqueous solution. It has significant influence on the storage and quality of red meat by affecting its WHC and color. Traditionally, pH is determined by inserting a pH meter into the muscle, but nowadays, there is the potential to predict pH using a hyperspectral imaging technique (Xiong et al., 2014). In more detail, Liu et al. (2014a) carried out the an experiment to investigate the utility of hyperspectral imaging (400–1000 nm) for predicting the pH of salted pork meat. The results from the PLSR model with a reduced number of wavelengths yielded R^2_{CV} of 0.86 and RMSECV of 0.073. Additionally, NIR hyperspectral imaging was employed to predict pH values of pork samples from the *longissimus dorsi* muscle and promising results were achieved with R^2_{CV} of 0.87 (Barbin et al., 2012b). The distribution maps of pH values were generated by applying the PLSR model to each pixel in the spectral image as shown in Figure 2.16c. The distribution maps illustrated how pH values varied drastically between different parts of the same sample.

Drip loss is of great importance in red meat production due to its financial implications. Generally speaking, meat with a high drip loss has an unattractive appearance and thus has low consumer preference, accounting for loss of sales. In addition to pH, Barbin et al. (2012b) used the same hyperspectral imaging system to predict drip loss of pork meat. The results from the PLSR model revealed that drip loss of pork meat could be well predicted with R^2_{CV} of 0.83 and the corresponding distribution maps are shown in Figure 2.16d. More recently, a hyperspectral imaging system was utilized to test frozen pork quality with drip loss as one of the quality indicators (Xie et al., 2015). In this study, hyperspectral images were acquired at the frozen state and PLSR was used to establish calibration models. The performance for drip loss prediction was acceptable with R^2_P of 0.762. Additionally, NIR hyperspectral imaging was explored for the prediction of some quality attributes of lamb meat including drip loss (Kamruzzaman et al., 2012c). As usual, PLSR models were developed and good performance was obtained for drip loss prediction with R^2 of 0.77 and RDP of 2.11.

Like drip loss, WHC also has a huge influence on the appearance of fresh red meat during retail and might affect the sensory attributes of cooked meat. From an economic point of view, high WHC is extremely desirable as red meat is normally sold by weight and any water loss accounts for a reduction in yield due to loss in the total weight of the meat (Hoving-Bolink et al., 2005). Past years have seen the potential of a hyperspectral imaging system for nondestructive and rapid evaluation of WHC of red meats (ElMasry et al., 2011; Kamruzzaman et al., 2012b). For instance, ElMasry et al. (2011) conducted an experiment for postmortem prediction of WHC in fresh beef using NIR hyperspectral imaging. Both PCA and PLSR models were developed to correlate spectral data with the reference

Downloaded From Vet-eBooks.com

WHC estimated by the drip loss method. The PLSR model resulted in an R^2_{CV} of 0.89 and standard error estimated by cross-validation (SECV) of 0.26%. Six spectral variables (940, 997, 1144, 1214, 1342, and 1443 nm) were selected as optimal wavelengths to establish a new PLSR prediction model with R^2_{CV} of 0.87 and SECV of 0.28%. More recently, a hyperspectral imaging system was tested for determination of effective wavelengths to be used in the design of a multispectral system for online monitoring of WHC in red meats including beef, lamb, and pork samples (Kamruzzaman et al., 2016a). Hyperspectral images of different red meat samples were acquired in the spectral range of 400–1000 nm and subsequently PLSR and LS-SVM models were constructed. Optimal wavelengths were collected using RC and CARS methods. Instead of selecting different sets of important wavelengths for different red meats, only eight spectral variables (545, 610, 705, 765, 805, 900, 940, and 970 nm) were selected to design a multispectral system for convenient industrial application of red meats. The RC-LS-SVM model demonstrated the best performance with an R^2_P of 0.93 and RPD of 4.09, confirming that this model is adequate for analytical purposes.

2.6 Conclusions

Hyperspectral imaging is a powerful and versatile technique and it is now under dynamic evaluation by researchers in many different fields. Among dozens of techniques that have been proposed for meat quality evaluation on the fresh intact form, the hyperspectral imaging technique has great potential advantages, including elimination of human error during subjective judgment, reduced labor costs, and the generation of product data in visualized forms in real time for documentation, labeling, and traceability. With the primary advantage of providing both spatial and spectral details, hyperspectral imaging not only provides a means of accurate quantification but also describes constituent variation within the tested meat sample, which consequently enables better characterization of the meat sample and improved description of meat quality and safety status. Additionally, hyperspectral imaging is able to measure multiple quality and/or safety attributes simultaneously without monotonous sample preparation. Therefore, the hyperspectral imaging technique has many applications in the meat industry, such as simple product inspection, full sample quantification, or the segregation of a subset of the measured batch for further manual inspection (Elmasry et al., 2012a). The results of the previous research works presented in this chapter confirm that hyperspectral imaging is a promising technology for rapid and noncontact evaluation of essential meat quality and safety attributes.

Downloaded From Vet-eBooks.com

However, there are two major challenges that may hamper widespread adoption in the meat industry. The first is the high purchase cost of hyperspectral imaging systems. There are few commercial suppliers as this technology is an emerging tool for meat quality evaluation. Future technological developments in hyperspectral imaging equipment manufacture are expected to promote the manufacture of low-cost systems. The second limitation arises from the relatively lengthy times necessary for hyperspectral image acquisition, processing, and modeling. Based on target size and image resolution, acquisition time can range from two to four minutes. Although a multispectral imaging system with a limited number of wavebands can largely reduce the prolonged time, it is normally very tricky to judge which subsets of selected wavelengths will provide the best performance, because applying different variable selection methods always leads to different optimal wavelengths combinations. It is of great importance for researchers to overcome technological challenges for performing hyperspectral imaging technology in the food industry for meat quality and safety assessment, so that the meat industry can realistically benefit from implementing this rapid and nondestructive technique at an early stage of processing without additional time-consuming, tedious, and laborious chemical analyses, enabling early sorting and grading of produce and thereby enhanced quality management.

Although it suffers from several disadvantages, considering the continuing improvements in hardware and software design, it is anticipated that hyperspectral imaging may progressively become a substantial and routine method for real-time meat safety and quality control.

References

Adam, E., Mutanga, O. and Rugege, D. (2010). Multispectral and hyperspectral remote sensing for identification and mapping of wetland vegetation: A review. *Wetlands Ecology and Management,* **18**(3), 281–296.

Amigo, J. M., Babamoradi, H. and Elcoroaristizabal, S. (2015). Hyperspectral image analysis. A tutorial. *Analytica Chimica Acta,* **896**, 34–51.

Anastasio, A., Vollano, L., Visciano, P., Miranda, E. and Cortesi, M. (1999). Correlations between pH, total volatile basic nitrogen, trimethylamine and sensory evaluation in fresh fish slices. *Archiv für Lebensmittelhygiene,* **50**(3), 63–66.

Bannon, D. and Thomas, R. (2005). Harsh environments dictate design of imaging spectrometer. *Laser Focus World,* **41**(8), 93–97.

Barbin, D., Elmasry, G., Sun, D.-W. and Allen, P. (2012a). Near-infrared hyperspectral imaging for grading and classification of pork. *Meat Science,* **90**(1), 259–268.

Barbin, D. F., ElMasry, G., Sun, D.-W. and Allen, P. (2012b). Predicting quality and sensory attributes of pork using near-infrared hyperspectral imaging. *Analytica Chimica Acta,* **719**, 30–42.

Downloaded From Vet-eBooks.com

Barbin, D. F., ElMasry, G., Sun, D.-W. and Allen, P. (2013a). Non-destructive determination of chemical composition in intact and minced pork using near-infrared hyperspectral imaging. *Food Chemistry,* **138**(2), 1162–1171.

Barbin, D. F., ElMasry, G., Sun, D.-W., Allen, P. and Morsy, N. (2013b). Non-destructive assessment of microbial contamination in porcine meat using NIR hyperspectral imaging. *Innovative Food Science and Emerging Technologies,* **17**, 180–191.

Barbin, D. F., Sun, D.-W. and Su, C. (2013c). NIR hyperspectral imaging as non-destructive evaluation tool for the recognition of fresh and frozen-thawed porcine longissimus dorsi muscles. *Innovative Food Science and Emerging Technologies,* **18**, 226–236.

Blanco, M., Coello, J., Iturriaga, H., Maspoch, S. and Pezuela, C. (1997). Effect of data preprocessing methods in near-infrared diffuse reflectance spectroscopy for the determination of the active compound in a pharmaceutical preparation. *Applied Spectroscopy,* **51**(2), 240–246.

Bro, R., Van den Berg, F., Thybo, A., Andersen, C. M., Jorgensen, B. M. and Andersen, H. (2002). Multivariate data analysis as a tool in advanced quality monitoring in the food production chain. *Trends in Food Science and Technology,* **13**(6), 235–244.

Brosnan, T. and Sun, D.-W. (2004). Improving quality inspection of food products by computer vision—A review. *Journal of Food Engineering,* **61**(1), 3–16.

Chao, K., Kim, M. S. and Chan, D. E. (2014). Control interface and tracking control system for automated poultry inspection. *Computer Standards and Interfaces,* **36**(2), 271–277.

Chao, K., Mehl, P. and Chen, Y. (2002). Use of hyper-and multi-spectral imaging for detection of chicken skin tumors. *Applied Engineering in Agriculture,* **18**(1), 113–120.

Chao, K., Yang, C., Chen, Y., Kim, M. and Chan, D. (2007). Hyperspectral-multispectral line-scan imaging system for automated poultry carcass inspection applications for food safety. *Poultry Science,* **86**(11), 2450–2460.

Chao, K., Yang, C., Kim, M. and Chan, D. (2008). High throughput spectral imaging system for wholesomeness inspection of chicken. *Applied Engineering in Agriculture,* **24**(4), 475–485.

Chau, A., Whitworth, M., Leadley, C. and Millar, S. (2009). Innovative sensors to rapidly and non-destructively determine fish freshness. *Seafish Industrial Authority*. Report No. CMS/REP/110284/1. References 233.

Chen, Q., Zhao, J., Fang, C. and Wang, D. (2007). Feasibility study on identification of green, black and Oolong teas using near-infrared reflectance spectroscopy based on support vector machine (SVM). *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy,* **66**(3), 568–574.

Cheng, J.-H., Dai, Q., Sun, D.-W. et al. (2013). Applications of non-destructive spectroscopic techniques for fish quality and safety evaluation and inspection. *Trends in Food Science and Technology,* **34**(1), 18–31.

Cheng, J.-H. and Sun, D.-W. (2015a). Data fusion and hyperspectral imaging in tandem with least squares-support vector machine for prediction of sensory quality index scores of fish fillet. *LWT—Food Science and Technology,* **63**(2), 892–898.

Cheng, J.-H. and Sun, D.-W. (2015b). Rapid quantification analysis and visualization of *Escherichia coli* loads in grass carp fish flesh by hyperspectral imaging method. *Food and Bioprocess Technology,* **8**(5), 951–959.

Chevallier, S., Bertrand, D., Kohler, A. and Courcoux, P. (2006). Application of PLS-DA in multivariate image analysis. *Journal of Chemometrics,* **20**(5), 221.

Downloaded From Vet-eBooks.com

Cho, B., Kim, M. S., Chao, K., Lawrence, K., Park, B. and Kim K. (2009). Detection of fecal residue on poultry carcasses by laser-induced fluorescence imaging. *Journal of Food Science,* **74**(3), E154–E159.

Cutler, D. R., Edwards, T. C., Beard, K. H. et al. (2007). Random forests for classification in ecology. *Ecology,* **88**(11), 2783–2792.

Damez, J.-L. and Clerjon, S. (2008). Meat quality assessment using biophysical methods related to meat structure. *Meat Science,* **80**(1), 132–149.

Doyle, M. P. and Buchanan, R. L. (2012). *Food Microbiology: Fundamentals and Frontiers*. Washington, D.C: American Society for Microbiology Press.

Du, Z., Jeong, M. K. and Kong, S. G. (2007). Band selection of hyperspectral images for automatic detection of poultry skin tumors. *IEEE Transactions on Automation Science and Engineering,* **4**(3), 332–339.

Elmasry, G., Barbin, D. F., Sun, D.-W. and Allen, P. (2012a). Meat quality evaluation by hyperspectral imaging technique: An overview. *Critical Reviews in Food Science and Nutrition,* **52**(8), 689–711.

Elmasry, G., Kamruzzaman, M., Sun, D.-W. and Allen, P. (2012b). Principles and applications of hyperspectral imaging in quality evaluation of agro-food products: A review. *Critical Reviews in Food Science and Nutrition,* **52**(11), 999–1023.

ElMasry, G. and Sun, D.-W. (2010). Meat quality assessment using a hyperspectral imaging system. *In: Hyperspectral Imaging for Food Quality Analysis and Control,* Da-Wen Sun (Ed.), Amsterdam : Academic Press, 273–294.

ElMasry, G., Sun, D.-W. and Allen, P. (2011). Non-destructive determination of water-holding capacity in fresh beef by using NIR hyperspectral imaging. *Food Research International,* **44**(9), 2624–2633.

ElMasry, G., Sun, D.-W. and Allen, P. (2012c). Near-infrared hyperspectral imaging for predicting colour, pH and tenderness of fresh beef. *Journal of Food Engineering,* **110**(1), 127–140.

ElMasry, G., Sun, D.-W. and Allen, P. (2013). Chemical-free assessment and mapping of major constituents in beef using hyperspectral imaging. *Journal of Food Engineering,* **117**(2), 235–246.

ElMasry, G. and Wold, J. P. (2008). High-speed assessment of fat and water content distribution in fish fillets using online imaging spectroscopy. *Journal of Agricultural and Food Chemistry,* **56**(17), 7672–7677.

Esquerre, C., Gowen, A. A., Burger, J., Downey, G. and O'Donnell, C. P. (2012). Suppressing sample morphology effects in near infrared spectral imaging using chemometric data pre-treatments. *Chemometrics and Intelligent Laboratory Systems,* **117**, 129–137.

Feng, Y.-Z., Elmasry, G., Sun, D.-W., Scannell, A. G., Walsh, D. and Morcy, N. (2013). Near-infrared hyperspectral imaging and partial least squares regression for rapid and reagentless determination of Enterobacteriaceae on chicken fillets. *Food Chemistry,* **138**(2), 1829–1836.

Feng, Y.-Z. and Sun, D.-W. (2013a). Determination of total viable count (TVC) in chicken breast fillets by near-infrared hyperspectral imaging and spectroscopic transforms. *Talanta,* **105**, 244–249.

Feng, Y.-Z. and Sun, D.-W. (2013b). Near-infrared hyperspectral imaging in tandem with partial least squares regression and genetic algorithm for non-destructive determination and visualization of Pseudomonas loads in chicken fillets. *Talanta,* **109**, 74–83.

Downloaded From Vet-eBooks.com

Feng, Y.-Z. and Sun, D.-W. (2014). Seeing the bacteria: Hyperspectral imaging for bacterial prediction and visualisation on chicken meat. *NIR news,* **25**(7), 4–6.

Gaston, E., Fras, J., Cullen, P., O'Donnell, C. and Gowen, A. (2010). Visible-near infrared hyperspectral imaging for the identification and discrimination of brown blotch disease on mushroom (*Agaricus bisporus*) caps. *Journal of Near Infrared Spectroscopy,* **18**(5), 341.

Gowen, A. A., Feng, Y., Gaston, E. and Valdramidis, V. (2015). Recent applications of hyperspectral imaging in microbiology. *Talanta,* **137**, 43–54.

He, H.-J., Wu, D. and Sun, D.-W. (2013). Non-destructive and rapid analysis of moisture distribution in farmed Atlantic salmon (Salmo salar) fillets using visible and near-infrared hyperspectral imaging. *Innovative Food Science and Emerging Technologies,* **18**, 237–245.

Hoving-Bolink, A., Vedder, H. W., Merks, J. W. et al. (2005). Perspective of NIRS measurements early post mortem for prediction of pork quality. *Meat Science,* **69**(3), 417–423.

Huang, H., Liu, L. and Ngadi, M. O. (2014). Recent developments in hyperspectral imaging for assessment of food quality and safety. *Sensors,* **14**(4), 7248–7276.

Ivorra, E., Girón, J., Sánchez, A. J. et al. (2013). Detection of expired vacuum-packed smoked salmon based on PLS-DA method using hyperspectral images. *Journal of Food Engineering,* **117**(3), 342–349.

Kamruzzaman, M., Barbin, D., ElMasry, G., Sun, D.-W. and Allen, P. (2012a). Potential of hyperspectral imaging and pattern recognition for categorization and authentication of red meat. *Innovative Food Science and Emerging Technologies,* **16**, 316–325.

Kamruzzaman, M., ElMasry, G., Sun, D.-W. and Allen, P. (2011). Application of NIR hyperspectral imaging for discrimination of lamb muscles. *Journal of Food Engineering,* **104**(3), 332–340.

Kamruzzaman, M., ElMasry, G., Sun, D.-W. and Allen, P. (2012b). Non-destructive prediction and visualization of chemical composition in lamb meat using NIR hyperspectral imaging and multivariate regression. *Innovative Food Science and Emerging Technologies,* **16**, 218–226.

Kamruzzaman, M., ElMasry, G., Sun, D.-W. and Allen, P. (2012c). Prediction of some quality attributes of lamb meat using near-infrared hyperspectral imaging and multivariate analysis. *Analytica Chimica Acta,* **714**, 57–67.

Kamruzzaman, M., ElMasry, G., Sun, D.-W. and Allen, P. (2013). Non-destructive assessment of instrumental and sensory tenderness of lamb meat using NIR hyperspectral imaging. *Food Chemistry,* **141**(1), 389–396.

Kamruzzaman, M., Makino, Y. and Oshita, S., (2016a). Hyperspectral imaging for real-time monitoring of water holding capacity in red meat. *LWT—Food Science and Technology,* **66**, 685–691.

Kamruzzaman, M., Makino, Y. and Oshita, S. (2016b). Parsimonious model development for real-time monitoring of moisture in red meat using hyperspectral imaging. *Food Chemistry,* **196**, 1084–1091.

Kim, I., Kim, M., Chen, Y. and Kong, S. (2004). Detection of skin tumors on chicken carcasses using hyperspectral fluorescence imaging. *Transactions-American Society of Agricultural Engineers,* **47**(5), 1785–1792.

Kirk, P. L. (1950). Kjeldahl method for total nitrogen. *Analytical Chemistry,* **22**(2), 354–358.

Downloaded From Vet-eBooks.com

Klein, M. E., Aalderink, B. J., Padoan, R., De Bruin, G. and Steemers, T. A. (2008). Quantitative hyperspectral reflectance imaging. *Sensors,* **8**(9), 5576–5618.

Kleynen, O., Leemans, V. and Destain, M.-F. (2005). Development of a multi-spectral vision system for the detection of defects on apples. *Journal of Food Engineering,* **69**(1), 41–49.

Konda Naganathan, G., Cluff, K., Samal, A. et al. (2016). Three dimensional chemometric analyses of hyperspectral images for beef tenderness forecasting. *Journal of Food Engineering,* **169**, 309–320.

Langeron, Y., Doussot, M., Hewson, D. J. and Duchêne, J. (2007). Classifying NIR spectra of textile products with kernel methods. *Engineering Applications of Artificial Intelligence,* **20**(3), 415–427.

Larsen, E., Heldbo, J., Jespersen, C., and Nielsen, J. (1992). Development of a method for quality assessment of fish for human consumption based on sensory evaluation. In: Huss, H H., Jakobsen, M., Liston, J., (eds.) *Quality Assurance in the Fish Industry.* Amsterdam: Elsevier Science Publishing, 351--358.

Lawrence, R. L., Wood, S. D. and Sheley, R. L. (2006). Mapping invasive plants using hyperspectral imagery and Breiman Cutler classifications (RandomForest). *Remote Sensing of Environment,* **100**(3), 356–362.

Li, Y., Shan, J., Peng, Y. and Gao, X. (2011). Nondestructive assessment of beef-marbling grade using hyperspectral imaging technology. In *Procs 2011 International Conference on New Technology of Agricultural Engineering (ICAE)*, Zibo, China. IEEE, 779–783.

Liu, D. Ma, J., Sun, D.-W. et al. (2014a). Prediction of color and pH of salted porcine meats using visible and near-infrared hyperspectral imaging. *Food and Bioprocess Technology,* **7**(11), 3100–3108.

Liu, D., Sun, D.-W., Qu, J., Zeng, X. A., Pu, H. and Ma, J. (2014b). Feasibility of using hyperspectral imaging to predict moisture content of porcine meat during salting process. *Food Chemistry,* **152**, 197–204.

Lohumi, S., Lee, S., Lee, H., Kim, M. S., Lee, W. H. and Cho, B.-K. (2016). Application of hyperspectral imaging for characterization of intramuscular fat distribution in beef. *Infrared Physics and Technology,* **74**, 1–10.

Luypaert, J., Heuerding, S., Vander Heyden, Y. and Massart, D. (2004). The effect of preprocessing methods in reducing interfering variability from near-infrared measurements of creams. *Journal of Pharmaceutical and Biomedical Analysis,* **36**(3), 495–503.

Ma, J., Sun, D.-W. and Pu, H. (2016). Spectral absorption index in hyperspectral image analysis for predicting moisture contents in pork longissimus dorsi muscles. *Food Chemistry,* 197, **Part A**, 848–854.

Martens, H. and Stark, E. (1991). Extended multiplicative signal correction and spectral interference subtraction: New preprocessing methods for near infrared spectroscopy. *Journal of Pharmaceutical and Biomedical Analysis,* **9**(8), 625–635.

Mendes, R., Cardoso, C. and Pestana, C. (2009). Measurement of malondialdehyde in fish: A comparison study between HPLC methods and the traditional spectrophotometric test. *Food Chemistry,* **112**(4), 1038–1045.

Mercier, G. and Lennon, M. (2003). Support vector machines for hyperspectral image classification with spectral-based kernels. In *Proc Geoscience and Remote Sensing Symposium*, *2003 (IGARSS'03)*, Toulouse, France. IEEE International, 288–290.

Downloaded From Vet-eBooks.com

Naganathan, G. K., Grimes, L. M., Subbiah, J., Calkins, C. R., Samal, A. and Meyer G. E. (2008). Visible/near-infrared hyperspectral imaging for beef tenderness prediction. *Computers and Electronics in Agriculture,* **64**(2), 225–233.

Nakariyakul, S. and Casasent, D. (2007). Fusion algorithm for poultry skin tumor detection using hyperspectral data. *Applied Optics,* **46**(3), 357–364.

Palmer, C.A., Loewen, E. G. and Thermo, R. (2005). *Diffraction Grating Handbook*. Springfield, OH: Newport Corporation.

Panagou, E. Z., Papadopoulou, O., Carstensen, J. M. and Nychas, G.-J. E. (2014). Potential of multispectral imaging technology for rapid and non-destructive determination of the microbiological quality of beef filets during aerobic storage. *International Journal of Food Microbiology,* **174**, 1–11.

Park, B., Lawrence, K., Windham, W. and Buhr, R. J. (2002). Hyperspectral imaging for detecting fecal and ingesta contaminants on poultry carcasses. *Transactions of the ASAE,* **45**(6), 2017–2026.

Park, B., Lawrence, K., Windham, W. and Smith, D. (2006). Performance of supervised classification algorithms of hyperspectral imagery for identifying fecal and ingesta contaminants. *Transactions of the ASABE,* **49**(6), 2017–2024.

Park, B., Lawrence, K. C., Windham, W. R. and Smith, D. P. (2004). Multispectral imaging system for fecal and ingesta detection on poultry carcasses. *Journal of Food Process Engineering,* **27**(5), 311–327.

Park, B., Lee, S., Yoon, S.-C. et al. (2011). AOTF hyperspectral microscopic imaging for foodborne pathogenic bacteria detection. In *SPIE Defense, Security, and Sensing*, April 2011, Orlando, Florida. International Society for Optics and Photonics, 802707-802707-11.

Park, B., Yoon, S.-C., Lawrence, K. and Windham, W. (2007). Fisher linear discriminant analysis for improving fecal detection accuracy with hyperspectral images. *Transactions of the ASABE,* **50**(6), 2275–2283.

Park, B., Yoon, S.-C., Windham, W. R. et al. (2010). Line-scan hyperspectral imaging for real-time poultry fecal detection. In *SPIE Defense, Security, and Sensing*, April 2010, Orlando, Florida. International Society for Optics and Photonics, 76760I-76760I-10.

Peng, Y., Zhang, J., Wang, W. et al. (2011). Potential prediction of the microbial spoilage of beef using spatially resolved hyperspectral scattering profiles. *Journal of Food Engineering,* **102**(2), 163–169.

Penha, R. and Hines, J. W. (2001). Using principal component analysis modeling to monitor temperature sensors in a nuclear research reactor. In *Proc 2001 Maintenance and Reliability Conference (MARCON 2001)*, Knoxville, Tennessee. Citeseer.

Piqueras, S., Duponchel, L., Tauler, R. and de Juan, A. (2011). Resolution and segmentation of hyperspectral biomedical images by Multivariate Curve Resolution-Alternating Least Squares. *Analytica Chimica Acta,* **705**(1–2), 182–192.

Qin, J., Chao, K. and Kim, M.S. (2011). Investigation of Raman chemical imaging for detection of lycopene changes in tomatoes during postharvest ripening. *Journal of Food Engineering,* **107**(3), 277–288.

Reich, G. (2005). Near-infrared spectroscopy and imaging: Basic principles and pharmaceutical applications. *Advanced Drug Delivery Reviews,* **57**(8), 1109–1143.

Savitzky, A. and Golay, M. J. (1964). Smoothing and differentiation of data by simplified least squares procedures. *Analytical Chemistry,* **36**(8), 1627–1639.

Downloaded From Vet-eBooks.com

Schaepman, M. E. (2007). Spectrodirectional remote sensing: From pixels to processes. *International Journal of Applied Earth Observation and Geoinformation*, **9**(2), 204–223.

Segtnan, V. H., Høy, M., Sørheim, O. et al. (2009). Noncontact salt and fat distributional analysis in salted and smoked salmon fillets using X-ray computed tomography and NIR interactance imaging. *Journal of Agricultural and Food Chemistry,* **57**(5), 1705–1710.

Sivertsen, A. H., Heia, K., Hindberg, K. and Godtliebsen, F. (2012). Automatic nematode detection in cod fillets (*Gadus morhua* L.) by hyperspectral imaging. *Journal of Food Engineering,* **111**(4), 675–681.

Sivertsen, A. H., Kimiya, T. and Heia, K. (2011). Automatic freshness assessment of cod (*Gadus morhua*) fillets by Vis/Nir spectroscopy. *Journal of Food Engineering,* **103**(3), 317–323.

Sone, I., Olsen, R. L., Sivertsen, A. H., Eilertsen, G. and Heia, K. (2012). Classification of fresh Atlantic salmon (*Salmo salar* L.) fillets stored under different atmospheres by hyperspectral imaging. *Journal of Food Engineering,* **109**(3), 482–489.

Song, Y., Luo, Y., You, J., Shen, H. and Hu, S. (2012). Biochemical, sensory and microbiological attributes of bream (*Megalobrama amblycephala*) during partial freezing and chilled storage. *Journal of the Science of Food and Agriculture,* **92**(1), 197–202.

Sun, D.-W. (2010). *Hyperspectral Imaging for Food Quality Analysis and Control*. Elsevier.

Sun, D. -W. (2007). *Computer Vision Technology for Food Quality Evaluation*. San Diego, CA: Academic Press, Amsterdam: Academic Press.

Tao, F. and Peng, Y. (2015). A nondestructive method for prediction of total viable count in pork meat by hyperspectral scattering imaging. *Food and Bioprocess Technology,* **8**(1), 17–30.

Tao, F., Peng, Y., Li, Y., Chao, K. and Dhakal, S. (2012). Simultaneous determination of tenderness and *Escherichia coli* contamination of pork using hyperspectral scattering technique. *Meat Science*, 90(3), 851–857.

Timmermans, A. (1995). Computer vision system for on-line sorting of pot plants based on learning techniques. *In*: van Meurs, W. T. H. M., Gieling, T. H. and Bennedsen, B. S. (eds.) *II International Symposium on Sensors in Horticulture 421,* March 1998, Tune Landboskole, Greve, Denmark, 91–98.

Wang, H.-H. and Sun, D.-W. (2001). Evaluation of the functional properties of cheddar cheese using a computer vision method. *Journal of Food Engineering,* **49**(1), 49–53.

Wang, W., Peng, Y., Huang, H. and Wu, J. (2011). Application of hyper-spectral imaging technique for the detection of total viable bacteria count in pork. *Sensor Letters,* **9**(3), 1024–1030.

Willits, C. (1951). Methods for determination of moisture-oven drying. *Analytical Chemistry,* **23**(8), 1058–1062.

Windham, W., Lawrence, K., Park, B. and Buhr, R. (2003). Visible/NIR spectroscopy for characterizing fecal contamination of chicken carcasses. *Transactions-American Society of Agricultural Engineers,* **46**(3), 747–754.

Wold, J. P., Jakobsen, T. and Krane, L. (1996). Atlantic salmon average fat content estimated by near-infrared transmittance spectroscopy. *Journal of Food Science,* **61**(1), 74–77.

Downloaded From Vet-eBooks.com

Wold, J. P., Johansen, I.-R., Haugholt, K. H. et al. (2006). Non-contact transflectance near infrared imaging for representative on-line sampling of dried salted coalfish (bacalao). *Journal of Near Infrared Spectroscopy,* **14**(1), 59.

Wold, J. P., Westad, F. and Heia, K. (2001). Detection of parasites in cod fillets by using SIMCA classification in multispectral images in the visible and NIR region. *Applied Spectroscopy,* **55**(8), 1025–1034.

Wu, D. and Sun, D.-W. (2013a). Advanced applications of hyperspectral imaging technology for food quality and safety analysis and assessment: A review—Part I: Fundamentals. *Innovative Food Science and Emerging Technologies,* **19**, 1–14.

Wu, D. and Sun, D.-W. (2013b). Advanced applications of hyperspectral imaging technology for food quality and safety analysis and assessment: A review—Part II: Applications. *Innovative Food Science and Emerging Technologies,* **19**, 15–28.

Wu, D. and Sun, D.-W. (2013c). Application of visible and near infrared hyperspectral imaging for non-invasively measuring distribution of water-holding capacity in salmon flesh. *Talanta,* **116**, 266–276.

Wu, D. and Sun, D.-W. (2013d). Potential of time series-hyperspectral imaging (TS-HSI) for non-invasive determination of microbial spoilage of salmon flesh. *Talanta,* **111**, 39–46.

Wu, D., Sun, D.-W. and He, Y. (2012). Application of long-wave near infrared hyperspectral imaging for measurement of color distribution in salmon fillet. *Innovative Food Science and Emerging Technologies,* **16**, 361–372.

Wu, D., Sun, D.-W. and He, Y. (2014). Novel non-invasive distribution measurement of texture profile analysis (TPA) in salmon fillet by using visible and near infrared hyperspectral imaging. *Food Chemistry,* **145**, 417–426.

Wu, D., Wang S., Wang, N. et al. (2013). Application of time series hyperspectral imaging (TS-HSI) for determining water distribution within beef and spectral kinetic analysis during dehydration. *Food and Bioprocess Technology,* **6**(11), 2943–2958.

Xie, A., Sun, D.-W., Xu, Z. and Zhu, Z. (2015). Rapid detection of frozen pork quality without thawing by Vis–NIR hyperspectral imaging technique. *Talanta,* **139**, 208–215.

Xiong, Z., Sun, D.-W., Dai, Q. et al. (2015a). Application of visible hyperspectral imaging for prediction of springiness of fresh chicken meat. *Food Analytical Methods,* **8**(2), 380–391.

Xiong, Z., Sun, D.-W., Xie, A., Han, Z. and Wang, L. (2015b). Potential of hyperspectral imaging for rapid prediction of hydroxyproline content in chicken meat. *Food Chemistry,* **175**, 417–422.

Xiong, Z., Sun, D.-W., Zeng, X.-A. and Xie, A. (2014). Recent developments of hyperspectral imaging systems and their applications in detecting quality attributes of red meats: A review. *Journal of Food Engineering,* **132**, 1–13.

Xu, J. L., Riccioli, C. and Sun, D.-W. (2015). An overview on nondestructive spectroscopic techniques for lipid and lipid oxidation analysis in fish and fish products. *Comprehensive Reviews in Food Science and Food Safety,* **14**(4), 466–477.

Xu, J.-L., Riccioli, C. and Sun, D.-W. (2016). Efficient integration of particle analysis in hyperspectral imaging for rapid assessment of oxidative degradation in salmon fillet. *Journal of Food Engineering,* **169**, 259–271.

Yoon, S., Lawrence, K., Smith, D., Park, B. and Windham, W. (2008). Embedded bone fragment detection in chicken fillets using transmittance image enhancement and hyperspectral reflectance imaging. *Sensing and Instrumentation for Food Quality and Safety,* **2**(3), 197–207.

Downloaded From Vet-eBooks.com

Zhu, F., Zhang, D., He, Y., Liu, F. and Sun, D.-W. (2013). Application of visible and near infrared hyperspectral imaging to differentiate between fresh and frozen–thawed fish fillets. *Food and Bioprocess Technology,* **6**(10), 2931–2937.

Zhu, F., Zhang, H., Shao, Y., He, Y. and Ngadi, M. (2014). Mapping of fat and moisture distribution in Atlantic salmon using near-infrared hyperspectral imaging. *Food and Bioprocess Technology,* **7**(4), 1208–1214.

Downloaded From Vet-eBooks.com



Taylor & Francis
Taylor & Francis Group
http://taylorandfrancis.com

Downloaded From Vet-eBooks.com

3

Raman Spectroscopy for Predicting Meat Quality Traits

Stephanie M. Fowler, Heinar Schmidt, Rico Scheier, and David L. Hopkins

CONTENTS

3.1 Introduction 83
3.2 Theory of Raman Spectroscopy 84
3.3 Raman Spectroscopic Devices 86
3.4 Applications in Meat Science 87
3.4.1 Pork 88
3.4.1.1 Prerigor Pork 88
3.4.1.2 Postrigor Pork 92
3.4.1.3 Heat-Treated Pork 93
3.4.1.4 Fatty Acid Composition of Pork Adipose Tissue 94
3.4.2 Lamb 96
3.4.3 Beef 100
3.4.4 Spoilage 102
3.4.5 Differentiation of Animal Species and Detection of Adulteration in Muscle Foods 103
3.5 Limitations 104
3.6 Conclusion 106
References 107

3.1 Introduction

Raman spectroscopy is a method of vibrational spectroscopy that was first discovered by Indian physicist Sir C. V. Raman in 1928. Initially used to provide the first catalog of molecular vibrational frequencies, the low sensitivity of the technique hindered its use as extensive methods were needed to measure relatively large volumes of highly concentrated samples in order to obtain quality Raman spectra. Consequently, the use of Raman spectroscopy for chemical analysis declined as infrared (IR) spectrophotometers became available. Since these early days, the advent of lasers in the 1960s facilitated the development of more

Downloaded From Vet-eBooks.com

simplified Raman spectroscopy equipment and increased the sensitivity of the technique. Further development of technologies in recent decades has continued to increase the efficiency of the equipment, which has enabled the construction of smaller and more robust devices (McCreery, 2000). As a result, Raman spectroscopy is now a widely used method of chemical analysis used in a number of applications including archaeology, forensics, biomedical, and food sciences.

3.2 Theory of Raman Spectroscopy

Raman spectroscopy is based on the principle that when light is directed at matter, there is an interaction and an exchange of energy occurs, which is referred to as inelastic scattering (Das and Agrawal, 2011). If the photons interact with the molecules and the net exchange of energy causes the photon to gain vibrational energy from the molecules and the backscattered frequency is greater than the incident frequency, this is called anti-Stokes Raman scattering. However, the reverse is more likely, where matter gains energy from the photons resulting in a backscattered frequency which is lower than the incident beam, which is referred to as Stokes Raman scattering (Ozaki, 1999). These interactions with the excitation photons are graphed for relative wave number (Raman shift) and intensity of the frequencies measured as a spectrum where the peaks indicate Raman scattering and the central peak indicates the Rayleigh scattered light (Figure 3.1).

Vibrations for different chemical bonds are distinct due to energy that excites them and the movement of atoms which are generated by the

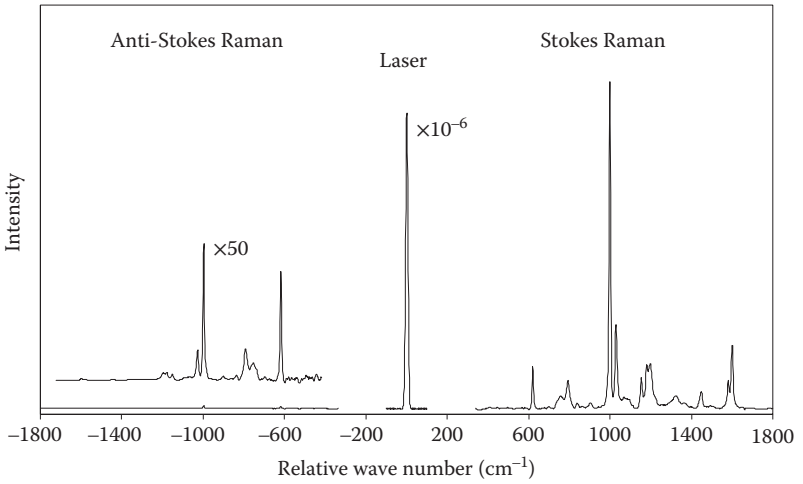


FIGURE 3.1
Representation of anti-Stokes and Stokes Raman spectra of polystyrene. Raman shifts are given in wave numbers relative to the laser line, which has been supressed by a notch filter.

Downloaded From Vet-eBooks.com

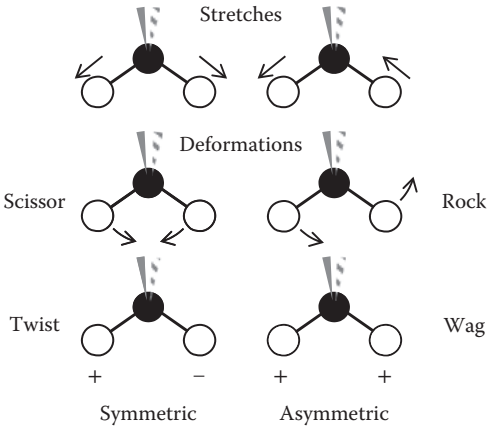


FIGURE 3.2
Examples of the chemical bond vibrations of the methylene group as characterized by Raman spectroscopy.

exchange as well as the frequency at which the atoms oscillate, which corresponds to the wave number. Consequently, vibrations are characterized by their type of motion including the stretch of bonds (symmetric and asymmetric) and deformation of bond angles including in-plane deformations (scissor and rock deformations) and out-of-plane deformations (twist and wagging), as illustrated in Figure 3.2 for the three-atom methylene group.

Although the structure of large molecules cannot be directly obtained from the spectrum, approximations have been determined using the atomic vibrations of small molecules (Krimm and Bandekar, 1986). Early Raman studies ascertained the character of the vibrations (intensity, wave number, and symmetry) and the determination of their relation to the structure of the molecule using various pure and denatured samples, isotopic substitution and excitation at various wavelengths (Frushour and Koenig, 1975; Carey, 1982; Hudson and Mayne, 1986; Krimm and Bandekar, 1986; Krimm, 1987).

Historically, Raman spectroscopy has many applications including chemical identification of materials in artworks (Breitman et al., 2007), quantitative analysis of fungicides, three-dimensional imaging of chemical composition, rapid identification of biological samples, pharmacology, dermatology, cosmetic development, and characterizing pigments as well as atomic and element composition (Hudson and Mayne, 1986; Das and Agrawal, 2011). With recent advances in charge-coupled devices (CCDs) and laser technologies, the use of Raman spectroscopy is expanding and its uses within commercial food systems are being explored.

Raman spectroscopy is suitable for use in food systems as it can provide information about the structure, function, and kinetics of biomolecules including proteins and lipids by identification of the vibrational bands (Das and Agrawal,

Downloaded From Vet-eBooks.com

2011). The advantages of using Raman spectroscopy, in lieu of other technologies, include no sample preparation so measurements can be taken in any physical state including gases, liquids, gels, amorphous solids, or crystals. Raman is less affected by varying water content compared to IR or near infrared (NIR); it is nondestructive, noninvasive, and can detect substances even if only small amounts are present. Furthermore, it is sensitive to structural changes that are induced by freezing, heating, mixing, aeration, fiber formation, denaturation, and gelatination (Li-Chan et al., 1994; Li-Chan, 1996; Ozaki, 1999; Beattie et al., 2004b; Afseth et al., 2006; Chen and Han, 2011). Consequently, Raman spectroscopy is ideal for application in the research and assessment of muscle foods.

3.3 Raman Spectroscopic Devices

Benchtop Raman devices are the most common Raman spectroscopic equipment currently in use. A schematic diagram (Figure 3.3) illustrates the arrangement, where a laser passes through the sample, and the scattered light is collected through a CCD detector, after it has passed through a notch filter, and focused onto the CCD detector. While the benchtop devices can be useful in suppressing fluorescence, which can be a limitation when measuring biological samples (Hudson and Mayne, 1986), they are limited for *in situ* measurement by the sampling required as well as the size and weight of the devices, and the power consumption of the lasers (Schmidt et al., 2009).

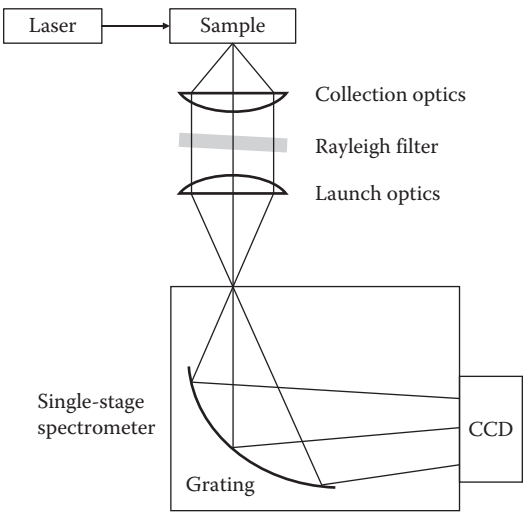


FIGURE 3.3
Schematic diagram of a benchtop single grating Raman spectrometer with a multichannel charge-coupled device (CCD) detector.

Downloaded From Vet-eBooks.com

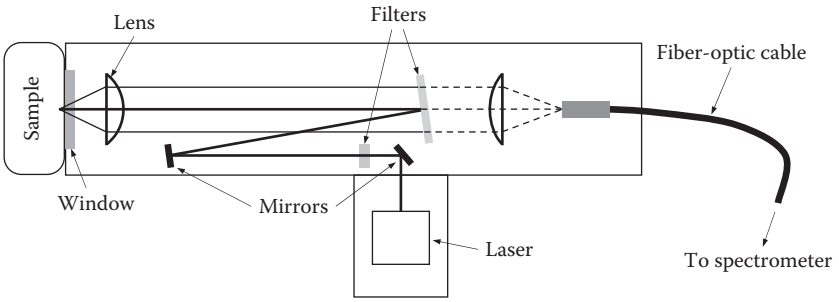


FIGURE 3.4
Schematic diagram of a Raman handheld probe head. (Adapted from Schmidt et al., *Proceedings of the Advanced Environment, Chemical and Biological Sensing Technology VI*, International Society for Optics and Photonics, Orlando, Florida, 2009.)

Miniaturization of the laser and implementation of fiber optics into Raman devices has enabled the development of ball probes which can be connected to Raman spectroscopy instruments via a fiber-optic cable (Olsen et al., 2007). The CCD and spectrograph technologies used with this probe are bulky and collected light needs to be filtered before it can be focused and collected with the CCD, while others have developed a handheld Raman sensor head for *in situ* characterization of meat quality (Schmidt et al., 2009). This technology uses the principles of backscattering geometry, lens optics, integration of laser, and Raman filter stage in one optical bench device. Miniaturization of the benchtop device required the laser, band pass, and the Raman edge filters to be combined into a single module (or optical bench), along with mirrors and lens optics as illustrated in Figure 3.4 (Schmidt et al., 2009). The Rayleigh scattered radiation is removed by the Raman filters and the Stokes-shifted signals is launched into an optical fiber within the same device (Schmidt et al., 2009).

Laboratory assessment of this technology suggested that a wavelength of 671 nm is optimal for pork meat, as it is a trade-off between the shorter wavelengths which allow for faster measuring times due to higher scattering intensities and the long wavelengths required to reduce fluorescence of the biological molecules (Schmidt et al., 2010). However, other Raman spectroscopic measurement parameters including laser power, integration time (the length of time used to collect photons on the detector), and the number of integrations (accumulations) acquired can be altered between samples to improve spectra, dependent on factors such as the rigor status of the meat.

3.4 Applications in Meat Science

The advantages of using Raman spectroscopy in food systems has not been overlooked in recent meat science research. Consequently, numerous studies

Downloaded From Vet-eBooks.com

have been conducted using both benchtop and handheld devices to determine whether Raman spectroscopy has the ability to predict a number of meat quality traits of beef, lamb, and pork.

3.4.1 Pork

Among the Raman publications in meat science, pork has the largest share (>40% between 2000 and 2015), reflecting that pork is a highly consumed meat in the world and especially preferred in Europe, large parts of Asia, and North America (Food and Agriculture Organization of the United Nations [FAO], 2014). Apart from the pioneering work in the 1970s (Carew et al., 1975; Pezolet et al., 1978), Raman spectroscopy was rarely used in the meat science field before 2000.

The first Raman papers on pork addressed sensory and technological parameters associated with warmed-over flavor due to preslaughter stress (Brøndum et al., 2000a) indicating high expectations for the fingerprinting capabilities of Raman spectroscopy, while the second tackled the prediction of the water holding capacity (WHC) of pork in the abattoir before rigor (Pedersen et al., 2003). However, this early work was carried out with benchtop laboratory instruments which were not suited to the measurement of meat or for fieldwork. Furthermore, these results were reported to be inferior due to lack of practicability and lack of sensitivity when compared to low-field nuclear magnetic resonance (NMR) (Brøndum et al., 2000a) or Fourier transform infrared (FTIR) predictions (Pedersen et al., 2003), which were conducted in parallel. It took nearly 10 years until the development of compact and sensitive Raman instrumentation allowed for a more practical and general use of Raman spectroscopy in meat science. Since then, predominantly technological and sensory quality parameters of pork have been investigated with Raman spectroscopy while the investigation of processed products is still an emerging field.

3.4.1.1 Prerigor Pork

The initial work of Pedersen et al. (2003) conducted Raman spectra of meat samples which were excised from freshly slaughtered pigs ($n = 14$) in an abattoir between 10 and 30 minutes *postmortem* (PM). While the authors found a correlation ($R^2 = 0.95$) between the spectra and the measured drip loss (DL), the number of samples was low and no understanding of the Raman spectra in the prerigor phase was reported, limiting further applications of the findings. However, Pedersen et al. (2003) did collect reference spectra of metabolites relevant to the development of meat quality traits during anaerobic glycolysis.

Although these early studies were limited, they highlighted the potential for Raman spectroscopy to monitor PM metabolism, particularly for pork

Downloaded From Vet-eBooks.com

which is susceptible to metabolic-related meat quality defects, such as dry firm and dark (DFD) and pale soft and exudative (PSE) meat (England et al., 2013). Indeed, further research conducted by Scheier and Schmidt (2013) used a prototype handheld Raman device over a period of 0.5–10 h PM to measure the *musculus semimembranosus* (SM) from 10 animals, while measuring pH and lactate concentration as reference. In this research, pH was shown to correlate with two phosphate signals which act as pH indicators (Scheier and Schmidt, 2013). The pH values were calculated from the net signal intensities at 980 $[A^-]$ and 1080 cm^{-1} [HA] with a standard error of 0.3 pH units using the Henderson–Hasselbalch equation (Equation 3.1):

$$\mathrm{pH} = \mathrm{p}K_a + \log_{10}\left(\frac{[A^-]}{[HA]}\right) \tag{3.1}$$

The observed pH values in this study gave a mean value of 5.8 and a standard deviation (SD) of 0.32, while the root-mean-squared error of calibration (RMSEC) was 0.14 pH units calculated with a multiple linear regression model based on the net intensities of 11 selected peaks. However, this error was reduced to 0.07 pH units with a partial least squares regression (PLSR) model (Scheier and Schmidt, 2013) demonstrating that different analyses can reduce the predictive error.

Using advanced spectral preprocessing and multivariate calibration techniques, such as multiplicative scattering correction and locally weighted regression, further studies have shown that the lactate concentration and pH values can be determined from the Raman spectra with an error of root mean square error of cross-validation (RMSECV) = 4.5 mmol/kg (mean 62.2 mmol/kg and SD 22.2 mmol/kg) and RMSECV = 0.06, respectively, highlighting the lactate concentration and pH predictions can be further improved (Nache et al., 2015).

The other observed changes in the spectra associated with pH decline in pork, however, were too complex for a direct assignment of the signals to metabolites (Scheier and Schmidt, 2013). The key to an understanding of the spectra was the calculation of difference spectra which reduced the complexity of the spectral changes in two defined phases: *prerigor* (spectra collected at <1 h PM minus the spectra collected at 2 h PM) and when the muscle enters into the *rigor* (spectra collected at 8.5 h PM minus spectra collected at 2 h PM). The subsequent measured difference spectra from both phases could then be well explained by calculating difference spectra using the scaled reference spectra of the involved metabolites (Figure 3.5) (Scheier et al., 2014b). To this end, the reference spectra were measured in aqueous solution under the same pH conditions which were observed in the meat samples and these spectra were scaled according to the measured or expected concentrations in normal meat (reddish, firm, nonexudative—RFN) (Scheier et al., 2014b).

Downloaded From Vet-eBooks.com

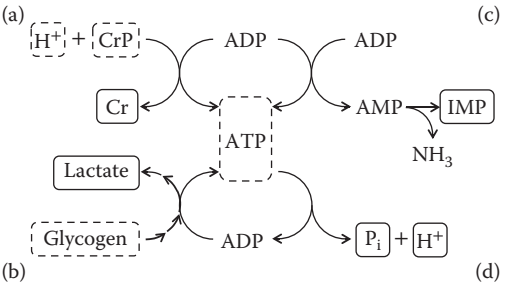


FIGURE 3.5
Scheme of postmortem energy metabolism in muscle cells showing (a) degradation of creatine phosphate (CrP) to creatine (Cr), (b) glycolysis and formation of lactate, (c) consumption of adenosine diphosphate (ADP) and subsequent deamination of adenosine monophosphate (AMP) to inosine monophosphate (IMP), and (d) hydrolysis of adenosine triphosphate (ATP) with formation of inorganic phosphate (Pi) and H^+ ions. Metabolites identified in the Raman spectra are framed: dashed and solid lines denote decreasing and increasing concentrations, respectively. (Modified from Scheier et al., *Vibrational Spectroscopy*, 70, 12–17, 2014b.)

The key metabolites of anaerobic glycolysis are summarized in Figure 3.6, where metabolites which were observed in the Raman spectra with decreasing concentration are marked with dashed lines, and compounds with increasing concentrations are marked with solid lines. Central to this process is the regeneration of adenosine triphosphate (ATP) by depleting stored creatine phosphate (CrP), reaction (a), or glycolysis and formation of lactate, reaction (b). Preslaughter stress can accelerate or inhibit these reactions causing deviations from normal meat quality (Scheffler and Gerrard, 2007; Adzitey and Nurul, 2011). If the muscle glycogen reserves were already exhausted before the death of the animal, glycolysis and formation of lactate are inhibited. If the metabolism is upregulated perimortem, regeneration from adenosine diphosphate (ADP) and subsequent deamination to inosine monophosphate (IMP), reaction (c), is significant and was shown to be an indicator of PSE and DFD (Honikel and Fischer, 1977). This information can be extracted from *prerigor* Raman spectra of pork. Finally, when ATP is hydrolyzed, reaction (d), protons are released which causes a fall in the muscle's pH. It is interesting to note that the proton is not released during glycolysis or upon reduction of pyruvate to lactate (this step is consuming a proton), but that the proton is virtually stored in polyphosphates (as acid anhydrides) until the hydrolysis of ATP (Robergs et al., 2004). These key metabolites and components with their most significant Raman peaks are summarized in Table 3.1.

Downloaded From Vet-eBooks.com

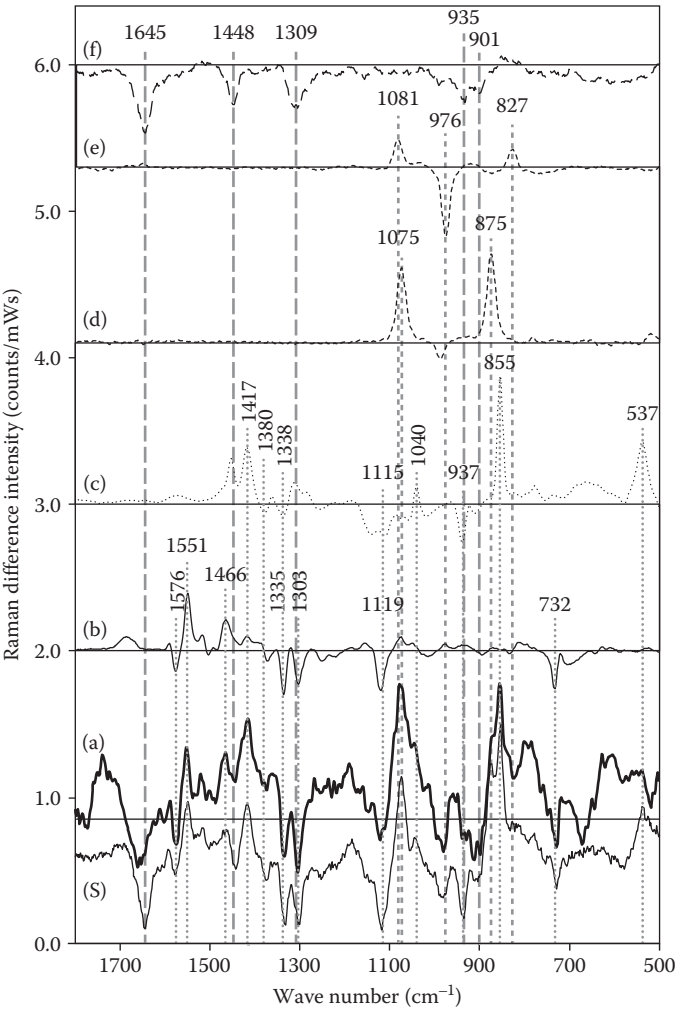


FIGURE 3.6
Raman difference spectra of (a) porcine *m. semimembranosus* from seven pigs measured 8.5 h and 2 h postmortem and (S) simulated sum spectrum comprising Raman spectra of (b) inosine monophosphate at pH 5.6 minus adenosine triphosphate (ATP) at pH 6.0, (c) lactate minus glycogen, (d) phosphate at pH 5.6 minus phosphate at pH 6.0, (e) glucose 6-phosphate (G6P) at pH 5.6 minus G6P at pH 6.0, and (f) α-helical proteins. (Modified from Scheier et al., *Vibrational Spectroscopy*, 70, 12–17, 2014b.)

Downloaded From Vet-eBooks.com

TABLE 3.1

Assignment of Most Significant Peaks to Metabolites Found in Different Raman Spectra of Early Postmortem Porcine Muscle

Peak Position/cm^{-1}	Assignment
732, 1119, 1303, 1335, 1576	Adenosine triphosphate
901, 935, 1309, 1448, 1645	α-helical proteins
826, 1045	Creatine
849, 978	Creatine phosphate
937, 1115	Glycogen
1466, 1551	Inosine monophosphate
537, 855, 1040, 1417	Lactate
875, 1075 (980)	Phosphate, monobasic (dibasic)
827, 1081 (976)	Sugar phosphates, monobasic (dibasic)

Source: Scheier, R. et al., *Vibrational Spectroscopy*, 70, 12–17, 2014b.

To transfer this approach from the laboratory to field tests, two experiments were performed using the portable Raman device to demonstrate the feasibility of using Raman spectra for the *prerigor* assessment of meat quality in a production environment (Scheier et al., 2014a, 2015b). In the first study, the Raman spectra of the SM from 96 pigs were recorded where the carcasses had been split, in the chilling room of an abattoir 1–2 h after slaughter. Ten Raman spectra with only 2.5 s integration times were averaged and correlated with pH_{45}, pH_{24}, DL, L*, a*, b*, and shear force (SF) measured after 24 (SF_{24}) and 72 h (SF_{72}) using PLSR. The animals were selected to cover all typical slaughter weights and grades and the sample contained PSE and DFD type animals. Coefficients of correlation of between 0.64 and 0.73 were obtained for pH_{45}, pH_{24}, DL, L*, b*, and SF_{72} (Scheier et al., 2014a). While the measurement in the chiller allowed for a good differentiation of the metabolic state of the carcasses, this point in time did not meet the requirements of the regular operation of the abattoir. Therefore, in the second study, the Raman spectra of the SM from 151 pigs were recorded at 25–40 min after slaughter before weighing and classification of the carcasses. The averaged Raman spectra with 2.5 s integration time, six accumulations and seven replicates were correlated with pH_{45}, pH_{24}, L*, and DL yielding good results for pH_5 ($R^2 = 0.75$), pH_{24} ($R^2 = 0.58$), and DL ($R^2 = 0.83$) (Scheier et al., 2015b).

In summary, the field studies demonstrated that Raman spectra recorded from porcine muscles before rigor (0.5–2 h PM) can be utilized to measure and predict technological quality traits such pH_1 (pH value at 1 h PM), ultimate pH (pHu), and DL (which will occur three days later) with one measurement. This is possible because the predictions are based on (known) differences of the early PM energy metabolism which is captured by the Raman spectra and which is related to deviating qualities such as dry (DFD) or exudative (PSE).

Downloaded From Vet-eBooks.com

3.4.1.2 Postrigor Pork

While anaerobic glycolysis is ending and the muscle is passing into *rigor mortis*, a large part of this information is lost, e.g., normal pork cannot be discriminated from PSE meat based on the pHu. Similarly, postrigor Raman spectra contain less spectral information which can be used for the prediction of DFD and PSE affected pork. For example, the Raman spectra of exudative (PSE) pork exhibits significantly lower signals of α-helical proteins (Table 3.1) in the *rigor* phase, but these pH-related signals cannot be distinguished from normal pork (Scheier and Schmidt, 2013).

Subsequently, a very weak relationship between Raman spectra and the meat quality defect warmed-over flavor was reported when the impact of preslaughter stress on the development of warmed-over flavor was studied by Brøndum et al. (2000b). However, we cannot exclude that this poor relationship could also be due to the instrumentation used in this study, as a 1064 nm laser was used which is less suited to applications in meat science. Alternatively, postrigor spectra from 95 pigs recorded with the 671 nm Raman device yielded correlations with pH and DL of $R^2 = 0.85$ and 0.7, respectively, showing that the spectra allowed for the identification of DFD meat, but not for a clear differentiation of PSE meat (Schmidt et al., 2012). Subsequent work with 137 *m. longissimus thoracis* yielded improved prediction errors for pH_{24} (RMSECV = 0.03 pH units), but not for DL (0.9%). In addition, L* (RMSECV = 1.2) and, to some extent, cooking loss (1.6%) could be correlated with the *postrigor* Raman spectra (Scheier et al., 2015a). However, the samples in this study were homogenous and contained neither PSE nor DFD animals so no conclusions can be drawn with respect to the *postrigor* detection of these qualities.

The ability to predict other meat quality traits of pork using Raman spectroscopy has also been investigated as further studies have measured pork to determine SF values (Beattie et al., 2008) and sensory attributes (Wang et al., 2012). In a study conducted by Beattie et al. (2008), aged and cooked pork was tested with a 785 nm Raman benchtop device to determine whether SF values could be predicted. Prediction models for this study yielded a prediction error of RMSEP = 4.4 N for animals with an average SF of 36.9 N. However, the number of samples measured in this study was low, as meat from only three pigs was measured. Therefore, the application of this study is limited. Further work to predict sensory attributes of pork using a Raman microscope equipped with a 780 nm excitation laser yielded poor accuracies in PLSR predictions of 23.2%, 9.6%, and 17.3% (with 5% error tolerance) for tenderness, chewiness, and juiciness which were assessed by a sensory panel, but showed >83% correct predictions with tenderness and chewiness when employing PLS discriminant analysis (PLSDA) (Wang et al., 2012) suggesting that Raman spectral data may be better suited to differentiating and classifying meat on tenderness.

Overall, these studies conducted on pork highlight the potential of *postrigor* Raman spectra to determine technological relevant pork quality traits SF, pH_{24}, and L* as well as sensory attributes.

Downloaded From Vet-eBooks.com

3.4.1.3 Heat-Treated Pork

Cooking meat leads to changes in protein structures including denaturation of myosin at 53°C, sarcoplasmic proteins and connective tissue at 62°C, as well as actin at 76°C (Xiong et al., 1987; Tornberg, 2005). Subsequently, the changes associated with cooking can be monitored using Raman spectroscopy as they alter the spectra of cooked meat (Beattie et al., 2008; Berhe et al., 2014, 2015). The signals indicating denaturation of the proteins predominantly originate from the protein backbone signals at 1640–1660 (amide I, α-helix), 1660–1670 (amide I, random coil and β-pleated sheet), 1330–1350 (CH-bending), 1230–1260 (amide III, random coil and β-pleated sheet), 1000 (C-C stretching β-pleated sheet) (Beattie et al., 2008), and 920–960 cm^{-1} (C-C stretching α-helix) (Berhe et al., 2014, 2015). Although broad signals in the regions 510–550 cm^{-1} have been assigned to disulfide bonds (Berhe et al., 2014), the observed intensities are too high in this study to account for the low cysteine content of the meat. Furthermore, loss of fat, which also occurs during cooking, has been identified by peaks at 1060, 1080, and 1128 cm^{-1} (Beattie et al., 2008).

It has been long established that protein conformation and denaturation are reflected by the Raman spectra (Frushour and Koenig, 1975). Consequently, several studies were completed to determine if Raman spectroscopy could predict end point temperature by using principal component analysis (PCA) to cluster spectra according to the end point cooking temperature (Berhe et al., 2014, 2015). These studies found that the temperature could be calculated using PLSR with a prediction error of RMSECV = 1.3°C (Berhe et al., 2014) and 1.3–1.6°C for freshly cooked meat (Berhe et al., 2015), and with 1.6–2.0°C for cooked meat stored for four or eight days (Berhe et al., 2014). Furthermore, classification of samples cooked below and above 65°C was achieved with <5% misclassification (Berhe et al., 2015) indicating that Raman spectroscopy could be useful to check the core temperature of cooked meat in a final product. However, the application of these studies is limited given that noninvasive methods of temperature determination are already available.

The spectral changes associated with cooking have also been studied to determine the potential for Raman spectra to predict the amount of intracellular fluid lost during cooking (cooking loss) and postcooking SF values of pork. Indeed, several studies have agreed that cooking loss of pork could be determined from Raman spectra with an RMSECV of 2.5% (Beattie et al., 2008) and 2.8% (Berhe et al., 2014). Interestingly, these studies were also able to predict cooking time with an RMSECV = 4.1 min (Beattie et al., 2008) and 1.4 h (Berhe et al., 2014). The correlation of SF with spectra of cooked pork samples was also promising, as models yielded a root-mean-squared error of prediction of RMSEP = 4.4 N using 54 samples for calibration and for validation with an averaged SF for all samples of 36.9 N (Beattie, 2015, pers. comm.).

Overall, these studies have shown that Raman spectra of cooked pork were able to provide information about conformational changes in the secondary structure of meat proteins and the hydrophobic environment at the molecular level. This spectral information can be used to predict the end point tem-

Downloaded From Vet-eBooks.com

perature, cooking loss, and cooking time with high accuracy. In addition, there is potential to assess tenderness with Raman spectra of cooked pork.

3.4.1.4 Fatty Acid Composition of Pork Adipose Tissue

Raman work on pork adipose tissue has focused on the fatty acid (FA) composition (Olsen et al., 2007, 2008, 2010) and the prediction of FA bulk parameters for adipose tissue to differentiate it from the fat of other meat species (Beattie et al., 2006). These studies demonstrated consistently good correlations between the Raman spectra and bulk unsaturation parameters of pork subcutaneous fat, including total unsaturated cis FAs ($R^2 = 0.97$) and polyunsaturated fatty acids (PUFAs) ($R^2 = 0.97$) (Beattie et al., 2006), iodine value (IV) ($R^2 = 0.89$ and 0.97), monounsaturated fatty acids (MUFAs) ($R^2 = 0.96$), and PUFAs ($R^2 = 0.98$) (Olsen et al., 2007, 2010).

The predictions of trans-FAs were not as promising ($R^2 = 0.52$), which was ascribed to the inherently low content of trans-FAs present in pork meat (Beattie et al., 2006). Similarly, the prediction of individual FAs with Raman spectroscopy was shown to be feasible for subcutaneous fat in general, but with different levels of accuracy for individual FAs (Beattie et al., 2006). As this is of considerable interest due to the importance of determining the nutritional value of meats, further work has addressed the determination of omega-3 und omega-6 FAs in adipose tissue from the neck of 73 pork carcasses (Olsen et al., 2008). This study found a high correlation between omega-6 and Raman spectra ($R^2 = 0.93$) yet the correlation was slightly lower between omega-3 FAs and Raman spectra ($R^2 = 0.91$). Further work on the prediction of individual FAs has revealed that prediction of individual FAs is likely to be indirectly but strongly correlated to other FAs, such as PUFA and IV, when predicted using Raman spectra (Berhe et al., 2016). The authors describe this as a "cage of covariance," which is also affected by the animal's diet and potentially breed. Consequently, the authors suggested that further analysis is required when assessing models to predict individual FAs to prevent model instability due to this covariance.

Further Raman spectroscopy research has also been conducted to discriminate between the inner and the outer layers of porcine subcutaneous adipose tissue, which is important due to the impact of chemical composition of fat on the cohesiveness and firmness of fat, visual appearance, oxidative stability, and nutritive value of value-added pork products (Lyndgaard et al., 2012). Discrimination between the layers is possible as there is a higher degree of unsaturation (higher IV) of the outer layer compared to the inner layer (Lyndgaard et al., 2012).

The ability of Raman spectra to reflect the bulk FA profile was also exploited when discriminating animal fats from different species (Beattie et al., 2007; Sowoidnich and Kronfeldt, 2012; Boyaci et al., 2014b) or animal fats from plant oils (Abbas et al., 2009). The differentiation of animal fats from different origins is possible using polymorphic features in the Raman spectra. Polymorphism is the ability of a compound to solidify in different crystalline structures. As an example, the polymorphs of tristearin can be discriminated

Downloaded From Vet-eBooks.com

by their Raman spectra (Da Silva et al., 2009). Thus, the 1417 cm^{-1} methylene scissoring vibration which is an indicator for the β′ polymorph can be used to discriminate porcine adipose tissue from bovine adipose tissue (Motoyama et al., 2010). Furthermore, Raman spectroscopy has been used to monitor physical changes of adipose tissue during the refrigeration process of a carcass. The degree of crystallinity and the fraction of β′ polymorph of porcine adipose tissue were shown to increase with refrigeration time and with lowering temperature (Motoyama et al., 2013). In this study, the degree of crystallinity was calculated from the methylene twisting vibrations attributed to crystalline and melted fat at 1297 cm^{-1} and 1305 cm^{-1}, respectively. Raman imaging was then applied to visualize and characterize the spatial distribution of the crystalline states of fats (Motoyama et al., 2016).

The ban on surgical castration of piglets in Europe has resulted in research into the detection of boar taint (EU, 2010). Boar taint is characterized as an off-flavor that is perceivable when fat of entire males is heated and is mainly ascribed to the compounds skatole (3-methyl-indole) and androstenone (5α-androst-16-en-3-one), which are enriched in adipose tissue. Three approaches have been reported to investigate the feasibility of using Raman spectroscopy for boar taint detection (Wang, 2013; Sorensen et al., 2015; Liu et al., 2016). The first method used a methanolic extract from subcutaneous fat and direct measurement of the extract (Wang, 2013). However, both skatole and androstenone occur at concentrations that are so low that they are not directly assessed with spontaneous Raman scattering. Therefore, this approach is not practical for a production environment due to the extraction process. The second approach also used a methanolic extract, but assessed the malodorous compounds directly with surface-enhanced Raman spectroscopy (SERS) (Sorensen et al., 2015). SERS is the amplification of Raman scattering by orders of magnitude when the compounds are adsorbed to gold or silver nanoparticles (Lee and Meisel, 1982). With SERS, quantification of skatole and androstenone was shown to be feasible with prediction errors (RMSECV) of 0.17 μg/g for skatole and 1.5 μg/g for androstenone in the fat (Sorensen et al., 2015). The third approach tested the indirect detection of boar taint based on variations in the FA profile of boars. The rationale behind this approach was that such a measurement can be recorded during the production process. Liu et al. (2016) showed that detection of extreme boar taint based on differences in the FA composition of subcutaneous fat was feasible with Raman spectroscopy and that the highest classification accuracy was achieved using only spectra from the inner layer (81% samples correctly classified). However, this approach had limitations as only extremely divergent fat samples were detectable and the classification may be affected by the diet of the animals as it influences the FA composition.

3.4.2 Lamb

The initial Raman spectroscopic research on lamb carcass quality conducted by Schmidt et al. (2013) used a Raman spectroscopic handheld device to pre-

Downloaded From Vet-eBooks.com

dict SF values, due to the importance of tenderness to the consumer acceptance of meat products (Thompson, 2002). Consequently, the *m. longissimus thoracis et lumborum* (LTL) was sampled from 140 mixed sex lambs that were bred and reared at two different research sites, and this muscle was measured using a Raman handheld device equipped with a 671 nm laser (Schmidt et al., 2009). Prediction models developed using PLSR yielded promising correlations of $R^2 = 0.79$ and 0.86, respectively, for the samples measured from the two sites. However, the methodology of this study limits the application of the findings to commercial meat processors, as the measurements were conducted on 3 cm sections of LTL that were aged, frozen at –20°C, and thawed. Furthermore, this study did not conduct the Raman spectroscopic measurements and determine the SF values from the same piece of meat. Subsequently, further research was conducted to investigate whether the same Raman handheld device could be used to predict SF values of fresh intact lamb measured on the same piece of meat (Fowler et al., 2014a,b).

The complementary studies conducted by Fowler et al. (Fowler et al., 2014a,b) reported for the first time the potential of Raman spectroscopy to predict the SF of fresh intact lamb, while also comparing the prediction using Raman spectroscopy against commonly used indicators of SF including particle size, sarcomere length, pH measured at 24 h PM (pH_{24}), and pHu. Given the promising correlations of the initial research predicting SF using a Raman handheld device (Schmidt et al., 2013), these studies moved toward developing Raman spectroscopy as a predictor of SF which could be used in industrial applications.

For the first time, these studies measured unrelated carcasses, collecting cuts from 80 carcasses selected from the same abattoir from different consignments over a four-day period to represent the animals typically processed in order to obtain a spread in SF values which would be expected by commercial processors. Furthermore, the Raman spectroscopy measurements were conducted on each intact sample at 24 h and five days PM in 12 positions parallel to the muscle fibers of the cuts after they were removed from the carcass using an integration time of 3.75 (topside) and 3 s (loin). Therefore, the area sampled and the measurement times of each muscle were shorter and more feasible for measurement in industrial applications compared to previous Raman spectroscopic research in lamb where the 3 cm thick samples were cut into quarters and the freshly cut surfaces of three of the quarters were measured in five different positions using integration times of 5 and 4 s for the two sample groups (Schmidt et al., 2013).

Despite the initial research suggesting there was an excellent correlation between SF of lamb and the Raman spectra, these subsequent studies (Fowler et al., 2014a,b) indicated otherwise, as the most fruitful model yielded a cross-validated coefficient of determination between the predicted and observed values (R^2_{cv}) equal to 0.22 (Fowler et al., 2014a). This model was obtained for the prediction of SF values measured on the SM at five days PM using Raman spectra collected at one-day PM. Although these studies yielded lower accu-

Downloaded From Vet-eBooks.com

racies for the prediction of SF values using Raman spectra, Fowler et al. (2014a) did demonstrate that compared to the laboratory- based indicators of SF, Raman spectroscopy had greater potential to predict SF values as the predictions using Raman spectra yielded lower prediction errors compared to using the indicators alone.

Comparing these Raman spectroscopic studies focused on predicting SF values highlights that there are numerous experimental design factors which vary between studies, including the spectral acquisition parameters, muscle and face measured, sample handling, number of independent samples, positioning of the sample under the laser, and the Raman spectroscopic device and wavelengths used for measurement as well as the methods of chemometric analysis. For example, multiple studies have been conducted all aimed to predict the SF values of meat determined by a Warner–Bratzler SF texture analyzer using Raman spectra (Beattie et al., 2008; Schmidt et al., 2013; Fowler et al., 2014a,b). Yet models from these studies have yielded a range of results from no correlation ($R^2_{cv} = 0.00$) (Fowler et al., 2014b) to strong correlations ($R^2 = 0.86$) (Schmidt et al., 2013) between predicted and observed values. However, as the experimental design varies between the cited studies, it is difficult to determine the reasons for the variation. Consequently, as the experimental design of studies have been altered to move toward development of Raman spectroscopy as a tool for processors to predict SF values, the impact of changes to the design of the experiments on the quality of spectral information and the robustness of the prediction is currently unknown.

As sample handling has varied greatly between earlier studies, Fowler et al. (2014a) hypothesized that freezing the meat may have improved the prediction of SF values found by Schmidt et al. (2013) as Raman spectroscopy is sensitive to the effects of freezing and thawing (Li-Chan et al., 1994). Subsequently, further research was conducted to test this hypothesis and determine the effects of freezing and thawing on the prediction of SF of intact lamb (Fowler et al., 2015b). However, this research also aimed to test whether Raman spectroscopy could predict several other meat quality traits given that other research in pork has demonstrated the ability to predict pHu ($R^2 = 0.85$) (Scheier et al., 2014a) and WHC ($r = 0.98$) (Pedersen et al., 2003) yet studies conducted on lamb had focused solely on the prediction of SF values.

The two complementary studies conducted by Fowler et al. (2015b) used similar experimental designs to previous research on fresh intact lamb (Fowler et al., 2014a); however, in these studies carcasses were subjected to electrical stimulation, Raman spectra were collected at 25-min PM and the 80 SM collected over two consecutive days for experiment two were frozen after the five-day PM measurements, held frozen for 24 h, and thawed prior to measurement. Furthermore, the data analysis for both of these studies also included models to determine the potential to predict other meat quality traits of lamb including sarcomere length, particle size, color (L*, a*, b* values), collagen content, purge loss, and pH values at 24 h PM (pH_{24}) as well as pHu.

Downloaded From Vet-eBooks.com

The results of these experiments indicated that there was no ability to predict SF values using spectra collected at 24 h, five days PM, or after freezing and thawing (Fowler et al., 2015b). Given the inconsistent results from experiments over multiple years (Schmidt et al., 2013; Fowler et al., 2014a,b, 2015b), it is likely that the measurement of intact lamb using the Raman spectroscopic handheld device is unable to provide a repeatable prediction of SF values as currently tested. Yet, the findings of Fowler et al. (2015b) indicated that there is a potential to predict pH_{24} and pHu using Raman spectra measured at 24 h ($R^2_{cv} = 0.59$ and $R^2_{cv} = 0.48$, respectively) as well as purge ($R^2_{cv} = 0.42$) and L* values measured at both 24 h PM ($R^2_{cv} = 0.32$) and five days PM ($R^2_{cv} = 0.33$).

Given that relationships between pH and purge were found and it has been well established that pH, purge, and color are interrelated (Hughes et al., 2014), it is plausible that the predictions of these meat quality traits using Raman spectroscopy are related to the biochemical changes which occur during the conversion of muscle to meat during the early PM period. Although there is a scarcity in literature which has reported on the prediction of lamb meat quality characteristics, spectral assignments to determine the biochemical characteristics that underlie the predictions for pH, purge, and color based on a comparison with existing literature on the determination of meat quality traits in pork (Scheier and Schmidt, 2013; Scheier et al., 2014b) agrees with this hypothesis. Although it was acknowledged that there are limitations in the transference of findings between the measurement of pH early PM in pork and the measurement of pHu at 24 h in lamb, some of the spectral changes were identified as metabolites involved in anaerobic glycolysis. These included spectral changes associated with inorganic phosphate (875, 970, and 1080 cm^{-1}), adenosine (1334 cm^{-1}), α-helical proteins (937 cm^{-1}), and the amino

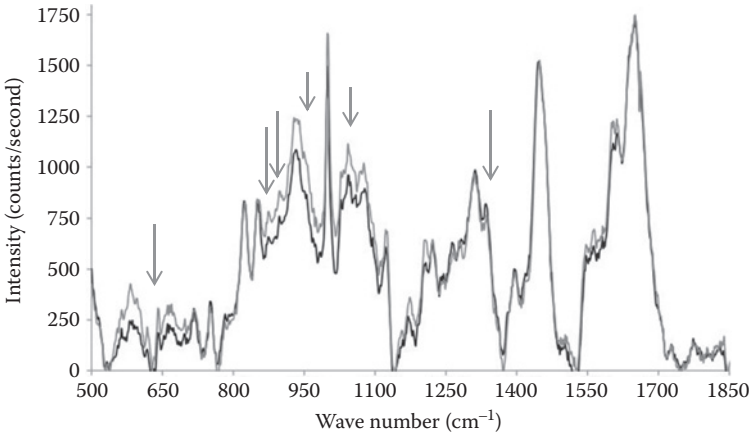


FIGURE 3.7
The averaged background corrected Raman spectra collected 24 h postmortem (PM) from the ovine *m. semimembranosus* with the five highest and five lowest pH values, highlighting the spectral changes associated with pH value.

Downloaded From Vet-eBooks.com

acid side chains (563–720 cm^{-1}) (Figure 3.7). Overall, it was concluded that the spectral changes noted suggested that lamb with lower pHu values may have undergone a more accelerated pH decline PM which could have been driven by glycogen concentrations at slaughter (Ferguson et al., 2008). However, the exact biochemical pathways which resulted in the spectral differences remained unclear due to the complexity of the spectra collected and a lack of reference spectra for comparison (Fowler et al., 2015b). Furthermore, it is yet to be determined whether these findings are repeatable over time and for independent data sets and subsequent research will need to address this.

As Raman spectroscopy has been used to classify adipose tissue by species (Beattie et al., 2007), and predict the FA composition of pork subcutaneous adipose tissue (Olsen et al., 2007, 2010; Lyndgaard et al., 2011) and intact salmon (Afseth et al., 2006), further research conducted by Fowler et al. (2015a) has also investigated its potential as a tool to predict the fat content and FA group composition of lamb loin including PUFA, MUFA, and saturated fatty acids (SFA). Despite finding strong correlations between the observed and predicted values for the prediction of PUFA ($R^2 = 0.93$) and MUFA ($R^2 = 0.54$), this study found that the models became less accurate when cross-validation procedures were used as the cross-validated correlations between the predicted and observed values dropped to $R^2_{cv} = 0.21$ and $R^2_{cv} = 0.16$ for PUFA and MUFA, respectively.

Being the first study to focus on the prediction of major FA group composition of intact meat, several challenges were identified with the approach undertaken to measure FA composition in intact meat which could be contributing to the reduction in the predictions when the models were cross validated. Fowler et al. (2015a) hypothesized that the discrimination between spectra containing lipid and protein signals and overlapping spectral signals from contributions of the phospholipid head groups present in intact muscle may be causing the models to under- or overestimate the major FA group concentrations when the models are cross validated. The optimal thresholds for discriminating between spectra containing mixed lipid and protein signals and the impact of contributions of phospholipid signals in spectra for meat with varying intramuscular fat content on the prediction models is currently unknown. Therefore, the current models do not have general applicability; however, the value of measuring FA group composition due to human health targets warrants further investigation.

3.4.3 Beef

To date, there has been very little research conducted on the potential of Raman spectroscopy to predict meat quality traits of beef, although a preliminary investigation on the application of Raman spectroscopy to predict sensory quality traits (Beattie et al., 2004a) and tenderness (Bauer et al., 2016) of beef have been completed. The study by Beattie et al. (2004a) was an exploratory study to determine if Raman spectroscopy could predict meat quality given that other technology-based methods including elastograms, fluores-

Downloaded From Vet-eBooks.com

cence emission spectroscopy, or NIR spectroscopy had not been adopted by industry for widespread use and high-performance push-button Raman spectroscopy equipment was becoming available at a reasonable cost. To this end, 52 samples of beef silverside were collected, cooked, and subsampled for Raman spectroscopic measurement on a rotating stage using a benchtop Raman device equipped with a 785 nm excitation laser, for determination of SF and sensory analysis. Despite achieving good correlation coefficients using Raman spectra to measure SF ($R^2 = 0.75$) and sensory traits including texture ($R^2 = 0.71$), juiciness ($R^2 = 0.62$), as well as overall liking ($R^2 = 0.67$), the application of these results to industry is limited as the samples were cooked prior to measurement and a benchtop Raman device was used which still requires a biopsy of the sample to be taken for measurement. Nonetheless, this experiment does highlight the potential for Raman spectroscopy to be applied to predict a broader range of eating quality traits.

A longer term study to determine the potential for Raman spectroscopy to predict the tenderness of beef *m. gluteus medius* (Bauer et al., 2016) demonstrated that there was an ability to predict beef tenderness with good accuracy ($R^2_{cal} = 0.94$) with the initial modeling. However, when this model was further validated on independent samples, this accuracy is reduced ($R^2_{val} = 0.23$) suggesting the initial model was overfitted or the biological variance of the samples included in the calibration model did not represent the samples collected in the validation model. Yet, the correlation between Raman spectra and tenderness was improved when a model was created using the validation data to create a new model ($R^2_{cv} = 0.65$). Further analysis for this data showed that reducing the number of spectral channels included and limiting the latent variables in the analysis resulted in more robust models but reduced the precision of the predictions ($R^2_{cv} = 0.33$) (Bauer et al., 2016) highlighting the need to determine the optimal processing and analysis techniques to obtain repeatable predictions of meat quality parameters to determine whether there is a potential for industry to apply this research.

Bauer et al. (2016) also suggested an alternative approach to predicting SF values as they demonstrated that Raman spectroscopy has potential to discriminate between tough and tender samples based on a given threshold, thereby eliminating the need for an exact prediction of the SF values. Thresholds of 30, 34, 40, 43, and 49 N were trialled as consumer studies in the United States have demonstrated SF values below these values met the 100%, 99%, 94%, 86%, and 25% consumer satisfaction scores for tenderness. Using partial least squares discriminate analysis (PLS-DA), Bauer et al. (2016) concluded that it was possible to classify samples with accuracies of between 59% and 80%. However, the precision increased with the threshold suggesting that Raman spectroscopy can be used as a screening tool to accurately identify tough beef.

While this study overcomes some of the limitations of earlier meat science research using Raman spectroscopy by using a handheld Raman device and measuring SF on the same areas of muscle which were scanned using

Downloaded From Vet-eBooks.com

the Raman device, more work is required to determine whether the accuracy and precision of predictive models are achievable using measurement protocols which more closely reflect commercial operations. Consequently, research is needed to determine whether an early PM Raman measurement in a commercial situation is able to predict the tenderness of the product when it reaches the consumer after aging and transport.

Tentative band assignment of the spectra collected from tough and tender beef indicates that while there are clear signals associated with α-helical proteins (1650, 1447, 1312, 935, and 906 cm^{-1}), amino acid side chains such as tryptophan (1550, 1353, 1336, 1009, and 759 cm^{-1}), and connective tissue (1665, 1453, 1270, 1248, and 855 cm^{-1}), overall intensity of the spectra were the main difference between tough and tender samples (Bauer et al., 2016; Figure 3.8). This disagrees with the research conducted in lamb, which found that spectra from tender samples had a higher overall intensity (Fowler et al., 2014a); however, reasons for these differences have not been well defined.

3.4.4 Spoilage

Meat is a very perishable food whose shelf life is dependent on the microbial flora, the packaging, and the storage conditions (Borch et al., 1996; Saucier, 2016). Food safety regulations require routine control of microbial status, but as the traditional microbiological methods are time-consuming and expensive, rapid methods are sought for more efficient monitoring (Ellis

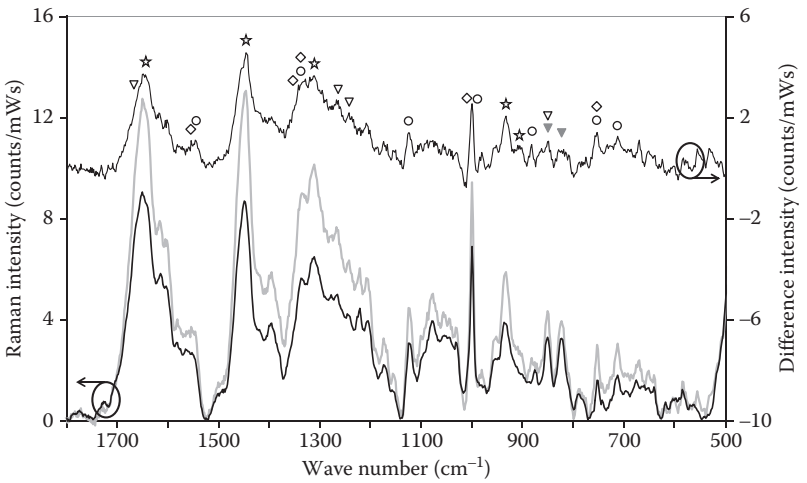


FIGURE 3.8
Averaged and preprocessed Raman spectra of the 10 most tender (black line) and the 10 toughest (gray line) bovine *gluteus medius* samples with shear force values <29 N and >48 N, respectively. The upper curve (thin line) represents the difference spectrum "tough minus tender." Selected signals of α-helical proteins (star), myoglobin (circle), tryptophan (diamond), tyrosine doublet (gray triangle), and connective tissue (triangle) are highlighted. (From Bauer et al., *Meat Science*, 115, 27–33, 2016.)

Downloaded From Vet-eBooks.com

and Goodacre, 2001). Spectroscopic methods are among those being trialled as they are rapid, noninvasive, and nondestructive (Damez and Clerjon, 2008). The first detection of spoilage of pork using Raman spectroscopy was reported with a 785 nm laboratory set up (Schmidt et al., 2008). In this work, principal components analysis (PCA) was applied for a qualitative discrimination between samples at a threshold of 10^6 colony forming units (cfu)/cm^2 total viable mesophilic aerobic plate counts. This discrimination of spoiled pork was confirmed with a handheld Raman sensor head operating at 671 nm which was developed for the characterization of meat quality (Schmidt et al., 2009). Subsequent research revealed that this detection was largely based on a laser-induced fluorescence which started beyond the threshold of 10^6 cfu/cm^2 (Sowoidnich et al., 2010) and that this detection could be performed through packaging (Schmidt et al., 2009). In these studies, however, the growth of the spoilage flora was intrinsically tied to the storage time. To separate both effects, the storage experiment was repeated by comparing the Raman spectra of pork chops (LTL) which were sterilized and which did not spoil over a period of 14 days and untreated pork chops (SM and LTL), which perished over a period of 21 days (Sowoidnich et al., 2012). In this instance, the PCA revealed that spoilage was detected by means of the first principal component as observed before and that the aging of the sterilized meat was monitored by the second principal component. Raman signals indicating storage, time, and aging were observed at 1650 cm^{-1} (amide I), 1446 and 1340 cm^{-1} (C–H deformation), and 902 and 935 cm^{-1} (C–C stretching α-helix), respectively (Sowoidnich, 2012; Sowoidnich et al., 2012).

Raman measurements using the polarization dependency of the spectra during a storage experiment with sterilized and untreated meat revealed that Raman spectra are anisotropic (with the muscle fiber direction). During storage changes in signals attributed to α-helical proteins indicated a loss of anisotropy as the surface spoiled; however, this did not occur with the sterilized samples. Hence, it was concluded that the bacteria on the surface obscure to some extent the spectra of the underlying meat (Al Ebrahim et al., 2013b). Quantification of the microbial surface contamination of pork has also been successfully completed using a portable Raman device, which resulted in a model with a reported prediction error of RMSEV = 1.6 log total viable counts (TVC)/cm² (Grimmler and Schmidt, 2015). Overall, this research demonstrates that Raman spectroscopy can be used below the critical threshold of 5 log TVC/cm² (Grimmler and Schmidt, 2015).

3.4.5 Differentiation of Animal Species and Detection of Adulteration in Muscle Foods

Given the species differences evident in Raman spectra, it is unsurprising that Raman spectroscopy has been used to distinguish between different species such as pork, chicken, turkey, mutton, goat, beef, and horse (Ellis et al., 2005; Sowoidnich, 2012; Sowoidnich and Kronfeldt, 2012; Boyaci et al.,

Downloaded From Vet-eBooks.com

2014a,b). This is achieved as the Raman spectra reflect differences in the protein and lipid composition, although heme signals from myoglobin can also be used as a marker for distinguishing between red and white muscles (Sowoidnich and Kronfeldt, 2012). Differences in muscle structure and minor components will also contribute to the classification of species using Raman spectroscopy, but current knowledge about the spectra and the identification of marker substances is inconsistent and incomplete.

Increasing attention is being paid to the detection of fraudulent addition of undesired meat, especially to minced meat products. Consequently, the addition of pork to minced lamb products has been investigated using a Raman microscope (Hassing et al., 2012; Hassing and Jernshøj, 2014). This work demonstrated classification of fat and meat from minced pork and lamb was possible, although it was stated that for a practical implementation, the 785 nm system which was used will require improvements in order to automatically detect areas of fat, to focus the laser, and to measure Raman spectra.

After the large-scale adulteration of beef mince by the addition of minced horse meat in Europe during 2013, the discrimination of horse meat and beef was investigated with Raman spectroscopy. Principal components analysis was shown to successfully discriminate Raman spectra of beef and horse which were measured with 671 nm excitation (Al Ebrahim et al., 2013a). FT-Raman measurements using 1064 nm excitation with ground beef mixtures with 0%, 25%, 50%, 75%, and 100% admixture of horse meat indicated a correlation with selected signals (937, 879, 856, and 829 cm^{-1}) which were normalized to the phenylalanine signal at 1003 cm^{-1} with the percentage of added horse meat (Zając et al., 2014). Similarly, the detection of horse meat in beef was also reported based on the 785 nm Raman spectra of the extracted fat using PCA (Boyaci et al., 2014b).

Dispersive Raman spectroscopy was used to detect beef offal adulterations (kidney, liver, heart, and lung) in beef burgers (Zhao et al., 2015). PLS-DA classifications with >89% correctly classified samples were reported and the authors stated a prediction accuracy acceptable for screening purposes when assessing total offal and added fat in the samples (Zhao et al., 2015).

3.5 Limitations

From the literature it is clear that Raman spectroscopy has the potential to be an online tool to predict meat quality traits of intact muscle in a nondestructive and rapid way. However, gaps in the understanding of existing literature including the reasons for inconsistencies between results of current studies predicting meat quality traits, the biochemical characteristics of the meat which underlie predictions, and the optimal muscle and measurement

Downloaded From Vet-eBooks.com

parameters for each species will need to be addressed before Raman spectroscopic technologies can be widely adopted within industry.

Lack of understanding of the causes of variation in predictability between studies is an ongoing challenge for adoption of Raman spectroscopy in industry as it is sensitive to variation in measurement parameters (Beattie et al., 2004a) yet there is little consistency in existing literature. For example, the research by Fowler et al. (2014a) was conducted by collecting 10 Raman spectra measured perpendicular to the muscle fiber over the largest face of the SM, where the *m. adductor* was removed, from 80 randomly selected lambs. However, Schmidt et al. (2013) measured 3 cm blocks of loin from 140 lambs that had been quartered before five different positions were measured on the freshly cut surfaces with integration times of 5 and 4 s for the two sample groups. Thus, Schmidt et al. (2013) collected 15 spectra per sample over a much smaller portion of muscle. Furthermore, other Raman spectroscopic studies predicting meat quality traits of pork (Beattie et al., 2008) and beef (Beattie et al., 2004a) have measured different faces of other muscles using integration times of six and three minutes which produced relatively high R^2_{cv} values of 0.75 and 0.77 (Beattie et al., 2004a, 2008). Consequently, the most appropriate measurement parameters, muscle, and face of the muscle for measurement for pork, beef, and lamb are yet to be determined.

The number of positions and locations measured on each independent sample needed to develop robust calibration models is also required as it determines the way in which the scattered light can be detected and the chemometric data can be analyzed. Despite the widespread use of PCA as a tool for spectral data analysis, when PCA is used in the analysis of data from a small number of samples the average spectra representing each sample may underrepresent subtle differences between spectral regions of the samples (Bonnier and Byrne, 2012). This occurs because PCA clusters data based on spectral similarity and variance. Consequently, it is expected that Raman spectra obtained from numerous subsections removed from a small number of independent samples would substantially impact the variance and loadings that differentiate groups in further data analysis. This is because the strength of this analysis technique is in the loadings, given that they represent the spectral origin of the variations (Bonnier and Byrne, 2012). As such, data sets with few truly independent samples, for example, Beattie et al. (2004a), who measured 168 pork *m. longissimus lumborum* (LL) sections from only 18 pigs, are at risk of artificially biasing the results from chemometric analysis and consequently underrepresenting subtle differences within more complex data sets. Consequently, the models generated from data sets with small numbers of independent samples or from more positions measured on fewer samples may then have a better accuracy of prediction than if the true spectral differences were represented.

Despite the strong advantages in using Raman within food systems, the presence of fluorescence is also a continuing challenge when measuring biological samples, particularly meat. Fluorescence occurs when light absorbed

Downloaded From Vet-eBooks.com

and emitted at a longer wavelength and therefore at lower energy (Austin et al., 1993). As the level of light scattered by the Raman process is generally weak in comparison to other optical processes, the vibrational spectrum can be completely obscured by other contributions including broad featureless fluorescence emission (Hudson and Mayne, 1986). Although the use of highly effective filters and measuring at a wavelength >800 nm can, to some extent, overcome these challenges (Hudson and Mayne, 1986), current Raman spectroscopic research on meat quality has focused on using shorter wavelengths (785 and 671 nm) to facilitate shorter measurement times (Schmidt et al., 2013). Although 785 nm excitation is considerably less impaired by fluorescence, measurements are slower compared to 671 nm excitation and *vice versa*. Consequently, losses of Raman spectral information due to fluorescence continues to be an ongoing challenge for the prediction of meat quality traits using Raman spectroscopy in red meat where higher levels of iron and heme are likely to generate higher amounts of fluorescence when meat is excited with a 671 nm laser.

Like fluorescence, the presence of non-Raman background signals can completely obscure the Raman vibration modes (Beattie, 2011). Any photons that aren't generated by the sample at the frequency of interest are considered background noise (McCreery, 2005). Background noise may arise from a range of optical phenomena (Beattie, 2011) including optic equipment, stray light from Rayleigh scattering, and reflections from optics or dust (McCreery, 2005). However, it also may arise from the samples due to differences in the myofibrils associated with background and age. After completing the chemometric analysis jointly and separately for the samples reared at two sites, Schmidt et al. (2013) noted that coefficients of determination for prediction models were increased when two models were created based on site of origin due to the differences in background noise and fluorescence in the spectra. Therefore, the impact of differences in background noise and fluorescence generated from samples randomly collected to generate calibration models needs to be determined.

3.6 Conclusion

Although there has been relatively limited research into the use of Raman spectroscopy for meat quality and eating quality assessment, the studies which have been completed demonstrate that there is potential to develop Raman spectroscopy into a carcass assessment tool which can be used in commercial situations. Predictions of meat quality traits including pHu, purge, SF, cooking loss, FA composition, and sensory traits including tenderness, juiciness, and flavor have been successfully completed in a number of species including pork, beef, and lamb. Furthermore, additional research has been able to determine microbiological loads and differentiate between species to identify muscle food adulteration.

Downloaded From Vet-eBooks.com

Before Raman spectroscopy can be adopted for widespread industry use, further research is required to determine the optimal measurement and analysis parameters methods, the factors which are contributing to differences between predictive models of species and traits and further identify the biochemical and biophysical characteristics of meat which are contributing to these predictions. However, as technological advancements continue, Raman spectroscopy equipment will continue to become more robust, providing repeatable measurements, and limitations associated with high fluorescence will be overcome making Raman spectroscopy as an ideal tool for carcass quality assessment.

References

Abbas, O., Fernández Pierna, J. A., Codony, R., Von Holst, C. and Baeten, V. (2009). Assessment of the discrimination of animal fat by FT-Raman spectroscopy. *Journal of Molecular Structure,* **924–962**, 294–300.

Adzitey, F. and Nurul, H. (2011). Pale soft exudative (PSE) and dark firm dry (DFD) meats: Causes and measures to reduce these incidences—A mini review. *International Food Research,* **18**, 11–20.

Afseth, N. K., Wold, J. P. and Segtnan, V. (2006). The potential of Raman spectroscopy for characterisation of the fatty acid unsaturation of salmon. *Analytica Chimica Acta,* **572**, 85–92.

Al Ebrahim, H., Sowoidnich, K. and Kronfeldt., H.-D. (2013a). Raman spectroscopic differentiation of beef and horse meat using a 671 nm microsystem diode laser. *Applied Physics B,* **113**, 159–163.

Al Ebrahim, H., Sowoidnich, K., Schmidt, H. and Kronfeldt, H.-D. (2013b). Polarization dependence of the Raman scattering of oriented porcine muscle fibers affected by storage time and spoilage. *Focusing on Modern Food Industry,* **2**, 1–9.

Austin, J. C., Jordan, T. and Spiro, T. G. (1993). Ultra violet reasonance Raman studies of proteins and related molecules. *In:* Clark, R. J. H. and Hester, R. E. (eds.) *Biomolecular Spectroscopy-Part A*. West Sussex: John Wiley and Sons.

Bauer, A., Scheier, R., Eberle, T. and Schmidt, H. (2016). Assessment of tenderness of aged bovine gluteus medius muscles using Raman spectroscopy. *Meat Science,* **115**, 27–33.

Beattie, J. R. (2011). Optimising reproducibility in low quality signals without smoothing: An alternative paradigm for signal processing. *Journal of Raman Spectroscopy,* **42**, 1419–1427.

Beattie, J. R., Bell, S. E. J., Borgaard, C., Fearon, A. M. and Moss, B. W. (2006). Prediction of adipose tissue composition using Raman spectroscopy: Average properties and individual fatty acids. *Lipids,* **41**, 287–294.

Beattie, J. R., Bell, S. E. J., Borggaard, C., Fearon, A. M. and Moss, B. W. (2007). Classification of adipose tissue species using Raman spectroscopy. *Lipids,* **42**, 679–685.

Beattie, J. R., Bell, S. E. J., Borggaard, C. and Moss, B. W. (2008). Preliminary investigations on the effects of ageing and cooking on the Raman spectra of porcine longissimus dorsi. *Meat Science,* **80**, 1205–1211.

Downloaded From Vet-eBooks.com

Beattie, J. R., Bell, S. E. J., Farmer, L. J., Moss, B. W. and Patterson, D. (2004a). Preliminary investigation of the application of Raman spectroscopy to the prediction of the sensory quality of beef silverside. *Meat Science,* **66**, 903–913.

Beattie, J. R., Bell, S. E. J. and Moss, B. W. (2004b). A critical evaluation of Raman spectroscopy for the analysis of lipids: Fatty acid methyl esters. *Lipids,* **39**, 407–419.

Berhe, D. T., Engelsen, S. B., Hviid, M. S. and Lametsch, R. (2014). Raman spectroscopic study of effect of the cooking temperature and time on meat proteins. *Food Research International,* **66**, 123–131.

Berhe, D. T., Eskildsen, C. E., Lametsch, R., Hviid, M. S., Van Den Berg, F. and Engelsen, S. B. (2016). Prediction of total fatty acid parameters and individual fatty acids in pork backfat using Raman spectroscopy and chemometrics: Understanding the cage of covariance between highly correlated fat parameters. *Meat Science,* **111**, 18–26.

Berhe, D. T., Lawaetz, A. J., Engelsen, S. B., Hviid, M. S. and Lametsch, R. (2015). Accurate determination of endpoint temperature of cooked meat after storage by Raman spectroscopy and chemometrics. *Food Control,* **52**, 119–125.

Bonnier, F. and Byrne, H. J. (2012). Understanding the molecular information contained in principal component analysis of vibrational spectra of biological systems. *Analyst,* **137**, 322–332.

Borch, E., Kant-Muermans, M. and Blixt, Y. (1996). Specific spoilage organisms bacterial spoilage of meat and cured meat products. *International Journal of Food Microbiology,* **33**, 103–120.

Boyaci, I. H., Temiz, H. T., Uysal, R. S., Velioglu, H. M., Yadegari, R. J. and Rishkan, M. M. (2014a). A novel method for discrimination of beef and horsemeat using Raman spectroscopy. *Food Chemistry,* **148**, 37–41.

Boyaci, İ. H., Uysal, R. S., Temiz, T. et al. (2014b). A rapid method for determination of the origin of meat and meat products based on the extracted fat spectra by using of Raman spectroscopy and chemometric method. *European Food Research and Technology,* **238**, 845–852.

Breitman, M., Ruiz-Moreno, S. and Gil, A. L. (2007). Experimental problems in Raman spectroscopy applied to pigment identification in mixtures. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy,* **68**, 1114–1119.

Brøndum, J., Byrne, D. V., Bak, L. S., Bertelsen, G. and Engelsen, S. B. (2000a). Warmed-over flavour in porcine meat—A combined spectroscopic, sensory and chemometric study. *Meat Science,* **54**, 83–95.

Brøndum, J., Munck, L., Henckel, P., Karlsson, A., Tornberg, E. and Engelsen, S. B. (2000b). Prediction of water-holding capacity and composition of porcine meat by comparative spectroscopy. *Meat Science,* **55**, 177–185.

Carew, E. B., Asher, I. M. and Stanley, H. E. (1975). Laser raman spectroscopy—New probe of myosin substructure. *Science,* **188**, 933–936.

Carey, P. R. (1982). *Biochemical Applications of Raman and Reasonance Raman Spectroscopies*. New York, NY: Academic Press.

Chen, H. and Han, M. (2011). Raman spectroscopic study of the effects of microbial transglutaminase on heat-induced gelation of pork myofibrillar proteins and its relationship with textural characteristics. *Food Research International,* **44**, 1514–1520.

Da Silva, E., Bresson, S. and Rousseau, D. (2009). Characterization of the three major polymorphic forms and liquid state of tristearin by Raman spectroscopy. *Chemistry and Physics of Lipids,* **157**, 113–119.

Downloaded From Vet-eBooks.com

Damez, J.-L. and Clerjon, S. (2008). Meat quality assessment using biophysical methods related to meat structure. *Meat Science,* **80**, 132–149.

Das, R. S. and Agrawal, Y. K. (2011). Raman spectroscopy: Recent advancements, techniques and applications. *Vibrational Spectroscopy,* **57**, 163–176.

Ellis, D. I., Broadhurst, D., Clarke, S. J. and Goodacre, R. (2005). Rapid identification of closely related muscle foods by vibrational spectroscopy and machine learning. *Analyst,* **130**, 1648–1654.

Ellis, D. I. and Goodacre, R. (2001). Rapid and quantitative detection of the microbial spoilage of muscle foods: Current status and future trends. *Trends in Food Science and Technology,* **12**, 414–424.EU. (2010). *European Declaration on alternatives to surgical castration of pigs.* Available at: https://ec.europa.eu/food/animals/welfare/practice/farm/pigs/castration_alternatives_en

England, E. M., Scheffler, T. L., Kasten, S. C., Matarneh, S. K. and Gerrard, D. E. (2013). Exploring the unknowns involved in the transformation of muscle to meat. *Meat Science,* **95**, 837–843.

Food and Agriculture Organization of the United Nations (FAO). (2014). Sources of meat. Available at http://www.fao.org/ag/againfo/themes/en/meat/backgr_sources.html (accessed 8 June 2016).

Ferguson, D. M., Daly, B. L., Gardner, G. E. and Tume, R. K. (2008). Effect of glycogen concentration and form on the response to electrical stimulation and rate of post-mortem glycolysis in ovine muscle. *Meat Science,* **78**, 202–210.

Fowler, S. M., Ponnampalam, E. N., Schmidt, H., Wynn, P. and Hopkins, D. L. (2015a). Prediction of intramuscular fat content and major fatty acid groups of lamb M. longissimus lumborum using Raman spectroscopy. *Meat Science,* **110**, 70–75.

Fowler, S. M., Schmidt, H., Van De Ven, R., Wynn, P. and Hopkins, D. L. (2014a). Predicting tenderness of fresh ovine semimembranosus using Raman spectroscopy. *Meat Science,* **97**, 597–601.

Fowler, S. M., Schmidt, H., Van De Ven, R., Wynn, P. and Hopkins, D. L. (2014b). Raman spectroscopy compared against traditional predictors of shear force in lamb m. longissimus lumborum. *Meat Science,* **98**, 652–656.

Fowler, S. M., Schmidt, H., Van De Ven, R., Wynn, P. and Hopkins, D. L. (2015b). Predicting meat quality traits of ovine m. semimembranosus, both fresh and following freezing and thawing, using a hand held Raman spectroscopic device. *Meat Science,* **108**, 138–144.

Frushour, B. G. and Koenig, J. L. (1975). Raman spectroscopy of proteins. *In:* Clark, R. J. H. and Hester, R. E. (eds.) *Advances in Infrared and Raman Spectroscopy.* London: Wiley & Sons.

Grimmler, C. and Schmidt, H. (2015). Quantifying bacteria on pork using a portable Raman scanner. 61st International Congress of Meat Science and Technology (ICoMST). Clermont-Ferrand, France.

Hassing, S. and Jernshøj, K. D. (2014). Benefits and challenges in applying Raman spectroscopy as a non-destructive tool in the analysis of food quality. *Agro Food Industry Hi Tech,* **25**, 63–69.

Hassing, S., Jernshøj, K. D. and Christensen, L. S. (2012). Raman spectroscopy: A non-destructive and on-site tool for control of food quality? InTech.

Honikel, K. O. and Fischer, C. (1977). A rapid method for the dectection of PSE and DFD porcine muscles. *Journal of Food Science,* **42**, 1633–1636.

Downloaded From Vet-eBooks.com

Hudson, B. and Mayne, L. (1986). Ultraviolet resonance Raman spectroscopy of biopolymers. *In:* Hirs, C. H. W. (ed.) *Methods in Enzymology.* New York, NY: Academic Press.

Hughes, J. M., Oiseth, S. K., Purslow, P. P. and Warner, R. D. (2014). A structural approach to understanding the interactions between colour, water-holding capacity and tenderness. *Meat Science,* **98**, 520–532.

Krimm, S. (1987). Peptides and proteins. *In:* Spiro, T. G. (ed.) *Biological Applications Raman Spectroscopy.* New York, NY: John Wiley and Sons.

Krimm, S. and Bandekar, J. (1986). Vibrational spectroscopy and conformation of peptides, polypeptides, and proteins. *In:* Anfinsen, C. B., Edsall, J. T. and Richards, F. M. (eds.) *Advances in Protein Chemistry.* New York, NY: Academic Press.

Lee, P. C. and Meisel, D. (1982). Adsorption and surface-enhanced Raman of dyes on silver and gold sols. *Journal of Physical Chemistry,* **86**, 3391–3395.

Li-Chan, E. C. Y. (1996). The applications of Raman spectroscopy in food science. *Trends in Food Science and Technology,* **7**, 361–370.

Li-Chan, E. C. Y., Nakai, S. and Hirotsuka, M. (1994). Raman spectroscopy as a probe of protein structure in food systems. *In:* Yada, R. Y., Jackman, R. L. and Smith, J. L. (eds.) *Protein Structure-Function Relationships in Food.* Glasgow: Blackie Academic and Professional.

Liu, X., Schmidt, H. and Mörlein, D. (2016). Feasibility of boar taint classification using a portable Raman device. *Meat Science,* **116**, 133–139.

Lyndgaard, L. B., Sorensen, K. M., Van Den Berg, F. and Engelsen, S. B. (2011). Depth profiling of porcine adipose tissue by Raman spectroscopy. *Journal of Raman Spectroscopy,* **43**, 482–489.

Lyndgaard, L. B., Sorensen, K. M., Van Den Berg, F. and Engelsen, S. B. (2012). Depth profiling of porcine adipose tissue by Raman spectroscopy. *Journal of Raman Spectroscopy,* **43**, 482–489.

McCreery, R. L. (2000). Raman spectroscopy for chemical analysis. *In:* Winefordner, J. D. (ed.) *A Series of Monographs of Analytical Chemistry and It's Applications.* New York, NY: Wiley-Interscience John Wiley & Sons.

McCreery, R. L. (2005). *Raman Spectroscopy for Chemical Analysis*. 1st ed. Hoboken, NJ: Wiley-Interscience.

Motoyama, M., Ando, M., Sasaki, K. and Hamaguchi, H.-O. (2010). Differentiation of animal fats from different origins: Use of polymorphic features detected by Raman spectroscopy. *Applied Spectroscopy,* **64**, 1244–1250.

Motoyama, M., Ando, M., Sasaki, K. et al. (2016). Simultaneous imaging of fat crystallinity and crystal polymorphic types by Raman microspectroscopy. *Food Chemistry,* **196**, 411–417.

Motoyama, M., Chikuni, K., Narita, T., Aikawa, K. and Sasaki, K. (2013). In situ Raman spectrometric analysis of crystallinity and crystal polymorphism of fat in porcine adipose tissue. *Journal of Agriculture and Food Chemistry,* **61**, 69–75.

Nache, M., Scheier, R., Schmidt, H. and Hitzmann, B. (2015). Non-invasive lactate- and pH-monitoring in porcine meat using Raman spectroscopy and chemometrics. *Chemometrics and Intelligent Laboratory Systems,* **142**, 197–205.

Olsen, E. F., Baustad, C., Egelandsdal, B., Rukke, E.-O. and Isaksson, T. (2010). Long-term stability of a Raman instrument determining iodine value in pork adipose tissue. *Meat Science,* **85**, 1–6.

Downloaded From Vet-eBooks.com

Olsen, E. F., Rukke, E.-O., Egelandsdal, B. and Isaksson, T. (2008). Determination of omega-6 and omega-3 fatty acids in pork adipose tissue with nondestructive Raman and fourier transform infrared spectroscopy. *Applied Spectroscopy,* **62**, 968–974.

Olsen, E. F., Rukke, E.-O., Flåtten, A. and Isaksson, T. (2007). Quantitative determination of saturated-, monounsaturated- and polyunsaturated fatty acids in pork adipose tissue with non-destructive Raman spectroscopy. *Meat Science,* **76**, 628–634.

Ozaki, Y. (1999). Raman spectroscopy. *In:* Mossoba, M. M. (ed.) *Spectral Methods in Food Analysis—Instrumentation and Applications.* New York, NY: Marcel Dekker.

Pedersen, D. K., Morel, S., Andersen, H. J. and Balling Engelsen, S. (2003). Early prediction of water-holding capacity in meat by multivariate vibrational spectroscopy. *Meat Science,* **65**, 581–592.

Pezolet, M., Pigeon-Gosselin, M. and Caille, J.-P. (1978). Laser Raman investigation of intact single muscle fibers: Protein conformations. *Biochimica et Biophysica Acta (BBA)—Protein Structure,* **533**, 263–269.

Robergs, R. A., Ghiasvand, F. and Parker, D. (2004). Biochemistry of exercise-induced metabolic acidosis. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology,* **287**, R502–R516.

Saucier, L. (2016). Microbial spoilage, quality and safety within the context of meat sustainability. *Meat Science,* **120**, 78–84.

Scheffler, T. L. and Gerrard, D. E. (2007). Mechanisms controlling pork quality development: The biochemistry controlling postmortem energy metabolism. *Meat Science,* **77**, 7–16.

Scheier, R., Bauer, A. and Schmidt, H. (2014a). Early postmortem prediction of meat quality traits of porcine semimembranosus muscles using a portable Raman system. *Food and Bioprocess Technology,* **7**, 2732–2741.

Scheier, R., Köhler, J. and Schmidt, H. (2014b). Identification of the early post mortem metabolic state of porcine m. semimembranosus using Raman spectroscopy. *Vibrational Spectroscopy,* **70**, 12–17.

Scheier, R., Scheeder, M. and Schmidt, H. (2015a). Post-rigor assessment of porcine meat quality applying a portable Raman system. In 61st International Congress of Meat Science and Technology (ICoMST). Clermont-Ferrand, France.

Scheier, R., Scheeder, M. and Schmidt, H. (2015b). Prediction of pork quality at the slaughter line using a portable Raman device. *Meat Science,* **103**, 96–103.

Scheier, R. and Schmidt, H. (2013). Measurement of the pH value in pork meat early postmortem by Raman spectroscopy. *Applied Physics B,* **111**, 289–297.

Schmidt, H., Blum, J., Sowoidnich, K. and Kronfeldt, H. D. (2008). Raman spectroscopic examinations of meat ripening: An approach to product-flanking meat monitoring using non-invasive optical measuring methods. *Fleischwirtschaft,* **88**, 111–113.

Schmidt, H., Scheier, R. and Hopkins, D. L. (2013). Preliminary investigation on the relationship of Raman spectra of sheep meat with shear force and cooking loss. *Meat Science,* **93**, 138–143.

Schmidt, H., Scheier, R., Octoviani, A., Agarkov, N. and Petzet, A. (2012). Recent results of measuring quality traits of pork meat using Raman spectroscopy. *Mitteilungsblatt der Fleischforschung Kulmbach,* **51**, 219–225.

Downloaded From Vet-eBooks.com

Schmidt, H., Sowoidnich, K. and Kronfeldt, H. D. (2010). A prototype hand-held Raman sensor for the in situ characterization of meat quality. *Applied Spectroscopy,* **64**, 888–894.

Schmidt, H., Sowoidnich, K., Maiwald, M., Sumpf, B. and Kronfeldt, H. D. (2009). Hand-held Raman sensor head for *in-situ* characterization of meat quality applying a mircosystem 671 nm diode laser. *In:* Vo-dinh, T., Lieberman, R. A. and Gauglitz, G. (eds.) *Proceedings of the Advanved Environment, Chemical and Biological Sensing Technology VI.* Orlando, FL: International Society for Optics and Photonics, 1–8.

Sorensen, K. M., Westley, C., Goodacre, R. and Engelsen, S. B. (2015). Simultaneous quantification of the boar-taint compounds skatole and androstenone by surface-enhanced Raman scattering (SERS) and multivariate data analysis. *Analytical and Bioanalytical Chemistry,* **407**, 7787–7795.

Sowoidnich, K. (2012). Multispektrale, Diodenlaser-basierte Raman-Untersuchungen zur In-situ-Analytik ausgewählter Fleischsorten. PhD dissertation, Technische Universität, Berlin.

Sowoidnich, K. and Kronfeldt, H.-D. (2012). Shifted excitation Raman difference spectroscopy at multiple wavelengths for in-situ meat species differentiation. *Applied Physics B,* **108**, 975–982.

Sowoidnich, K., Schmidt, H., Kronfeldt, H.-D. and Schwägele, F. (2012). A portable 671 nm Raman sensor system for rapid meat spoilage identification. *Vibrational Spectroscopy,* **62**, 70–76.

Sowoidnich, K., Schmidt, H., Maiwald, M., Sumpf, B. and Kronfeldt, H.-D. (2010). Application of diode-laser Raman spectroscopy for in situ investigation of meat spoilage. *Food and Bioprocess Technology,* **3**, 878–882.

Thompson, J. M. (2002). Managing meat tenderness. *Meat Science,* **62**, 295–308.

Tornberg, E. (2005). Effects of heat on meat proteins—Implications on structure and quality of meat products. *Meat Science,* **70**, 493–508.

Wang, Q. 2013. Raman spectroscopic characterization and analysis of agricultural and biological systems. PhD dissertation, Iowa State University, Ames, Iowa.

Wang, Q., Lonergan, S. M. and Yu, C. (2012). Rapid determination of pork sensory quality using Raman spectroscopy. *Meat Science,* **5**, 4069–4085.

Xiong, Y. L., Brekke, C. J. and Leung, H. K. (1987). Thermal denaturation of muscle proteins from different species and muscle types as studied by differential scanning calorimetry. *Canadian Institute of Food Science and Technology Journal,* **20**, 357–362.

Zając, A., Hanuza, J. and Dymińska, L. (2014). Raman spectroscopy in determination of horse meat content in the mixture with other meats. *Food Chemistry,* **156**, 333–338.

Zhao, M., Downey, G. and O'Donnell, C. P. (2015). Dispersive Raman spectroscopy and multivariate data analysis to detect offal adulteration of thawed beefburgers. *Journal of Agricultural and Food Chemistry,* **63**, 1433–1441.

Downloaded From Vet-eBooks.com

4

Real-Time PCR for the Detection of Pathogens in Meat and Meat Products

Alicia Rodríguez, María J. Andrade, Mar Rodríguez, and Juan J. Córdoba

CONTENTS

4.1 Principles and Characteristics of Real-Time Polymerase Chain Reaction for Detecting and Quantifying Pathogens in Meat and Meat Products 114
4.1.1 Sample Preparation 114
4.1.2 Main Considerations for qPCR 116
4.1.3 Targets 117
4.1.4 Controls of the qPCR 118
4.1.5 Validation 118
4.2 qPCR for Detecting Bacterial Pathogens in Meat and Meat Products 119
4.2.1 qPCR for Detecting Bacteria Foodborne Infection 119
4.2.1.1 *Salmonella* 119
4.2.1.2 *Listeria monocytogenes* 122
4.2.1.3 Verotoxigenic *E. coli* 124
4.2.1.4 *Campylobacter* 127
4.2.1.5 *Yersinia* 127
4.2.2 qPCR for Detecting Bacteria Foodborne Intoxications 129
4.2.2.1 *Staphylococcus aureus* 129
4.2.2.2 *Clostridium botulinum* 131
4.3 qPCR for Detecting Toxigenic Molds 132
4.4 Reverse Transcription Quantitative PCR as a Tool for Evaluating Gene Expression of Foodborne Pathogens in Meat and Meat Products 134
4.4.1 *Salmonella* 135
4.4.2 *Listeria monocytogenes* 139
4.4.3 Shiga Toxin-Producing *E. coli* 140
4.4.4 *Campylobacter jejuni* 140
4.4.5 *Staphylococcus aureus* 141
4.4.6 Viruses 141
4.4.7 Ochratoxin A-Producing Penicillia 142
4.5 Conclusions 142
Acknowledgments 143
References 143

Downloaded From Vet-eBooks.com

4.1 Principles and Characteristics of Real-Time Polymerase Chain Reaction for Detecting and Quantifying Pathogens in Meat and Meat Products

In the meat industry, there is a necessity of having available rapid microbiological methods to ensure the safety of products. For such purposes, a wide range of protocols for detecting and/or quantifying pathogenic microorganisms has been developed. Despite the fact that the results yielded by classical microbiological techniques based on the enrichment, isolation, and/or enumeration using selective culture media and the subsequent confirmation by biochemical and/or serological tests are generally adequate, they are laborious and time consuming. In recent years, many molecular techniques have been optimized for the detection of pathogens in meat and meat products. The most valuable molecular methods rely on the polymerase chain reaction (PCR). They have demonstrated to be faster than the traditional techniques and allow a more specific and sensitive detection of pathogenic microorganisms. Within such methods, the real-time PCR (quantitative PCR [qPCR]) ones are the most valuable since they allow quantifying the level of pathogens apart from being more sensitive and less prone to cross-contamination risks than conventional PCR and have potential for automation (Hernández et al., 2009). Besides, qPCR procedures developed for detecting and quantifying pathogenic microorganisms in meat and meat products requires less time for the analyses, less than 24 h in some cases, while this time is much higher (to seven days or even more depending on the pathogen) when using traditional methods. In addition, qPCR methods are able to detect and quantify different pathogens in the same reaction. Thus, the current trend is to develop multiplex qPCR methods to detect several pathogenic microorganisms. They are also very useful for detecting closely related species that display genetic variations requiring the use of different sets of primers for reliable detection (Postollec et al., 2011).

This chapter focuses on current qPCR methods that are available for detecting and quantifying microbial pathogens of interest in meat and meat products.

4.1.1 Sample Preparation

DNA isolation is the first critical step of qPCR procedures to detect and/or quantify pathogenic microorganisms in meat and meat products. Due to the fact that the DNA to be amplified is directly isolated from the meat matrix, its components can inhibit the subsequent amplification, which can cause false negative results or an underestimation of pathogen levels. Consequently, the elimination of such inhibitory substances from DNA is a paramount prerequisite for the success of the qPCR procedure. Thus, qPCR protocols have to be adapted for each meat or meat product type since each one has a different nature mainly based on their composition. Different methods of sample preparation to eliminate PCR inhibitors from meat matrices have been reported included filtration, centrifugation,

Downloaded From Vet-eBooks.com

the use of detergents and organic solvents, enzyme treatment, dilution of the sample, the use of PCR components, flotation, and immunomagnetic separation (IMS) (Botteldoorn et al., 2008; Gordillo et al., 2014; Melero et al., 2011; Rodríguez et al., 2012b; Wolffs et al., 2005). Several commercial DNA extraction kits as well as in-house developed protocols are currently available to obtain DNA with high purity and quantitative yields. For instance, Gattuso et al. (2014) used a DNA extraction protocol based on a washing step with phosphate saline buffer (PBS) combined with a Chelex resin for detecting *Listeria monocytogenes* in pork meat. For the same pathogenic bacterium, Rodríguez-Lázaro et al. (2014) compared simple boiling of the culture after washing the bacterial pellet with PBS, the use of a Chelex resin, and the QIAamp DNA Mini kit (QIAGEN), which contains a silica column, obtaining the best results with the commercial kit. In general, many commercial kits have proved to be adequate for a pathogen's DNA extraction in meat and meat products.

After isolating the DNA, its quantity and quality should be checked. Measurement of absorbance is the most commonly used method for such purposes. For determining DNA purity, the OD_{260}/OD_{280} ratio should be calculated. When this ratio is around 1.8, it indicates a good purity of the obtained DNA. The concentration of DNA can also be quickly estimated by agarose gel electrophoresis (Thermo Scientific. 2016).

Due to the fact that one of the limitations of qPCR methods consists of their high limit of quantification considering the microbiological criteria required by international legislations, nowadays their application for quantifying pathogens in meat and meat products generally requires a sample enrichment of a few hours prior to further preparation and thus yields appropriate sensitivity. Besides it has to be kept in mind that in most cases low levels of pathogenic bacteria are able to cause disease. Apart from allowing the concentration of the target microorganism, such an enrichment step diminishes the probability of detecting dead microorganisms and dilutes the inhibitory food matrix components. However, it should be considered that the disadvantage of using an initial enrichment step is the impossibility to quantify the initial loads of pathogenic microorganisms (Postollec et al., 2011). Regarding the enrichment medium, both nonselective and selective media have been proposed. For example Gattuso et al. (2014) and Rodríguez-Lázaro et al. (2014) successfully used the selective Half Fraser Broth for enrichment of pork and poultry meat for *L. monocytogenes*. For *Campylobacter* spp., Bolton broth with selective supplements has been normally used. However, the presence of blood in this medium could be problematic because of its PCR inhibitors. The omission of such a component in Bolton enrichment broth has not been reported to have a negative influence in *Campylobacter* recovery by qPCR in poultry samples (Melero et al., 2011). Rodríguez et al. (2016) used Mannitol Salt broth (MSB) for selective enrichment of *Staphylococcus aureus* from different kinds of meat products. On the contrary, the nonselective tryptone soy broth (TSB) modified by the addition of yeast extract has been used for detecting pathogenic *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* (Thisted Lambertz et al., 2008a,b)

Downloaded From Vet-eBooks.com

Another important limitation of qPCR consists of the lack of differentiation between viable and dead cells. To circumvent this disadvantage, propidium monoazide (PMA) coupled with qPCR has been used in meat and meat products (Josefsen et al., 2010). Such DNA intercalating dye can only penetrate membranes of damaged cells, thus preventing the following PCR reaction (Barbau-Piednoir et al., 2014). However, this PMA treatment should not replace the initial enrichment for a rapid and reliable foodborne bacteria detection (Barbau-Piednoir et al., 2014). Controversial results have been obtained when performing treatment of samples with PMA. Therefore, Josefsen et al. (2010) obtained a good correlation between *Campylobacter* counts obtained by qPCR with PMA sample treatment and counts from culture-based enumeration in chicken carcass rinse and Pacholewicz et al. (2013) did not obtain concordant results when testing the same protocol in broiler chicken carcass rinse samples. On the other hand, Wolffs et al. (2005) developed a flotation method to be used prior to qPCR capable of selecting viable and viable nonculturable *Campylobacter* and *Y. enterocolitica* cells for being subsequently quantified by qPCR.

4.1.2 Main Considerations for qPCR

In qPCR methods, the accumulation of amplification products is continuously monitored through the reaction by using fluorescent reporters without the necessity of post-PCR handling of the obtained products. The change in fluorescence during the reaction is measured by an instrument that combines thermal cycling with fluorescent dye scanning capability. The fluorescence intensity during qPCR is proportional to the amount of amplification products (Law et al., 2015). By plotting fluorescence against the number of cycles, the qPCR instrument generates an amplification curve that represents the accumulation of PCR product over the duration of the entire reaction (Invitrogen, 2008). The quantification cycle (Cq), also known as threshold cycle (Ct), is determined from the amplification plot and used to calculate the initial concentration of the target DNA because this value is inversely related to the amount of starting template (Invitrogen, 2008). Cq is the cycle number at which the fluorescent signal of the reaction crosses the threshold (Invitrogen, 2008).

Many fluorescence chemistries are available including nonspecific dyes, such as SYBR® Green, and sequence-specific primers or probes coupled to fluorescent dyes, including hydrolysis probes, fluorescence resonance energy transfer (FRET) probes, molecular beacons, and scorpions primers. SYBR Green chemistry and TaqMan® hydrolysis probes are the most commonly used technologies for detecting and quantifying pathogenic microorganism in meat and meat products by qPCR. As SYBR Green binding is not specific for a target DNA sequence this system can be readily used for different gene assays, it is inexpensive and flexible (Postollec et al., 2011). Regarding the TaqMan chemistry, this is more expensive than DNA-binding dye assays, but the presence of the hydrolysis probe ensures that only specific amplification products are measured (Postollec et al., 2011). Furthermore, the last technology

Downloaded From Vet-eBooks.com

allows the development of multiplex PCR reactions, although an important optimization procedure is needed (Postollec et al., 2011). When using SYBR Green dyes melting curve analysis should be performed to determine the specificity of qPCR procedure and assure accurate results. This is normally generated by the qPCR instrument and allows the calculation of the *Tm* value of the amplification products.

To estimate the initial DNA concentration and consequently the unknown target pathogen load in the analyzed meat or meat product, a linear standard curve based on tenfold serial dilutions (know concentrations) of the DNA should be set up. At least four but preferably six or more points should be included in the curve (Kavanagh et al., 2011). It has to be kept in mind that the concentrations of DNA chosen for the standard curve should cover the expected concentration range of the target DNA (Invitrogen, 2008). For constructing the standard curve, the Cq values of diluted reference material are plotted against the logarithm of the samples' concentrations, number of template copies, or dilution factor (Raymaekers et al., 2009). When the Cq values of the analyzed meat or meat product samples are determined, they are correlated to a certain concentration by placement on the standard curve. It is recommended to use the same matrix as the one containing samples to be tested and the same target DNA sequence for constructing the standard curve as well as to include it on each experiment (Postollec et al., 2011).

Standard curves also allow for calculating the efficiency of the amplification reaction by using their slope (S) in the formula $E = [10^{(-1/S)}]-1$ (Bustin et al., 2009). The acceptable range is generally between 90% and 110% (Raymaekers et al., 2009). Furthermore, the coefficient of correlation (R^2) derived from the standard curve must be between 0.99 and 0.999 (Raymaekers et al., 2009). Despite the fact that it is possible to automatically elaborate standard curves and calculate the efficiency using the software of the qPCR instruments, it can be manually determined.

4.1.3 Targets

The basis of the qPCR methods is the detection of a characteristic and specific DNA sequence in the DNA of the pathogen. Thus the design or selection of the primers and probes is a key factor for the correct detection of a pathogenic microorganism (Chapela et al., 2015). Although a wide variety of DNA targets have been selected for developing qPCR methods to detect microbial pathogens in meat and meat products, multicopy ribosomal RNA (rRNA) genes and those encoding virulence microbial factors have been the most commonly employed. rRNA genes have been commonly used because they span both variable and highly conserved sequences, are easily available from public databases for many species, and often result in sensitive detection due to their multicopy nature (Chapela et al., 2015; Postollec et al., 2011). Housekeeping genes involved in essential cellular processes have also been considered adequate for detecting pathogens (Bonjoch et al., 2010).

Downloaded From Vet-eBooks.com

4.1.4 Controls of the qPCR

Owing to the fact that the qPCR is an instrumental technique, false negative and false positive results can be produced (Rodríguez-Lázaro and Hernández, 2013). Consequently, proper controls must be carried out for monitoring the critical steps and assuring the validity of the results. Controls necessary for assessing the analytical performance of PCR methods have been summarized (Hernández et al., 2009). Process or amplification controls can be used to check the entire method or only the PCR, respectively. Positive and negative PCR controls and internal amplification control (IAC) are highly recommended. Positive PCR control consists of a negative sample spiked with target DNA (Hernández et al., 2009; Postollec et al., 2011). Negative or nontemplate PCR control does not contain the target DNA and water is used instead of it (Hernández et al., 2009; Postollec et al., 2011). IAC consists of a nontarget DNA sequence present in the same sample reaction tube which is coamplified simultaneously with the target sequence, but with an amplicon size visually distinguishable from the target sequence (Hoorfar et al., 2004a,b). In a qPCR with an IAC, a control signal will be always produced when there is no target sequence present. When neither IAC nor target signal is produced, it indicates that the amplification reaction has been inhibited due to malfunction of the thermal cycler, incorrect PCR mixture, poor polymerase activity, and/or the presence of inhibitors in the sample matrix (Hoorfar et al., 2004a,b). Postollec et al. (2011) have proposed a Cq shift ≥1 between qPCR performed with the IAC alone and with the IAC and the sample as cut-off inhibition value.

The use of the abovementioned analytical controls also assures the accurate quantification of the target pathogen. Additionally, to avoid false positive results due to sample contamination, precautionary measurements are required in the laboratory (Rodríguez-Lázaro and Hernández, 2013).

The most used controls for detecting pathogens in meat and meat products are IAC (Bonjoch et al., 2010; Josefsen et al., 2010; Thisted Lambertz et al., 2008a,b). The controls are processed throughout the whole protocol in parallel with samples to be analyzed (Postollec et al., 2011).

4.1.5 Validation

Despite the fact that many qPCR protocols have been reported for detecting and quantifying different pathogenic microorganisms in meat and meat products, they have not normally been implemented in the meat industry for routine analysis. This could be due to the lack of validation and/or standardization.

Validation of qPCR methods is necessary to ensure that their performance is equal or better when comparing with the conventional culture-based reference methods (Ceuppens et al., 2014). Validation of qPCR methods assures that the target pathogens are detected without false negative or positive results.

During the validation process, the performance characteristics and limitations of a method are established. Validation also allow identification of the influences that may change these characteristics (Jones and Marengo, 2016).

Downloaded From Vet-eBooks.com

Raymaekers et al. (2009) proposed practical guidelines for the validation of qPCR procedures. Apart from studying the amplification efficiency and using appropriate controls as previously mentioned, checking of several parameters such as precision, linearity, trueness, limit of detection, and specificity, among others, was recommended when validating qPCR methods before their application for routine analysis (Raymaekers et al., 2009). Additionally, the use of the specific meat matrix to be checked is vital for detecting potential inhibitors and determining the competition of the target pathogen in the presence of the natural microbial population (Gorski and Csordas, 2010).

There are some commercial qPCR methods that have been validated by official independent organizations, such as the Association of Official Analytical Chemists (AOAC), for the detection of foodborne bacterial pathogens including shiga toxin-producing *Escherichia coli, S. aureus, L. monocytogenes, Salmonella*, and *Campylobacter.*

On the other hand, the International Standardization Organization (ISO) has provided guidelines to detect foodborne pathogens by qPCR. Concretely, ISO 22119:2011 (Anonymous, 2011) is focused on the general requirements for food testing by means of qPCR.

4.2 qPCR for Detecting Bacterial Pathogens in Meat and Meat Products

Microorganisms that are involved in foodborne illness include *Salmonella* spp., *L. monocytogenes*, *Campylobacter* spp., and *E. coli* O157:H7 are known to be responsible for the majority of foodborne infections outbreaks (Velusamy et al., 2010). A TaqMan qPCR procedure to simultaneously detect 20 foodborne pathogens, including all of the abovementioned pathogenic bacteria, in complex food matrices such as meat has been reported (Cremonesi et al., 2014). Although no meat matrix interferences were observed, the sensitivity of this method in artificially contaminated meat matrices ranged between 10^8 and 10^4 cfu/g. Thus, this qPCR has the potential to be a powerful tool for a high-throughput screening for multiple pathogens, enabling simultaneous processing of several samples and reducing the total time required for analysis. However, for a more sensitive quantification specific qPCR should be conducted to analyze each of the most common and important pathogenic bacteria. In this section, qPCR methods to detect bacteria involved in foodborne infections and foodborne intoxications in meat and meat products will be separately analyzed.

4.2.1 qPCR for Detecting Bacteria Foodborne Infection

4.2.1.1 Salmonella

The European Commission through regulation No. 2073/2005 (European Commission. 2005) established EN/ISO 6579 (Anonymous, 2002) as the

Downloaded From Vet-eBooks.com

official analytical reference method for detection of *Salmonella* in foods. This procedure is a culture-based method that includes pre-enrichment and selective enrichment steps and subsequent plating in selective media and final confirmatory tests. This takes four to five days of analysis. The scientific community, in response to the demands of the European Commission and the industrial sector, aimed to identify novel molecular-based methods of high accuracy, specificity, sensitivity, and reduced time of analysis for *Salmonella* detection (Pasquali et al., 2014). Thus, several qPCR methods based on the target sequences the virulence *hil*A, *inv*A, *Sal*, and *ttrRSBCA* genes have been developed for rapid and sensitive detection of *Salmonella* in meat carcasses (Krämer et al., 2011; Löfström et al., 2009; McCabe et al., 2011; Zheng et al., 2014) and pork and meat products (Delibato et al., 2013; Pasquali et al., 2014).

Most of the developed PCR methods include an enrichment step of 7–24 h at 37°C (Figure 4.1) to increase the number of the target *Salmonella* at detectable levels (Martin et al., 2012; Zheng et al., 2014). Although most of the reported

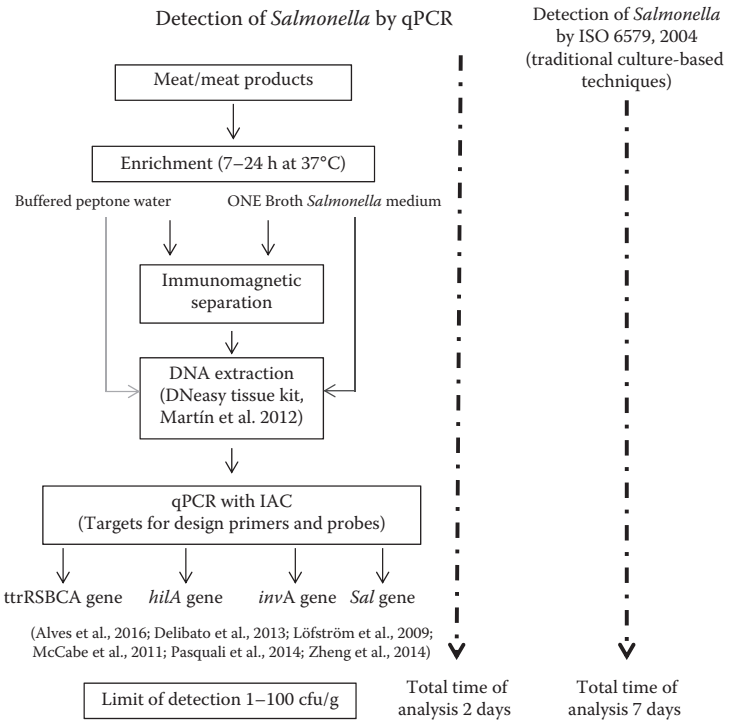


FIGURE 4.1
Flow diagram of the detection of *Salmonella* in meat and meat products by quantitative polymerase chain reaction (qPCR) protocols validated with ISO 6579–2004. IAC, internal amplification control; ONE, oxoid noel enrichment. (From Rodríguez, A. et al, *Food Control*, 60, 302–308, 2016.)

Downloaded From Vet-eBooks.com

qPCR methods include buffered peptone water as culture medium for enrichment, it has been demonstrated that oxoid noel enrichment (ONE) Broth *Salmonella* medium increases the growth of *Salmonella* in the presence of competitive microbial population in meat products compared with buffered peptone water (Delibato et al., 2013). Thus, ONE Broth could be used in enrichment protocol in order to minimize the effect of the competitive microflora naturally present in meat samples. In addition, in meat and meat products, a pre-PCR treatment consisting of a treatment with the DNeasy Tissue kit could be recommended to reduce the risk of false negative results and increase the probability of *Salmonella* detection, since PCR inhibitors present in these complex food matrices or in the enrichment media would be eliminated (Martin et al., 2012). Some of the developed qPCR methods have been validated against the reference culture-based method, ISO 6579 (Anonymous, 2002), providing an alternative testing protocol to this reference microbiological method. Thus, Löfström et al. (2009) reported a qPCR method that included an IAC, making it useful for detecting *Salmonella* in meat. This method was validated in comparative and collaborative trials, based on the recommendations from the Nordic organization for validation of alternative microbiological methods (NordVal) of a same-day, noncommercial qPCR method for detection of *Salmonella* in meat and carcass swabs. This method includes a short pre-enrichment of 18 h in buffered peptone water followed by automated DNA purification and targets a part of the ttrRSBCA locus specific for *Salmonella*. This locus, located in the *Salmonella* pathogenicity island 2, is essential for tetrathionate-based anaerobic respiration and significant for *Salmonella* survival and outgrowth in anaerobic competitive environments (Hensel et al., 1999; Pasquali et al., 2014). The detection level of this method was between 1 and 100 cfu/25 g of sample. The ttrRSBCA locus has been used to design new primers and a hydrolysis fluorescent probe for accurate and validated detection of *Salmonella* spp. by qPCR using IAC (Delibato et al., 2013).

Furthermore, McCabe et al. (2011) developed a DNA and a RNA qPCR validated assays amplifying a 270 bp region of the *hil*A gene of *Salmonella enterica* serovars which includes an IAC that coamplified with the *hil*A gene to monitor potential PCR inhibitors and ensure successful amplification. The *hil*A target gene is located in the *Salmonella* pathogenicity island 1 (SPI1) and regulates the expression of *Salmonella* invasion genes (Pasquali et al., 2014). Both DNA and RNA PCR methods include an adapted two-step enrichment protocol before DNA or RNA extraction that is able to detect between 1 and 10 cfu per carcass swab.

Different validated qPCR methods for accurate detection of *Salmonella* using as the target the *inv*A, *ttrRSBCA*, and *Sal* genes (Figure 4.1) have been also reported (Zheng et al., 2014). The former authors reported qPCR protocols combined with IMS for detecting healthy and heat-injured *Salmonella typhimurium* on raw duck wings increasing sensitivity of the methods. From the developed qPCR-IMS protocols that used primers targeting *Sal* genes showed lower limit of detection and higher amplification efficiency than those obtained with the *invA* and *ttrRSBCA*-based primers (Zheng et al., 2014).

Downloaded From Vet-eBooks.com

Apart from simplex qPCR methods to detect *Salmonella*, a multiplex protocol that detect this pathogen and other worrying pathogenic microorganism in chicken meat such as *Campylobacter* spp. has been recently developed (Alves et al., 2016). For the *Salmonella* gene, the *invA* was targeted. This method showed a sensitivity of 1 cfu of each of these pathogens per milliliter of rinse after 24 h of the selective enrichment of *Campylobacter* spp. and the nonselective enrichment of *Salmonella* spp.

When the sensitivity and specificity of validated qPCR methods for *Salmonella* detection are tested possible differences between artificially and naturally contaminated meat and meat products should be considered, since during their shelf life, the number of *Salmonella*-stressed cells and the related positive deviation might be increased (Pasquali et al., 2014). To avoid differences on sensitivity of the new qPCR validated method due to stressed cells, Pasquali et al. (2014) evaluated the relative accuracy, sensitivity, and specificity of a qPCR targeting the *ttrRSBCA* locus, for detection of potentially stressed *Salmonella* in naturally contaminated pork cuts from packaging, during storage at retail and at the consumer's house, in comparison with ISO 6579:2004. The same authors reported that the qPCR assay targeting the *ttrRSBCA* locus is an accurate, sensitive, and specific method for the rapid and routine detection of *Salmonella* spp. also in naturally contaminated pork cuts subject to physical stresses. Thus, the reference ISO method might be applied only on positive samples for confirmatory and isolation purposes, which are mandatory in epidemiological investigations.

Finally, the investigation of *Salmonella* serotypes in meat and meat products could be needed, especially when the traditional culture-based method according to ISO 6579 (Anonymous, 2002) is followed. Recently, Maurischat et al. (2015) developed a validated multiplex qPCR assay targeting the partial sequences of the *saf*A, *fli*AIS*200*, *fljB-hin*, and *hin-iroB* genes and the pUC18/19 plasmid as IAC. This protocol detects *Salmonella enteritidis* and *Salmonella typhimurium* and differentiates *Salmonella* serovars in 24 h after sampling with a detection probability of 100% between 3 and 5 cfu in 25 g of sample, showing a direct correlation with serovars determined by the traditional method using slide agglutination according to the White–Kauffman–Le Minor scheme (Grimont and Weill, 2007). Thus, this qPCR assay could be used to serotype *Salmonella* isolates from meat and meat products.

4.2.1.2 Listeria monocytogenes

The current ISO 11290-1 standard method (Anonymous, 2004) for the detection of *L. monocytogenes* that includes two consecutive enrichment steps in the selective medium Half Fraser Broth and full concentrations, followed by plating out on two selective solid media (PALCAM and ALOA) and subsequent presumptive colonies confirmation through hemolysis and biochemical tests (Anonymous, 2004), usually takes more than seven days. The availability of rapid methods such as qPCR is one of the most important

Downloaded From Vet-eBooks.com

advancements to managing *L. monocytogenes* along the meat chain, allowing faster hazard analysis critical control points (HACCP) verification and positive release of finished food products (Gattuso et al., 2014). Several qPCR methods have been developed for rapid and sensitive detection of *L. monocytogenes* in meat and meat products targeting different virulence *hlyA*, *inlA*, *prfA* genes (Figure 4.2; Bolocan et al., 2016; Dalmasso et al., 2014; De Cesare et al., 2014; Heo et al., 2014). From these target sequences, the *hlyA* gene encoding for the virulence factor listeriolisin O has been the most frequently used to design primers and probes for developing qPCR protocols (Rodríguez-Lázaro et al., 2004; De Cesare et al., 2014). Until now, the developed methods do not have sufficient sensitivity for direct detection of such pathogens in meat samples without the use of an enrichment procedure (Bolocan et al., 2016; Dalmasso et al., 2014).

Thus, qPCR methods for accurate and sensitive detection of *L. monocytogenes* in meat and meat products should include an enrichment step around 24–48 h at 37°C to increase the number of the target *L. monocytogenes* to

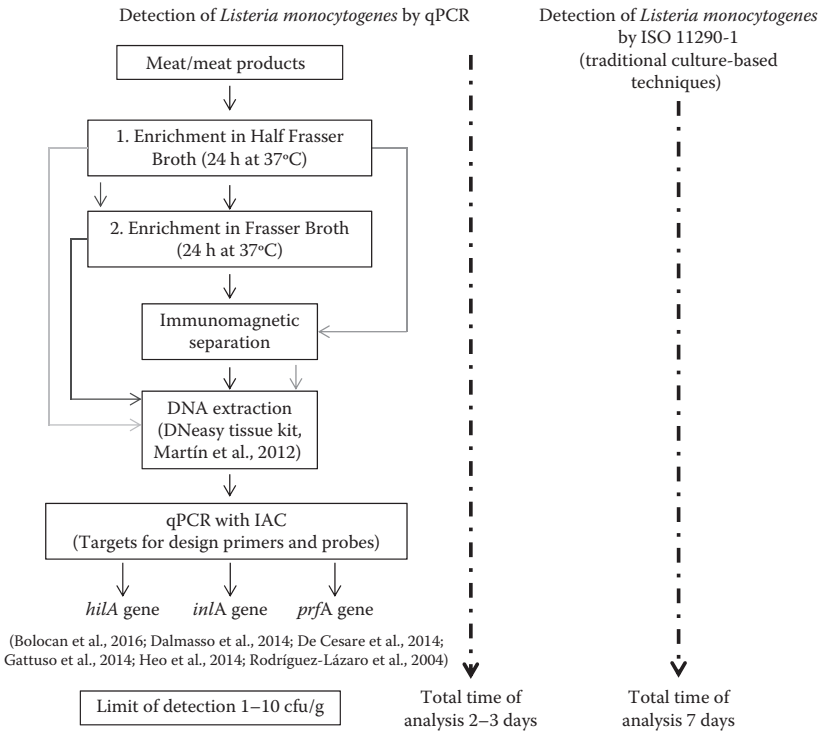


FIGURE 4.2
Flow diagram of the detection of *Listeria monocytogenes* in meat and meat products by qPCR validated with International Standardization Organization (ISO) 11290-1. IAC, internal amplification control; ONE, oxoid noel enrichment. (From Rodríguez, A. et al, *Food Control*, 60, 302–308, 2016.)

Downloaded From Vet-eBooks.com

detectable levels (Figure 4.2). Dalmasso et al. (2014) demonstrated that the use of the second enrichment broth in the qPCR reduces time for a confirmed result from seven days needed for the ISO standard procedure performance to three days. Besides, Gattuso et al. (2014) reported that a combination of an incubation step in 100 mL of Half Fraser Broth for 24 h coupled to a DNA extraction and a qPCR assay could detect about 8 cfu of *L. monocytogenes* per sample in less than 27 h. This approach is fully compatible with the ISO standard for detection of *L. monocytogenes* if 1:10 and 1:5 dilutions are used, providing results more rapidly (27 h vs. 7 days) and cost-effectively (five times cheaper, Gattuso et al., 2014). Recently, Bolocan et al. (2016) demonstrated that the analysis of the second enrichment of the ISO method by qPCR was more sensitive for the detection of *L. monocytogenes* than when the ISO method was used alone.

4.2.1.3 Verotoxigenic E. coli

Verotoxin-producing *E. coli* (VTEC) are a group of foodborne pathogenic *E. coli* strains that produce two cytotoxins called shiga-like toxins or verotoxins (Stx1 and Stx2). VTEC can cause diarrhea and hemorrhagic colitis and may lead to hemolytic uremic syndrome. Although *E. coli* O157:H7 is the serotype that has been linked to most of the outbreaks of foodborne diseases, other VTEC serotypes such as O26, O103, O145, O91, O146, and O111 are related to sporadic cases (EFSA, 2015). This diversity of microorganisms is a difficulty when designing primers for qPCR. Despite the fact that it would be easier to screen for the presence of the shiga-like toxins gene sequences, its value remains controversial, since not all VTEC have been proven to be clinically significant in humans. Furthermore, each shiga-like toxin contains several subtypes which differ in their sequence gene, biological activity, and association with disease which complicates the design of appropriate targets (Gyles, 2007). The Stx1 type is divided into three subtypes: Stx1a, Stx1c, and Stx1d, and the Stx2 have been classified into seven subtypes: Stx2a, Stx2b, Stx2c, Stx2d, Stx2e, Stx2f, and Stx2g (Scheutz et al., 2012). Therefore, for a correct Stx screening, the greatest amount of Stx subtypes should be considered. For all these reasons, there are many methods of qPCR for the detection of VTEC in meat products based on different targets. Different virulence genes such as *stx1*, *stx2,* and *eae*, which encode the bacterial outer-membrane protein intimin, have been targeted to assess the presence of VTEC in minced beef (Verhaegen et al., 2016), processed meat product (Bardasi et al., 2015), and lamb (Osés et al., 2010). Other authors have developed multiplex qPCR procedures to detect all known *stx* gene variants in ground and minced beef (Brusa et al., 2015; Derzelle et al., 2011).

A commercial method known as the "BAX® System real-time PCR Assay" was approved by the United States Department of Agriculture-Food Safety and Inspection Service (USDA-FSIS) and AOAC for VTEC detection in raw ground beef and beef trim (DuPont, 2014). The system amplifies and detects

Downloaded From Vet-eBooks.com

the virulence genes *stx* and *eae* in less than an hour, and allows the screening of samples containing any of the top six non-O157 serogroups. Furthermore, there is a specific kit for detecting *E. coli* O157:H7 certified by AENOR and another kit, the "BAX System Real-Time PCR STEC Suite" for detecting O26, O45, O103, O111, O121, and O145 serogroups recommended by USDA-FSIS.

The ISO/TS 13136:2012 (Anonymous, 2012) describes a multiplex qPCR targeting the virulence genes *eae*, *stx1* and *stx2*, and after the samples result positive for the presence of *stx2* gene are tested for *E. coli* O104 serogroup-associated gene. Positive samples for the presence of the *stx1* and/or *stx2* in association with the *eae* gene were tested for the detection of *E. coli* O103, O111, O145, O157, O26, serogroup-associated genes. When such genes are detected, the isolation of the strain is needed to confirm the presence of the *stx* genes in addition to relevant virulence factor in the same live cell while excluding the presence of free DNA or free *stx* phages in the enrichment culture (EFSA, 2013). A sensitive multiplex qPCR using a melting curve analysis for simultaneously detecting eight serogroups of VTEC has been developed to sensitive detection of specific VTEC serotypes during preliminary screenings (Singh and Mustapha, 2015).

In this sense, methods in which the combination of virulence genes with serogroup-specific genes such as the *wzx* (O antigen flippase) and *wzy* (O antigen polymerase) genes in minced meat and ground beef have been developed (Kagkli et al., 2011; Perelle et al., 2007; Hara-Kudo et al., 2016). Nevertheless, other authors have published a duplex-specific mRNA-based qPCR tests for detection and quantification of viable *E. coli* O157:H7 in ready-to-eat (RTE) meat products. This assay includes a combination of serotype-specific markers of *E. coli* O157:H7 such as the *rfbE* and $fliC_{h7}$ genes, encoding the O157 antigen and the H7 flagellar antigen, respectively, to ensure specificity in the detection of this pathogenic microorganism (Gordillo et al., 2014). Recently, these two targets have been used in the commercial validated "Thermo Scientific SureTect *Escherichia coli* O157:H7" procedure through the AOAC as well (Cloke et al., 2015).

Finally, another *E. coli* O157:H7-specific qPCR assay targeting the highly conserved mutation at position +93 of the *uidA* (β-glucuronidase) gene in such pathogen has been used in raw ground meat for its accurate detection (Miszczycha et al., 2012).

Detection limits of the listed methods are variable (Table 4.1), ranging between 0.25 and 10^4 cfu/g, but most of them are below 100 cfu/g, the advisable limit of this microbial group in most foods. All assays include a previous enrichment ranging between 6 and 24 h (Table 4.1). From those methods the one developed by Gordillo et al. (2014) may be the most suitable for the detection of *E. coli* O157:H7 in meat and meat products. Regarding the assay proposed by Singh and Mustapha (2015), it seems to be the most appropriate qPCR for simultaneously detecting eight serogroups of VTEC in different meat products. Finally, the multiplex qPCR developed by Brusa et al. (2015) is the most appropriate to detect all known *stx* gene variants in meat.

Downloaded From Vet-eBooks.com

TABLE 4.1

Target Sequence, Meat Matrix, Sample Preparation, Technology, and Limit of Detection for qPCR Detection of *E. Coli* VTEC in Meat and Meat Products

Target Sequence	Matrix	Sample Preparation	qPCR Technology	Limit of Detection/ Quantification	References
stx1 and *stx2* genes	Minced beef and cattle carcass	Enrichment 20 h	TaqMan	0.25 cfu/g	Verhaegen et al. (2016).
stx1, *stx2*, and *eae* genes	Meat product Lamb Ground beef, beef trim	Enrichment 18–24 h Enrichment 18 h Enrichment 9–12 h	TaqMan TaqMan Scorpion/ TaqMan	1–5 copies per reaction 3–4 log cfu/g 4 log cfu/mL	ISO/TS 13136:2012 Osés et al. (2010) 0BAX System (DuPont, 2014)
stx1a–stx1d genes	Ground beef	Enrichment 20 h	SYBR Green	2 log cfu/mL	Brusa et al. (2015)
stx2a–stx2g genes	Minced beef	Enrichment 18 h	TaqMan	2 log cfu/g	Derzelle et al. (2011)
wzx_{O26}, wzx_{O45}, wzx_{O103}, wzx_{O111}, wzx_{O121} genes, glycosyl transferase gene (O104), *uidA* gene (O157)	Ground beef, ground chicken, ground turkey	Enrichment 6 h	SYTO 9, HRM dye	10 cfu/25 g	Singh and Mustapha (2015)
stx, wzx_{O26}, wzx_{O103}, wzx_{O141}, wzx_{O145}, $wbdI_{O111}$, $rfbE_{O157}$ genes	Ground beef	Enrichment 20–24 h	TaqMan	10 cfu/25 g	Hara-Kudo et al. (2016)
stx1a, stx2a–stx2d, eae, *wzxO26*, wzx_{O103}, $ihp1_{O145}$, $wbdI_{O111}$, $rfbE_{O157}$ genes	Minced meat	Enrichment 18–24	TaqMan	2–10 cfu/g	Kagkli et al. (2011)
$rfbE_{O157}$ *and* $fliC_{h7}$ genes	Raw beef Dry-cured ham, dry-cured pork loin, dry-fermented sausages ("chorizo," "salchichón" and "salami"), cooked ham, cooked turkey breast, chopped, mortadella	Enrichment 9–24 h Enrichment 6 h	TaqMan SYBR Green	– 1 cfu/g	Thermo Sci. Sure Test (Cloke et al., 2015) Gordillo et al. (2014)
uidA gene (O157)	Raw ground meat	Enrichment 24 h	TaqMan	2 log cfu/mL	Miszczycha et al. (2012)

HRM, high-resolution melting; ISO, International Standardization Organization; VTEC, verotoxin-producing *E. coli*.

Downloaded From: Vet-eBooks.com

4.2.1.4 Campylobacter

Since *Campylobacter* detection by traditional culture-based methods is problematic owing to its presence in low concentration and its sensitivity to culture conditions (Botteldoorn et al., 2008), qPCR methods seem to be more adequate for its detection.

As illustrated in Table 4.2, most qPCR methods were developed for chicken due to the fact that poultry is often implicated as a main source of human infections with *Campylobacter* spp.

For the detection of *Campylobacter* spp., two different alternatives have been proposed depending on the whole genus or the most important species (*Campylobacter jejuni, Campylobacter Coli*, and *Campylobacter lari*). The detection of *Campylobacter* genus without differentiating the species may be an ideal approach from a risk assessment point of view, and it is particularly based on the thermotolerant *C. jejuni, C. coli,* and *C. lari* (Chapela et al., 2015).

rRNA gene sequences, mainly 16S, have been applied for specific detection of *Campylobacter* genus and/or the three species (Table 4.2).

In the case of the differentiation of the thermotolerant species, the design of appropriate primers and probes is a challenge since such species are remarkably similar to each other in their genome sequences (He et al., 2010). Different genes have been targeted for each species (Table 4.2).

Botteldoorn et al. (2008) evaluated the ability of three qPCR methods, two of them already described, to quantify *Campylobacter* genus, thermotolerant *Campylobacter* (Josefsen et al., 2004), and *C. jejuni* in chicken carcass rinses. The most sensitive and specific method was the first one, based on the TaqMan technology (Table 4.2). The other two methods used SYBR Green but they were not appropriate because of cross-reactions with other species closely related with *Campylobacter*. Consistently, most of the published qPCR for detecting and quantifying *Campylobacter* in meat and meat products have used the TaqMan technology (Table 4.2).

On the other hand, Botteldoorn et al. (2008) compared the qPCR results with those from traditional culture-based methods, obtaining lower detection sensitivity than from the latter. They suggested the presence of viable but non-culturable or dead *Campylobacter* spp. and the sensitivity and difficulty of enumerating them as the reason for such underestimation. This feature has also been established by other authors working with *Campylobacter* detection.

The described qPCR methods for detecting *Campylobacter* in meat and meat products are generally characterized for not having low detection limits, although a few of them are able to detect as low as 1 cfu/g (Table 4.2).

4.2.1.5 Yersinia

Several qPCR protocols mainly using TaqMan technology have been reported for detecting and quantifying pathogenic *Yersinia* in meat and meat products (Table 4.2). Most of them have been developed for *Y. enterocolitica* and employ

Downloaded From Vet-eBooks.com

TABLE 4.2

Target Sequence, Meat Matrix, Sample Preparation, Technology, and Limit of Detection for qPCR Detection of Pathogenic *Campylobacter* and *Yersinia* Species in Meat and Meat Products

Microorganisms	Target Genes	Matrix	Sample Preparation	qPCR Technology	Limit of Detection/ Quantification	References
Campylobacter jejuni, Campylobacter coli, Campylobacter lari	*hipO, cdtA, pepT*	Chicken (skin)	24 h selective enrichment	TaqMan IAC	1 cfu/g	He et al. (2010)
C. jejuni, C. coli, C. lari	16S rRNA	Chicken rinse (skin)	Flotation	Hybridization probes	8.6×10^2 cfu/mL	Wolffs et al. (2005)
C. jejuni, C. coli, C. lari, Campylobacter upsaliensis	*bipA, cje0832*	Meat and meat products	Selective enrichment	TaqMan IAC	1–5 cfu/g	Bonjoch et al. (2010)
Campylobacter spp.	16S rRNA	Chicken carcass rinse		TaqMan	2×10^3 cfu/carcass	Botteldoorn et al. (2008)
C. jejuni, C. coli, C. lari	16S rRNA	Chicken carcass rinse	PMA	TaqMan IAC	10^2 cfu/mL	Josefsen et al. (2010)
C. jejuni	*rpoB*	Chicken, turkey	18–48 h selective enrichment	SYBR® Green	1–10 cfu/g or mL	Rantsiou et al. (2010); Melero et al. (2011)
Yersinia enterocolitica	*ail*	Ground beef, cold-smoked sausage, raw pork	18–20 h nonselective enrichment	TaqMan IAC	5.5–55 cfu/10g	Thisted Lambertz et al. (2008)
Yersinia enterocolitica	*ail*	Ground pork	24 h selective enrichment	TaqMan	≤1 cfu/g	Jourdan et al. (2000)
Y. enterocolitica	*ail*	Pork	16–18 h nonselective enrichment	TaqMan	1–10 cfu/g	Fredriksson-Ahomaa et al. (2007)
Yersinia pseudotuberculosis	*ail*	Ground beef	18–24 h nonselective enrichment	TaqMan IAC	28 cfu/10g	Thisted Lambertz et al. (2008)
Yersinia pestis	Virulence plasmids (pPCP1, pMT1), *cnp60*	Ground beef		TaqMan	10^2 cfu/g	Amoako et al. (2010)

IAC, internal amplification control; PMA, propidium monoazide; qPCR, quantitative polymerase chain reaction.

Downloaded From: Vet-Books.com

the chromosomally located virulence-associated gene *ail* (Table 4.2). Such protocols are of great interest since some difficulties are associated with the isolation of pathogenic *Y. enterocolitica* consisting of its low number in the samples and the presence of competing microbial population (Fredriksson-Ahomaa and Korkeala, 2003). The ability of the *ail* gene to detect *Y. pseudotuberculosis* has been also proven (Table 4.2). Thisted Lambertz et al. (2008a,b) reported a multiplex qPCR assay for the simultaneous detection and differentiation of *Y. enterocolitica* and *Y. pseudotuberculosis* based on the *ail* gene. However, the utility of this gene seems to be limited due to the fact that it is also found in some nonpathogenic strains of *Y. enterocolitica* (Fredriksson-Ahomaa and Korkeala, 2003).

For the detection of *Y. enterocolitica*, its specific region of the 16SrRNA gene and the plasmid-borne virulence gene *yadA* have also been described in pork and chicken samples (Wolffs et al., 2005).

qPCR methods for detecting pathogenic *Yersinia* spp. other than *Y. enterocolitica* and *Y. pseudotuberculosis* in meat and meat products are scarce (Table 4.2). Concretely, Amoako et al. (2010) described qPCR assays involving four primer sets and their corresponding probes based on two virulence plasmids able to distinguish *Yersinia pestis* from the various *Yersinia* and other bacterial species tested. They designed an additional primer and probe set based on the *cnp60* chromosomal gene for distinguishing *Y. pestis* and *Y. pseudotuberculosis* from the various *Yersinia* and other bacterial species tested.

4.2.2 qPCR for Detecting Bacteria Foodborne Intoxications

4.2.2.1 Staphylococcus aureus

Staphylococcal foodborne poisoning is an intoxication produced by consumption of foods containing high enough amounts of preformed staphylococcal enterotoxins (Argudín et al., 2010). *Staphylococcus aureus* is a recognized causative agent of this poisoning and has been considered the main representative of the genus *Staphylococcus* capable of producing enterotoxins (SEs) (Podkowik et al., 2013). However, some coagulase negative *Staphylococci* strains showing enterotoxigenic capacity have been found in Spanish dry-cured hams (Rodríguez et al., 1996) and even in starter cultures (Zell et al., 2008).

The correct choice of the target sequence to design primers is essential for a good power of discrimination and sensitivity of a qPCR method. Several qPCR procedures to detect *S. aureus* on meat and meat products are based on different staphylococcal genes, coding genes for thermonuclease (*nuc*), chromosomal DNA fragment (*Sa442*), methicillin resistance (*mecA*), and heat shock protein (*htra*) (Chiang et al., 2007; Cremonesi et al., 2014; Martinon and Wilkinson, 2011; Table 4.3). Nevertheless, these target genes are not related to the production of SEs, and not all *S. aureus* are enterotoxin-producers. In this sense, other simplex or multiplex qPCR procedures based on genes involved

Downloaded From Vet-eBooks.com

TABLE 4.3

Target Sequence, Meat Matrix, Sample Preparation, Technology, and Limit of Detection for qPCR Detection of Toxin-Producing *Staphylococcus aureus* and *Clostridium botulinum* in Meat and Meat Products

Microorganisms	Target Sequence	Matrix	Sample Preparation	qPCR Technology	Limit of Detection/ Quantification	References
Staphylococcus aureus	*nuc* gene	Ham, chicken, raw minced meat	Enrichment 18 h	SYBR Green	2 cfu/g	Martinon and Wilkinson (2011)
	16S rRNA, *nuc*, *mecA*, *PVL* genes	Meat (beef, pork, poultry) and deli meat (chicken, ham. turkey)	Primary enrichment 18–20 h Second enrichment 18–20 h	TaqMan	–	Velasco et al. (2014)
	htrA gene	Pork sausage	Direct qPCR	TaqMan	1pg genomic DNA	Cremonesi et al. (2014)
	Sa422 gene	Pork	Inmunomagnetic separation 30 min	SYBR Green	9.6 cfu/g	Ma et al. (2014)
	conserved regions of enterotoxin genes	Dry-cured ham, dry-cured pork loin, dry-fermented sausages, cooked ham, mortadella	Enrichment 8 h	SYBR Green	2–40 cfu/g	Rodríguez et al. (2016)
Clostridium botulinum	*BoNT/A*,	sausage	–	TaqMan	100 spores/mL	Yoon et al. (2005)
	BoNT/A, *BoNT/B*, *BoNT/E*, and *BoNT/F* gene	"foie gras" salami, meat juice and homogenized meat Sausages, canned meat, salami, meat juice and homogenized meat, blood sausage	Enrichment 48 h Enrichment 4 days	TaqMan TaqMan	10^3–10^4 cfu/mL 10^3–10^4 cfu/mL	Fach et al. (2009, 2011) Fenicia et al. (2011)
	NTNH gene	"foie gras"	Enrichment 48 h	TaqMan	10^3–10^4 cfu/mL	Fach et al. (2009)

qPCR, quantitative polymerase chain reaction.

Downloaded From: Vet-eBooks.com

in the production of several SEs have been developed (Letertre et al., 2003; Rodríguez et al., 2016; Table 4.3).

The qPCR methods designed for screening single genes encoding each SE require several sets of specific primers targeting each SE gene. Therefore, a rapid and specific method is still necessary able to detect the potential production of all the described SEs using universal primers. Sharma et al. (2000) reported a single-reaction multiplex PCR assay using a universal toxin gene forward primer (SA-U) in combination with various toxin-specific reverse primers for detecting SE genes between A and E from pure cultures of *S. aureus*. Letertre et al. (2003) designed a qPCR using such a forward primer and another universal reverse primer suitable to check for SE genes from A to J but not for the remaining SE genes. Rodríguez et al. (2016) developed a SYBR Green-based qPCR procedure for detecting most of the described staphylococcal SE (A to V) in meat products.

Detection limits of the listed methods are variable (Table 4.3) but all are below 100 cfu/g, the advisable limit of this microbial group in most foods. Considering all the abovementioned methods, it seems that the method developed by Rodríguez et al. (2016) may be the most suitable for the detection of enterotoxigenic *Staphylococci* in meat and meat products. In this procedure, the detection limits were about 2–40 cfu/g after an 8 h enrichment period at 30°C, and the total time for assay completion was approximately 12 h. This qPCR method offers a useful, rapid, and efficient tool for screening SEs-producing *Staphylococci* in meat products.

4.2.2.2 Clostridium botulinum

Foodborne botulism is caused by the ingestion of foods containing botulinum neurotoxin (BoNT). BoNT is produced by some species of the genus *Clostridium*, particularly *Clostridium botulinum*, but some strains of *Clostridium baratii* and *Clostridium bytiricum* can also produce it. BoNTs can also be separated into one of seven distinct serotypes (A–G) on the basis of the immunological characteristics of its botulinum toxin. Moreover, five immunologically or genetically distinct BoNT/A subtypes, five BoNT/B subtypes, six BoNT/E subtypes, and three BoNT/F subtypes have been identified (Satterfield et al., 2010).

Several qPCR procedures to detect *C. botulinum* on meat and meat product rely on different BoNT genes. Yoon et al. (2005) have designed primers for detecting only the *BoNT/A* gene, while other authors have proposed qPCR methods for identifying and typing BoNT-producing *C. botulinum* types A, B, E, and F using a specific target based on the *BoNT/A, BoNT/B, BoNT/E*, and *BoNT/F* genes on meat and meat products (Fach et al., 2009, 2011; Fenicia et al., 2011).

Another strategy consists of using universal primers that recognize all *BoNT* genes, but the nucleotide diversity among the *BoNT* genes may present a potential problem (Hauser et al., 1995). Nonetheless, BoNTs are generated

Downloaded From Vet-eBooks.com

as part of a progenitor toxin complex and a conserved component among serotypes is nontoxic nonhemagglutinin (NTNH) (East and Collins, 1994). These authors demonstrated that the gene encoding NTNH reveals a high level of similarity and is present in all strains that produce BoNTs while it is missing in nontoxic strains. Nucleotide sequence analysis of the cluster of genes associated with the *BoNT* gene demonstrates that the presence of the NTNH gene is directly upstream of the *BoNT* gene in all tested toxin types (Raphael and Andreadis, 2007). A qPCR assay based on the detection of the *NTNH* gene has been developed; its specificity in naturally contaminated samples of "foie gras" has been also shown (Fach et al., 2009).

Detection limits of the listed methods are higher than methods for detecting *C. botulinum* (Table 4.3), ranging between 10^2 and 10^4 cfu/g. All assays include a previous enrichment ranging between 48 h and four days, excepting the protocol of Yoon et al. (2005). This assay was the most sensitive and fast, but only detected the *BoNT/A* gene (Table 4.3). Fenicia et al. (2011) included an external amplification control that gives more robustness to the technique, but the disadvantage was that the enrichment period is too long.

4.3 qPCR for Detecting Toxigenic Molds

Only a few qPCR methods have been developed for detecting and quantifying foodborne molds in meat and meat products. Generally, molds do not grow on meats, which do not have a ripening step within their processing. However, the processing of cured meat products such as dry-cured ham and dry-cured fermented sausages have a ripening step which favors mold colonization on their surface. Normally, this mycobiota only has positive effects on these cured meats, nevertheless some mold species may contaminate such products with mycotoxins (Núñez et al., 1996), which are secondary metabolites produced by molds which have toxic effects in both animals and humans. Among them, ochratoxin A (OTA) is the most important mycotoxin found in dry-cured meat products (Bertuzzi et al., 2013; Comi and Iacumin, 2013; Markov et al., 2013; Perši et al., 2014; Pleadin et al., 2013; Rodríguez et al., 2011). Rodríguez et al. (2011) developed two new qPCR methods to detect and quantify ochratoxigenic molds in dry-cured meat products by using both TaqMan and SYBR Green methodologies (Table 4.4). For these qPCR protocols primers and probe target the *otanps* gene involved in OTA biosynthesis were developed. The specificity of the methods has been tested on 75 mold strains distinguishing between producing and nonproducing strains. The efficiencies and R^2 values of the standard curves were within the recommended range (Rodríguez et al., 2015b) so they can be used for quantification purposes in dry-cured meat products. Therefore, such methods have been widely used in further stud-

Downloaded From Vet-eBooks.com

TABLE 4.4

Target Sequence, Meat Matrix, Sample Preparation, Technology, and Limit of Detection for qPCR Detection of Toxigenic Molds in Dry-Cured Meat Products

Toxigenic Mold	Target Genes	Matrix	Sample Preparation	qPCR Technology	Limit of Detection/ Quantification	References
Ochratoxin A-producing mold	*otanps*	Dry-cured ham, dry-fermented sausage	Direct qPCR	SYBR® Green/ TaqMan®	10 cfu/g	Rodríguez et al. (2011)
Aflatoxin-producing mold	*aflP*	Dry fermented sausages "salchichón" and "chorizo," dry-cured ham, dry-cured pork loin	Direct qPCR	SYBR Green/ TaqMan	10 cfu/g	Rodríguez al. (2012c)
Cyclopiazonic acid-producing mold	*dmaT*	Dry-cured ham	Direct qPCR	TaqMan/ Competitive IAC	10–100 cfu/g	Rodríguez et al. (2012e)

IAC, internal amplification control; qPCR, quantitative polymerase chain reaction.

ies to quantify OTA-producing molds in the dry-cured ham industry (Rodríguez et al., 2012d), to test different DNA extraction methods from inoculated meat matrices (Rodríguez et al., 2012b) and to evaluate the efficacy of different antagonist microorganisms to control ochratoxigenic mold growth on dry-cured ham and dry-fermented sausages (Andrade et al., 2014; Bernáldez et al., 2013; Rodríguez et al., 2015a). Besides, other types of toxigenic molds can be quantified in cured meat products using qPCR. Thus, Rodríguez et al. (2012c) optimized two qPCR methods, which use both SYBR Green and TaqMan methodologies for detection and quantification of aflatoxigenic molds in such products (Table 4.4). Specific primers and probe based on the *aflP* gene involved in aflatoxin biosynthesis have been designed. The specificity of the two methods has been tested on 53 producing and nonproducing strains and their efficiencies of around 90% allows using both methods to quantify the load of aflatoxigenic molds contaminating cured meat products. These methods can be carried out in any laboratory/equipment and performed by different operators since they also show good repeatability and reproducibility. On the other side, two TaqMan-based qPCR methods have been reported for detection and quantification of cyclopiazonic acid (CPA)- and verrucosidin-producing molds in dry-cured meat products (Rodríguez et al., 2012a,e; Table 4.4). Both methods utilize IAC to assess the validity of qPCR and avoid false negative results as has been explained in Section 4.1. However, the IAC methodology used for both methods is different. The qPCR for detecting producers of CPA includes a competitive IAC, while the method for producers of

Downloaded From Vet-eBooks.com

verrucosidin uses a noncompetitive IAC. For the qPCR for CPA-producing mold detection, the design of primers and probe is based on the *dmaT* gene that encodes the enzyme dimethylallyl tryptophan synthase involved in the biosynthesis of CPA. The competitive IAC consists of a 105 bp chimeric DNA fragment containing a region of the *hly* gene of *L. monocytogenes*. The qPCR method for quantifying verrucosidin-producing molds was developed with primers and probes designed from the SVr1 probe sequence of a verrucosidin-producing *Penicillium polonicum* (Aranda et al., 2002). The conserved regions of the β-*tubulin* gene have been used to design primers and probe of the noncompetitive IAC included in such qPCR. Both methods are specific, able to quantify only CPA- or verrucosidin-producing molds between other toxigenic molds, which normally contaminate dry-cured ham and dry-fermented sausages. The functionality of both methods is demonstrated by high linear relationships of the standard curves relating to $\log_{10}$ cfu/g and the Cq values obtained from the different producers of CPA and verrucosidin tested, respectively. The described qPCR for detecting toxigenic molds in cured meat products are characterized as having detection limits between 1 and 2 cfu/g (Table 4.4).

4.4 Reverse Transcription Quantitative PCR as a Tool for Evaluating Gene Expression of Foodborne Pathogens in Meat and Meat Products

Reverse transcription quantitative PCR (RT-qPCR) could also be a useful technique for evaluation of meat and meat products safety. RT-qPCR has been proven to be a powerful method to study population dynamics and activities through quantification of gene expression (Gadkar and Filion, 2013), which is increasingly important in a variety of food research fields (Ishii et al., 2007). qPCR when combined with reverse transcription (RT) can also estimate transcript amounts, providing data on microbial activity (Postollec et al., 2011). The main difference between the qPCR and RT-qPCR methods is that in the latter mRNA is used as a template. Altered levels of specific mRNAs may indicate a change in the level of the proteins encoded by the mRNA required in response to specific environmental conditions. Such a technique can also be used in foodborne pathogen viability studies. However, the major drawback is that mRNA itself cannot be stable for a quite long time after cell death (Birch et al., 2001; Rijpens and Herman, 2004). Design of RT-qPCR methods should be based on the targeting sequences of virulence genes and/or stress-related genes instead of housekeeping genes.

Target detection chemistries for RT-qPCR are the same as those used for qPCR and they are detailed in depth in Section 4.1. RT-qPCR can be conducted in one step within a single reaction, or in two steps with cDNA synthesis done

Downloaded From Vet-eBooks.com

independently of qPCR (Nolan et al., 2006). Despite the fact that both RT-qPCR procedures are acceptable, it is preferable to choose the two-step protocol when several qPCR analyses will be carried out from the same RNA sample. Analysis of data derived from RT-qPCR can be performed either at absolute levels to determine the absolute transcript copy number or at relative levels to measure differences in the expression level of a specific target between different samples (VanGuilder et al., 2008). For absolute quantification, a RNA standard curve of the gene of interest is required in order to calculate the number of copies. However, for the relative one, an endogenous control (e.g., housekeeping gene) and a calibrator (control or reference sample) are needed. Although several mathematical models have been set up for relative quantification, the most common one is the $2^{-\Delta\Delta C}{}_{T}$ method (Livak and Schmittgen, 2001), which relies on two assumptions. The first one is that the amplification efficiencies of the target and the reference must be approximately equal. The second assumption of the $2^{-\Delta\Delta C}{}_{T}$ method is that the endogenous gene should be expressed at a constant level between the samples. This endogenous control will be used to correct any difference in sample loading. Depending on the chosen quantification method, different results can be observed. Compared to absolute quantification, relative quantification is simpler. However, it can only be applied to the samples run within the same PCR.

Generally, gene expression studies in foodborne bacteria and molds normally growing in meat and meat products have been conducted in culture media and not in such complex matrices. This makes it difficult to analyze the real gene expression of pathogens in food environments due to several reasons: (a) differences between growth medium and meat matrices, (b) the isolation of stable mRNA from complex matrices such as meat and meat products that contains nucleases and PCR inhibitors, and (c) the substantial technique challenges associated with accurately measuring bacteria and mold-related gene expression in complex matrices. Due to the fact that gene expression measurements can vary depending on the substrate that the pathogen grows on, only studies which have investigated quantitative gene expression of foodborne pathogens on complex meat matrices are discussed in Sections 4.4.1 to 4.4.7.

This section provides brief descriptions of the currently available RT-qPCR-based methods for gene expression studies of the most relevant foodborne pathogens in meat and meat products: *Salmonella*, *Campylobacter*, shiga toxin-producing *E. coli*, *S. aureus*, *L. monocytogenes*, viruses, and OTA-producing *Penicillia*. RT-qPCR methods to analyze gene expression responses by *Yersinia* and *C. botulinum* in meats have not been developed yet. A review of RT-qPCR methods developed to quantify gene expression of the most relevant foodborne pathogens in meat and meat products is summarized in Table 4.5.

4.4.1 *Salmonella*

Several RT-qPCR methods have been developed to analyze gene expression of *Salmonella* spp. contaminating meat and meat products. Most of them

Downloaded From Vet-eBooks.com

TABLE 4.5

Target Sequence and Sample Preparation for Gene Expression by RT-qPCR of Pathogenic Microorganisms in Meat and Meat Products

Foodborne Pathogen	Target Sequence (Reference Gene)	Matrix	Sample Preparation	Detection Chemistry	Gene Expression Quantification Method	Factor Analyzed	References
Salmonella	*rpoE, rpoH, rpoS, htrA, uspA, uspB (rpoD)* *dnaK, otsB (rpoD)* *rpoS, nlpD, clpD (gmk)* *adrA, bapA, csgB, csgD, csrA, rpoS, invA, sipB-C, sdiA, luxS (16S rRNA)*	Poultry Minced beef Meat extract broth Meat-based growth medium	Enrichment Direct qPCR Enrichment Enrichment	NA SYBR Green SYBR Green SYBR Green	Relative Relative Relative Relative	Temperature/time Heat/salt stress Tolerance to acid (lactic acid and acetic acid) Biofilm formation	Yadav et al. (2016) Kjeldgaard et al. (2011) Burin et al. (2014) Wang et al. (2016)
Escherichia coli	*dnaK, ostB (gapA)* *asnB, rbsB, rplD, uhpT, asr, melA, osmY, melB, melR, grxB (16S rRNA)* *fliCh7, rfbE*	Minced beef Beef Dry-cured meat products, dry-fermented sausages and cooked meat products	Direct qPCR Enrichment Enrichment	SYBR Green SYBR Green SYBR Green/ TaqMan	Relative Relative Absolute	Heat/salt stress Beef composition Viability	Kjeldgaard et al. (2011) Fratamico et al. (2011) Gordillo et al. (2014)
Campylobacter jejuni	*rplA, cheA, Cj0006, hslV, glyA (Cj0402)* *dps (Cj0402)* *dnaJ, dnaK, groEL (rpoA)*	Chicken Chicks Chicken	*In vivo* inoculation and direct qPCR *In vivo* inoculation and direct qPCR *In vivo* inoculation and direct qPCR	SYBR Green SYBR Green SYBR Green	Relative Relative Relative	Interaction between chicken-*C. jejuni* Biofilm formation Heat shock response, motility, and maintenance of cell length homogeneity	Hu et al. (2014) Theoret et al. (2012) Apel et al. (2012)

(*Continued*)

Downloaded From: Vet-eBooks.com

TABLE 4.5 (*Continued*)

Target Sequence and Sample Preparation for Gene Expression by RT-qPCR of Pathogenic Microorganisms in Meat and Meat Products

Foodborne Pathogen	Target Sequence (Reference Gene)	Matrix	Sample Preparation	Detection Chemistry	Gene Expression Quantification Method	Factor Analyzed	References
Staphylococcus aureus	*sec (rpoB and hu)* *sea (16S rRNA)*	Pepper beef salami, turkey ham, chicken ham, pork ham Frankfurter type sausage	Direct qPCR Direct qPCR	EvaGreen TaqMan	Relative Relative	Gene expression through various meat products processing Gene expression through sausage storage and lactic acid effect	Alibayov et al. (2015) Zeaki et al. (2014)
Listeria monocytogenes	*sigB, lmo0669, lmo2434, gbuA, gbuB, lmo1421, betL, opuCA, lmo1038, lmo0442, lmo0115, lmo0938, prfA (rpoB, rplD, gap, bglA and tuf)* *prfA, inlA, sigB, clpC (gap and rpoB)* *hly, sigB, iap, plcA (IGS)* *hly*	Salami Liver pâtés Fermented sausage, minced meat Chilled pork	Direct qPCR Enrichment Direct qPCR Direct qPCR	SYBR Green TaqMan TaqMan SYBR Green	Relative Relative Relative Absolute	Potential stress resistance NaCl content Common (4°C) and abuse (12°C) refrigeration conditions Viability	Mataragas et al. (2015) Olesen et al. (2010) Rantsiou et al. (2012) Ye et al. (2012)

(*Continued*)

Downloaded From: Vet-Books.com

TABLE 4.5 (*Continued*)

Target Sequence and Sample Preparation for Gene Expression by RT-qPCR of Pathogenic Microorganisms in Meat and Meat Products

Foodborne Pathogen	Target Sequence (Reference Gene)	Matrix	Sample Preparation	Detection Chemistry	Gene Expression Quantification Method	Factor Analyzed	References
Viruses	*HEV (MNV-1)* *HEV (MS2 phage)* Murine norovirus and F-RNA coliphage MS2	Pork liver sausages/Raw pig liver products Liver raw sausages Pork chop	Direct qPCR Direct qPCR Direct qPCR	TaqMan TaqMan TaqMan	Absolute Detection Absolute	Viability of hepatitis E virus Viability of hepatitis E virus Viability of hepatitis E virus Survival of viruses during meat processing	Di Bartolo et al. (2015); Martin-Latil et al. (2014) Szabo et al. (2015) Brandsma et al. (2012)
Ochratoxigenic molds	*otapks, otanps* *otapks*	Dry-cured ham-based medium Salami	Direct qPCR Direct qPCR	SYBR Green SYBR Green	Relative Absolute	NaCl content on temporal gene expression Seasoning process of salami on temporal gene expression	Rodríguez et al. (2014) Ferrara et al. (2016)

MNV, murine norovirus; RT-qPCR, reverse transcription quantitative polymerase chain reaction.

Downloaded From: Via eBooks.com

use the nonspecific dye SYBR Green to monitor the fluorescence through the qPCR reactions and the relative quantification method is the method of choice to analyze gene expression data. Only a few studies have investigated quantitative gene expression of *Salmonella* in meat matrices (Table 4.5). The majority of them have examined the behavior of such foodborne pathogens in culture media (González-Gil et al., 2012).

Depending on the objective of the study, different genes have been used as targets of the RT-qPCR designed to analyze gene expression of *Salmonella* in meat and meat products. For studying the cue of genes involved in imparting thermotolerance or thermal stress response of *S. typhimurium* and *S. enteritidis* on dressed poultry skin surface, relative expression of heat stress-related genes has been analyzed (Yadav et al., 2016). The target genes of such RT-qPCR are the heat stress-related sigma factor (*rpoH*), alternative sigma factor (*rpoE*), universal stress protein (*uspA* and *uspB*), and the serine protease *htrA*. However, for in-depth analysis on heat and salt stress changes of *S. enterica* in minced beef, a RT-qPCR method has been devised based on the targeting sequences of the *dnaK* related to many cellular processes (e.g., DNA replication of the bacterial chromosome, RNA synthesis, and autoregulation of the heat shock response) and *ostB* genes involved in trehalose biosynthesis connected to osmotic stress (Kjeldgaard et al., 2011). Both investigations use the gene encoding the RNA polymerase sigma factor (*rpoD*) as the reference gene to normalize quantification of the mRNA targets.

Other authors use model systems such as meat extract broth and agar to simulate meat matrix composition to study the influence of lactic acid and acetic acid and biofilm formation on expression of related genes by *Salmonella*, respectively (Burin et al., 2014; Wang et al., 2016). Burin et al. (2014) have developed a RT-qPCR method targeting three genes involved in a complex tolerance mechanism of survival under acid stress: *rpoS* and *nlpD,* which are responsible for protein expression, and *clpP,* which is associated with the regulation of these proteins inside the cell (Foster, 2001). The essential *gmk* gene encoding guanylate kinase is used as endogenous control in such RT-qPCR. Wang et al. (2016) have investigated the expression of the attachment (*adrA*, *bapA*, *csgB*, *csgD*, and *csrA*), virulence (*invA* and *sipB-C*), and quorum sensing (*sidA* and *luxS*) genes in *Salmonella* biofilm formed on meat-based medium. They have also identified the relationships between each tested gene involved in biofilm formation. In this case, 16s rRNA has been used as endogenous control.

4.4.2 *Listeria monocytogenes*

Different meat products including fermented sausage "salami," liver pâtés, minced meats, and chilled pork have been used as meat matrices to study expression of different stress- and virulence-related genes of *L. monocytogenes*. Mataragas et al. (2015) examined the gene expression profiling of the pathogen in response to the conditions encountered during the fermentation and ripening of sausages (salami). For this, SYBR Green-based RT-qPCR

Downloaded From Vet-eBooks.com

methods based on one gene related to general stress (*sigB*) and eleven genes relative to various stresses commonly found during fermented sausage production, such as acid (*lmo0669* and *lmo2434* or *gadD*), osmotic (*gbuA*, *gbuB*, *lmo1421*, *betL*, and *opuCA*) and competition for nutrients (*lmo1038*, *lmo0442*, *lmo0115*, and *lmo0938*), are used as targets. Five housekeeping genes (*rpoB, rplD*, *gap*, *bglA*, and *tuf*) are included as reference genes for performing relative quantification. Ye et al. (2012) have also used the nonspecific SYBR Green dye to detect rapidly viable *L. monocytogenes* in chilled pork by RT-qPCR. This method is able to quantify the absolute expression of the *L. monocytogenes*-specific *hly* gene encoding listeriolysin.

Other researchers have used the sequence-specific double-labeled probes for their gene expression studies of such pathogens in meat products. Olesen et al. (2010) analyzed the effect of NaCl content on relative transcription of several *L. monocytogenes* virulence genes such as *prfA, inlA*, *sigB*, and *clpC* in liver pâtés. However, Rantsiou et al. (2012) studied the influence of common (4°C) and abuse (12°C) refrigeration conditions on the relative expression profile of a stress response gene (*sigB*) and three virulence genes (*hly*, *iap*, and *plcA*) of this foodborne pathogen in fermented sausages and minced meat.

4.4.3 Shiga Toxin-Producing *E. coli*

A limited number of RT-qPCR methods have been devised to analyze gene expression of verocytotoxic *E. coli* contaminating meat and meat products. Two of them have analyzed the relative expression of virulence-related genes of *E.coli* in beef using the SYBR Green chemistry as the detection system (Kjeldgaard et al., 2011; Fratamico et al., 2011). One of them analyses the effect of heat and salt stress on the *dnaK* and *ostB* genes using the *gapA* gene as the reference gene. The other one evaluates the influence of 10 genes (*asnB, rbsB, rplD, uhpT, asr, melA, osmY, melB, melR*, and *grxB*) with the nonribosomal 16s rRNA region as the housekeeping gene. On the other hand, Gordillo et al. (2014) quantifies the viability of *E. coli* 0157:H7 in meat products by means of duplex qPCR assays based on two serotype-specific markers such as the $fliC_{h7}$ and *rfbE* genes in RTE meat products. Both detection qPCR methodologies, SYBR Green and TaqMan, are proposed to be used to quantify expression of verocytotoxic *E. coli* in meat products.

4.4.4 *Campylobacter jejuni*

Chicken is the only meat matrix used for *C. jejuni* gene expression studies by RT-qPCR. In such studies, chickens were *in vivo* inoculated by *C. jejuni* through oral or intramuscular administration (Hu et al., 2014; Theoret et al., 2012; Apel et al., 2012). In addition, regarding RT-qPCR performance, SYBR Green chemistry is utilized and Cq data from qPCR are analyzed using

Downloaded From Vet-eBooks.com

the $2^{-\Delta\Delta C_T}$ method (Table 4.5). Although earlier studies have several similarities, they set different research goals. Hu et al. (2014) have studied the genes expressed *in vivo* during interaction between chicken and the host infection by the pathogen. For this, the virulence-associated genes *plA*, *cheA*, *Cj0006*, *hslV*, and *glyA* have been used as target genes. However, Theoret et al. (2012) have analyzed the expression of the *dps* gene for a role in biofilm formation and cecal colonization in poultry. The housekeeping gene *Cj0402* was used as endogenous control in both abovementioned RT-qPCR methods. On the other hand, the RT-qPCR method, reported by Apel et al. (2012), targets three genes (*dnaJ*, *dnaK*, and *groEL*) to analyze their role in the heat shock response of *C. jejuni* when it colonizes the cecum of birds. The *rpoA* gene is used to normalize cDNA quantities in the qPCR reactions.

4.4.5 *Staphylococcus aureus*

Scarce RT-qPCR methods have been developed to examine gene expression of *S. aureus* in meat products. In all studies, expression of genes encoding enterotoxin A (SEA) and C (SEC) has been analyzed throughout processing of different meat products. In addition, Zeaki et al. (2014) investigated the effect of lactic acid on the *sea* gene expression through sausage storage. A detection method using hydrolysis probes and relative gene expression measurements are used by Zeaki et al. (2014). The *sea* gene, which has previously shown that regulates the expression and production of SEA, and 16s rRNA are utilized as target and endogenous genes, respectively. The main difference between the abovementioned two methods were the different types of meat matrix used. The first one evaluates gene expression on several pork meat products (boiled ham, hot-smoked ham, Serrano ham, and black pepper salami), and the second one checked expression of the *sea* gene on frankfurter type sausage. Recently, Alibayov et al. (2015) studied differences in transcription and expression of the *sec* gene associated with SEC formation in processed meat products such as pepper beef salami and turkey, chicken, and pork ham. This method used two genes (*rpoB* and *hu*) as endogenous control to calculate relative expression of the target gene. Fluorescence changes occurring during qPCR are measured by the DNA-binding EvaGreen® dye.

4.4.6 Viruses

Human foodborne viruses are not usually found in meat and meat products. However, the meat industry and laboratories should be prepared to detect viruses in meat matrices due to the increase in recent years of foodborne outbreaks involving viruses. Detection of worrying foodborne viruses requires preferably RT-qPCR instead of qPCR. All the RT-qPCR methods developed to check gene expression of viruses in meat products use the TaqMan chemistry. Most of these methods target hepatitis E virus

Downloaded From Vet-eBooks.com

and their purpose was to study the viability of the pathogen in different meats and meat products such as pigs, pork liver sausages, and raw pig liver products (Di Bartolo et al., 2015; Martin-Latil et al., 2014; Szabo et al., 2015). On the other hand, Brandsma et al. (2012) have devised a method to evaluate the survival of murine norovirus and F-RNA coliphage MS2 on pork chop during storage and retail display.

4.4.7 Ochratoxin A-Producing Penicillia

Only two RT-qPCR methods have been developed to study expression of genes involved in mycotoxin of unwanted molds in cured meat products. Both protocols have used the nonspecific SYBR Green dye to monitor fluorescence during qPCR reactions. Rodríguez et al. (2014) have evaluated the effect of NaCl content on relative temporal expression of the *otapks* and *otanps* genes associated with OTA biosynthesis of *Penicillium nordicum* on dry-cured ham-based medium. However, Ferrara et al. (2016) have analyzed the influence of the seasoning process of a dry-fermented sausage (salami) on the absolute expression of the *otapks* gene.

4.5 Conclusions

Recently, several qPCR protocols have been reported as accurate, specific, and sensitive methods to detect pathogenic microorganisms of concern in meat and meat products. Some of these methods such as those reported for detecting *Salmonella* and *L. monocytogenes* are validated against the corresponding ISO methods and thus they could be used in the meat industry for routine analysis. Although in most of the qPCR protocols an enrichment step of 7–48 h could be included, time of analysis is greatly reduced in comparison to traditional culture-based techniques proposed by ISO methods. The limit of detection of these methods is around 1–100 cfu/g. The high sensitivity and reasonably low time of analysis of the reported qPCR protocols make them very useful tools to evaluate raw material and preprocessed and finished products; they allow for the use of rapid and efficient corrective actions during processing in the meat industry. In addition, RT-qPCR could be also a valuable technique for evaluation of meat and meat products safety. RT-qPCR has proved to be a powerful method to evaluate population dynamics and activities through quantification of gene expression. Several RT-qPCR protocols have been reported for accurate and sensitive evaluation of gene expression of the most important foodborne pathogenic bacteria and mold in meat products. These protocols could be used to detect live pathogenic microorganisms in meat and meat products, which could allow for the use of corrective actions during processing in the meat industry. Although further

Downloaded From Vet-eBooks.com

studies should be developed to reduce the time of analysis and protocols validation, the current qPCR and RT-qPCR methods allow a rapid and sensitive detection and gene expression analysis respectively of concern microorganisms in the meat industry.

Acknowledgments

This work has been funded by the Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA), the Spanish Ministry of Economy and Competitiveness, the Government of Extremadura, and FEDER (INIA-RTA-2013-00070-C03-03, AGL2013-45729-P, GR15108). Dr. Alicia Rodríguez is a recipient of a *Juan de la Cierva* senior fellowship from the Spanish Ministry of Economy and Competitiveness (IJCI-2014-20666).

References

Alibayov, B., Karamonova, L., Hollerova, R., Zdenkova, K. and Demnerova, K. (2015). Differences in transcription and expression of staphylococcal enterotoxin C in processed meat products. *LWT–Food Science and Technology,* **64**, 578–585.

Alves, J., Hirooka, E. Y. and Oliveira, T. C. R. M. D. (2016). Development of a multiplex real-time PCR assay with an internal amplification control for the detection of *Campylobacter* spp. and *Salmonella* spp. in chicken meat. *LWT—Food Science and Technology,* **72**, 175–181.

Amoako, K. K., Goji, N., Macmillan, T. et al. (2010). Development of multitarget real-time PCR for the rapid, specific, and sensitive detection of *Yersinia pestis* in milk and ground beef. *Journal of Food Protection,* **73**, 18–25.

Andrade, M. J., Thorsen, L., Rodríguez, A., Córdoba, J. J. and Jespersen. L. (2014). Inhibition of ochratoxigenic moulds by *Debaryomyces hansenii* strains for biopreservation of dry-cured meat products. *International Journal of Food Microbiology,* **170**, 70–77.

Anonymous. (2002). International Standard Organisation 6579. Microbiology of food and animal feeding stuffs: horizontal method for the detection of *Salmonella*. Available at: https://www.iso.org/standard/29315.html (Accessed on 19 June 17).

Anonymous. (2004). International Standard Organisation 11290-1. Microbiology of food and animal feeding stuffs—Horizontal method for the detection and enumeration of *Listeria monocytogenes*—Part 1: Detection method. Available at: https://www.iso.org/standard/19268.html (Accessed on 19 June 17).

Anonymous. (2011). International Standard Organisation 22119:2011. Microbiology of food and animal feeding stuffs—Real-time polymerase chain reaction (PCR) for the detection of food-borne pathogens—General requirements and definitions. Available at:https://www.iso.org/standard/45035.html (Accessed on 19 June 17).

Downloaded From Vet-eBooks.com

Anonymous. (2012). International Standard Organisation 13136. Microbiology of food and animal feed—Real-time polymerase chain reaction (PCR)-based method for the detection of food-borne pathogens—Horizontal method for the detection of shiga toxin-producing *Escherichia coli* (STEC) and the determination of O157, O111, O26, O103 and O145 serogroups (ISO/TS 13136:2012). Available at: https://www.iso.org/standard/53328.html (Accessed on 19 June 17).

Apel, D., Ellermeier, J., Pryjma, M., DiRita, V. J. and Gaynor, E. C. (2012). Characterization of *Campylobacter jejuni* RacRs reveals roles in the heat shock response, motility, and maintenance of cell length homogeneity. *Journal of Bacteriology,* **194**, 2342–2354.

Aranda, E., Rodríguez, M., Benito, M. J., Asensio, M. A. and Córdoba, J. J. (2002). Molecular cloning of verrucosidin-producing *Penicillium polonicum* genes by differential screening to obtain a DNA probe. *International Journal of Food Microbiology,* **76**, 55–61. Available at http://www.ncbi.nlm.nih.gov/pubmed/12038578

Argudín, M. Á., Mendoza, M. C. and Rodicio, M. R. (2010). Food poisoning and *Staphylococcus aureus* enterotoxins. *Toxins,* **2**, 1751–1773.

Barbau-Piednoir, E., Mahillon, J., Pillyser, J., Coucke, W., Roosens, N. H. and Botteldoorn, N. (2014). Evaluation of viability-QPCR detection system on viable and dead *Salmonella* serovar *enteritidis*. *Journal of Microbiological Methods,* **103**, 131–137.

Bardasi, L., Taddei, R., Nocera, L., Ricchi, M. and Merialdi, G. (2015). Shiga toxin-producing *Escherichia coli* in meat and vegetable products in Emilia Romagna region, years 2012–2013. *Italian Journal of Food Safety,* **4**, 33–35.

Bernáldez, V., Córdoba, J. J., Rodríguez, M., Cordero, M., Polo, L. and Rodríguez, A. (2013). Effect of *Penicillium nalgiovense* as protective culture in processing of dry-fermented sausage "salchichón." *Food Control,* **32**, 69–76.

Bertuzzi, T., Gualla, A., Morlacchini, M. and Pietri, A. (2013). Direct and indirect contamination with ochratoxin A of ripened pork products. *Food Control,* **34**, 79–83.

Birch, L., Dawson, C. E., Cornett, J. H. and Keer, J. T. (2001). A comparison of nucleic acid amplification techniques for the assessment of bacterial viability. *Letters in Applied Microbiology,* **33**, 296–301.

Bolocan, A. Nicolau, S. A. I., Alvarez-Ordóñez, A. et al. (2016). Dynamics of *Listeria monocytogenes* colonisation in a newly-opened meat processing facility. *Meat Science,* **113**, 26–34.

Bonjoch, X., Calvó, L., Soler, M., Ruiz-Rueda, O. and Garcia-Gil, L. J. (2010). A new multiplexed real-time PCR assay to detect *Campylobacter jejuni, C. coli, C. lari*, and *C. upsaliensis*. *Food Analytical Methods,* **3**, 40–46.

Botteldoorn, N., Van Coillie, E., Piessens, V. et al. (2008). Quantification of *Campylobacter* spp. in chicken carcass rinse by real-time PCR. *Journal of Applied Microbiology,* **105**, 1909–1918.

Brandsma, S. R., Muehlhauser, V. and Jones, T. H. (2012). Survival of murine norovirus and F-RNA coliphage MS2 on pork during storage and retail display. *International Journal of Food Microbiology,* **159**, 193–197.

Brusa, V., Galli, L., Linares, L. H., Ortega, E. E., Lirón, J. P. and Leotta, G. A. (2015). Development and validation of two SYBR Green PCR assays and a multiplex real-time PCR for the detection of shiga toxin-producing *Escherichia coli* in meat. *Journal of Microbiological Methods,* **119**, 10–17.

Burin, R., Konrad, C., Silva, A. and Nero, L. A. (2014). Influence of lactic acid and acetic acid on *Salmonella* spp. growth and expression of acid tolerance-related genes. *Food Research International,* **64**, 726–732.

Downloaded From Vet-eBooks.com

Bustin, S. A., Benes, V., Garson, J. A. et al. (2009). The MIQE guidelines: Minimum information for publication of quantitative real-time PCR experiments. *Clinical Chemistry,* **55**, 611–622.

Ceuppens, S., Li, D., Uyttendaele, M. et al. (2014). Molecular methods in food safety microbiology: Interpretation and implications of nucleic acid detection. *Comprehensive Reviews in Food Science and Food Safety,* **13**, 551–577.

Chapela, M. J., Garrido-Maestu, A. and Cabado, A. G. (2015). Detection of foodborne pathogens by qPCR: A practical approach for food industry applications. *Cogent Food and Agriculture,* **1**, 1013771.

Chiang, Y.-C., Fan, C.-M., Liao, W.-W., Lin, C.-K. and Tsen, H.-Y. (2007). Real-time PCR detection of *Staphylococcus aureus* in milk and meat using new primers designed from the heat shock protein gene htrA sequence. *Journal of Food Protectection,* **70**, 2855–2859.

Cloke, J., Crowley, E., Bird, P. et al. (2015). Validation of the Thermo Scientific SureTect *Escherichia coli* O157: H7 real-time PCR assay for raw beef and produce matrixes. *Journal of AOAC International,* **98**, 1301–1314.

Comi, G. and Iacumin, L. (2013). Ecology of moulds during the pre-ripening and ripening of San Daniele dry cured ham. *Food Research International,* **54**, 1113–1119.

Cremonesi, P., Pisani, L. F., Lecchi, C. et al. (2014). Development of 23 individual TaqMan® real-time PCR assays for identifying common foodborne pathogens using a single set of amplification conditions. *Food Microbiology,* **43**, 35–40.

Dalmasso, M., Bolocan, A. S., Hernandez, M. et al. (2014). Comparison of polymerase chain reaction methods and plating for analysis of enriched cultures of *Listeria monocytogenes* when using the ISO11290-1 method. *Journal of Microbiological Methods,* **98**, 8–14.

De Cesare, A., Pasquali, F., Lucchi, A. and Manfreda, G. (2014). Relative accuracy, specificity and sensitivity of a 5'nuclease real-time PCR assay for *Listeria monocytogenes* detection in naturally contaminated pork cuts. *Food Analytical Methods,* **7**, 1359–1365.

Delibato, E., Anniballi, F., Sinibaldi Vallebona, P. et al. (2013). Validation of a 1-day analytical diagnostic real-time PCR for the detection of *Salmonella* in different food meat categories. *Food Analytical Methods,* **6**, 996–1003.

Derzelle, S., Grine, A., Madic, J. et al. (2011). A quantitative PCR assay for the detection and quantification of shiga toxin-producing *Escherichia coli* (STEC) in minced beef and dairy products. *International Journal of Food Microbiology,* **151**, 44–51.

Di Bartolo, I., Angeloni, G., Ponterio, E., Ostanello, F. and Ruggeri, F. M. (2015). Detection of hepatitis E virus in pork liver sausages. *International Journal of Food Microbiology,* **193**, 29–33.

DuPont (2014). Dupont™ BAX® system real-time PCR assays e STEC suite. Available at: http://www2.dupont.com/Qualicon/en_US/assets/downloads/BAX%20product%20descrip-RealTimeSTEC.pdf (Accessed on 15 June 17).

East, A. K. and Collins, M. D. (1994). Conserved structure of genes encoding components of botulinum neurotoxin complex-m and the sequence of the gene coding for the nontoxic component in nonproteolytic *Clostridium botulinum* type-F. *Current Microbiology,* **29**(2), 69–77.

EFSA. (2013). Scientific opinion on VTEC-Seropathotype and scientific criteria regarding pathogenicity assessment. *EFSA Journal*, **11**(4), 3138.

EFSA. (2015). The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2011 has been published. *Euro*

Downloaded From Vet-eBooks.com

Surveillance: Bulletin Europe en Sur Les Maladies Transmissibles European Communicable Disease Bulletin, **18**(15), 20449.

European Commission. (2005). Commission regulation (EC) No. 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. *Official Journal of the European Union,* **338**, 1–26.

Fenicia, L., Fach, P., van Rotterdam, B. J. et al. (2011). Towards an international standard for detection and typing botulinum neurotoxin-producing clostridia types A, B, E and F in food, feed and environmental samples: A European ring trial study to evaluate a real-time PCR assay. *International Journal of Food Microbiology,* **145**(Suppl. 1), S152–S157.

Fach, P., Fenicia, L., Knutsson, R. et al. (2011). An innovative molecular detection tool for tracking and tracing *Clostridium botulinum* types A, B, E, F and other botulinum neurotoxin producing Clostridia based on the GeneDisc cycler. *International Journal of Food Microbiology,* **145**(Suppl. 1), S145–S151.

Fach, P., Micheau, P., Mazuet, C., Perelle, S. and Popoff, M. (2009). Development of real-time PCR tests for detecting botulinum neurotoxins A, B, E, F producing *Clostridium botulinum, Clostridium baratii* and *Clostridium butyricum*. *Journal of Applied Microbiology,* **107**, 465–473.

Ferrara, M., Magistà, D., Epifani, F. et al. (2016). Study of gene expression and OTA production by *Penicillium nordicum* during a small-scale seasoning process of salami. *International Journal of Food Microbiology,* **227**, 51–55.

Foster, J. W. (2001). Acid stress responses of *Salmonella* and *E. coli*: Survival mechanisms, regulation, and implications for pathogenesis. *Journal of Microbiology,* **39**, 89–94.

Fratamico, P. M., Wang, S., Yan, X., Zhang, W. and Li. Y. (2011). Differential gene expression of *E. coli* O157:H7 in ground beef extract compared to tryptic soy broth. *Journal of Food Science,* **76**, 79–87.

Fredriksson-Ahomaa, M., Hartmann, B., Scheu, P. and Stolle. A. (2007). Detection of pathogenic *Yersinia enterocolitica* in meat using real-time PCR. *Journal Für Verbraucherschutz Und Lebensmittelsicherheit*, **2**, 202–208.

Fredriksson-Ahomaa, M. and Korkeala, H. (2003). Low occurrence of pathogenic *Yersinia enterocolitica* in clinical, food, and environmental samples: A methodological problem. *Society,* **16**, 220–229.

Gadkar, V. J. and Filion, M. (2013). New developments in quantitative real-time polymerase chain reaction technology. *Current Issues in Molecular Biology,* **16**, 1–6.

Gattuso, A., Gianfranceschi, M. V. and Sonnessa, M. (2014). Optimization of a real time PCR based method for the detection of *Listeria monocytogenes* in pork meat. *International Journal of Food Microbiology,* **184**, 106–108.

González-Gil, F., Le Bolloch, A., Pendleton, S., Zhang, N., Wallis, A. and Hanning. I. (2012). Expression of hila in response to mild acid stress in *Salmonella enterica* is serovar and strain dependent. *Journal of Food Science,* **77**, 292–297.

Gordillo, R., Rodríguez, A., Werning, M. L., Bermúdez, E. and Rodríguez, M. (2014). Quantification of viable *Escherichia coli* O157: H7 in meat products by duplex real-time PCR assays. *Meat Science,* **96**, 964–970.

Gorski, L. and Csordas, A. (2010). Molecular detection: Principles and methods. *In:* Liu, D. (ed.) *Molecular Detection of Foodborne Pathogens*. Boca Raton, FL: CRC Press (Taylor and Francis), pp. 1–20.

Grimont, P. and Weill, F. X. (2007). Antigenic formulae of the *Salmonella* serovars. *WHO collaborating centre for reference and research on Salmonella*, 1–167. Available

Downloaded From Vet-eBooks.com

at: http://www.serotest-thailand.com/upload/news/download/9-8310-0.pdf (Accessed on 15 June 17).

Gyles, C. L. (2007). Shiga toxin-producing *Escherichia coli*: An overview. *Journal of Animal Science,* **85**(Suppl. E), E45–E62.

Hara-Kudo, Y., Konishi, N., Ohtsuka, K. et al. (2016). An interlaboratory study on efficient detection of shiga toxin-producing *Escherichia coli* O26, O103, O111, O121, O145, and O157 in food using real-time PCR assay and chromogenic agar. *International Journal of Food Microbiology,* **230**, 81–88.

Hauser, D., Gibert, M., Marvaud, J. C., Eklund, M. W. and Popoff, M. R. (1995). Botulinal neurotoxin C1 complex genes, clostridial neurotoxin homology and genetic transfer in *Clostridium botulinum*. *Toxicon,* **33**, 515–526.

He, Y., Yao, X. and Gunther, N. W. et al. (2010). Simultaneous detection and differentiation of *Campylobacter jejuni*, *C. coli*, and *C. lari* in chickens using a multiplex real-time PCR assay. *Food Analytical Methods,* **3**, 321–329.

Hensel, M., Nikolaus, T. and Egelseer, C. (1999). Molecular and functional analysis indicates a mosaic structure of *Salmonella* pathogenicity island 2. *Molecular Microbiology,* **31**, 489–498.

Heo, E. J., Song, B. R., Park, H. J. et al. (2014). Rapid detection of *Listeria monocytogenes* by real-time PCR in processed meat and dairy products. *Journal of Food Protection,* **77**, 453–458.

Hernández, M., Hansen, F., Cook, N. and Rodríguez-Lázaro, D. (2009). Real-time PCR methods for detection of foodborne bacterial pathogens in meat and meat products. *In:* Toldrá, F. (ed.) *Safety of Meat and Processed Meat*. New York, NY: Springer, pp. 427–446.

Hoorfar, J., Cook, N., Malorny, B. et al. (2004a). Diagnostic PCR: Making internal amplification control mandatory. *Letters in Applied Microbiology,* **38**, 79–80.

Hoorfar, J., Malorny, B., Abdulmawjood, A., Cook, N., Wagner, M. and Fach, P. (2004b). Practical considerations in design of internal amplification controls for diagnostic PCR assays. *Journal of Clinical Microbiology,* **42**, 1863–1868.

Hu, Y., Huang, J. and Jiao, X. A. (2014). Screening of genes expressed in vivo during interaction between chicken and *Campylobacter jejuni*. *Journal of Microbiology and Biotechnology,* **24**, 217–224.

Invitrogen. (2008). Real-time PCR. *Real-Time PCR: From Theory to Practice*. Available at: www.invitrogen.com (Accessed on 15 June 17).

Ishii, T., Sootome, H., Shan, L. and Yamashita, K. (2007). Validation of universal conditions for duplex quantitative reverse transcription polymerase chain reaction assays. *Analytical Biochemistry,* **362**, 201–212.

Jones, M. and Marengo, S. (2016). Laboratory validation, verification and accreditation of molecular methods. *In:* Cook, N., D'Agostino, M. and Thompson, K. C. (eds.) *Molecular Microbial Diagnostic Methods: Pathways to Implementation for the Food and Water Industries*. London: Academic Press, pp. 107–133.

Josefsen, M. H., Jacobsen, N. R. and Hoorfar, J. (2004). Enrichment followed by quantitative PCR both for rapid detection and as a tool for quantitative risk assessment of food-borne thermotolerant *Campylobacters*. *Applied and Enviromental Microbiology,* **70**, 3588–3592.

Josefsen, M. H., Löfström, C., Hansen, T. B., Christensen, L. S., Olsen, J. E. and Hoorfar, J. (2010). Rapid quantification of viable *Campylobacter* bacteria on chicken carcasses, using real-time PCR and propidium monoazide treatment, as a tool for quantitative risk assessment. *Applied and Environmental Microbiology,* **76**, 5097–5104.

Downloaded From Vet-eBooks.com

Jourdan, A. D., Johnson, S. C. and Wesley, I. V. (2000). Development of a fluorogenic 5'nuclease PCR assay for detection of the ail gene of pathogenic *Yersinia enterocolitica*. *Applied and Environmental Microbiology,* **66**, 3750–3755.

Kagkli, D. M., Weber, T. P., Van den Bulcke, M. et al. (2011). Application of the modular approach to an in-house validation study of real-time PCR methods for the detection and serogroup determination of verocytotoxigenic *Escherichia coli*. *Applied and Environmental Microbiology,* **77**, 6954–6963.

Kavanagh, I., Jones, G. and Nayab, S. N. (2011). Significance of controls and standard curves in PCR. *In:* Kennedy, S. and Oswald, N. (eds.) *PCR Troubleshooting and Optimization: The Essential Guide*. Norfolk, VA: Caister Academic Press, pp. 67–78.

Kjeldgaard, J., Henriksen, S., Cohn, M. T. Aabo, S. and Ingmer, H. (2011). Method enabling gene expression studies of pathogens in a complex food matrix. *Applied and Environmental Microbiology,* **77**, 8456–8458.

Krämer, N., Vigre, C. L. H., Hoorfar, J., Bunge, C. and Malorny, B. (2011). A novel strategy to obtain quantitative data for modelling: Combined enrichment and real-time PCR for enumeration of *Salmonellae* from pig carcasses. *International Journal of Food Microbiology,* **145**, S86–S95.

Law, J. W. F., Ab Mutalib, N. S., Chan, K. G. and Lee, L. H. (2015). Rapid methods for the detection of foodborne bacterial pathogens: Principles, applications, advantages and limitations. *Frontiers in Microbiology,* **5**, 1–19.

Letertre, C., Perelle, S., Dilasser, F. and Fach, P. (2003). Detection and genotyping by real-time PCR of the staphylococcal enterotoxin genes *sea* to *sej*. *Molecular and Cellular Probes,* **17**, 139–147.

Livak, K. J. and Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2(-delta delta C(T)) method. *Methods,* **25**, 402–408.

Löfström, C., Krause, M., Josefsen, M. H., Hansen, F. and Hoorfar, J. (2009). Validation of a same-day real-time PCR method for screening of meat and carcass swabs for *Salmonella*. *BMC Microbiology,* **9**, 85.

Ma, K., Deng, Y., Bai, Y. et al. (2014). Rapid and simultaneous detection of *Salmonella, Shigella*, and *Staphylococcus aureus* in fresh pork using a multiplex real-time PCR assay based on immunomagnetic separation. *Food Control,* **42**, 87–93.

Markov, K., Pleadin, J., Bevardi, M. et al. (2013). Natural occurrence of aflatoxin B1, ochratoxin A and citrinin in Croatian fermented meat products. *Food Control,* **34**, 312–317.

Martin, B., Garriga, M. and Aymerich. T. (2012). Pre-PCR treatments as a key factor on the probability of detection of *Listeria monocytogenes* and *Salmonella* in ready-to-eat meat products by real-time PCR. *Food Control,* **27**, 163–169.

Martin-Latil, S., Hennechart-Collette, C., Guillier, L. and Perelle, S. (2014). Method for HEV detection in raw pig liver products and its implementation for naturally contaminated food. *International Journal of Food Microbiology,* **176**, 1–8.

Martinon, A. and Wilkinson, M. G. (2011). Selection of optimal primer sets for use in a duplex SYBR green-based, real-time polymerase chain reaction protocol for the detection of *Listeria monocytogenes* and *Staphylococcus aureus* in foods. *Journal of Food Safety,* **31**, 297–312.

Mataragas, M., Rovetto, F., Bellio, A. et al. (2015). Differential gene expression profiling of *Listeria monocytogenes* in cacciatore and felino salami to reveal potential stress resistance biomarkers. *Food Microbiology,* **46**, 408–417.

Downloaded From Vet-eBooks.com

Maurischat, S., Baumann, B., Martin, A. and Malorny, B. (2015). Rapid detection and specific differentiation of *Salmonella enterica* subsp. *enterica Enteritidis, Typhimurium* and its monophasic variant 4,[5],12:i: By real-time multiplex PCR. *International Journal of Food Microbiology,* **193**, 8–14.

McCabe, E. M., Burgess, C. M., Walsh, D. et al. (2011). Validation of DNA and RNA real-time assays for food analysis using the *hila* gene of *Salmonella enterica* serovars. *Journal of Microbiological Methods,* **84**, 19–26.

Melero, B., Cocolin, L., Rantsiou, K., Jaime, I. and Rovira, J. (2011). Comparison between conventional and qPCR methods for enumerating *Campylobacter jejuni* in a poultry processing plant. *Food Microbiology,* **28**, 1353–1358.

Miszczycha, S. D., Ganet, S., Duniere, L., Rozand, C., Loukiadis, E. and Thevenot-Sergentet, D. (2012). Novel real-time PCR method to detect *Escherichia coli* O157: H7 in raw milk cheese and raw ground meat. *Journal of Food Protection,* **75**, 1373–1381.

Nolan, T., Hands, R. E. and Bustin, S. (2006). Quantification of mRNA using real-time RT-PCR. *Nature Protocols,* **1**, 1559–1582.

Núñez, F., Rodríguez, M., Bermúdez, M. E., Córdoba, J. J. and Asensio, M. A. (1996). Composition and toxigenic potential of the mould population on dry-cured Iberian ham. *International Journal of Food Microbiology,* **32**, 185–197.

Olesen, I., Thorsen, L. and Jespersen, L. (2010). Relative transcription of *Listeria monocytogenes* virulence genes in liver pates with varying NaCl content. *International Journal of Food Microbiology,* **141**, S60–S68.

Osés, S. M., Rantsiou, K., Cocolin, L., Jaime, I. and Rovira, J. (2010). Prevalence and quantification of shiga-toxin producing *Escherichia coli* along the lamb food chain by quantitative PCR. *International Journal of Food Microbiology,* **141**, S163–S169.

Pacholewicz, E., Swart, A., Lipman, L. J., Wagenaar, J. A., Havelaar, A. H. and Duim, B. (2013). Propidium monoazide does not fully inhibit the detection of dead *Campylobacter* on broiler chicken carcasses by qPCR. *Journal of Microbiological Methods,* **95**, 32–38.

Pasquali, F., De Cesare, A., Bovo, F., Serraino, A. and Manfreda, G. (2014). Relative accuracy, specificity and sensitivity of a 5′nuclease real-time PCR assay for *Salmonella* detection in naturally contaminated pork cuts. *Molecular and Cellular Probes,* **28**, 133–137.

Perelle, S., F. Dilasser, J. Grout, and P. Fach. 2007. Screening food raw materials for the presence of the world's most frequent clinical cases of shiga toxin-encoding *Escherichia coli* O26, O103, O111, O145 and O157. *International Journal of Food Microbiology* 113, 284–88.

Perši, N., Pleadin, J., Kovačević, D., Scortichini, G. and Milone, S. (2014). Ochratoxin A in raw materials and cooked meat products made from OTA-treated pigs. *Meat Science,* **96**, 203–210.

Pleadin, J., Perši, N., Kovačević, D., Vahčić, N., Scortichini, G. and Milone, S. (2013). Ochratoxin A in traditional dry-cured meat products produced from subchronic-exposed pigs. *Food Additives and Contaminants: Part A,* **30**, 1827–1836.

Podkowik, M, Park, J. Y, Seo, K. S., Bystroń, J. and Bania, J. (2013). Enterotoxigenic potential of coagulase-negative *Staphylococci*. *International Journal of Food Microbiology,* **163**, 34–40.

Postollec, F., Falentin, H., Pavan, S., Combrisson, J. and Sohier, D. (2011). Recent advances in quantitative PCR (qPCR) applications in food microbiology. *Food Microbiology,* **28**, 848–861.

Downloaded From Vet-eBooks.com

Rantsiou, K., Lamberti, C. and Cocolin, L. (2010). Survey of *Campylobacter jejuni* in retail chicken meat products by application of a quantitative PCR protocol. *International Journal of Food Microbiology,* **141**, S75–S79.

Rantsiou, K., Mataragas, M., Alessandria, V. and Cocolin, L. (2012). Expression of virulence genes of *Listeria monocytogenes* in food. *Journal of Food Safety,* **32**, 161–168.

Raphael, B. H. and Andreadis, J. D. (2007). Real-time PCR detection of the nontoxic nonhemagglutinin gene as a rapid screening method for bacterial isolates harboring the botulinum neurotoxin (A-G) gene complex. *Journal of Microbiological Methods,* **71**, 343–346.

Raymaekers, M., Smets, R., Maes, B. and Cartuyvels, R. (2009). Checklist for optimization and validation of real-time PCR assays. *Journal of Clinical Laboratory Analysis,* **23**, 145–151.

Rijpens, N. and Herman, L. (2004). Comparison of selective and nonselective primary enrichments for the detection of *Listeria monocytogenes* in cheese. *International Journal of Food Microbiology,* **94**, 15–22.

Rodríguez, A., Bernáldez, V., Rodríguez, M., Andrade, M. J., Núñez, F. and Córdoba, J. J. (2015a). Effect of selected protective cultures on ochratoxin A accumulation in dry-cured Iberian ham during its ripening process. *LWT—Food Science and Technology,* **60**, 923–928.

Rodríguez, A., Córdoba, J. J., Werning, M. L., Andrade, M. J. and Rodríguez, M. (2012a). Duplex real-time PCR method with internal amplification control for quantification of verrucosidin producing molds in dry-ripened foods. *International Journal of Food Microbiology,* **153**, 85–91.

Rodríguez, A., Gordillo, R., Andrade, M. J., Córdoba, J. J. and Rodríguez, M. (2016). Development of an efficient real-time PCR assay to quantify enterotoxin-producing staphylococci in meat products. *Food Control,* **60**, 302–308.

Rodríguez, A., Medina, Á., Córdoba, J. J. and Magan, N. (2014). The influence of salt (NaCl) on ochratoxin A biosynthetic genes, growth and ochratoxin A production by three strains of *Penicillium nordicum* on a dry-cured ham-based medium. *International Journal of Food Microbiology,* **178**, 113–119.

Rodríguez, M., Núñez, F., Córdoba, J. J., Bermúdez, E. and Asensio, M. A. (1996). Gram-positive, catalase-positive cocci from dry cured Iberian ham and their enterotoxigenic potential. *Applied and Environmental Microbiology,* **62**, 1897–1902.

Rodríguez, A., Rodríguez, M., Córdoba, J. J. and Andrade, M. J. (2015b). Design of primers and probes for quantitative real-time PCR methods. In *PCR Primer Design (Series: Methods in Molecular Biology)*, Chhandak Basu (ed). New York, NY: Springer.pp. 189–191.

Rodríguez, A., Rodríguez, M., Luque, M. I., Justesen, A. F. and Córdoba, J. J. (2011). Quantification of ochratoxin A-producing molds in food products by SYBR Green and TaqMan real-time PCR methods. *International Journal of Food Microbiology,* **149**, 226–235.

Rodríguez, A., Rodríguez, M., Luque, M. I., Justesen, A. F. and Córdoba, J. J. (2012b). A comparative study of DNA extraction methods to be used in real-time PCR based quantification of ochratoxin A-producing molds in food products. *Food Control,* **25**, 666–672.

Rodríguez, A., Rodríguez, M., Luque, M. I., Martín, A. and Córdoba, J. J. (2012c). Real-time PCR assays for detection and quantification of aflatoxin-producing molds in foods. *Food Microbiology,* **31**, 89–99.

Downloaded From Vet-eBooks.com

Rodríguez, A., Rodríguez, M., Martín, A., Delgado, J. and Córdoba, J. J. (2012d). Presence of ochratoxin A on the surface of dry-cured Iberian ham after initial fungal growth in the drying stage. *Meat Science,* **92**, 728–734.

Rodríguez, A., Werning, M. L., Rodríguez, M., Bermúdez, E. and Córdoba, J. J. (2012e). Quantitative real-time PCR method with internal amplification control to quantify cyclopiazonic acid producing molds in foods. *Food Microbiology,* **32**, 397–405.

Rodríguez-Lázaro, D., Gonzalez-García, P., Gattuso, A., Gianfranceschi, M. V. and Hernandez, M. (2014). Reducing time in the analysis of *Listeria monocytogenes* in meat, dairy and vegetable products. *International Journal of Food Microbiology,* **184**, 98–105.

Rodríguez-Lázaro, D. and Hernández, M. (2013). Real time PCR in food science: Introduction. *Current Issues in Molecular Biology,* **15**, 25–38.

Rodríguez-Lázaro, D., Jofre, A., Aymerich, T., Hugas, M. and Pla, M. (2004). Rapid quantitative detection of *Listeria monocytogenes* in meat products by real-time PCR. *Society,* **70**, 6299–6301.

Satterfield, B. A., Stewart, A. F., Lew, C. S. et al. (2010). A quadruplex real-time PCR assay for rapid detection and differentiation of the *Clostridium botulinum* toxin genes A, B, E and F. *Journal of Medical Microbiology,* **59**, 55–64.

Scheutz, F., Teel, L. D., Beutin, L. et al. (2012). Multicenter evaluation of a sequence-based protocol for subtyping shiga toxins and standardizing stx nomenclature. *Journal of Clinical Microbiology,* **50**, 2951–2963.

Sharma, N. K, Rees, C. E. D., Christine, E. R. and Dodd, C. E. R. (2000). Development of a single-reaction multiplex PCR toxin typing assay for *Staphylococcus aureus* strains. *Applied and Environmental Microbiology,* **66**, 1347–1353.

Singh, P. and Mustapha, A. (2015). Multiplex real-time PCR assays for detection of eight shiga toxin-producing *Escherichia coli* in food samples by melting curve analysis. *International Journal of Food Microbiology,* **215**, 101–108.

Szabo, K., Trojnar, E., Anheyer-Behmenburg, H. et al. (2015). Detection of hepatitis E virus RNA in raw sausages and liver sausages from retail in Germany using an optimized method. *International Journal of Food Microbiology,* **215**, 149–156.

Theoret, J. R., Cooper, K. K., Zekarias, B. et al. (2012). The *Campylobacter jejuni* Dps homologue is important for in vitro biofilm formation and cecal colonization of poultry and may serve as a protective antigen for vaccination. *Clinical and Vaccine Immunology,* **19**, 1426–1431.

Thermo Scientific. (2016). T042-TECHNICAL BULLETIN NanoDrop Spectrophotometers, Assessment of Nucleic Acid Purity. Available at http://www.nanodrop.com/Library/T042-NanoDrop-Spectrophotometers-Nucleic-Acid-Purity-Ratios.pdf (accessed 23 May 2016).

Thisted Lambertz, S., Nilsson, C. and Hallanvuo, S. (2008a). TaqMan-based real-time PCR method for detection of *Yersinia pseudotuberculosis* in food. *Applied and Environmental Microbiology,* **74**, 6465–6469.

Thisted Lambertz, S., Nilsson, C., Hallanvuo, S. and Lindblad, M. (2008b). Real-time PCR method for detection of pathogenic *Yersinia enterocolitica* in food. *Applied and Environmental Microbiology,* **74**, 6060–6067.

VanGuilder, H. D., Vrana, K. E. and Freeman, W. M. (2008). Twenty-five years of quantitative PCR for gene expression analysis. *BioTechniques,* **44**, 619–626.

Velasco, V., Sherwood J.S., Rojas-García P.P., Logue, C.M. Multiplex Real-Time PCR for Detection of *Staphylococcus aureus, mecA* and Panton-Valentine Leukocidin (PVL) Genes from Selective Enrichments from Animals and Retail Meat. *PLOS ONE* 9, e97617.

Downloaded From Vet-eBooks.com

Velusamy, V., Arshak, K., Korostynska, O., Oliwa, K. and Adley, C. (2010). An overview of foodborne pathogen detection: In the perspective of biosensors. *Biotechnology Advances,* **28**, 232–254.

Verhaegen, B., Van Damme, I., Heyndrickx, M. et al. (2016). Evaluation of detection methods for non-O157 shiga toxin-producing *Escherichia coli* from food. *International Journal of Food Microbiology,* **219**, 64–70.

Wang, H., Dong, Y., Wang, G., Xu, X. and Zhou, G. (2016). Effect of growth media on gene expression levels in *Salmonella typhimurium* biofilm formed on stainless steel surface. *Food Control,* **59**, 546–552.

Wolffs, P., Norling, B., Hoorfar, J., Griffiths, M. and Radström, P. (2005). Quantification of *Campylobacter* spp. in chicken rinse samples by using flotation prior to real-time PCR. *Applied and Environmental Microbiology,* **71**, 5759–5764.

Yadav, A. S., Saxena, G. K., Saxena, V. K. and Kataria, J. M. (2016). Study on heat stress response in *Salmonella typhimurium* and *Salmonella enteritidis* and its impact on their attachment to dressed broiler skin surface. *Asian Journal of Animal and Veterinary Advances,* **11**, 114–121.

Ye, K., Zhang, Q., Jiang, Y., Xu, X., Cao, J. and Zhou, G. (2012). Rapid detection of viable *Listeria monocytogenes* in chilled pork by real-time reverse-transcriptase PCR. *Food Control,* **25**, 117–124.

Yoon, S. Y., Chung, G. T., Kang, D. H., Ryu, C., Yoo, C. K. and Seong, W. K. (2005). Application of real-time PCR for quantitative detection of *Clostridium botulinum* type A toxin gene in food. *Microbiology and Immunology,* **49**, 505–511.

Zeaki, N., Cao, R., Skandamis, P. N., Rådström, P. and Schelin, J. (2014). Assessment of high and low enterotoxin a producing *Staphylococcus aureus* strains on pork sausage. *International Journal of Food Microbiology,* **182**, 44–50.

Zell, C., Resch, M., Rosenstein, R., Albrecht, T., Hertel, C. and Götz, F. (2008). Characterization of toxin production of coagulase-negative staphylococci isolated from food and starter cultures. *International Journal of Food Microbiology,* **127**, 246–251.

Zheng, Q., Mikš-Krajnik, M., Yang, Y., Xu, W. and Gyun Yuk, H. (2014). Real-time PCR method combined with immunomagnetic separation for detecting healthy and heat-injured *Salmonella typhimurium* on raw duck wings. *International Journal of Food Microbiology,* **186**, 6–13.

Downloaded From Vet-eBooks.com

5

Sensors and Biosensors for Meat Safety: Recent Advances in Nanotechnology Integration

Rosa Pilolli, Nicoletta Ditaranto, and Linda Monaci

CONTENTS

5.1 Introduction 154
5.1.1 Nanomaterial Implementation for Enhanced Sensing Performance 156
5.2 Advances in Screening Methods for Meat Contamination Monitoring 157
5.2.1 Microbial Contamination 157
5.2.1.1 Optical Fibers and Microarrays 159
5.2.1.2 FTIR Spectroscopy 164
5.2.1.3 Light-Scattering Sensors 166
5.2.1.4 Hyperspectral Imaging 166
5.2.1.5 Latest Trends in Optical Sensors 166
5.2.1.6 Electrochemical and Electromechanical Sensors 167
5.2.1.7 Electronic Nose-Based Sensors 169
5.2.1.8 Advances in Nanotechnology Integration for Microbial Contamination 171
5.2.2 Chemical Contamination 172
5.2.2.1 Veterinary Drug Residues: Overview and Legislative Frame 173
5.2.2.2 Optical Sensors 177
5.2.2.3 Electromechanical Sensors 183
5.2.2.4 Advances in Nanotechnology Integration for Chemical Contamination 184
5.3 Concluding Remarks 187
References 187

Downloaded From Vet-eBooks.com

5.1 Introduction

Meat is an important constituent of the human diet and is consumed worldwide mainly because of its valuable nutrients such as protein, fat, iron, zinc, niacin, and vitamins B6 and B12, all of which are essential for balanced and healthy nutrition. However, both meat and poultry can also become a vehicle of human foodborne diseases, mainly due to the potential contamination of food from chemical and biological hazards. In spite of extensive food safety regulations and excellent monitoring systems in the food industry, one of the greatest challenges for authorities is the control of foodborne diseases. The number of foodborne outbreaks associated with eating meat and poultry products is still raising intense consumer concern about meat safety. The continuous developments in livestock practices have posed newer threats in terms of physical, chemical, and biological hazards for the final product quality and related consumer health. New risks, in addition to the most common pathogens, toxins, drugs, pesticides, and heavy metals, have emerged in recent years due to indiscriminate agriculture and animal husbandry practices causing serious health implications in terms of causing illness.

The more quality and safety are challenged, the more stringent tools are needed to monitor effectively the contamination and to provide marketable products according to prescribed standard parameters. Traditional analytical methods, such as culture and colony counting for pathogen monitoring or liquid chromatography (LC)–tandem mass spectrometry, for the detection of chemical contaminants are still widely used in the meat industry to preserve food safety and quality. However, most of them are time consuming (up to several days), and they are sometimes expensive and require strict adherence to protocol, complicated procedures, and trained personnel. Increase in meat production and threat of contamination have led the industry to pursue rapid and innovative methods of analysis to safeguard the health and safety of consumers. In this context, sensor technology is nowadays gaining more interest, as it is perceived to comprise several advantages over conventional methods such as cost-effectiveness and the possibility for real-time and on-site analysis.

Physical and biological sensors (biosensors) are the two most promising technologies that might fit this purpose. According to the International Union of Pure and Applied Chemistry, a chemical sensor is "a device that transforms chemical information, ranging from the concentration of a specific sample component to total composition analysis, into an analytically useful signal. The chemical information, mentioned above, may originate from a chemical reaction of the analyte or from a physical property of the system investigated" (Hulanicki et al., 1991). Biosensors are "chemical sensors in which the recognition system utilizes a biochemical mechanism" (Thevenot et al., 1999). The recognition mechanism is crucial in biosensing performance. For a long time, recognition elements, such as enzymes or antibodies, isolated from living organisms were preferred for their unquestionable selectivity and

Downloaded From Vet-eBooks.com

specificity, but new artificial and/or engineered recognition elements, such as aptamers, molecularly imprinted polymers (MIPs), phages, and affibodies, were recently tested and improved for their implementation in chemical sensors and biosensors (Table 5.1). Most of these alternative recognition elements

TABLE 5.1

Advantages and Limitations of Recognition Elements in Chemical Sensors and Biosensors

Recognition Elements		Sensor Designation	Advantages	Limitations
Classical	Enzymes	Enzymatic biosensor	Specificity	Purification is costly and time consuming
		–	Simple apparatus and procedures	Poor stability
		–	–	Efficient only at optimum pH and temperature
	Antibodies	Immunosensor	High affinity	Limited target (protein)
		–	Specificity	Laborious production
		–	–	Production requires use of animals
		–	–	Lack of stability
	Nucleic acid	Genosensor	Stability	Limited target (complimentary nucleic acid)
	Whole cells	Whole cell biosensor	Low-cost preparation	–
			Reduced purification requirements	–
Recent	Phages	Phage biosensor	Specificity and sensitivity	Laborious selection
			Stability	Skilled personnel required for selection
	Aptamers	Aptasensor	Easy to modify	Laborious selection
		–	Possibility to design structure	Affinity constant not always comparable to the relevant antibody
		–	Possibility to denaturalize and to rehybridize	–
		–	Possibility to distinguish targets with different functional groups	–
		–	Thermally stable	–
		–	In vitro synthesis	–

(*Continued*)

Downloaded From Vet-eBooks.com

TABLE 5.1 (*Continued*)

Advantages and Limitations of Recognition Elements in Chemical Sensors and Biosensors

Recognition Elements	Sensor Designation	Advantages	Limitations
MIPs	MIP sensor	High thermal, chemical, and mechanical tolerance	Complex fabrication methodology
	–	Reusability	Time-consuming process
	–	Low cost	Incompatibility with aqueous media
	–	–	Leakage of template molecules
Affibodies	Affibody sensor	Lack of disulfide bonds that enables intracellular applications	–
	–	Long shelf life	–

MIPs, molecularly imprinted polymers.

were initially devised to reduce the cost of production but a few of them were proved comparable in term of selectivity with traditional solutions.

Together with the discovery of new recognition elements, the integration of nanotechnology has contributed to progress in sensor performance (Inbaraj and Chen, 2016; Cho et al., 2014; Warriner et al., 2014). In the following sections, an overview of the most recent and relevant achievements in the development of analytical methods for chemical and biological contamination monitoring in meat samples is provided. The contribution will start from the critical discussion of consolidated sensing platforms, and then widen to the new trends and advances achieved by nanotechnology integration.

5.1.1 Nanomaterial Implementation for Enhanced Sensing Performance

The development of novel sensors and biosensors targeted to the food industry has gained a growing interest over the years and represents one of the key fields that has pushed the development of nanobiotechnology and nanomaterial science. The sensing capacity of the detection systems is being improved by employing nanomaterials due to their unique physical and chemical properties. When used in electrical biosensors, they possess a very high capacity for charge transfer, which makes them suitable to reach lower detection limits and higher sensitivity values. Nanomaterials can also contribute as labels or transducers modifiers so as to improve the performance of the biosensor. Some of the reported nanomaterials are quantum dots: they are crystalline clusters in the nanometer range that can be

Downloaded From Vet-eBooks.com

synthesized from semiconductor materials. If so, their exceptional optical properties (such as high quantum yield, high molar extinction coefficients, high resistance to photobleaching, and exceptional resistance to photo and chemical degradation) make them one of the most interesting materials for bioimaging, labeling, and sensing. Similarly, carbon nanotubes (CNTs) and metal nanoparticles (NPs) (AuNPs, AgNPs, etc.) have attracted considerable attention for the assembling of novel electrochemical biosensing systems due to their excellent conductivity and electrocatalytic activity, high surface/volume ratio, and their ability to be functionalized. Thanks to these incomparable properties, nanobiosensors can achieve very low detection limits (even single molecule or cell), can be easily implemented in portable devices, and may ensure higher stability compared to the usual enzymes or dyes employed in the classical methods/devices. In recent years, novel nanomaterials have been greatly used in modifying electrode surfaces to achieve a faster electron transfer of biomolecules. The introduction of nanomaterials into electrochemical sensors also brings further advantages such as decreased overpotentials, ensuring the reversibility of some redox reactions, which are irreversible at unmodified electrodes, and novel labeling opportunities including multidetection capabilities (Pérez-López and Merkoçi, 2011).

5.2 Advances in Screening Methods for Meat Contamination Monitoring

5.2.1 Microbial Contamination

Foodborne diseases can often be caused by the consumption of food contaminated by pathogenic microorganisms such as bacteria, viruses, parasites, and related toxins. Conventional methods for detecting pathogens include microscopy-, nucleic acid-, and immunoassay-based techniques. The gold standard for food pathogen detection is colony counting (CFU) on an agar plate, which takes two to three days for initial results, and up to one week for confirming pathogen specificity. In addition, with the progress made in DNA management and polymerase chain reaction (PCR) researcher attention was moved from targeting the microorganism itself to targeting specific genes. Although the PCR-based techniques and several other molecular diagnostics are highly sensitive and selective, they require undamaged DNA, experienced personnel, and expensive equipment as well as reagents, thus making the overall cost of detection high enough to prevent wide-scale application. As an alternative, immunoassays can be preferred, such as enzyme-linked immunosorbent assays (ELISAs) and Western blot analyses, targeting specific proteins or carbohydrate moieties related to pathogens (Gopinath et al., 2014), providing high sensitivity and molecular fingerprinting of the pathogen. Still, the major enduring limitation of such traditional detection methods is time to results. In Figure 5.1, a scheme of conventional and innovative methods for pathogen detection is presented as

Downloaded From Vet-eBooks.com

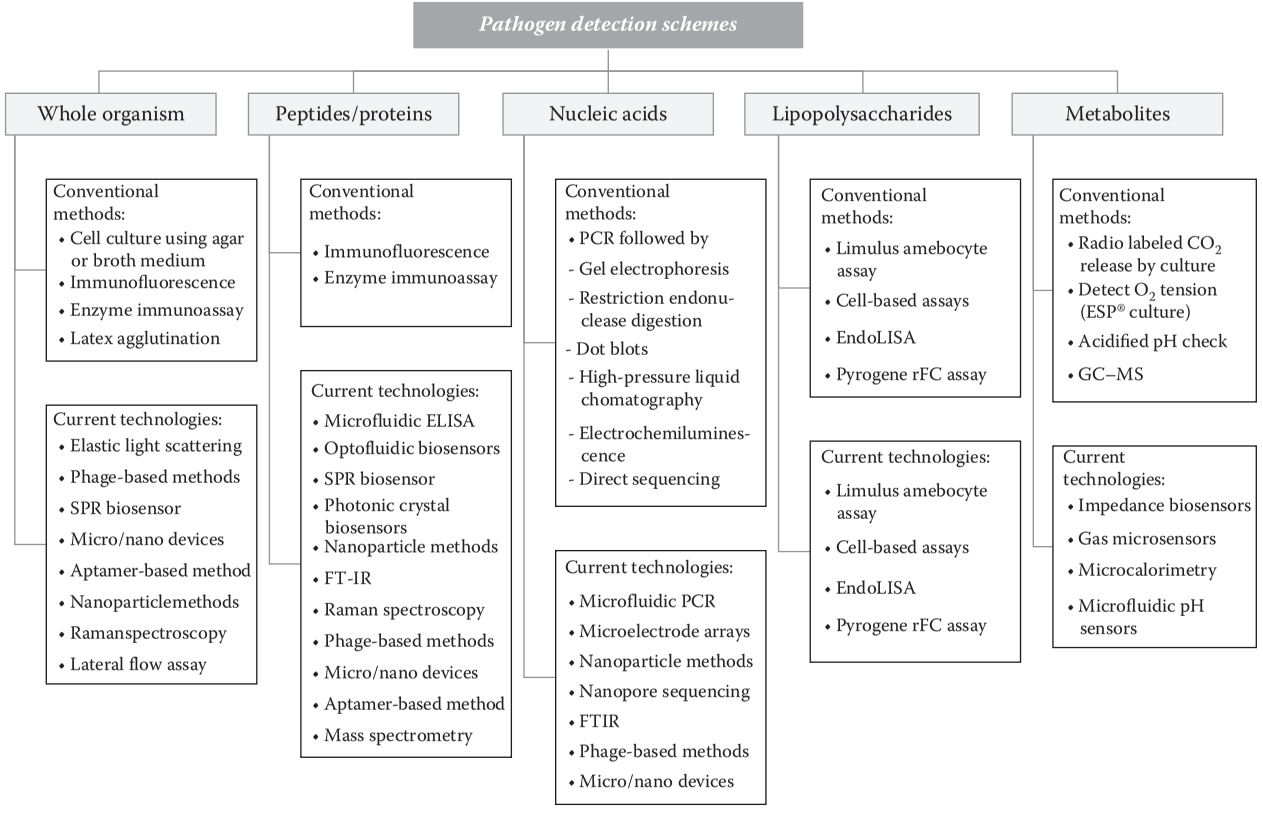


FIGURE 5.1
Overview of pathogen detection techniques. ELISA, enzyme-linked immunosorbent assay; FTIR, Fourier transform infrared spectroscopy; GC–MS, gas chromatography–mass spectrometry; PCR, polymerase chain reaction; rFC, recombinant factor C; SPR, surface plasmon resonance.

Downloaded From: Vet-eBooks.com

a general overview. Rapid detection of food pathogens is challenging because microorganisms need to be recovered from food matrices, differentiated from nonpathogenic microorganisms, and then detected and quantified. This is a big issue for both industry and regulatory agencies because products with short shelf life may spoil before test results are available and because time is critical when identifying and controlling foodborne disease outbreaks.

Food contamination can be spoted by direct targeting of the pathogen itself or by indirect detection of specific metabolites produced by the biochemical processes occurring into the microorganism. In the following sections, some of the most recent and representative investigations on the development of sensing platforms for microbial contamination in meat samples are presented; particular attention is devoted to sensors already tested on real samples and on enhancement. The works have been classified based on the transduction mechanism employed for the detection, and the main features of each investigation are itemized in Table 5.2.

5.2.1.1 Optical Fibers and Microarrays

Among the various phenomena exploited for optical detection, fluorescence, chemiluminescence and absorbance measurements by optical fibers or microarray configurations, and Fourier transform infrared (FTIR) spectroscopy represent the most well-established approaches for meat contamination monitoring. More recently, light scattering and hyperspectral imaging (HI) have been proposed as alternative optical transductions in sensing platforms for their potential to combine spectral and spatial information of the food sample under investigation.

Biosensors based on optical fiber exploit a tapered fiber in total internal reflection operating mode, to send excitation laser light signals to the detection surface and receive emitted light. Light propagation through a fiber or waveguide can be a very sensitive phenomenon, which makes the optical fibers excellent detectors for a variety of applications in the industry, especially the detection of pathogenic organisms. A portable evanescent-wave fiber-optic biosensor to detect *Escherichia coli* O157:H7 in seeded ground beef samples was demonstrated by DeMarco and Lim (2002), reporting 100% correct identification of positive samples obtained at 9.0×10^3 CFU/g for 25 g and at 5.2×10^2 CFU/g for 10 g ground beef samples with silica and polystyrene waveguides, respectively. Similarly, Liu et al. (2003) developed a biosensor, consisting of a chemiluminescence reaction cell, a fiber-optic light guide, and a luminometer linked to a personal computer, in conjunction with immunomagnetic separation for rapid detection of *E. coli* O157:H7. The chemiluminescence biosensor was selective toward *E. coli* O157:H7 even in the presence of other bacteria in the sample, including *Salmonella typhimurium*, *Campylobacter jejuni*, and *Listeria monocytogenes*. The authors could detect *E. coli* O157:H7 in ground beef and chicken carcass, with detection limits of 3.2×10^2 and 4.4×10^2 CFU/g, respectively, and time to results of 1.5 hours without any enrichment. Another fiber-optic portable

Downloaded From Vet-eBooks.com

TABLE 5.2

Summary of Sensors for Microbial/Fungal Contamination Detection

Target Analyte	Transduction System	Nanomaterial Integration	Food Matrix	Recognition Element	Reference
Salmonella typhimurium	Optical fiber (fluorescence)	None	Ground pork samples	Antibody	Ko and Grant (2006)
S. typhimurium	e-nose	None	Fresh beef	None	Balasubramanian et al. (2008)
S. typhimurium	SPR biosensor	None	Chicken carcass	Antibody	Lan et al. (2008)
Salmonella enterica	Optical fiber (fluorescence)	None	Shell egg and chicken breast	Antibody	Valadez et al. (2009)
Listeria monocytogenes	Optical fiber (fluorescence)	None	Ready-to-eat beef, chicken, and turkey	Antibody–aptamer	Ohk et al. (2010)
L. monocytogenes, *Escherichia coli* O157:H7 and *S. enterica*	Optical fiber (fluorescence)	None	Ready-to-eat beef, chicken, and turkey meats	Antibody	Ohk and Bhunia (2013)
E. coli O157:H7	FTIR	None	Ground beef	None	Davis et al. (2010a)
S. typhimurium	QCM	None	Chicken	Antibody	Su and Li (2005)
S. typhimurium	e-nose	None	Beef strip loins	None	Balasubramanian et al. (2005)
S. typhimurium	e-nose	None	Beef strip loins	None	Balasubramanian et al. (2008)
E. coli O157:H7	FTIR	None	Ground beef	None	Davis et al. (2010b)
S. typhimurium	FTIR	None	Beef	None	Amamcharla et al. (2010)
S. typhimurium, *Salmonella dublin*, *Salmonella thompson*, *Staphylococcus aureus*, *Yersinia enterocolitica*, *L. monocytogenes*, *Shigella flexneri*, *Shigella bogdii*, *Campylobacter jejuni*, *E. coli* O157:H7, *Vibrio parahaemolyticus*, *Vibrio cholerae*, *Enterobacter sakazakii*, *Pseudomonas aeruginosa*	Optical microarray	None	Pork	ss-DNA	Bai et al. (2010)

(*Continued*)

Downloaded From: Vet-Books.com

TABLE 5.2 (*Continued*)

Summary of Sensors for Microbial/Fungal Contamination Detection

Target Analyte	Transduction System	Nanomaterial Integration	Food Matrix	Recognition Element	Reference
Toxigenic *Penicillum nordicum*	e-nose	None	Ham	None	Leggieri et al. (2011)
L. monocytogenes	BARDOT light-scattering sensor	None	Hotdog	Protein 60	Koo et al. (2011)
Salmonella	QCM	None	Beef	Odorant-binding protein	Sankaran et al. (2011)
S. typhimurium	e-nose	None	Beef strip loin	None	Balasubramanian et al. (2012)
Salmonella	e-nose	None	Beef	Peptide	Panigrahi et al. (2012)
S. typhimurium	Electrochemical impedence spectroscopy	None	Pork	Antibody	Kim et al. (2013)
E. coli K12	Mie scattering (smartphone)	None	Ground beef	None	Liang et al. (2014)
Salmonella and *Campylobacter*	DVD microarray	None	Chicken	ss-DNA	Tortajada-Genaro et al. (2015)
Campylobacter	Square wave voltammetry	None	Chicken	ss-DNA	Morant-Miñana and Elizalde (2015)
C. jejuni and *Campylobacter coli*	BARDOT light-scattering sensor	None	Chicken	None	He et al. (2015)
P. nordicum	e-nose	Nose	Dry-cured meats	None	Lippolis et al. (2016)
Y. enterocolitica	Differential pulse voltammetry	Chitosan/nano-V2O5/MWCNTs	Pork	ss-DNA	Sun et al. (2010)

(*Continued*)

Downloaded From Vet-Books.com

TABLE 5.2 (*Continued*)

Summary of Sensors for Microbial/Fungal Contamination Detection

Target Analyte	Transduction System	Nanomaterial Integration	Food Matrix	Recognition Element	Reference
Salmonella	Electrochemical impedence spectroscopy	GO and gold NPs	Pork	Aptamer	Ma et al. (2014)
S. aureus	Differential pulse voltammetry	PbS NPs MWCNTs	Beef	ss-DNA	Abdalhai et al. (2014)
E. coli O157:H7	Differential pulse voltammetry	CdS NPs MWCNTs	Beef	ss-DNA	Abdalhai et al. (2015)
Shiga toxin-producing *E. coli*	Microarray (absorbance)	Gold NPs	Ground beef	ss-DNA	Quintela et al. (2015)
E. coli O157:H7	Portable evanescent-wave fiber-optic	None	Beef	Antibody	De Marco and Lim (2002)
E. coli O157:H7	Chemilumines-cence fiber-optic biosensor	None	Ground beef, chicken carcass, and lettuce samples	Antibody	Liu et al. (2003)
S. typhimurium	FRET-based optical fiber	None	Pork	Antibody	Ko and Grant (2006)
Salmonella	Electrochemical impedance spectroscopy	Gold NPs	Pork	Antibody	Yang et al. (2009)

BARDOT, bacterial rapid detection using optical scattering technology; CNT, carbon nanotubes; FRET, fluorescence resonance energy transfer; FTIR, Fourier transform infrared spectroscopy; GO, graphene oxide; MWCNTs, multiwalled carbon nanotubes; NP, nanoparticle; SPR, surface plasmon resonance; QCMs, quartz-crystal microbalances.

Downloaded From: Vet-Books.com

biosensor working on the principle of fluorescence resonance energy transfer (FRET) for rapid detection of *S. typhimurium* in pork samples was reported in 2006 by Ko and Grant. Valadez et al. (2009) also achieved a limit of detection (LOD) of 10^2 CFU/mL for *Salmonella enterica* in egg and chicken after six hours enrichment by a fiber-optic biosensor integrating a fluorescently labeled secondary antibody. Ohk et al. (2010) described an alternative, optical (fluorescence) based, fiber-optic approach in which they used a capture antibody and a fluorescently labeled aptamer to sandwich *L. monocytogenes* with an LOD of 10^2 CFU/25 g in ready-to-eat meat samples. The investigation was recently updated with the same authors achieving multiplexing: *L. monocytogenes*, *E. coli* O157:H7, and *S. enterica* were simultaneously detected in a single assay (Ohk and Bhunia, 2013). Optical waveguides were functionalized with polyclonal antibodies and exposed to bacterial suspensions or enriched food samples (ready-to-eat beef, chicken, and turkey meats) for two hours. Pathogens were detected after reacting with Alexa-Fluor 647-labeled monoclonal antibodies (McAbs). The biosensor was able to detect each pathogen, individually or in a mixture with very little cross-reactivity. The LOD for the sensor was 10^3 CFU/mL for all three pathogens. Furthermore, the biosensor successfully detected each pathogen, grown in a mixture from enriched meat samples in under 24 hours.

Optical microarrays have been quite popular sensing solutions in recent decades for several application fields mainly due to the high multiplexing capabilities and easy read-out not requiring expensive equipment. A few investigations dealt with the detection of microbiological contamination in meat samples, mainly based on the detection of specific DNA target genes preliminarily amplified by PCR.

Bai et al. (2010) described the development of a silicon-based optical thin-film biosensor for the detection of 11 foodborne pathogens, in pork meat, using a microarray approach with biospecific DNA probes immobilized on a sensor surface. Specific probes with aldehyde moieties were arrayed and covalently attached to a hydrazine-derivatized chip surface, and then the biotinylated PCR products were hybridized with their relevant probes. To complete the sandwich assay format, anti-biotin IgG conjugated to horseradish peroxidase (HRP) was then passed over the surface, followed by substrate, which allowed the detection of the target DNA sequence as a concentration-dependent color change on the surface of the optical biosensor. Interestingly, the detection of PCR fragment targets took only 30 minutes. Very recently, a novel microarray biosensor was presented for identification and genotyping of *Salmonella* and *Campylobacter* in meat products (Tortajada-Genaro et al., 2015). Also, this method was based on a DNA microarray developed on a standard DVD as support for the hybridization assay and a DVD driver as scanner; a scheme of the set up was presented in Figure 5.2. This approach was found to be highly sensitive, reproducible, and, interestingly, high working capacity (20 samples per disc). One hundred isolates from meat samples, collected in a poultry factory, were analyzed by the DVD microarraying and fluorescent real-time PCR. An excellent correlation was observed for both generic and specific.

Downloaded From Vet-eBooks.com

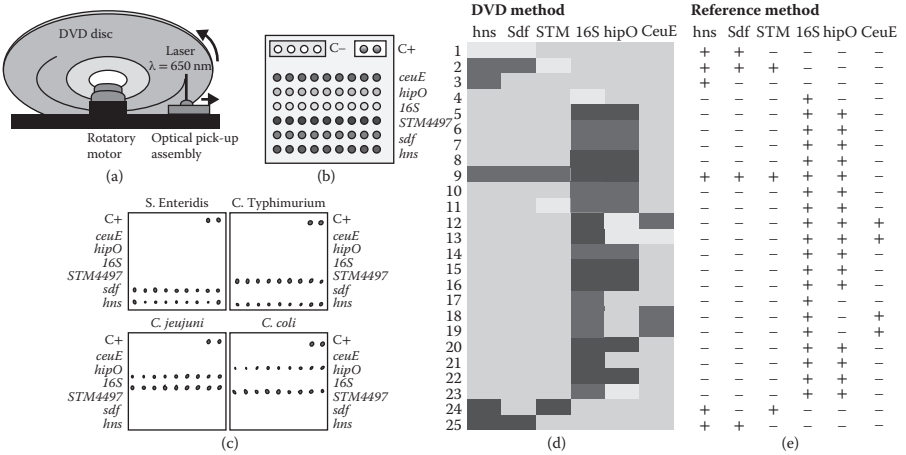


FIGURE 5.2
(a) Scheme of reading principle by a DVD drive: the reflections of the laser beam following the spiral track are converted into electrical pulses and collected by a data acquisition card. (b) Microarray layout: inter-spot distance 1.5 mm, spot diameter 0.55 mm. C–: negative control, noncomplementary probe; C+: positive control, digoxigenin-labeled probe. (c) Microarray images obtained for pure cultures (pathogen concentration 16 × 103 CFU/μL). (d) Results for 25 poultry samples with positive presence of pathogens (color scheme based on optical densities registered by DVD drive: mid-grey-positive, light-grey positive with a low signal (concentration $<10^2$), grey-positive with an intermediate signal (concentration 10^2–10^4), dark grey-positive with a high signal (concentration $>10^4$). (e) Results of reference methods (quantitative polymerase chain reaction [qPCR] and microbiological/biochemical analysis) are expressed as positive presence (+) or negative presence. (With kind permission from **Springer Science+Business Media**: *Analytical and Bioanalytical Chemistry*, Microarray on digital versatile disc for identification and genotyping of *Salmonella* and *Campylobacter* in meat products, 407, 2015, 7285–7294, Tortajada-Genaro, L. A. et al.)

5.2.1.2 FTIR Spectroscopy

Fourier transform infrared (FTIR) spectroscopy is another technique with considerable potential for application in foodborne pathogen optical detection. Among the first applications, in 2002 Ellis et al. reported on an FTIR-based method used directly on the food surface to produce biochemically interpretable fingerprints of meat substrate. The strength of this approach relies on the potential to enhance and accelerate the detection of microbial spoilage. Quantitative interpretation of FTIR spectra was possible using partial least squares (PLSs) regression, which provided accurate estimates of bacterial loads calculated directly from the meat surface in 60 s (Ellis et al., 2002).

Since then, the use of FTIR techniques coupled with different chemometric analyses has been expanded and successfully applied to detect, discriminate, identify, and classify bacteria belonging to different species, including foodborne pathogens such as *Listeria* (Davis et al., 2011), *E. coli* (Kim et al., 2006a;

Downloaded From Vet-eBooks.com

Mura et al., 2012; Davis et al., 2012), and *Salmonella* (Kim et al., 2006b). Detecting pathogenic bacteria in food using FTIR has been done using direct and indirect methods, although the indirect approach is most common. In the direct method, infrared (IR) spectra of the contaminated food are collected directly from the sample and compared with the spectra of a blank (uncontaminated) sample. If successful, direct analyses could enable near real-time food analysis because spectral acquisition requires at most five minutes. The presence of a new absorbance peak and/or a change in peak intensity compared to baseline spectra of uncontaminated sample may indicate the presence of a pathogen. However, in many cases, the spectra collected in a direct method contain a complex spectral background owing to the presence of food matrix, which challenges the spectral interpretation. Indirect detection reduces or eliminates this spectral background from food by using a bacterial separation step such as filtration or immunomagnetic separation prior to spectral acquisition.

Concerning microbial contamination monitoring, beside the total bacterial amount, specific information about their viability is also of major concern in the food industry because injured bacteria pose a significant health threat if they propagate during food distribution and storage. Since FTIR methods rely on the analysis of the biochemical composition of cells, they may be used to differentiate *live* and *dead* microorganisms. As an example, Davis et al. (2010b) used an FTIR method coupled with filtration and immunomagnetic separation to discriminate heat-killed *E. coli* O157:H7 in ground beef. Differences in the spectra were very small and occurred mainly in the amide and nucleic acid regions, likely generated by heat-induced denaturation of these biomolecules in the killing step. For discrimination studies, second derivative spectra were generated to increase the number of distinctive spectral features, and principal component analysis was used to successfully classify live and dead *E. coli* O157:H7. A similar approach was also successfully employed by the same authors for the detection and differentiation of live and heat-treated *S. enterica* serovars inoculated onto chicken breast (Davis et al., 2010a).

In general, microbial contamination can be spotted not only by detection of the offending microorganism itself but also via detection of volatile compounds produced by its metabolism. As an example of such an approach, Amamcharla et al. (2010) proposed an interesting investigation based on the FTIR fingerprinting of headspace volatile compounds in packaged meat to discriminate *Salmonella*-contaminated packed commodities. A suitable headspace sampling setup was designed to collect the headspace volatiles from the packed meat to the FTIR gas cell (Amamcharla et al., 2010). The whole FTIR spectrum was divided into several regions, based on the absorbance properties of various volatiles components in the headspace of meat package. Principal component analysis was performed on the entire spectrum (4000–500 cm^{-1}) as well as on the selected subregions of entire spectrum. Two statistical classification techniques (linear and quadratic discriminate analysis) were used to develop classification models for nondestructive discrimination of *Salmonella*-contaminated packed beef samples from uncontaminated ones.

Downloaded From Vet-eBooks.com

5.2.1.3 Light-Scattering Sensors

Light-scattering sensors measure the spatial distribution of the scattered light from an object, captured by a photodetector, photomultiplier tube (PMT), or charge-coupled device (CCD): the PMT provides high signal gain through signal amplification and is typically used as a point detector, whereas a CCD collects the two-dimensional (2D) spatial distribution of scattered light, allowing the sample imaging. A light-scattering device called BARDOT (bacterial rapid detection using optical scattering technology) that utilizes a 635 nm laser and a CCD detector was firstly developed by Purdue University to capture 2D scattering patterns from bacterial colonies grown on agar medium (Bae et al., 2007; Banada et al., 2007). It directly applies the laser beam to the center of a colony and creates a circular symmetric scatter pattern without destroying the cells; the resulting forward light-scattered image is automatically captured by a CCD sensor. Depending on the colony's physical characteristics (size, shape, and refractive index), cell surface chemical compositions, and the interactions with different agar media, scatter patterns distinctive among bacterial species can be produced. These light-scattering images are automatically processed via software for image features extraction and analysis associated with the BARDOT instrument and bacterial classification can be accomplished at the genus, species, and serovar level based on their similarity to known scatter patterns in a library. Very recently, a novel BARDOT-based sensor was proposed for high-throughput screening of *Campylobacter* colonies in spiked ground chicken and naturally contaminated fresh chicken cuts. Combined with real-time PCR verification, BARDOT was able to identify *Campylobacter* isolates from retail chicken. Moreover, applying passive filtration to food samples facilitated the isolation of pure *Campylobacter* colonies and therefore overcame the interference from the food matrix (He et al., 2015).

5.2.1.4 Hyperspectral Imaging

HI represents an emerging technology based on light scattering, raising interest for its ability of performing real-time assessment and spectral 2D mapping. This technique integrates digital imaging and traditional spectroscopy into a single system to achieve simultaneously both spatial and spectral characterization from an object; such an approach is not detailed in this section, because it is already the subject of Chapter 2 of this book.

5.2.1.5 Latest Trends in Optical Sensors

Among the latest trends in optical sensors, great attention has been paid to smartphone integration in the sensing platform, due to its portability and ubiquitous availability. The ubiquitous availability guarantees a convenient and cost-effective acquisition of smartphones and a large number of potential subscribers for smartphone-based sensor systems. Smartphones can be

Downloaded From Vet-eBooks.com

integrated in biosensing approaches as optical detectors for imaging and optical intensity measurements thanks to their integrated cameras. Several studies suggested that Mie scattering from a biomodified microparticle scan be digitally imaged to report concentrations of proteins and bacteria (San Park et al., 2013). Starting from this idea, Liang et al. (2014) developed a reagentless approach using a smartphone to detect microbial spoilage on ground beef (Figure 5.3). In their work, scanning electron microscopy (SEM) and fluorescence microscopy experiments revealed that *E. coli* could change the size and morphology of fat particles in beef, which modulated Mie scattering in color. The smartphone was also used to record the color at different angles for microbial spoilage detection without antibodies, microbeads, or any other reagents. This study provided a promising approach to apply smartphone-based color quantification in practical monitoring of microbial contamination on food products.

5.2.1.6 Electrochemical and Electromechanical Sensors

By definition, an electrochemical biosensor is a self-contained integrated device, which is capable of providing specific quantitative or semiquantitative

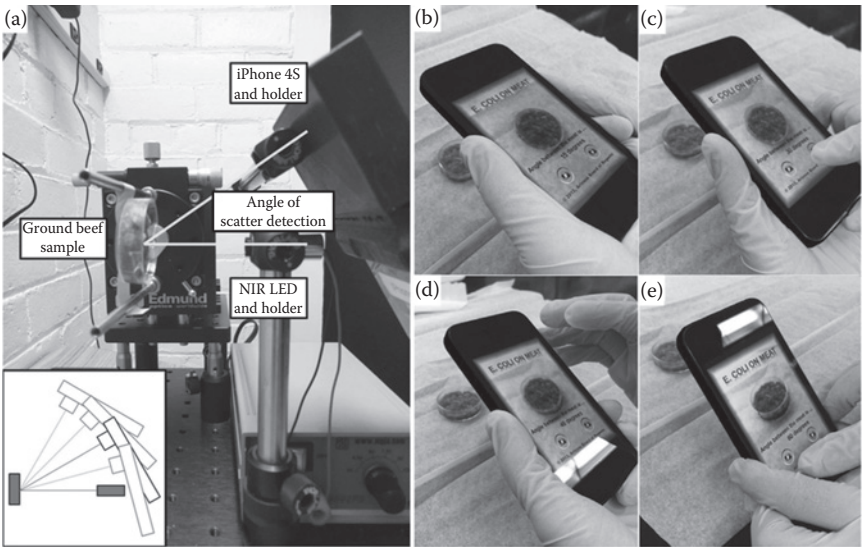


FIGURE 5.3
(a) The benchtop system consists of an iPhone 4S and its holder, a near-infrared light-emitting diode (NIR LED) and its holder, and a ground beef sample and its holder. The angle of scatter detection refers to the angle between the iPhone camera and the NIR LED light source. Photographs showing the operation of the smartphone application at the four specific angles of scatter detection: (b) 15°, (c) 30°, (d) 45°, and (e) 60°. (Reprinted by permission from Macmillan Publishers Ltd., *Scientific Reports*, Liang et al., 2014, copyright 2014.)

Downloaded From Vet-eBooks.com

analytical information using a biological-recognition element, which is retained in direct spatial contact with an electrochemical transduction element (Pilolli et al., 2013). Due to the low cost and ease of miniaturization, electrochemical biosensors hold great promise for particular applications where minimizing size and cost is crucial, such as online contamination monitoring and point-of-care (POC) diagnosis.

Electrochemical biosensors can be classified into potentiometric, amperometric, voltammetric, and impedance types. Potentiometric sensors have been traditionally defined as zero-current devices that measure the potential across an interface, often a membrane. By contrast, both amperometry and voltammetry are based on the measurement of current as a function of applied electrode-solution voltage. During amperometric measurement, the working electrode (i.e., the sensing electrode) is held at constant potential, while the current is monitored as a function of time and related to the concentration of the analyte. During voltammetric measurement, the current of the working electrode is recorded as a function of the potential. Several types of experiment may be performed to gather information from voltammetry (e.g., cyclic, linear sweep, square wave, stripping, and pulse). Recently, Morant-Miñana and Elizalde (2015) reported on the first electrochemical genosensor based on thin-film gold electrodes deposited onto cyclo-olefin polymer (COP) substrates for the detection of *Campylobacter* spp. in poultry meat. This was presented as the last step for the fabrication of a Lab on a Chip, integrating DNA sensor technology into a microfluidic system, believed to perform an automated and complete assay, including sample preparation, PCR amplification, and electrochemical detection of *Campylobacter* spp. in real samples. The sensing element was characterized by several surface techniques and provided a good linear relationship for the concentrations of PCR amplicon of *Campylobacter* spp. between 1 and 25 nM with an LOD of 90 pM.

Impedance biosensors measure the electrical impedance of an interface by imposing a small sinusoidal voltage at a particular frequency and measuring the resulting alternating current. The current–voltage ratio gives the impedance. The impedance of the interface may be measured at a single frequency or at different frequencies, the latter approach known as electrochemical impedance spectroscopy (EIS). An example of an impedimetric biosensor to quantify *S. typhimurium* in meat was developed by Kim et al. in 2013. To allow impedimetric biosensing, an interdigitated microelectrode was fabricated by using a semiconductor fabrication process and a functionalization with anti-*Salmonella* antibodies. A multivariate data analysis was performed on the impedimetric responses. It was assessed that the PLS regression model based on the whole frequency range produced better results than the PLS model based on part of the frequency range or the univariate regression model for the quantitative determination of *S. typhimurium* in PBS and pork samples. The PLS model built with impedance data improved R^2 to 0.85 and 0.89, and reduced the root mean square of error in prediction to 1.13 and 1.02 log CFU/mL for PBS and pork samples, respectively.

Downloaded From Vet-eBooks.com

As electromechanical platforms, quartz crystal microbalances (QCMs) demonstrated remarkable performance in biosensing such as high sensitivity and label-free detection. However, only a few examples of the application of such approaches to meat contamination management can be found. In QCM biosensors, the biological-recognition event generates a mass change of the sensing layer, giving rise to a change in the resonant frequency of the microbalance. Su and Li in 2005 demonstrated a QCM immunosensor for the detection of *S. typhimurium* with simultaneous measurements of significant changes in the resonance frequency and motional resistance. In the direct detection of *S. typhimurium* in chicken meat sample, resonance frequency and motional resistance were proportional to the cell concentration in the range of 10^5–10^8 and 10^6–10^8 cells/mL, respectively. Using anti-*Salmonella* magnetic beads as a separator/concentrator for sample pretreatment as well as a marker for signal amplification, the detection limit was lowered to 10^2 cells/mL based on the resistance measurement.

5.2.1.7 Electronic Nose-Based Sensors

Electronic noses (e-noses) have been applied to microbial contamination detection as artificial olfactory systems to sense the volatile metabolites profile that arise from various food products during bacteria growth. Four major sensor technologies are currently used in commercially available e-noses—metal–oxide–semiconductors (MOSs), metal–oxide–semiconductor field-effect transistors (MOSFETs), conducting organic polymers (CPs), and piezoelectric crystals (Loutfi et al., 2015). In food applications, MOS and CP sensors are most commonly used. The output of MOS and CP sensors to a target compound is represented by a resistance or voltage change caused by two phenomena—one related to chemical interaction of the volatile component with the sensor surface and the other related to electron transport. The rate of change occurring due to these two phenomena defines the magnitude of the sensor responses. Typically, the sensing element in MOS e-noses is usually sensitive to a specific volatile organic compound (VOC) while those of CP sensors are mostly nonspecific. The success and reliability of e-nose systems for safety of food products through their headspace monitoring depend to a large extent on appropriate statistical data analysis and pattern recognition. These pattern recognition techniques help to construct a reliable algorithm for discerning the "smell patterns" acquired from food products for classification or prediction purposes. Smell patterns obtained from the e-nose sensors can be analyzed using various statistical-based pattern recognition tools such as principal component regression, PLSs, factorial discriminant analysis, cluster analysis, and linear and quadratic discriminant analysis (Loutfi et al., 2015).

MOS- and CP-based sensors have been used individually with moderate success in identifying spoilage and contamination in stored beef. Balasubramanian et al. (2005) used a commercially available Cyranose-320™ e-nose

Downloaded From Vet-eBooks.com

system to identify *S. typhimurium* in inoculated beef samples. An e-nose containing an array of 32 conducting polymer sensors was used to obtain the odor patterns of meat headspace emanated from vacuum-packaged beef strip. The system was able to identify meat samples contaminated with *S. typhimurium* at a population concentration level ≥0.7 log CFU/g. Starting from these achievements, the same research group deepened the focus on engineering intelligent olfactory sensors using various sensor techniques to detect *Salmonella* contamination in packaged beef (Panigrahi et al., 2006a,b; Balasubramanian et al., 2008, 2009). More recently, they also investigated the potential of integrating the information from different sensing elements to enhance the discriminatory power of the detection system. As a fact, the reliability and efficiency in olfactory pattern recognition depend on the quality of information obtained by the sensing system and the ability to discard unnecessary information; thus combining information from different sensing systems can increase the prediction capabilities (Steinmetz et al., 1996). In 2012, they proposed the combination of sensor responses from two different e-nose systems, MOS and CP based, as a screening tool in order to identify the presence of *S. typhimurium* in fresh beef strip loins (stored at two temperatures) (Balasubramanian et al., 2012). The data from the two systems were ranked based on their Fisher criteria to evaluate their importance in discriminant analysis. The most informative sensors were then used to develop linear discriminant analysis and quadratic discriminant analysis-based classification models.

In the last two decades, e-noses have been also successfully applied to early detection of fungal growth and potential discrimination between toxigenic and nontoxigenic strains in food samples. Focusing on meat contamination, some examples can be found concerning fungal detection on the surface of dry-cured meat products. Although the fungal colonization contributes to the improvement of the final product quality, some uncontrolled fungal development may also occur on these products causing undesirable alteration and/or synthesis of mycotoxins, such as ochratoxin A (OTA). OTA is a strong nephrotoxic agent and it has been shown to be teratogenic, mutagenic, hepatotoxic, and immunosuppressive to animal species (EFSA, 2006). Clearly, it becomes crucial to prevent and monitor possible contamination of meat by OTA-producing species to avoid undesirable negative economic impact and a potential health hazard to consumers. *Penicillium nordicum* is the most important OTA-producing species frequently isolated from dry-cured meats. Leggieri et al. (2011) demonstrated the potential use of e-nose analysis to analyze qualitatively volatile patterns produced by *P. nordicum* and discriminate between OTA-producing and nonproducing strains on a ham-based medium. Very recently, Lippolis et al. (2016) presented another investigation, which aimed at developing and validating an e-nose-based method for early detection of OTA-producing and nonproducing strains of *Penicillium* during the seasoning process of dry-cured meat. In particular, a MOS e-nose was initially trained by using yeast extract sucrose and

Downloaded From Vet-eBooks.com

meat-based agar media inoculated with OTA-producing and nonproducing strains of *Penicillium.* Afterwards multivariate statistical analysis was used to discriminate the inoculated samples based on the pattern of VOCs. Finally, the approach was validated in dry-cured meat samples produced both at the laboratory (240 e-nose analyses carried out on inoculated sausages, seasoned and sampled at 5, 7, 10, and 14 days) and at an industrial scale (60 dry-cured meat samples produced by an industrial-scale seasoning process) showing a total recognition percentage of 73%.

5.2.1.8 Advances in Nanotechnology Integration for Microbial Contamination

The development of nanoscale materials such as nanowires (NWs), nanofibers, NPs, nanobelts or nanorods (NRs), and nanotubes (NTs) has dramatically changed clinical and molecular biology thanks to their significant use as bioanalyzers. When nanomaterials are being used for the analysis of pathogens, the recognition element in the (bio) sensing platform is typically bound to the surface of the nanomaterial, and the interaction of this hybrid with a pathogen is monitored through a signal transduction mechanism, which explicitly detects the interaction between the pathogen and the recognition element, improving the sensitivity of the analysis. Among the others, *Salmonella, E. coli*, and *Staphylococcus aureus* are the most detected pathogens with such an approach in food matrices. Yang et al. (2009) immobilized *Salmonella* spp. McAbs on AuNPs fabricating a capacitive immunosensor. They detected the interaction using the EIS for the recognition of *Salmonella* spp. in pork samples. The linear relationship between the relative change in capacitance and logarithm of *Salmonella* concentration was obtained in the range of 1.0×10^2 to 1.0×10^5 CFU/mL. The lowest detection limit of *Salmonella* antigen concentration was 1.0×10^2 CFU/mL. This capacitive immunosensor proved to have the advantage of high nonspecific interactions and short analysis time (40 minutes) in comparison with the PCR method. More recently, an electrochemical biosensor was developed for *Salmonella* detection using a *Salmonella*-specific recognition aptamer. The biosensor was based on a glassy carbon electrode modified with graphene oxide (GO) and gold NPs. Then, the aptamer ssDNA sequence was linked to the electrode. After incubation of the modified electrode with *Salmonella*, the electrochemical impedance spectrum was measured to quantify the *Salmonella*. The results revealed an inverse correlation between *Salmonella* added to the reaction system and the current between the electrode and electrolyte, with a detection limit as low as 3 CFU/mL. In particular, the introduction of GO and AuNPs conferred biocompatibility and high electron transfer to the sensor; moreover, the aptamer sensor proved to have a very good selectivity and specificity in the presence of *L. monocytogenes*, *Bacillus subtilis*, *S. aureus*, *Streptococcus pyogenes*, *E. coli*, and *Enterobacter sakazakii* (Ma et al., 2014). Abdalhai et al. (2014, 2015) developed electrochemical genosensors to detect *S. aureus* by

Downloaded From Vet-eBooks.com

using lead sulfide NPs and *E. coli* O157:H7 using cadmium sulfide NPs. The genosensors were prepared by immobilization of complementary DNA on the gold electrode surface, which hybridizes with a specific fragment gene from pathogenic to make a sandwich structure. The conductivity and sensitivity of the sensor were increased by using multiwalled carbon nanotubes (MWCNTs). The peak currents of microorganisms correlated in a linear fashion with the concentration of tDNA. The detection limit was 1.97×10^{-14} M and 3.17×10^{-14} M for *E. coli* and *S. aureus*, respectively. Both the DNA sensors were successfully applied to the pathogens detection in beef samples, after enrichment, extraction, and DNA amplification.

A direct detection of STEC was obtained by Quintela at al. (2015) by an optical biosensing method using oligonucleotide-functionalized AuNPs. This approach allowed a simultaneous visual discrimination and identification of STEC DNA samples following DNA hybridization with highly specific thiol-modified probes immobilized on the surface of AuNPs. Stability and reproducibility of this optical method were demonstrated using artificially inoculated pooled and individual ground beef and blueberry samples. The detection limit was <1 log CFU/g requiring less than 1 hour to complete and achieved 100% specificity.

The biocompatibility of some nanostructures and the excellent electron transfer of carbon NTs have been properly exploited by Sun et al. (2010) in the fabrication of an electrochemical DNA biosensor by using V_2O_5 nanobelts (nano-V_2O_5), MWCNTs, and chitosan (CTS) nanocomposite materials modified carbon ionic liquid electrode to detect *Yersinia enterocolitica* gene sequence in pork meat. Differential pulse voltammetry (DPV) was used to record the electrochemical response of the specific ssDNA sequence with a detection limit as low as 1.76×10^{-12} mol/L.

Overall, the effective detection of pathogens in food samples can be facilitated by nanomaterial-based sensors. Although several classical and modified methods have considerably reduced the incubation time, the nanomaterial-based sensors can detect pathogens and toxins at very low concentrations as they can react and produce a strong signal in a very short incubation time. However, there is still a need for critical evaluation of nanomaterials toxicity as pointed out in several papers (Inbaraj and Chen, 2016, and references therein).

5.2.2 Chemical Contamination

Chemical contamination of meat products can be caused by a large variety of potential health hazards like veterinary drug residues, pesticide residues, toxins, heavy metals, and anions. In this chapter, we focused the discussion only on the first category given the wider existing literature devoted to sensing platforms for detection of such contaminants. In fact, a lot of effort has been spent in developing screening methods for drug residue detection in real meat samples, achieving successful integration of nanotechnology for enhancement of the performance.

Downloaded From Vet-eBooks.com

5.2.2.1 Veterinary Drug Residues: Overview and Legislative Frame

Veterinary drugs are used in livestock to treat disease, promote growth, and improve meat quality in terms of reducing fat and increasing lean meat yield. Very small amounts of veterinary medicines or any related degradation product could accumulate in animal products and enter the food chain, posing a risk factor for public health. The chemical structures of veterinary drugs available are as diverse as their applications, but two main categories can be identified for classification purposes basing on their function, antibiotics, and hormones. The extensive use of antibiotics in food-producing animals has triggered the development of bacterial resistance (Novais et al., 2010), which in recent years has become an international concern as a potential source of antibiotic-resistant bacteria in humans. In addition, the misuse of hormone and growth promoters can cause drastic side effects in humans, such as cramps or tremors in muscles.

Government regulatory authorities control the use of veterinary drugs by approving or registering safe uses and monitoring food for unsafe or prohibited residues, with a quite complex legislative frame. Within the European Union (EU), legislation regarding the control of such drugs is given in Council Directive 96/23/EC (European Commission, 1996), which establishes measures to monitor certain substances and residues thereof, mainly veterinary medicinal products, in live animals and animal products, integrated by Commission Decision 97/747/EC which lays down levels and frequencies of sampling for certain animal products (European Commission, 1997). In order to protect public health, concerns have been raised about the need for a clear definition of the maximum concentration of residues resulting from the use of a veterinary medicinal product that may be accepted by the Community as "legally permitted." With this aim, regulation no. 470/2009 defined procedures for the establishment of maximum residue limits (MRLs) of veterinary medicinal products in food-producing animals and animal products (European Commission, 2009), and regulation no. 37/2010 reported a list of pharmacologically active substances and their classification regarding MRLs (European Commission, 2010). Efforts toward harmonization of regulatory systems across the world led to the agreement among members of Codex Alimentarius Commission concerning the MRLs and Risk Management Recommendations (RMRs) for residues of veterinary drugs in foods (CAC, 2015), but significant differences remain on how risk assessment principles should be placed into practice.

In addition, for those veterinary drugs that have no permitted limit by law (zero-tolerance), minimum required performance limits (MRPLs, defined as the minimum content of an analyte in a sample which has to be detected and confirmed) have been identified according to Commission Decision 2002/657/EC in order to harmonize the analytical performance of specific methods under development and validation (European Commission, 2002). MRPLs for chloramphenicol (CAP), nitrofurans metabolites, and medroxyprogesterone acetate were established by Commission Decision 2003/181/EC (European Commission, 2003).

Downloaded From Vet-eBooks.com

Effective drug residue control requires cost-effective routine screening with the capability of high sample throughput, eventually followed by more expensive chemical confirmation. False negative results are not acceptable in screening assays, although a limited number of false positives may be tolerated. Sensors and particular biosensors that can meet these requirements are probably the most promising solutions for this need; in fact in the last 15 years, a large variety of screening methods were developed (Cháfer-Pericás et al., 2010; Samsonova et al., 2012). Note that only a few of them achieved detection on real samples.

In the following section, an overview of the most interesting and recent achievements in the development of sensing and biosensing platforms for detection of veterinary drugs residues in meat samples is provided; only analytical approaches whose applicability was already assessed on real food matrix are discussed.

5.2.2.1.1 Antibiotics

The antibiotic category includes substances or compounds that either kill bacteria, and are therefore defined as bactericidal, or inhibit their growth, and are therefore defined as bacteriostatic. Antibiotics that target the bacterial cell wall, cell membrane, or interfere with essential bacterial enzymes are usually bactericidal in nature; among these are β-lactams (penicillins and cephalosporins), nitrofurans, polymixins, quinolones, and sulfonamides. Those that target protein synthesis such as the aminoglycosides, macrolides, tetracyclines, and amphenicols are usually bacteriostatic. Some antibiotics occur naturally and can be isolated by fungi, though nowadays most of them are produced industrially, either synthetically or semisynthetically. Among the aforementioned veterinary drugs, the most frequently investigated antibiotic compounds can be identified due to their widespread use, toxicity, and resulting concerns about consumers safety (Baynes et al., 2016).

β-lactam antibiotics have a long history in the treatment of infectious diseases; for nearly six decades, penicillins have been widely used to treat bacterial infections. However, the development of multidrug resistance has reduced their effectiveness. This type of antibiotic can be classified into several groups according to their structural characteristics, but their unique structural feature is the presence of the four-membered β-lactam (2-azetidinone) ring (Figure 5.4). They include penicillins, cephalosporins, and more recently, carbapenems. Many of these feed additives have therapeutic indications such as use for the treatment of erysipelas in turkeys (e.g., penicillin G potassium) or they may have indications for increased rate of weight gain and improved feed efficiency in poultry or swine (e.g., penicillin G).

Quinolones are a family of synthetic broad-spectrum antibiotic drugs, which share the basic structure and a number of common functional groups reported in Figure 5.4. Various modifications of the end moieties have produced compounds with different physical, chemical, pharmacokinetic, and antimicrobial

Downloaded From Vet-eBooks.com

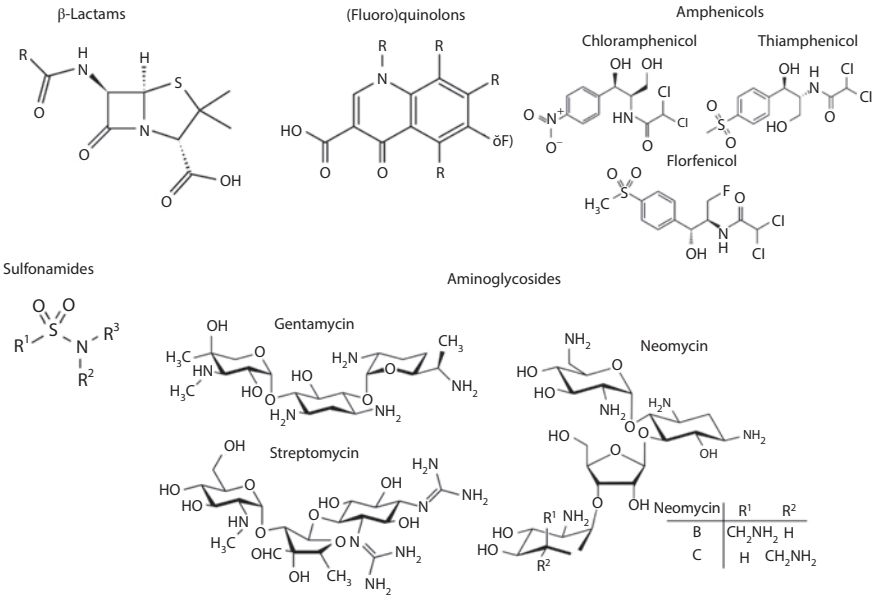


FIGURE 5.4
Chemical structures of the main drugs or drug categories occurring as chemical contaminants in food-producing animals.

properties. For example, substitution at position 6 with a fluorine moiety (fluoroquinolones) markedly enhances activity against gram-negative pathogens and broadens the spectrum of activity against gram-positive pathogens. Ciprofloxacin is a powerful antibiotic belonging to the fluoroquinolone class and is used to treat severe and life-threatening bacterial infections. It interferes with bacterial cell division by inhibition of the enzyme DNA gyrase (necessary for the separation of replicated DNA). However, ciprofloxacin also affects mammalian cell replication and can result in adverse side effects. In most cases, ciprofloxacin causes tendinitis and tendon rupture; other side effects may include central nervous system toxicity, cardiovascular toxicity, renal failure, seizures, etc. Ciprofloxacin is the active metabolite of enrofloxacin, a veterinary antibiotic used in poultry, cattle, pigs, and aquaculture. This wide distribution has raised concern over the possible emergence of fluoroquinolones-resistant strains and resistance transfer between animal and human pathogens, such as *E. coli*, *Salmonella*, and *Campylobacter* in humans.

CAP, thiamphenicol (TAP), and florfenicol (FF) are members of the *amphenicol* family of broad-spectrum antibacterial agents. They are effective against a wide variety of gram-positive and gram-negative bacteria, including most anaerobic organisms. CAP was first isolated from *Streptomyces venezuelae* in 1947 but it is relatively cheap to produce by industrial processes. CAP is highly effective as a bacteriostatic, and may also be bactericidal at high concentrations. It diffuses readily into all body tissues and it is rather rapidly

Downloaded From Vet-eBooks.com

metabolized by conjugation with glucuronic acid. These properties underline the reasons why CAP was widely used for many years in veterinary practice both therapeutically and prophylactically. However, CAP is hematotoxic for humans and may cause life-threatening side effects such as bone marrow aplasia (loss of ability to produce blood cells) and therefore aplastic anemia or fetotoxicity. Since the size of the CAP dose that may lead to these pathologies is still not defined, the use of this antibiotic for the treatment of animals and livestock meant for food production in many countries has been prohibited (EU, United States, Canada, and others). Currently, TAP and FF are used as alternatives to CAP for animal treatment. However, because of its high efficiency, broad spectrum of action, relatively low cost, and availability, CAP is still in use, often illegally.

The sulfonamides have been used in food animal production for over 60 years and they are still utilized in cattle, swine, and poultry; however, their use has somewhat declined, this drug class being the third most commonly used antimicrobial used in food animals (Baynes et al., 2016). A recent study across 25 European countries found that sulfonamides were the third most popular class of antimicrobials used in veterinary medicine (behind tetracyclines and penicillins) and that sulfonamides represented 11% of the total sales of veterinary antimicrobial drugs across Europe in 2011 (Grave et al., 2014). Oral sulfonamides are commonly given to calves with diarrhea, to pigs as a treatment for septicemia and bacterial pneumonia, and to turkeys for the treatment of *E. coli* and *Pasteurella multocida* (fowl cholera). Occasionally, potentiated sulfonamides are used in the poultry industry to control coccidiosis.

Gentamicin, neomycin, and streptomycin make up the majority of aminoglycosides used in livestock production. The immunogenicity of these antimicrobials has not been deeply investigated, but the amino sugars appear to be important epitopes for aminoglycosides. They are employed for treatment of infectious keratoconjunctivitis caused by *Moraxella bovis* in cattle, and for treatment or control of bacterial enteritis in piglets or young chickens and turkeys.

5.2.2.1.2 Hormones Acting as Growth Promoters

A hormone is a chemical messenger produced by all multicellular organisms. They are released by localized cells in the organism and can affect cells in different parts of the organism. Hormones are used for animal breeding to enhance body protein accumulation, metabolize fat stores, and increase lean growth rate. Growth promotion using anabolic hormone treatment rises up to 20% compared to nontreated animals. The β-agonists group (salbutamol [SAL], clenbuterol [CLB], and ractopamine [RAC]) are proven repartitioning agents in meat-producing animals, causing a reduction in fat content and improved production of lean meat. Another group of hormones is corticosteroids (Dexamethasone, Triamcinolone), which help control metabolism, inflammation, immune functions, salt and water balance, development of sexual characteristics, and the ability to withstand illness and injury.

Downloaded From Vet-eBooks.com

β-agonists such as β2-adrenergic receptor agonists are sympathomimetic drugs that mimic the action of epinephrine and norepinephrine. They act on the target cell via membrane-bound G protein-coupled receptors, and are used therapeutically in humans, as well as in veterinary medicine for the treatment of asthmatic respiratory diseases, for tocolysis, and for the treatment of peripheral disturbed blood flow. In higher doses, β2-adrenergic agonists (BAAs) show effects as partitioning agents in food animals, whereby they cause a modification of growth by increasing accretion of skeletal muscle and decreasing fat stores (Mersmann, 1998). Use of BAAs late in the feeding period results in consistent increases in the rate of weight gain without an increase in feed consumption, thereby increasing feed efficiency and cost-effectiveness (Loneragan et al., 2014). The most prominent example is CLB; other β-agonists are RAC, salbumatol, terbutaline, fenoterol, zilpaterol, brombuterol, cimaterol, and mabuterol. The misuse of these agents can cause drastic side effects in humans, such as cramps or tremors in muscles. The more common symptoms shown are aggression, agitation, increased blood pressure, dizziness, and nausea. Typical side effects also include tachycardia, arrhythmia, tremor, hyperglycemia, and hypocalcemia.

CLB is a sympathomimetic amine and β2 agonist with some structural and pharmacological similarities with epinephrine and SAL. It works mostly through increasing the production and secretion of catabolic hormones and leads to increased aerobic capacity. When the central nervous system is stimulated, blood pressure and oxygen transportation increase and this in turn raises the rate of body fat metabolism. It is most commonly available as hydrochloride salt, but is currently prohibited from use as a growth-promoting agent worldwide. RAC is another very common BAA used as a feed additive to promote growth and leanness in pigs. Food safety concerns about RAC divide the global community. Although RAC is not as toxic to humans compared to other β-agonists such as SAL and CLB, long-term misuse of RAC may still result in potentially harmful side effects. In toxicology studies, the primary side effects of RAC are those classically related to BAA, such as dose-dependent increases in heart rate and decreases in diastolic blood pressure, and it is unlikely to be carcinogenic. It should be noted that there have been no reported cases of adverse health effects in humans exposed to livestock products containing RAC residues.

5.2.2.2 Optical Sensors

The most common optical biosensors tailored to antibiotics detection are based on surface plasmon resonance (SPR) transduction (see Table 5.3). SPR biosensors utilize the principle of label-free interaction analysis, with interactions between molecules such as antigen and antibody being monitored in real time. Various assay formats are available (Pilolli et al., 2013); however, as for antibiotics detection, usually indirect assays (mainly inhibition) are preferred given the low molecular weight of the target analyte.

Downloaded From Vet-eBooks.com

TABLE 5.3

Summary of Sensors for Chemical Contamination Detection

Target Analyte	Transduction System	Nanomaterial Integration	Food Matrix	Recognition Element	Reference
Clenbuterol	Differential pulse voltammetry	None	Bovine liver	MIP	Pizzariello et al. (2001)
Streptomycin, dihydrostreptomycin	SPR	None	Milk, honey, and meat	Antibody	Ferguson et al. (2002)
Sulfonamides (sulfamethazine and N4-acetyl-sulfamethazine)	SPR	None	Chicken serum	Antibody	Haasnoot et al. (2003)
Multisulfonamides (sulfamethoxazole and sulfadiazine)	SPR	None	Chicken serum and plasma	Antibody	Haasnoot et al. (2005)
Multisulfonamides (sulfamethazine, sulfadiazine, sulfamethoxazole, sulfaquinoxaline, sulfachloropyridazine)	SPR	None	Chicken serum	Antibody and binding protein	Bienenmann-Ploum et al. (2005)
CAP, CAP glucuronide	SPR	None	Poultry muscle, honey, Prawn, and milk	Antibody	Ferguson et al. (2005)
CAP, CAP glucuronide	SPR	None	Honey, prawns, and porcine kidney	Antibody	Ashwin et al. (2005)
Multisulfonamides	SPR	None	Porcine muscles	Binding protein	McGrath et al. (2005)

(*Continued*)

Downloaded From: Vet-Books.com

TABLE 5.3 (*Continued*)

Summary of Sensors for Chemical Contamination Detection

Target Analyte	Transduction System	Nanomaterial Integration	Food Matrix	Recognition Element	Reference
CAP	Chemiluminescence	None	Pork, beef, chicken, shrimp, and milk	Antibody	Park and Kim (2006)
Multi-(fluoro)quinolones	SPR	None	Egg, fish, and poultry meat	Antibody	Huet et al. (2008)
Multi-(fluoro)quinolones	SPR	None	Egg, fish, and poultry meat	Antibody	Huet et al. (2009)
Tetracyclines	Luminescence	None	Poultry muscle	Cell (bacteria)	Pikkemaat et al. (2010)
Florfenicol	Fluorescence	None	Liver, pork, chicken, and fish	MIP	Ge et al. (2010)
CAP	QCM	None	Chicken muscles	Hapten–protein conjugate and antibody	Karaseva and Ermolaeva (2012)
Ractopamine	SPR	None	Pork	Antibody	Lu et al. (2012)
Ractopamine	Differential pulse voltammetry	None	Pork	None	Yang et al. (2014a)
PenicillinG, ampicillin	QCM	None	Milk, pork, beef, liver	Hapten–protein, antibody	Karaseva and Ermolaeva (2014)
CLB hydrochloride	SPR	None	Pork	Antibody	Li et al. (2014)
Neomycin	Amperometry	Gold NP-decorated CNTs	Beef, pork, chicken	Antibody	Zhu et al. (2010)
CAP	Amperometry	Cadmium sulfide NPs modified-dendrimer	Beef, chicken, pork	Antibody	Kim et al. (2010)
CLB, SAL, terbutaline, ractopamine, dopamine, dobutamine, adrenaline, isoprenaline	Linear sweep voltammetry	GO	Pork (spiked only with CLB)	None	Lin et al. (2013)

(*Continued*)

Downloaded From: Vet-eBooks.com

TABLE 5.3 (*Continued*)

Summary of Sensors for Chemical Contamination Detection

Target Analyte	Transduction System	Nanomaterial Integration	Food Matrix	Recognition Element	Reference
Ractopamine, SAL, CLB	Cyclic voltammetry	Reduced GO and silver–palladium alloy NPs	Pork	Antibody	Wang et al. (2013)
Enrofloxacin	Fluorescence	$Ru(phen)_3^{2+}$–doped silica FN	Chicken	Antibody	Huang et al. (2013)
Streptomycin	Differential pulse voltammetry	Gold–silica nanostructures	Honey, milk, and porcine kidney and muscle	Antibody	Liu et al. (2011)
Quinoxaline-2-carboxylic acid (metabolites of carbadox)	Square wave voltammetry	GO	Pork and chicken muscles	MIP	Yang et al. (2014b)
CLB	Linear sweep voltammetry	Poly(sodium 4-styrenesulfonate) functionalized graphene	Pork	None	Wang et al. (2014)
Tetracycline	Differential pulse voltammetry	Iron/zinc cation–exchanged montmorillonite	Fish, chicken, shrimp	None	Gan et al. (2014)
Penicillin G, ampicillin	QCM	Nanoparticulate MIPs	Chicken meat	MIPs	Karaseva et al. (2016)
Florfenicol	Fluorescence	Mn-doped ZnS quantum dots	Chicken and fish	MIPs	Sadeghi et al. (2016)
Ractopamine and CLB	Differential pulse voltammetry	GO and reduced GO	Pork	None	Wu et al. (2012)
Ractopamine	Differential pulse voltammetry	MWCNT	Pork and liver	None	Liu et al. (2012)

(*Continued*)

Downloaded From: Vet-eBooks.com

TABLE 5.3 (*Continued*)

Summary of Sensors for Chemical Contamination Detection

Target Analyte	Transduction System	Nanomaterial Integration	Food Matrix	Recognition Element	Reference
Ractopamine and SAL	Linear sweep voltammetry	Poly taurine/zirconian NPs	Pig meat (and human urine)	None	Rajkumar et al. (2013)
Ractopamine and SAL	Differential pulse voltammetry	Hybrid CNTs (single- and multiwalled) and Nafion composites	Pork	None	Lin et al. (2013)
Ractopamine	Fluorescence	CdSe quantum dots	Pork	MIP	Liu et al. (2014)
Ractopamine	Electromotive force	Ractopamine–tetraphenylborate complexed NPs	Pork	None	Zhang et al. (2014)
Multisulfonamides	SPR	None	Milk and porcine muscle	Antibody	Gaudin et al. (2007)
Flumequine	SPR	None	Chicken serum and muscles	Antibody	Haasnoot et al. (2007)

CAP, chloramphenicol; CLB, clenbuterol; CNT, carbon nanotubes; FN, fluorescent nanoparticles; GO, graphene oxide; MIPs, molecularly imprinted polymers; MWCNT, multiwalled carbon nanotube; NP, nanoparticles; SAL, salbutamol; SPR, surface plasmon resonance; QCMs, quartz-crystal microbalances.

Downloaded From: Vet-eBooks.com

One of the first examples of SPR-based detection of antibiotics dates back 2002 and reported on the development of an immunosensor inhibition assay for detection of aminoglycoside antibiotics residues (streptomycin and dihydrostreptomycin) in different food matrices (whole bovine milk, honey, porcine kidney, and porcine muscle) (Ferguson et al., 2002). After a few years, the same authors used and validated a commercially available Qflex® kit for Biacore® Q biosensor for the highly accurate and reliable detection of CAP and CAP glucuronide in poultry muscle, honey, prawns, and milk in concentrations below the MRPL (Ferguson et al., 2005). Analogously, another SPR-based screening method for the same analytes was developed and validated according to the EU legislation (European Commission, 2002). This included the determination of decision limits (CCα) and detection capabilities (CCβ) and associated data such as stability of the analyte in matrix. Incurred tissues were used in this study to evaluate the long-term performance and agreement between the screening and a confirmatory LC–mass spectrometry (LC–MS)-based procedure (Ashwin et al., 2005).

As for the screening methods tailored to the specific detection of sulfonamides in food-producing animals, several investigations were carried for single and multiple detection of such drugs in chicken serum (as a predictor of contamination levels in edible tissues) (Haasnoot et al., 2003, 2005; Bienenmann-Ploum et al., 2005) or porcine muscles (McGrath et al., 2005; Gaudin et al., 2007). However, among these, only the latter authored by Gaudin et al. (2007) was subjected to a full validation. In their work, a multisulfonamide antibody was used for the development of two different Biacore protocols, one for the screening of milk samples, the other for muscle samples. The Biacore 3000 system, which is completely automated, was used for high-throughput screening in muscle protocol, widening the applicability assessment to porcine, bovine, and poultry muscle. The different performance characteristics, according to European Decision 2002/657/EC (detection capability CC, specificity/selectivity, precision, stability, and applicability) were determined in relation to the European Union MRL of 100 g/kg for sulfonamides.

Focusing on quinolones monitoring, after the first investigation by Haasnoot et al. (2007), achieving the specific quantitative detection of flumequine in broiler serum and muscle, the research trend pushed toward the development of high-throughput platforms for multiple detection of chemicals belonging to the quinolones category.

With this aim, Huet et al. (2008) developed an optical biosensor inhibition immunoassay, based on the SPR principle, for the screening of 13 quinolones (norfloxacin, sarafloxacin, difloxacin, ciprofloxacin, enrofloxacin, flumequine, danofloxacin, marbofloxacin, pefloxacin, enoxacin, lomefloxacin, ofloxacin, and oxolinic acid). An innovative feature of this investigation was the recourse to bi-active antibody, specifically produced by a dual immunogen prepared after combining features of two complexes (i.e., norfloxacin-BSA and flumequine-BSA). The assay was optimized for detection in three

Downloaded From Vet-eBooks.com

matrices (poultry muscle, fish, and egg), and tested on incurred samples prepared by liquid extraction followed by two washing steps. The biosensor was validated afterward according to criteria specified by European legislation, through assessment of the performance characteristics, that is, detection capability, specificity/selectivity, decision limit, repeatability, ruggedness, and stability (Huet et al., 2009).

Researchers have also described a membrane-based immunosensor for the detection of CAP by chemiluminescence phenomenon in samples of pork, beef, chicken, and shrimp (Park and Kim, 2006). A flow-through cell was devised, connected to an injector and a peristaltic pump inside a dark box, and facing a PMT as light detector in front of it; it exploits competition between CAP and a CAP–HRP conjugate for binding to an anti-CAP antibody immobilized on the membrane. Addition of the peroxidase substrate luminol catalyzed a light-emitting reaction which was measured for the quantification.

Ge et al. (2010) proposed an online coupling of molecular imprinted solid-phase extraction with flow-injection fluorescence sensing for the detection of FF. The method described an FF-imprinted polymer as the recognition part followed by a solid-phase extraction to remove potential interferences from the matrix component. Fluorescence intensity in fluorescence energy transfer (FET) based sensing was inhibited by FF. The proposed sensor was applied successfully to the determination of FF in liver and meat samples.

Focusing on the most recent investigations tailored to the detection of hormone and growth promoters, two different works were reported on the development of SPR-based biosensors for the label-free detection of CLB hydrochloride and RAC, respectively (Li et al., 2014; Lu et al., 2012). In particular, in the latter the authors achieved good results in pork matrix by using a low cost SPR-2004 biosensor and chip created by the Chinese Academy of Sciences, proposed as an alternative to the most common but expensive commercial instruments.

5.2.2.3 Electromechanical Sensors

The application of electromechanical gravimetrical sensors allows for the detection of drug residues in composite matrixes at quite a low level, usually carried out in a competitive format with the preliminary introduction of fixed antibody quantities into the samples. The sensing performance (detection limit, range of detection, accuracy, and reproducibility) of the assay would be mainly influenced by the quality of the sensor electrode coating, which includes the formation of the substrate and the immobilization of the receptor. The use of direct electropolymerization on the gold electrode provides a promising solution for the immobilization of biomolecules since it is possible to control the mass and thickness of the coating in real time by tuning the potential and the scan rate (Cosnier, 2005). Electrogeneration of polyelectrolyte films provides strong binding with the surface of a

Downloaded From Vet-eBooks.com

gold electrode, and hence the immunoaffinity layer resistance to detection/regeneration cycles increases improving the sensor lifetime.

Recently, Karaseva and Ermolaeva (2012, 2014) proposed, in two different investigations, the development and application of a piezoelectric immunosensor prepared by electrogenerated polymer for single and multiresidual contamination monitoring in several food matrixes. In 2012, they developed a competitive assay for CAP detection in meat, milk, eggs, and honey. In 2014, the same authors proposed a QCM-based immunosensor for detecting penicillin G, ampicillin, and the total content of penicillin antibiotics by integration of homologous and group-specific antibodies. The receptor coating of the sensor was obtained by the immobilization of penicillin G or ampicillin hapten–protein conjugates on the polypyrrole film obtained by electropolymerization and activated by glutaraldehyde. The sensors were tested in detecting penicillins in milk, pork, beef, and liver.

5.2.2.4 Advances in Nanotechnology Integration for Chemical Contamination

One of the main concerns in the real samples analysis is the drug extraction procedure, especially when the matrix is a complex one, like food in general and meat in particular. Therefore, sensors able to achieve good performances upon simple treatment procedures are highly desirable. Among them, electrochemical approaches using properly modified electrodes are able to combine the high sensitivity, low cost, and simplicity of the methods with the selectivity induced from the specific nanomaterials employed. This strategy has also the great advantage of eliminating the use of enzymes and mediators, and all the possible negative effects coming from them.

The use of the "rising star" graphene has been described by Wang et al. to successfully determine CLB in pork meat (Wang et al., 2014). They propose poly (sodium 4-styrenesulfonate) (PSS) functionalized graphene nanocomposite as the electrode modifier material for the fabrication of an electrochemical sensor. It was found not only to enhance the enrichment of CLB on the electrode surface by strong π–π interaction, but also had an extraordinarily fast electron transfer rate. Moreover, the PSS functionalized graphene is water-soluble, which facilitates its application in many fields. Applying linear sweep voltammetry, a good linear relationship of the oxidation peak current with respect to concentrations of CLB was achieved with a detection limit of 2.2×10^{-8} mol/L.

A further approach based on the use of graphene is the development of a multiplexed electrochemical sensor for the simultaneous detection of RAC, SAL, and CLB, three β-agonists with similar structure (Wang et al., 2013). Graphene with high conductivity was used as an electrode material to immobilize artificial antigens and amplify electrochemical signal. AgPdNPs are used to label antibodies and generate a strong electrochemical signal in phosphate-buffered saline without any other substrates. Artificial antigens

Downloaded From Vet-eBooks.com

of RAC, SAL, and CLB are, respectively, immobilized onto three working electrodes. RAC, SAL and CLB are separately pre-reacted with the corresponding antibody functionalized AgPd NPs (Ab/AgPdNPs). The free Ab/AgPdNPs are then captured by the corresponding antigens in the competitive electrochemical biosensors. A simple pork extract solution was therefore used, with good recovery percentages and detection limits of 1.52, 1.44, and 1.38 pg/mL for RAC, SAL, and CLB, respectively.

Since RAC contains phenolic hydroxyl group, it could be oxidized at the electrode surface. However, sensors based on direct electrochemical detection of RAC are still limited, maybe due to the poor response activity on ordinary electrode surface. Recently, ordered mesoporous carbon (OMC) has attracted great attention as a novel advanced carbon material, due to its uniform and tailored pore structure, high specific surface area, large pore volume, chemical inertness, and good conductivity; such properties suggest its implementation in electrochemical sensors for the enhancement of sensitivity. In particular, Yang et al. (2014) proposed an electrochemical sensor based on OMC modified glass carbon electrode (OMC/GCE) to detect toxic RAC, demonstrating that the mesoporous film increases the oxidation signal of RAC, given the remarkably enhanced electrocatalytic activity compared with bare GCE surface (Yang et al., 2014). The signal for the determination of RAC was recorded using DPV and after optimization of the experimental conditions, the analytical performances of the electrochemical sensor were evaluated by the determination of RAC in pork samples, obtaining a good linear correlation over drug concentration, with a detection limit of 0.06 μM (Yang et al., 2014a).

In their work, Wu et al. also gave a rationale for the electrochemical behavior of RAC and CLB on the surface of different carbon materials electrode. The presence of graphene resulted in a strong enhancement effect and an increased oxidation signal due to the presence of a large number of oxygen-containing groups responsible for high electrochemical activities (Wu et al., 2012).

Recently, an interesting study has been published reporting the electrocatalytic activity of eight different β-agonists at the surface of a glassy carbon electrode modified with GO and a sodium salt (acid chrome blue K [ACBK]) possessing good electrocatalytic activity toward metal ions and proteins. Electrochemical oxidation of CLB, SAL, adrenaline, RAC, terbutaline, dopamine, dobutamine, and isoprenaline was investigated by cyclic voltammetry at differently modified electrodes (Lin et al., 2014). Briefly, no redox peaks were observed on the simple GCE; the current somewhat increased when measuring at GO/GCE or at ACBK/GCE, while it significantly increased at the ACBK/GO/GCE modified electrode. The detection limits for the eight drugs were in the range 0.58–1.46 ng/mL; the method proved to be reproducible and stable and successfully applicable for quantitative analysis of CLB in pork samples.

Along with the excellent electrochemical properties of graphene, other carbon-based materials like CNTs have gained attention as novel electrode

Downloaded From Vet-eBooks.com

materials due to their fascinating structural, mechanical, physical properties, and effective catalytic activity for the promotion of charge transfer processes. Liu et al. (2012) reported the use of MWCNT water dispersion as a GCE modifier to detect RAC at an LOD of 20 μg/L, with high recovery percentages in pork samples. Hybrid single-walled and MWCNT composites have been also investigated, where the synergistic effect allowed for a more compact structure between single-walled carbon nanotube (SWCNT) and MWCNT, resulting in higher electroactive CNTs concentration on the electrode surface. Amperometric sensors using these electrodes were able to simultaneously detect RAC and SAL in pork samples with LOD as low as 0.05 and 0.1 μM, respectively (Lin et al., 2013).

Following a diverse approach, the NPs can also be intercalated into the electrode modifier film, achieving the same enhancement effect of the drug catalytic activity: the determination of tetracycline in chicken meat samples with a detection limit of 0.10 μM (Gan et al., 2014) and the detection of RAC in pork samples in the range 1–114 μM (Rajkumar et al., 2013) are valuable examples.

As an alternative solution to reduce the effect of the matrices and improve sensitivity, molecularly imprinted technology has attracted increasing attention due to a possible predetermined recognition ability. In particular, MIPs may be used as a synthetic alternative for biomolecular recognition elements (Wei et al., 2006; Dickert et al., 2000; Piletsky and Turner, 2006; Wackerlig and Lieberzeit, 2015). The synthesis of MIPs is carried out in the presence of the molecules that selectivity should be entailed for, ideally establishing selective binding sites within a polymer matrix. Hence, MIPs constitute porous materials providing complementary functional groups capable of specifically recognizing the "template" molecules and subsequently binding them with similar affinities as natural antibodies. Very recently, the introduction of nanomaterials either as the support matrix (Zhang et al., 2011) or as the nanostructured polymer itself (Karasevaa et al., 2016; Liu et al., 2014) further increased selectivity toward determination of antibiotics like erythromycin and penicillins in meat and chicken muscle, respectively.

In the design of a biosensor, NPs can also play the role of the antibody-bearing constituent that is the immunosensor probe. The main advantage is the significant amplification of the response, allowing for the fabrication of ultrasensitive sensors. Usually, metal NPs are employed, such as gold, thanks to the enhancement of catalytic properties at nanometer size. Zhu et al. (2010) reported AuNP-decorated CNTs for the determination of neomycin in real meat samples at an LOD of 6.76 ng/mL; melamine functionalized AuNPs were successfully employed for the simultaneous detection of RAC and SAL in swine meat at an LOD of 10^{-11} mol/L (Zhou et al., 2013). Moreover an ultrasensitive electrochemical immunoassay for streptomycin was accomplished by gold–silica nanostructures (LOD = 5 pg/mL, in kidney and muscle) (Liu et al., 2011). Examples of different nanostructures have been reported as well, such as cadmium sulfide NPs modified-dendrimer for CAP detection were

Downloaded From Vet-eBooks.com

investigated by Kim et al. for real meat samples analysis (LOD = 45 pg/mL) (Kim et al., 2010).

The real goal in the field of food control would be a total portable device sensor, able to perform a fast and selective screening analysis. In this respect, there are very few published papers presenting real tests already performed on food matrices (Chen et al., 2012; Zhao et al., 2008). The Huang research group reported a aRu(phen)$_3^{2+}$-doped silica fluorescent nanoparticle (FN)-based immunochromatographic test strip sensor (ICTS) for rapid, high sensitivity, easy to use, and low-cost quantitative detection of enrofloxacin residues in chicken meat. The fluorescence signal intensity of the FNs at the test line (FIT) and control line (FIC) was determined with a prototype of a portable fluorescent strip reader. The signal was based on FIT/FIC ratio to effectively eliminate strip-to-strip variation and matrix effects (Huang et al., 2013).

5.3 Concluding Remarks

In this chapter, an overview of the most recent and relevant achievements in the development of screening methods for chemical and biological contamination monitoring in meat samples was provided, including critical discussion of consolidated sensing platforms and new trends and advances achieved by nanotechnology integration. A variety of platforms has been reported, and many have shown good promise for high sensitivity and low detection limits, eventually enhanced by the inclusion of NPs and nanostructures. A massive recourse to nanomaterials in the development of routine sensor platforms for the marketplace would require a preventive evaluation of the potential toxicity and proper disposal strategies. Effective success of this technology in the food safety and regulatory field requires rigorous validation with conventional methodologies, testing of real samples with statistically relevant sample numbers, careful evaluation of interferences, and interlaboratory studies; this represents the most important enduring challenge which prevents the achievement of full industry acceptance and regulatory approvals.

References

Abdalhai, M. H., Fernandes, A. M., Bashari, M., Ji, J., He, Q. and Sun, X. (2014). Rapid and sensitive detection of foodborne pathogenic bacteria (*Staphylococcus aureus*) using an electrochemical DNA genomic biosensor and its application in fresh beef. *Journal of Agricultural and Food Chemistry*, **62**, 12659–12667.

Downloaded From Vet-eBooks.com

Abdalhai, M. H., Fernandes, A. M., Xia, X., Musa, A., Ji, J. and Sun, X. (2015). Electrochemical genosensor to detect pathogenic bacteria (*Escherichia coli* O157:H7) as applied in real food samples (fresh beef) to improve food safety and quality control. *Journal of Agricultural and Food Chemistry,* **63**, 5017−5025.

Amamcharla, J. K., Panigrahi, S., Logue, C. M., Marchello, M. and Sherwood, J. S. (2010). Fourier transform infrared spectroscopy (FTIR) as a tool for discriminating *Salmonella typhimurium contaminated beef. Sensing and Instrumentation Food Quality and Safety,* **4**, 1–12.

Ashwin, H. M., Stead, S. L., Taylor, J. C. et al. (2005). Development and validation of screening and confirmatory methods for the detection of chloramphenicol and chloramphenicol glucuronide using SPR biosensor and liquid chromatography–tandem mass spectrometry. *Analytica Chimica Acta,* **529**, 103–108.

Bae, E., Banada, P. P., Huff, K., Bhunia, A. K., Robinson, J. P. and Hirleman, E. D. (2007). Biophysical modeling of forward scattering from bacterial colonies using scalar diffraction theory. *Applied Optics,* **46**, 3639–3648.

Bai, S., Zhao, J., Zhang, Y. et al. (2010). Rapid and reliable detection of 11 foodborne pathogens using thin-film biosensor chips. *Applied Microbiology and Biotechnology,* **86**, 983–990.

Balasubramanian, S., Amamcharla, J., Panigrahi, S., Logue, C. M., Marchello, M. and Sherwood, J. S. (2012). Investigation of different gas sensor-based artificial olfactory systems for screening *Salmonella typhimurium* contamination in beef. *Food and Bioprocess Technology,* **5**, 1206–1219.

Balasubramanian, S., Panigrahi, S., Logue, C. M., Doetkott, C., Marchello, M. and Sherwood, J. S. (2008). Independent component analysis-processed electronic nose data for predicting *Salmonella typhimurium* populations in contaminated beef. *Food Control,* **19**, 236–246.

Balasubramanian, S., Panigrahi, S., Logue, C. M., Gu, H. and Marchello, M. (2009). Neural networks-integrated metal oxide-based artificial olfactory system for meat spoilage identification. *Journal of Food Energy,* **91**, 91–98.

Balasubramanian, S., Panigrahi, S., Logue, C. M., Marchello, M. and Sherwood, J. S. (2005). Identification of *Salmonella*-inoculated beef using a portable electronic nose system. *Journal of. Rapid Methods and Automation in Microbiology,* **13**, 71–95.

Baynes, R. E., Dedonder, K., Kissell, L. et al. (2016). Health concerns and management of select veterinary drug residues. *Food and Chemical Toxicology,* **88**, 112–122.

Banada, P. P., Guo, S., Bayraktar, B. et al. (2007). Optical forward-scattering for detection of *Listeria monocytogenes* and other *Listeria* species. *Biosensors and Bioelectronics,* **22**, 1664–1671.

Bienenmann-Ploum, M., Korpimäki, T., Haasnoot, W. and Kohen, F. (2005). Comparison of multi-sulfonamide biosensor immunoassays. *Analytica Chimica Acta,* **529**, 115–122.

Codex Alimentarius Commission. (2015). Joint FAO/WHO Food Standard Program Codex Alimentarius Commission Thirty-Eighth Session. Maximum Residue Limits (MRLs) and Risk Management Recommendations (RMRs) for Residues of Veterinary Drugs in Foods. Geneva. Available at: http://www.fao.org/fao-who-codexalimentarius/standards/vetdrugs/en/ (accessed 21 June 2016).

Cháfer-Pericás, C., Maquieira, A. and Puchades, R. (2010). Fast screening methods to detect antibiotic residues in food samples. *Trends in Analytical Chemistry,* **29**, 1038–1049.

Downloaded From Vet-eBooks.com

Chen, X., Xu, H., Lai, W., Chen, Y., Yang, X. and Xiong, Y. (2012). A sensitive chromatographic strip test for the rapid detection of enrofloxacin in chicken muscle. *Food Additives and Contaminants: Part A,* **29**, 383–391.

Cho, I.-H., Radadia, A. D., Farrokhzad, K. et al. (2014). Nano/micro and spectroscopic approaches to food pathogen detection. *Annual Review of Analytical Chemistry,* **7**, 65–88.

Cosnier, S. (2005). Affinity biosensors based on electropolymerized films. *Electroanalysis,* **17**, 1701–1712.

Davis, R., Burgula, Y., Deering, A., Irudayaraj, J., Reuhs, B. L. and Mauer, L. J. (2010a). Detection and differentiation of live and heat-treated *Salmonella enterica* serovars inoculated onto chicken breast using Fourier transform infrared (FT-IR) spectroscopy. *Journal of Applied Microbiology,* **109**, 2019–2031.

Davis, R., Irudayaraj, J., Reuhs, B. L. and Mauer, L. J. (2010b). Detection of *E. coil* O157:H7 from ground beef using Fourier transform infrared (FT-IR) spectroscopy and chemometrics. *Journal of Food Science,* **75**, M340–M346.

Davis, R. and Mauer, L. J. (2011). Subtyping of *Listeria monocytogenes* at the haplotype level by Fourier transform infrared (FT-IR) spectroscopy and multivariate statistical analysis. *International Journal of Food Microbiology,* **150**, 140–149.

Davis, R., Paoli, G. and Mauer, L. J. (2012). Evaluation of Fourier transform infrared (FT-IR) spectroscopy and chemometrics as a rapid approach for sub-typing *Escherichia coli* O157:H7 isolates. *Food Microbiology,* **31**, 181–190.

De Marco, D. R. and Lim, D. V. (2002). Detection of *Escherichia coli* O157:H7 in 10- and 25-gram ground beef samples with an evanescent-wave biosensor with silica and polystyrene waveguides. *Journal of Food Protection,* **65**, 596–602.

Dickert, F. L., Lieberzeit, P. and Tortschanoff, M. (2000). Molecular imprints as artificial antibodies—A new generation of chemical sensors. *Sensors and Actuators B,* **65**, 186–189.

Ellis, D. I., Broadhurst, D., Kell, D. B., Rowland, J. J. and Goodacre, R. (2002). Rapid and quantitative detection of the microbial spoilage of meat by Fourier transform infrared spectroscopy and machine learning. *Applied and Environmental Microbiology,* **68**, 2822–2828.

European Commission. (1996). Council Directive 96/23/EC of 29 April 1996 on measures to monitor certain substances and residues thereof in live animals and animal products and repealing Directives 85/358/EEC and 86/469/EEC and Decision 89/187/EEC and 91/664/EEC. *Official Journal of the European Union,* **L125**, 10–32.

European Commission. (1997). Commission Decision of 27 October 1997 fixing the levels and frequencies of sampling provided for by Council Directive 96/23/EC for the monitoring of certain substances and residues thereof in certain animal products. *Official Journal of the European Union,* **L303**, 12–15.

European Commission. (2002). Commission Decision 2002/657/EC of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results. *Official Journal of the European Union,* **L221**, 8–36.

European Commission. (2003). Commission Decision 2003/181/EC of 13 March 2003 amending Decision 2002/657/EC as regards the setting of minimum required performance limits (MRPLs) for certain residues in food of animal origin. *Official Journal of the European Union,* **L71**, 17–18.

Downloaded From Vet-eBooks.com

European Commission. (2009). Regulation (EC) No 470/2009 of the European Parliament and of the Council of 6 May 2009 laying down Community procedures for the establishment of residue limits of pharmacologically active substances in foodstuffs of animal origin, repealing Council Regulation (EEC) No 2377/90 and amending Directive 2001/82/EC of the European Parliament and of the Council and Regulation (EC) No 726/2004 of the European Parliament and of the Council. *Official Journal of the European Union,* **L152**, 11–22.

European Commission. (2010). Commission Regulation (EU) No 37/2010 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin. *Official Journal of the European Union,* **L15**, 1–72.

European Food Safety Authority. (2006). Opinion of the scientific panel on contaminants in the food chain of the EFSA on a request from the Commission related to ochratoxinA in food. *EFSA Journal*, **365**, 1–56.

Ferguson, J. P., Baxter, G. A., McEvoy, J. D. G., Stead, S., Rawlings E. and Sharman, M. (2002). Detection of streptomycin and dihydrostreptomycin residues in milk, honey and meat samples using an optical biosensor. *Analyst,* **127**, 951–956.

Ferguson, J., Baxtera, A., Young, P. et al. (2005). Detection of chloramphenicol and chloramphenicol glucuronide residues in poultry muscle, honey, prawn and milk using a surface plasmon resonance biosensor and Qflex® kit chloramphenicol. *Analytica Chimica Acta,* **529**, 109–113.

Gan, T., Shi, Z., Sun, J. and Liu, Y. (2014). Simple and novel electrochemical sensor for the determination of tetracycline based on iron/zinc cations-exchanged montmorillonite catalyst. *Talanta,* **121**, 187–193.

Gaudin, V., Hdou, C. and Sanders, P. (2007). Validation of a Biacore method for screening eight sulfonamides in milk and porcine muscle tissues according to European decision 2002/657/EC. *Journal of AOAC International,* **90**, 1706–1715.

Ge, S., Yan, M., Cheng, X. et al. (2010). On-line molecular imprinted solid-phase extraction flow-injection fluorescence sensor for determination of florfenicol in animal tissues. *Journal of Pharmaceutical and Biomedical Analysis,* **52**, 615–619.

Gopinath, S. C. B., Tang, T.-H., Chen, Y., Citartan, M. and Lakshmipriya, T. (2014). Bacterial detection: From microscope to smartphone *Biosensors and Bioelectronics,* **60**, 332–342.

Grave, K., Torren-Edo, J., Muller, A., Greko, C., Moulin, G. and Mackay, D. (2014). Variations in the sales and sales patterns of veterinary antimicrobial agents in 24 European countries. *Journal of Antimicrobial Chemotherapy,* **69**, 2284–2291.

Haasnoot, W., Bienenmann-Ploum, M. and Kohen, F. (2003). Biosensor immunoassay for the detection of eight sulfonamides in chicken serum. *Analytica Chimica Acta,* **483**, 171–180.

Haasnoot, W., Bienenmann-Ploum, M., Lamminmäki, U., Swanenburg, M. and van Rhijn, H. (2005). Application of a multi-sulfonamide biosensor immunoassay for the detection of sulfadiazine and sulfamethoxazole residues in broiler serum and its use as a predictor of the levels in edible tissue. *Analytica Chimica Acta,* **552**, 87–95.

Haasnoot, W., Gerçek, H., Cazemier, G. and Nielen, M. W. (2007). Biosensor immunoassay for flumequine in broiler serum and muscle. *Analytica Chimica Acta,* **586**, 312–318.

Downloaded From Vet-eBooks.com

He, Y., Reed, S., Bhunia, A. K., Gehring, A., Nguyen, L. H. and Irwin, P. L. (2015). Rapid identification and classification of *Campylobacter* spp. Using laser optical scattering technology. *Food Microbiology,* **47**, 28–35.

Huang, X., Aguilar, Z. P., Li, H. et al. (2013). Fluorescent Ru $(phen)_3^{2+}$-doped silica nanoparticles-based ICTS sensor for quantitative detection of enrofloxacin residues in chicken meat. *Analytical Chemistry,* **85**, 5120–5128.

Huet, A. C., Charlier, C., Singh, G. S. et al. (2008). Development of an optical surface plasmon resonance biosensor assay for (fluoro) quinolones in egg, fish, and poultry meat. *Analytica Chimica Acta,* **623**, 195–203.

Huet, A. C., Charlier, C., Weigel, S., Godefroy, S. B. and Delahaut, P. (2009). Validation of an optical surface plasmon resonance biosensor assay for screening (fluoro) quinolones in egg, fish and poultry. *Food Additives & Contaminants. Part A, Chemistry, Analysis, Control, Exposure & Risk Assessment,* **26**, 1341–1347.

Hulanicki, A., Glab, S. and Ingman, F. (1991). Chemical sensors. Definitions and classification. *Pure and Applied Chemistry,* **63**, 1247–1250.

Inbaraj, B. S. and Chen, B. H. (2016). Nanomaterial-based sensors for detection of foodborne bacterial pathogens and toxins as well as pork adulteration in meat products. *Journal of Food and Drug Analysis,* **24**, 15–28.

Karaseva, N. A. and Ermolaeva, T. N. (2012). A piezoelectric immunosensor for chloramphenicol detection in food. *Talanta,* **93**, 44–48.

Karaseva, N. A. and Ermolaeva, T. N. (2014). Piezoelectric immunosensors for the detection of individual antibiotics and the total content of penicillin antibiotics in foodstuffs. *Talanta,* **120**, 312–317.

Karasevaa, N., Ermolaevaa, T. and Mizaikoff, B. (2016). Piezoelectric sensors using molecularly imprinted nanospheres for the detection of antibiotics. *Sensors and Actuators B: Chemical,* **225**, 199–208.

Kim, S., Burgula, Y., Ojanen-Reuhs, T., Cousin, M. A., Reuhs, B. L. and Mauer, U. J. (2006a). Differentiation of crude lipopolysaccharides from *Escherichia coli* strains using Fourier transform infrared spectroscopy and chemometrics. *Journal of Food Science,* **71**, M57–M61.

Kim, S., Kim, H., Reuhs, B. L. and Mauer, L. J. (2006b). Differentiation of outer membrane proteins from *Salmonella enterica* serotypes using Fourier transform infrared spectroscopy and chemometrics. *Letters in Applied Microbiology,* **42**, 229–234.

Kim, G., Moon J. H. and Morgan, M. (2013). Multivariate data analysis of impedimetric biosensor responses from *Salmonella typhimurium*. *Analytical Methods,* **5**, 4074–4080.

Kim, D. M., Rahman, M. A., Do, H., Ban, C. and Shim, Y. B. (2010). An amperometric chloramphenicol immunosensor based on cadmium sulfide nanoparticles modified-dendrimer bonded conducting polymer. *Biosensors and Bioelectronics,* **25**, 1781–1788.

Ko, S. H. and Grant, S. A. (2006). A novel FRET-based optical fiber biosensor for rapid detection of *Salmonella typhimurium*. *Biosensors and Bioelectronics,* **21**, 1283–1290.

Koo, O.K., Aroonnual, A., Bhunia, A.K. (2011). Human heat-shock protein 60 receptor-coated paramagnetic beads show improved capture of Listeria monocytogenes in the presence of the other Listeria in food. *Journal of Applied Microbiology*, 111, 93-104.

Downloaded From Vet-eBooks.com

Lan, Y.B., Wang, S.-Z., Yin, Y.-G., Hoffmann W.C., Zheng X. (2008) Using a surface plasmon resonance biosensor for rapid detection of *Salmonella Typhimurium* in Chicken Carcass. *Journal of Bionic Engineering*, 5, 239–246.

Leggieri, M. C., Pont, N. P., Battilani, P. and Magan, N. (2011). Detection and discrimination between ochratoxin producer and non-producer strains of *Penicillium nordicum* on a ham-based medium using an electronic nose. *Mycological Research,* **27**, 29–35.

Li, Y., Qi, P., Ma, X. and Zhong, J. (2014). Quick detection technique for clenbuterol hydrochloride by using surface plasmon resonance biosensor. *European Food Research and Technology,* **239**, 195–201.

Liang, P. S., San Park, T. and Yoon, J.-Y. (2014). Rapid and reagentless detection of microbial contamination within meat utilizing a smartphone-based biosensor. *Scientific Reports,* **4**, 5953.

Lin, K. C., Hong, C. P. and Chen, S. M. (2013). Simultaneous determination for toxic ractopamine and salbutamol in pork sample using hybrid carbon nanotubes. *Sensors and Actuators B: Chemical,* **177**, 428–436.

Lin, X., Ni, Y. and Kokot, S. (2014). A novel electrochemical sensor for the analysis of β-agonists: The poly (acid chrome blue K)/graphene oxide-nafion/glassy carbon electrode. *Journal of Hazardous Materials,* **260**, 508–517.

Lippolis, V., Ferrara, M., Cervellieri, S. et al. (2016). Rapid prediction of ochratoxin A-producing strains of Penicillium on dry-cured meat by MOS-based electronic nose. *International Journal of Food Microbiology,* **218**, 71–77.

Liu, H., Fang, G. and Wang, S. (2014). Molecularly imprinted optosensing material based on hydrophobic CdSe quantum dots via a reverse microemulsion for specific recognition of ractopamine. *Biosensors and Bioelectronics,* **55**, 127–132.

Liu, Y., Ye J. and Li, Y. (2003). Rapid detection of *Escherichia coli* O157:H7 inoculated in ground beef, chicken carcass, and lettuce samples with an immunomagnetic chemiluminescence fiber-optic biosensor. *Journal of Food Protection,* **66**, 512–517.

Liu, B., Zhang, B., Cui, Y., Chen, H., Gao, Z. and Tang, D. (2011). Multifunctional gold silica nanostructures for ultrasensitive electrochemical immunoassay of streptomycin residues. *ACS Applied Materials and Interfaces,* **3**, 4668–4676.

Liu, Z., Zhou, Y., Wang, Y., Cheng, Q. and Wu, K. (2012). Enhanced oxidation and detection of toxic ractopamine using carbon nanotube film-modified electrode. *Electrochimica Acta,* **74**, 139–144.

Loneragan, G. H., Thomson, D. U. and Scott, H. M. (2014). Increased mortality in groups of cattle administered the beta-adrenergic agonists ractopamine hydrochloride and zilpaterol hydrochloride. *PLOS ONE,* **9**, e91177, 1–13.

Loutfi, A., Coradeschi, S., Mani, G. K., Shankar, P., Bosco J. and Rayappan, B. (2015). Electronic noses for food quality: A review. *Journal of Food Engineering,* **144**, 103–111.

Lu, X., Zheng, H., Li, X. Q. et al. (2012). Detection of ractopamine residues in pork by surface plasmon resonance-based biosensor inhibition immunoassay. *Food Chemistry,* **130**, 1061–1065.

Ma, X., Jiang, Y., Jia, F., Yu, Y., Chen, J. and Wang, Z. (2014). An aptamer-based electrochemical biosensor for the detection of *Salmonella*. *Journal of Microbiological Methods,* **98**, 94–98.

McGrath, T., Baxter, A., Ferguson, J., Haughey, S. and Bjurling, P. (2005). Multi sulfonamide screening in porcine muscle using a surface plasmon resonance biosensor. *Analytica Chimica Acta,* **529**, 123–127.

Downloaded From Vet-eBooks.com

Mersmann, H. J. (1998). Overview of the effects of beta-adrenergic receptor agonists on animal growth including mechanisms of action. *Journal of Animal Science,* **76**, 160–172.

Morant-Miñana, M. C. and Elizalde, J. (2015). Microscale electrodes integrated on COP for real sample *Campylobacter* spp. detection. *Biosensors and Bioelectronics,* **70**, 491–497.

Mura, S., Greppi, G., Marongiu, M. L. et al. (2012). FT-IR nanobiosensors for *Escherichia coli* detection. *Beilstein Journal of Nanotechnology,* **3**, 485–492.

Novais, A., Comas, I., Baquero, F. et al. (2010). Evolutionary trajectories of beta-lactamase CTX-M-1 cluster enzymes: Predicting antibiotic resistance. *PLOS Pathogens,* **6**, e1000735, 1–16.

Ohk, S. H. and Bhunia, A. K. (2013). Multiplex fiber optic biosensor for detection of *Listeria monocytogenes*, *Escherichia coli* O157:H7 and *Salmonella enterica* from ready-to-eat meat samples. *Food Microbiology,* **33**, 166–171.

Ohk, S. H., Koo, O. K., Sen T., Yamamoto, C. M. and Bhunia, A. K. (2010). Antibody aptamer functionalized fibre-optic biosensor for specific detection of *Listeria monocytogenes* from food. *Journal of Applied Microbiology,* **109**, 808–817.

Panigrahi, S., Balasubramanian, S., Gu, H., Logue, C. M. and Marchello, M. (2006a). Neural network-integrated electronic nose system for identification of spoiled beef. *LWT Food Science and Technology,* **39**, 135–145.

Panigrahi, S., Balasubramanian, S., Gu, H., Logue, C. M. and Marchello, M. (2006b). Design and development of a metal oxide based electronic nose for spoilage classification of beef. *Sensors and Actuators B: Chemical,* **119**, 2–14.

Panigrahi, S., Sankaran, S., Mallik, S., Gaddam, B. and Hanson, A. A. (2012). Olfactory receptor-based polypeptide sensor for acetic acid VOC detection. *Materials Science and Engineering: C,* **32**, 1307–1313.

Park, I. S. and Kim, N. (2006). Development of a chemiluminescent immunosensor for chloramphenicol. *Analytica Chimica Acta,* **578**, 19–24.

Pérez-López, B. and Merkoçi, A. (2011). Nanomaterials based biosensors for food analysis applications. *Trends in Food Science & Technology,* **22**, 625–639.

Pikkemat, M.G., Rapallini, M.L.B.A., Karp M.T., Elferink, J.W.A. (2010). Application of a luminescent bacterial biosensor for the detection of tetracyclines in routine analysis of poultry muscle samples, *Food Additives & Contaminants: Part A: Chemistry, Analysis, Control, Exposure & Risk Assessment*, 27, 1112–1117.

Piletsky, S. and Turner, A. (2006). New generation of chemical sensors based on MIPs. *In: Molecular Imprinting of Polymers*, Piletsky, S. and Turner, A.P.F. (Eds.), United States: 1–16.

Pilolli, R., Monaci, L. and Visconti, A. (2013). Advances in biosensor development based on integrating nanotechnology and applied to food-allergen management. *Trends in Analytical Chemistry,* **47**, 12–26.

Pizzariello, A., Stred'ansky, M., Stred'anska, S., Stanislav, M. (2001). A solid binding matrix/molecularly imprinted polymer-based sensor system for the determination of clenbuterol in bovine liver using differential-pulse voltammetry. *Sensors and Actuators* B, 76, 286–294.

Quintela, I. A., de los Reyes, B. G., Lin, C. S. and Wu, V. C. H. (2015). Simultaneous direct detection of Shiga-toxin producing *Escherichia coli* (STEC) strains by optical biosensing with oligonucleotide-functionalized gold nanoparticles. *Nanoscale,* **7**, 2417–2426.

Downloaded From Vet-eBooks.com

Rajkumar, M., Li, Y. S. and Chen, S. M. (2013). Electrochemical detection of toxic ractopamine and salbutamol in pig meat and human urine samples by using poly taurine/zirconia nanoparticles modified electrodes. *Colloids and Surfaces. B, Biointerfaces,* **110**, 242–247.

Sadeghi, S., Jahani, M. and Belador, F. (2016). The development of a new optical sensor based on the Mn doped ZnS quantum dots modified with the molecularly imprinted polymers for sensitive recognition of florfenicol. *Spectrochimica Acta. Part A, Molecular and Biomolecular Spectroscopy,* **159**, 83–89.

Samsonova, J. V., Cannavan A. and Elliott, C. T. (2012). A critical review of screening methods for the detection of chloramphenicol, thiamphenicol, and florfenicol residues in foodstuffs. *Critical Reviews in Analytical Chemistry,* **42**, 50–78.

San Park, T., Li, W., McCracken, K. E. and Yoon, J. Y. (2013). Smartphone quantifies *Salmonella* from paper microfluidics. *Lab on a Chip,* **13**, 4832–4840.

Sankaran, S., Suranjan, P., Mallik, S. (2011). Odorant binding protein based biomimetic sensors for detection of alcohols associated with Salmonella contamination in packaged beef. *Biosensors and Bioelectronics*, 26, 3103–3109.

Su, X. L. and Li, Y. B. (2005). A QCM immunosensor for *Salmonella* detection with simultaneous measurements of resonant frequency and motional resistance. *Biosensors and Bioelectronics,* **21**, 840–848.

Steinmetz, V., Crochon, M., BellonMaurel, V., Garcia Fernandez, J. L., Barreiro Elorza, P. and Verstreken, L. (1996). Sensors for fruit firmness assessment: Comparison and fusion. *Journal of Agricultural Engineering Research,* **64**, 15–28.

Sun, W., Qin, P., Gao, H., Li, G. and Jiao, K. (2010). Electrochemical DNA biosensor based on chitosan/nano-V2O5/MWCNTs composite film modified carbon ionic liquid electrode and its application to the LAMP product of *Yersinia enterocolitica* gene sequence. *Biosensors and Bioelectronics,* **25**, 1264–1270.

Thevenot, D. R., Toth, K., Durst, R. A. and Wilson, G. S. (1999). Electrochemical biosensors: recommended definitions and classification. *Pure and Applied Chemistry,* **71**, 2333–2348.

Tortajada-Genaro, L. A., Rodrigo, A., Hevia, E., Mena, S., Niñoles, R. and Maquieira, Á. (2015). Microarray on digital versatile disc for identification and genotyping of *Salmonella* and *Campylobacter* in meat products. *Analytical and Bioanalytical Chemistry,* **407**, 7285–7294.

Valadez, A. M., Lana, C. A., Tu, S.-I., Morgan, M. T. and Bhunia, A. K. (2009). Evanescent wave fiber optic biosensor for *Salmonella* detection in food. *Sensors,* **9**, 5810–5824.

Wackerlig, J. and Lieberzeit, P. A. (2015). Molecularly imprinted polymer nanoparticles in chemical sensing—synthesis, characterisation and application. *Sensors and Actuators B Chemical,* **207**, 144–157.

Wang, L., Yang, R., Chen, J., Li, J., Qu, L. and Harrington, P. B. (2014). Sensitive voltammetric sensor based on isopropanol–Nafion–PSS–GR nanocomposite modified glassy carbon electrode for determination of clenbuterol in pork. *Food Chemistry,* **164**, 113–118.

Wang, H., Zhang, Y., Li, H. et al. (2013). A silver–palladium alloy nanoparticle-based electrochemical biosensor for simultaneous detection of ractopamine, clenbuterol and salbutamol. *Biosensors and Bioelectronics,* **49**, 14–19.

Warriner, K., Reddy, S. M., Namvar A. and Neethirajan, S. (2014). Developments in nanoparticles for use in biosensors to assess food safety and quality. *Trends in Food Science & Technology,* **40**, 183–199.

Downloaded From Vet-eBooks.com

Wei, S., Jakusch, M. and Mizaikoff, B. (2006). Capturing molecules with templated materials—Analysis and rational design of molecularly imprinted polymers. *Analytica Chimica Acta,* **578**, 50–58.

Wu, C., Sun, D., Li, Q. and Wu, K. (2012). Electrochemical sensor for toxic ractopamine and clenbuterol based on the enhancement effect of graphene oxide. *Sensors and Actuators B Chemical,* **168**, 178–184.

Yang, Y., Fang, G., Wang, X. et al. (2014b). Sensitive and selective electrochemical determination ofquinoxaline-2-carboxylic acid based on bilayer of novel poly (pyrrole) functional composite using one-step electro-polymerization and molecularly imprinted poly (o-phenylenediamine). *Analytica Chimica Acta,* **806**, 136–143.

Yang, X., Feng, B., Yang, P., Ding, Y., Chen, Y. and Fei, J. (2014a). Electrochemical determination of toxic ractopamine at an ordered mesoporous carbon modified electrode. *Food Chemistry,* **145**, 619–624.

Yang, G. J., Huang, J. L., Meng, W. J., Shen, M. and Jiao, X. A. (2009). A reusable capacitive immunosensor for detection of *Salmonella* spp. based on grafted ethylene diamine and self-assembled gold nanoparticle monolayers. *Analytica Chimica Acta,* **647**, 159–166.

Zhang, J., Shao, X., Yue, J., Li, D. and Chen, Z. (2014). Preparation of ractopamine-tetraphenylborate complexed nanoparticles used as sensors to rapidly determine ractopamine residues in pork. *Nanoscale Research Letters,* **9**, 639(1–7).

Zhang, Z., Yang, X., Zhang, H. et al. (2011). Novel molecularly imprinted polymers based on multi-walled carbon nanotubes with binary functional monomer for the solid-phase extraction of erythromycin from chicken muscle. *Journal of Chromatography. B, Analytical Technologies in the Biomedical and Life Sciences,* **879**, 1617–1624.

Zhao, Y., Zhang, G., Liu, Q., Teng, M., Yang, J. and Wang, J. (2008). Development of an immunochromatography strip for the rapid detection of 12 fluoroquinolones in chicken muscle and liver. *Journal of Agricultural and Food Chemistry,* **56**, 12138–12142.

Zhou, Y., Wang, P., Su, X., Zhao, H. and He, Y. 2013. Colorimetric detection of ractopamine and salbutamol using gold nanoparticles functionalized with melamine as a probe. *Talanta,* **112**, 20–25.

Zhu, Y., Son, J. I. and Shim, Y. B. (2010). Amplification strategy based on gold nanoparticle-decorated carbon nanotubes for neomycin immunosensors. *Biosensors and Bioelectronics,* **26**, 1002–1008.

Downloaded From Vet-eBooks.com



Taylor & Francis
Taylor & Francis Group
http://taylorandfrancis.com

Downloaded From Vet-eBooks.com

6

Meat Decontamination by Irradiation

Dong U. Ahn, Eun Joo Lee, and Aubrey Mendonca

CONTENTS

6.1 Introduction 198
6.2 Food Irradiation 199
6.2.1 History of Food Irradiation 199
6.2.2 Irradiation Process 201
6.3 Microbial Decontamination of Meat by Irradiation 204
6.3.1 Factors Affecting Radiation Destruction of Microorganisms in Meat 205
6.3.1.1 Irradiation Dose 205
6.3.1.2 Meat Composition 205
6.3.1.3 Temperature 205
6.3.1.4 Microbial Factors 206
6.3.2 Combinations of Irradiation and Other Antimicrobial Interventions 207
6.4 Quality Changes in Meat by Irradiation 209
6.4.1 Lipid Oxidation 209
6.4.2 Off-Odor Production 210
6.4.2.1 Sources of Off-Odor Production in Irradiated Meat 210
6.4.2.2 Mechanism of Off-Odor Production in Irradiated Meat 211
6.4.3 Color Change 212
6.4.3.1 Color Changes in Irradiated Raw and Cooked Meat 212
6.4.3.2 Mechanism of Color Changes in Irradiated Meat 213
6.4.4 Texture Change 215
6.4.5 Consumer Acceptance of Irradiated Meat 215
6.5 Control of Quality Changes in Meat by Irradiation 216
6.5.1 Additives 216
6.5.2 Packaging 217
6.5.3 Packaging and Additive Combinations 217
6.6 Future Research Needed 218
References 219

Downloaded From Vet-eBooks.com

6.1 Introduction

Meat is one of the major sources of pathogens that cause foodborne illness in humans. The intervention strategies for pathogens in meat can be divided into preharvest reduction of microorganisms in livestock and postharvest decontamination on carcass and meat. The reduction of bacteria in animal is achieved by priming their immune system via the use of dietary supplementation with known immune stimulants. Postharvest interventions are traditional meat decontamination methods that use various physical and chemical methods during slaughtering and processing steps (Farkas, 2006). Irradiation is among the most effective physical decontamination technologies for inactivating foodborne pathogens and improving the safety of meats (Roberts, 2014). Irradiation of meat for the purpose of killing indigenous microflora, and thereby extending shelf life, has been known as a preservation technique for several decades. The major advantages of irradiating meat are it is a nonthermal processing, maintains the integrity of products, and leaves no chemical residues. Also, the products can be treated after final packaging, which prevents further cross-contamination during postprocessing handling.

The bacteriocidal action of ionizing irradiation is through the damage of bacterial DNA by the free radicals produced during the irradiation process. The effect of irradiation in inhibiting foodborne pathogens and spoilage bacteria in meat products is dose dependent. The survival of microbial cells upon irradiation treatments is influenced by the nature and extent of direct damage produced inside the cell; the number, nature, and longevity of irradiation-induced chemical species; and the inherent ability of cells to withstand the assaults and undergo repair. Extracellular conditions such as pH, temperature, and chemical composition of the food, in which the microorganisms are also suspended, have significant effects on the microcidal efficiency of irradiation. Although very effective for controlling pathogens, irradiation can deplete antioxidants in muscle, induce color change, increase production of off-odor volatiles, and negatively alter the sensory characteristics of meat products. Formation of 2-alkylcyclobutanones, benzene, and methyl benzene (toluene) in irradiated foods is also another important issue that consumers are concerned about.

This chapter discusses the history, principles, and microcidal effects of irradiation as well as how irradiation influences quality, sensory characteristics, and consumer acceptance of meat products. The combinations of physical and/or chemical treatments that can improve the efficacy of irradiation and the quality and consumer acceptance of irradiated meat products are also discussed.

Downloaded From Vet-eBooks.com

6.2 Food Irradiation

6.2.1 History of Food Irradiation

Food irradiation has been studied for over 70 years. Most of the food products approved for irradiation have been investigated for more than 40 years before approval in the United States. To date, no other food technology has as long a history of scientific research and testing before gaining approval. Food irradiation research has been comprehensive, and included toxicological, microbiological evaluation, and wholesomeness (WHO, 1994). Although two patents were filed in 1905 and x-ray treatment was applied to kill *Trichina* in pork in 1921, food irradiation was economically unfeasible in the United States until World War II because of the high cost of ionizing radiation sources. In the 1940s, machines that can produce high-energy electron beams of up to 24 million electron volts (eV) became available. This energy was strong enough to penetrate six inches deep into food when electron beams were "fired" from both sides of the product. Also, man-made radionuclides such as Co-60 and Cs-137, which emit gamma rays during their radioactive decay, became available through the development of atomic energy. The availability of these sources stimulated research in food irradiation aimed at the development of commercial processes. In the mid-1940s, the use of irradiation to preserve foods was suggested. From 1940 to 1953, exploratory research on food irradiation in the United States was sponsored by the Department of the Army, the Atomic Energy Commission, and private industry. In the late 1940s and early 1950s, researchers investigated the potential of ultraviolet light, x-rays, electrons, neutrons, and alpha particles for food preservation and concluded that only electrons had the necessary characteristics of efficiency, safety, and practicality. They considered x-rays to be impractical because of very low conversion efficiency from electron to x-ray at that time (Hayashi, 1991). Ultraviolet light and alpha particles were also considered to be impractical because of their limited ability to penetrate matter. Neutrons exhibited an excellent penetration capability and were very effective in the inactivation of bacteria, but were considered inappropriate for use because of the potential for inducing radioactivity in food. Proctor and Goldblith (1951) found that the physical characteristics of food products were important factors in determining the correct dose of radiation for bacterial inactivation. Enzymes were more resistant to ionizing radiation than bacteria, and irradiation in frozen state minimized the development of off-flavor.

Most in-depth studies in food irradiation since 1952 have been government-sponsored because of military interest in this type of food processing. Much of the early research to sterilize foods was done by the Quartermaster Corps of the U.S. Army at the Food and Container Institute in Chicago

Downloaded From Vet-eBooks.com

because of its need to provide high-quality, shelf-stable field rations for troops. The Army Quartermaster Corps concluded that wholesome, economical, shelf-stable field rations could be provided through irradiation. Department began to assess the safety of irradiated foods in 1955, petitions to the Food and Drug Administration (FDA) for the approval of irradiation of specific foods followed, and commercial radiation equipment and sources were developed. In the meantime, the International Atomic Energy Agency (IAEA) that promoted nuclear technologies was working on the global acceptance of food irradiation. In 1959, IAEA signed an agreement with the World Health Organization (WHO) giving IAEA "the primary responsibility for encouraging, assisting and coordinating research on, and development and practical application of atomic energy for peaceful uses throughout the world." As a result, IAEA has had authority over nuclear energy programs, has played a major role in encouraging people to accept irradiated food, and has organized scientific committees that promote the wholesomeness of irradiated food (IAEA, 1991).

In 1962, the U.S. Army built a food irradiation facility at the Army's research laboratories and conducted research in sterilizing meat products using high-dose irradiations; it developed shelf-stable bacon, ham, pork, beef, hamburger, corned beef, pork sausage, codfish cakes, and shrimp, but the first products approved for irradiation by the FDA were wheat and wheat powder in 1963. In the early 1970s, the National Aeronautics and Space Administration (NASA) adopted an irradiation process to sterilize meats for astronauts in space, and added them to the flight menu of Apollo 17 in 1972. In 1975, irradiated ham, turkey, beef steak, and corned beef were used on the Apollo–Soyuz Test Project (ASTP) where irradiated foods were shared with the Russian cosmonauts.

In 1980, the Food and Agriculture Organization (FAO) of the United Nations, the IAEA, and the WHO stated that "irradiation of any food commodity up to an overall average dose of 1 Mrad (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" (WHO, 1981). During the 1980s, the FDA approved petitions for irradiation of spices and seasonings, pork, fresh fruits, and dry or dehydrated substances. The U.S. Department of Agriculture (USDA) approved irradiation of pork to destroy *Trichinella spirallis* (USDA-FSIS, 1986), and pathogen control for poultry and red meats (USDA-FSIS, 1999). More recently, WHO convened a Study Group to review all data on products irradiated above the 10-kGy ceiling and concluded the products to be safe and wholesome. As a result, WHO has recommended removing the dose limit so that irradiation can be used to sterilize foods commercially as in canning (WHO, 1999). During the past six decades, the commercial development of food irradiation has been delayed because of consumers' notion that food irradiation is linked to the atomic bomb and nuclear radiation. More than 60 countries have permitted irradiation of food, and more than 600,000 tons of food is irradiated annually in the

Downloaded From Vet-eBooks.com

world (Kume et al., 2009). The United States also has more than 40 licensed irradiation facilities, most of which are used to sterilize medical and pharmaceutical supplies. The approval date, dose, and purpose of food irradiation in the United States are listed in Table 6.1.

6.2.2 Irradiation Process

Atoms are made up of three types of particles: protons, neutrons, and electrons. These particles are held together by energy, and atomic nuclei contain protons (+ charge) and neutrons (uncharged) in about 1:1–1:1.5 ratio (Thakur and Singh, 1994). If any changes of number and arrangements in the forces of the nuclear particles occur, they lose balance and consequently become

TABLE 6.1

Approval of Food Irradiation in the United States

Date	Products	Dose (kGy)	Purpose
1963	Wheat and wheat powder	0.2–0.5	Disinfect insects
1964, 1965	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, 1986	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, 1999	Red meat, chilled	<4.5	Control of microorganisms
	Red meat, frozen	<7.5	
2000	Shell eggs	<3.0	Control of *Salmonella enteriditis*
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

NASA, National Aeronautics and Space Administration.

Downloaded From Vet-eBooks.com

an unstable or "radioactive" atom. This unstable atom can be restabilized by emitting energy to rebalance the nucleus. To emit energy, electrons are removed from the outer shell of atoms and the energy levels of electrons are changed as the electrons return to their original energy levels. This energy is electromagnetic and its emission, as particles or waves, is termed "radiation" (Halliwell and Gutteridge, 1989).

The amounts of emission energy depend on the level of energy being released. Low-energy electromagnetic radiations occur in TV, radio, and microwave as long waves; intermediate radiations occur in visible light, heat, and solar energy; high-energy radiations occur in x-ray and gamma ray; very high-energy radiations occur in the radioactive decay of radionuclides like uranium (Lagunas-Solar, 1995). If radiation has sufficient energy to move atoms in another material without chemical changes it is called a "nonionizing radiation," and if it also has sufficient energy to break chemical bonds it is called "ionizing radiation." The amount of energy to break a carbon–carbon bond is about ~33 eV and a dose of 1 kGy has been estimated to break <1 chemical bond in 1 million bonds present (CAST, 1986).

Radionuclides, such as ^{60}Co, ^{137}Cs, and uranium, are defined as the atoms that contain excess energy in the nucleus due to an excess of either proton or neutron. They emit energy as alpha particles, beta particles, and photons, and emitting the energy in these atoms is called "decay." Alpha particles are emitted from very large atoms such as radium, uranium, and plutonium when the neutron:proton ratio is too low. Alpha particles travel slowly and lose energy rapidly due to their charge and mass, expanding it in a few centimeters. Beta particles are emitted when the neutron:proton ratio is too high, and ^{60}Co, ^{137}Cs, and ^{14}C release beta particles. They have ~1/2000 mass of a neutron or proton. So, they can travel several feet from their source but are stopped by solid materials. The movement of a neutron does not reduce the nuclear energy level enough, and the extra energy is released as a gamma photon (Efiok, 1996).

Photons such as gamma rays and x-rays are emitted when the energy of atoms (^{60}Co and ^{137}Cs) is exhausted: gamma rays are emitted from nucleus and x-rays are from electron fields. Gamma rays have such high energy and are so small that they pass through living tissues without interacting with them (Jarrett, 1982). These gamma rays do not ionize atoms directly. They transfer energy to secondary electrons, which then interact with other materials to form ions. When a photon or an accelerated electron enters material, the energy can be transferred or absorbed by an electron of an atom in the material. The electron of an atom in the material increases in energy level and leaves its orbit. The ejected electron, called a "Compton electron," transfers its energy to a secondary electron and reduces the total energy of the Compton electron (Diehl, 1995). By the same token, Compton electrons cause further excitation and ionization in the material and this primary effect is called the "Compton effect." Because of this effect, energy is passed through a cascade of electrons until not enough energy to cause electrons to escape their orbitals is left (Venugopal et al., 1999).

Downloaded From Vet-eBooks.com

As the basic principle of the radiation process, irradiation energy applied to biological materials ejects electrons from the atoms/molecules of the material and produces ions and free radicals (Woods and Pikaev, 1994). The first target of highly energized electrons is water molecules in biological substances. The dispersion of ions and free radicals is greater when water is present in free form than in bound form (dried products) or in crystalline form (frozen products) (Thakur and Singh, 1994). The hydroxyl radical (HO·), the primary radiolytic product of water, is a powerful oxidizing agent, and this free radical tends to recombine to form stable products (Taub et al., 1979). Because the dispersion and capture of electrons are purely random, large molecules and compounds have a greater probability of being affected than smaller molecules. The cellular components such as DNA, pigments, fatty acids, and membrane lipids can be damaged by ionizing radiation. When the DNA of living cells is exposed to OH· by radiation, both single and double strands in the molecule are broken.

Radionuclides such as ^{137}Cs and ^{60}Co are used as the major sources of gamma rays, and a linear accelerator is used to generate high-energy electrons and x-rays. ^{137}Cs is produced when uranium and plutonium absorb neutrons and a undergo fission in nuclear reactors. ^{137}Cs produces 0.66 MeV gamma radiations, and it decays to nonradioactive barium ($_{56}Ba^{137}$) by emitting beta particles and strong gamma rays (Lagunas-Solar, 1995). ^{60}Co, the most common energy source for irradiation, produces 1.33 MeV gamma radiations as it decays to nonradioactive nickel by emitting beta particles and powerful gamma rays. ^{60}Co is a man-made radionuclide produced in linear accelerators and as a nuclear reactor by-product by bombarding ^{59}Co with neutrons. Gamma rays are highly penetrable and can be used to treat food contained inside a package. From a practical point of view, ^{60}Co is preferred to ^{137}Cs because the latter, apart from having weaker gamma rays, is also water soluble and thus poses environmental hazards (Venugopal et al., 1999).

An electon beam is a stream of high-energy electrons that are generated electrically then accelerated through an electron gun. The electrons can be accelerated to different energy levels including particles of high-energy electrons and x-rays that are produced when high-energy electrons strike a thin metal film (Josephson and Peterson, 2000). Electron beam accelerators accelerate electrons to a beam (up to 10 MeV) with minimal penetrating power into thin foods (5–10 cm). Electron beams are with single-sided treatment, and 10 MeV electrons can give adequate treatment for thicknesses up to about 35 mm of unit density material. Using a conveyor belt with double-sided treatment can give a bit more than double the single-sided depth because of the way the two depth-dose curves superimpose; hence, a product thickness of 8 cm can be used (Satin, 2002). Although electrons are less penetrable than gamma rays, they can be useful for irradiating large volumes of free flowing food items such as grains or packages of fish fillets with no more than 8–10 cm thickness with a density of 1 g/cm^3.

Downloaded From Vet-eBooks.com

TABLE 6.2

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)

Source: Kwon, 2010, Korea Food Safety Research Institute.

Each of these sources has specific advantages and disadvantages (Jarrett, 1982). The advantages of ^{60}Co include high penetration and dose uniformity, allowing treatment of products of variable sizes, shapes, and densities, a long history of satisfactory use in similar applications, ready availability, and low environmental risk. The disadvantages include 12% of the source must be replaced annually because of its short half-life (5.3 years) and a rather slow processing rate compared with electron beam irradiation. The advantages of a linear accelerator compared with the gamma irradiators are the following: can simply be turned off when not in use, does not need to be replenished, established history of use, and a high throughput rate. The disadvantages are the complexity of the machine and the consequent need for regular maintenance and the large requirements for power and cooling. Currently, e-beam and gamma rays are used as radiation sources for commercial food irradiation. Although x-rays have relatively high penetrating power, x-rays are not used in food irradiation due to poor conversion of accelerated electrons to x-rays (Hayashi, 1991). The comparative characteristics of ionizing radiation sources used for food irradiation are shown in Table 6.2.

The quantity of energy absorbed by something (food) as it passes through a radiation field is called "radiation absorbed dose." The unit (SI) for irradiation dose is Gray (Gy), which is equal to the absorption of energy equivalent to one Joule per kilogram of absorbing material (1 Gy = 1 J·kg^{-1} = 6200 billion MeV absorbed/kg of food = 0.01 calorie/lb of food = 100 rad, 1 rad = 100 erg/g).

6.3 Microbial Decontamination of Meat by Irradiation

Food irradiation is an effective technology for microbial decontamination of foods including meats. The antimicrobial efficacy of food irradiation against pathogenic microorganisms has been recognized for decades. The use of this technology with the aim of destroying meat-borne pathogenic microorganisms will also bring a reduction in the number of spoilage microorganisms to increase shelf life of meats (Olson, 1998).

Downloaded From Vet-eBooks.com

6.3.1 Factors Affecting Radiation Destruction of Microorganisms in Meat

6.3.1.1 Irradiation Dose

Significant reductions in microbial populations in meats can be achieved by using high radiation doses; however, such an approach can have adverse effects on the desirable sensory attributes of meats. Therefore, emerging trends in the application of irradiation include the use of this technology and other interventions (e.g., antimicrobial food preservatives, heat, and high hydrostatic pressure) as part of a hurdle technology approach to control meat-borne pathogens. This approach allows the use of relatively low doses of irradiation to improve microbial safety of meats while maintaining the desirable sensory attributes of these nutritious food products.

6.3.1.2 Meat Composition

Meat composition affects the destruction of microorganisms by irradiation. The proteins in the meat may protect microorganisms against the damaging effects of irradiation by neutralizing free radicals (Diehl, 1995). This neutralizing effect of proteins may explain the relatively high radiation resistance of microorganisms in meats and dairy products compared to nonprotein foods of similar moisture content (Soladoye et al., 2015). Proteins and other meat constituents, including natural antioxidants such as carnosine and vitamin E, compete for free radicals formed by the radiolysis of water (Galan et al., 2011). This competition for free radicals decreases the antimicrobial efficacy of ionizing radiation. Carnosine has been reported to increase the radiation resistance of *Aeromonas hydrophila* in minced turkey meat (Stecheni et al., 1998). Irradiation produces peroxides from dextrose, and free fatty acids, carbonyl compounds, hydrogen peroxide, and hydroperoxides from fat. However, dextrose added in processed meat and fat content of meat had no effect on microbial inactivation by irradiation (Sommers and Fan, 2002), probably because other meat constituents such as proteins may have protected bacteria and other microorganisms from the antimicrobial products of fats and dextrose (Diehl, 1995).

6.3.1.3 Temperature

The temperature of meat during irradiation is an important factor that affects the extent of irradiation destruction of microorganisms. Microbial resistance to irradiation increases with decreases in temperature below the freezing point of water. The impact of meat temperature on the survival of pathogenic bacteria following irradiation has been reported for *Campylobacter jejuni* (Clavero et al., 1994), *Salmonella* (Thayer and Boyd, 1991), *Escherichia coli* O157:H7 (Arthur et al., 2005), *Staphylococcus aureus* (Thayer and Boyd, 1992), *Listeria monocytogenes* (Thayer and Boyd, 1995), and *Clostridium botulinum* spores (El-Bisi et al., 1966). Microorganisms exhibit a greater sensitivity

Downloaded From Vet-eBooks.com

to irradiation at ambient temperatures than at subfreezing temperatures: D_{10}-values (dose required to destroy 90% of the bacterial population) for *E. coli* O157:H7 in mechanically deboned chicken meat were 0.28 and 0.44 kGy at 5°C and at –5°C, respectively (Thayer and Boyd, 1993). Significantly higher D_{10}-values have been reported for *E. coli* O157:H7 in ground beef patties irradiated at –15°C than at 5°C. Freezing meat reduces water activity by converting the water to ice, drastically reduce the generation of free radicals from the radiolysis of water (Diehl, 1995). Additionally, the frozen state impedes the migration of free radicals to other parts of the frozen product beyond those areas of limited free radical production (Taub et al., 1979).

6.3.1.4 Microbial Factors

Microbial factors including numbers and types of microorganisms in meats as well as the physiological state of the microorganisms can affect the extent of microbial destruction by irradiation. As observed with other food preservation processes, the presence of large populations of microorganisms reduces the effectiveness of a given irradiation dose. Therefore, decontamination of meat using irradiation would be more efficient if the meat to be treated is of good microbial quality. With regard to types of microorganisms, microbial sensitivity to irradiation in meats, as in other foods, can vary among microbial types. For example, viruses have much higher radiation resistance than bacterial spores, which in turn show a higher radiation resistance than vegetative bacterial cells. Bacterial vegetative cells are more radiation resistant than fungi (yeast and molds). More complex life forms have a higher sensitivity to irradiation than simpler life forms. This phenomenon is supported by the observation that a dose as high as 40 kGy is necessary for destroying viruses; however, a dose as low as 0.01 kGy could cause death in humans (Satin, 1993). Gram-negative bacteria are more sensitive to ionizing radiation than gram-positive bacteria. In fact irradiation doses of at least 1.0 kGy, which could virtually destroy gram-negative bacteria in food, exhibit a much less destructive effect on gram-positive bacteria such as the lactic acid producing bacteria (Lambert et al., 1992). Nonspore-forming bacteria exhibit a greater sensitivity to irradiation than spore formers.

With regard to the physiological state of bacteria, exponential phase cells are more sensitive to irradiation than lag phase cells or stationary phase cells. More importantly, meat-borne bacteria that have adapted to certain environmental stress demonstrate even greater radiation resistance than stationary-phase bacteria. More recently, significant increases in the radiation resistance (D_{10}-value) of starved *L. monocytogenes* cells in ground pork at 4°C have been reported (Mendonca et al., 2004). Irradiation of ground pork at 2.5 kGy decreased initial populations of nonstarved cells (control) by about 6.0 log, whereas starved cells were decreased by only 3.8 log. D_{10}-values for exponential, stationary, and starved *L. monocytogenes* cells in ground pork were reported to be 0.35, 0.42, and 0.66 kGy, respectively. Kundu et al. (2014)

Downloaded From Vet-eBooks.com

reported that 1 kGy irradiation treatment of beef reduced ≤1.9 log of *Salmonella* and ≤4.0 log of *E. coli* O157:H7. Xavier et al. (2014) applied 2.5 kGy irradiation doses to beef and found 2 log cfu/g for *L. monocytogenes* and 5 log cfu/g for *E. coli* O157: H7. The D_{10}-values of foodborne pathogens, parasite, and spoilage bacteria are listed in Table 6.3.

6.3.2 Combinations of Irradiation and Other Antimicrobial Interventions

Doses of irradiation used alone for microbial decontamination of meat may result in adverse sensory changes in these food products. Combinations of relatively mild antimicrobial treatments and preservative hurdles can enhance each other's antimicrobial activity. In this regard, combinations of marginally effective antimicrobials may result in enhanced microbial safety and improved quality of foods. Food preservation methods applied to meats including acidification, heating, and the addition of chemical food preservatives can improve the decontamination efficacy of irradiation by increasing the radiation sensitivity of meat-borne microorganisms and by inhibiting the proliferation of microbial survivors following irradiation.

Various acidifying agents have been widely used in combination with irradiation for controlling pathogens and extending the microbial shelf life of meat products. Farkas and Andrassy (1993) examined the antimicrobial effects of gamma irradiation (2 kGy) and acidulants (0.1% ascorbic acid or 0.5% glucono-delta-lactone) in vacuum-packaged minced meat and found

TABLE 6.3

D_{10}-Values of Foodborne Pathogens, Parasite, and Spoilage Bacteria

Pathogen	D_{10} (kGy)	Medium	Pathogen	D_{10} (kGy)	Medium
Trichinella spiralis	0.3–0.6	Pork	*Yersinia enterocolitica*	0.11	Beef
Bacillus cereus (vegetative)	0.14–0.19	Beef	*Clostridium botulinum* (spore)	3.56	Chicken
Campylobacter jejuni	0.18	Beef	*Clostridium perfringens*	0.58	Meat
Escherichia coli O157:H7	0.25	Beef	*Clostridium sporogenes* (spore)	6.3	Beef fat
Listeria monocytogenes	0.51–059	Beef	*Moraxella phenylpyruvica*	0.63–0.88	Chicken
Salmonella spp.	0.48–0.70	Meat	*Pseudomonas putida*	0.08–0.11	Chicken
Staphylococcus aureus	0.42	Chicken	*Streptococcus faecalis*	0.65–0.7	Chicken

Downloaded From Vet-eBooks.com

that acidulants delayed the growth of Enterobacteriaceae in nonirradiated meat for two weeks at 0°C–2°C. However, a combination of acidulant with irradiation completely prevented the growth of this microbial group during refrigerated storage and temperature abuse (10°C). Bhide et al. (2001) sprayed sheep carcasses with 1% propionic acid, 2% lactic acid, or 2% acetic acid at a pressure of 3 kg/cm^2 for two to four minutes. The meat harvested from the carcasses was packaged and exposed to gamma irradiation at 1, 2, or 3 kGy. All organic acids used increased the sensitivity of *Bacillus cereus* to irradiation. Acetic acid (2%) plus irradiation at 3 kGy was the most effective in reducing total viable count and *B. cereus* count during refrigerated storage (5°C–7°C) of the meat samples. Citric acid, used as a surface treatment for frankfurters, has been shown to decrease the irradiation resistance of *L. monocytogenes* in this ready-to-eat (RTE) meat product. The D_{10}-values for *L. monocytogenes* on frankfurters dipped in 0, 1, 5, or 10% (w/v) citric acid were 0.61, 0.60, 0.54, and 0.53 kGy, respectively (Sommers et al., 2003). Alahakoon et al. (2015) reported that 2% citrus peel extract treatment was effective to extend the shelf life of 2 kGy irradiated chicken breast meat. Combinations of organic acid and irradiation were more effective than each intervention used alone for controlling the growth of total microbial counts and coliforms in pork during storage at 4°C for 14 days.

Various other antimicrobial treatments have shown potential in enhancing destruction of microorganisms by irradiation. Kim and Thayer (1996) reported that irradiation and heating combination significantly decreased survival of *Salmonella typhimurium* irrespective of the order of application of each treatment. Interestingly, application of irradiation before rather than after heating was consistently more lethal to the pathogen. Huq et al. (2015) indicated that microencapsulation of oregano and cinnamon essential oils combined with nisin showed an antimicrobial effect to reduce *L. monocytogenes* in RTE ham. By using a combination of hurdles (low a_w, vacuum packaging, and irradiation), Kanatt et al. (2002) developed a few RTE shelf-stable intermediate moisture (IM) spiced mutton and spiced chicken products. Those researchers reduced the a_w of the meat products to 0.80 by grilling or hot-air drying. The IM meat products were then vacuum sealed, treated with gamma irradiation at 0 (control), 2.5, 5.0, and 10.0 kGy, then stored at 30°C for six months (spiced chicken cubes) or nine months (mutton kababs). No viable microorganisms were detected in meat products treated with 10 kGy and those products retained acceptable sensory quality for up to nine months. More recently, Chen et al. (2004) demonstrated a synergistic effect between the bacteriocin pediocin (in ALTA 2341) and electron beam irradiation for inhibiting *L. monocytogenes* in frankfurters. Storage of the frankfurters at 4°C enhanced the antilisterial effects of the combined treatments; little or no growth of the pathogen occurred in packages of frankfurters during 12 weeks of storage. The combined treatments did not alter the sensory characteristics of the frankfurters negatively.

Downloaded From Vet-eBooks.com

6.4 Quality Changes in Meat by Irradiation

The main goal of irradiating meat is eliminating pathogens and improving the safety and storage stability of meat. However, the application of irradiation technology by the meat industry is limited because of quality concerns about irradiated meat products. Irradiation produces a characteristic odor as well as alters meat flavor and color, which significantly influences 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, developing methods that can prevent these quality changes in meat by irradiation is essential for implementing irradiation technology in the meat industry.

6.4.1 Lipid Oxidation

Lipid is one of the main meat components, and polyunsaturated fatty acids are the most susceptible to oxidation by irradiation. Hydroxyl radicals are the most reactive oxygen species that can initiate lipid oxidation in meat. Thakur and Singh (1994) reported that ionizing radiation generates hydroxyl radicals in aqueous systems. Because meat contains near 75% water, irradiation is expected to accelerate oxidative changes in meat. Irradiation-induced lipid oxidation in meat is dose dependent (Ahn et al., 1997). The presence of oxygen also has a significant effect on the development of oxidation and odor production (Merritt et al., 1975; Badr, 2004). Without oxygen, lipid oxidation in raw and cooked meat did not progress and thiobarbituric acid reactive substances (TBARS) and volatiles of vacuum-packaged irradiated raw and cooked meat did not correlate well. Under aerobic conditions, however, TBARS had very high correlations with the amounts of aldehydes, total volatiles, and ketones in aerobically packaged irradiated meat. Therefore, excluding oxygen from meat and meat products, whether they are irradiated or not, is critical to stop oxidative chain reactions (Ahn et al., 2000b). Ahn et al. (1998b) reported that preventing oxygen, especially for cooked meat, was more important than irradiation dose and storage time (Ahn et al., 2000a). Aerobically packaged sausages irradiated at a higher dose produced greater amounts of TBARS than those irradiated at lower doses (Kanatt et al., 2015b). The TBARS of sausages with higher polyunsaturated fatty acids was greater than those with lower polyunsaturated fatty acids.

Diehl (1995) reported that irradiation of aqueous systems produced hydrogen peroxide, particularly in the presence of oxygen. During postirradiation storage, hydrogen peroxide gradually disappears while other constituents of the system are oxidized. A series of dienes, trienes, and tetraenes were formed from unsaturated fatty acids by irradiation at high dose (60 kGy) under vacuum conditions (Nawar, 1986). Shahidi and Pegg (1994) reported that aldehydes contributed the most to oxidation flavor and rancidity in

Downloaded From Vet-eBooks.com

cooked meat, and hexanal was the major volatile aldehyde. Lee and Ahn (2003) reported that the TBARS values of oil emulsion immediately after irradiation were lower than those of the nonirradiated ones, but rapidly increased during storage. Especially, arachidonic acid, linolenic acid, and fish oil, which had a large proportion of polyunsaturated fatty acids, had accelerated lipid oxidation after irradiation.

Under frozen conditions, irradiation increased the TBARS of pork patties and turkey breast but storage time had no effect on lipid oxidation even under aerobic packaging conditions (Nam and Ahn, 2002b). Taub et al. (1979) reported that with less mobility in the frozen state, free radicals tend to recombine to form the original substances rather than diffuse through the food and react with other food components. Thus, the minimal lipid oxidation detected in frozen turkey after irradiation should be due to the limited mobility of free radicals in frozen states. During the warming process, however, they tend to react with each other rather than with the substrates (Javanmard et al., 2006).

6.4.2 Off-Odor Production

6.4.2.1 Sources of Off-Odor Production in Irradiated Meat

All irradiated meat produces a characteristic, readily detectable, irradiation odor regardless of the degree of lipid oxidation (Ahn et al., 1997). Meat sterilized through irradiation develops a characteristic odor, which has been described as "metallic," "sulfide," "wet dog," "wet grain," or "burnt." These investigators assumed that the off-odor was the result of free-radical oxidation, which was initiated by the irradiation process. Others described the irradiated meat odor as "bloody and sweet" (Hashim et al., 1995) and "barbecued corn-like" (Ahn et al., 2000a). Batzer and Doty (1955) found that methyl mercaptan and hydrogen sulfide were important to irradiation odor, and the precursors of the undesirable odor compounds in irradiated meat were sulfur-containing compounds that were water soluble. Others postulated that hydrocarbons and carbonyl from fats were the major source of irradiation off-odor. Sensory results, however, clearly indicated that the major source of irradiation off-odor was sulfur compounds. More recent studies showed that irradiation greatly increased or newly produced many volatile compounds such as 2-methyl butanal, 3-methyl butanal, 1-hexene, 1-heptene, 1-octene, 1-nonene, *cis*-3- and *trans*-6-nonenals, oct-1-en-3-one, hydrogen sulfide, sulfur dioxide, mercaptomethane, bis(methylthio-)methane, methyl thioacetate, dimethyl disulfide, and trimethyl sulfide from meat (Patterson and Stevenson, 1995; Fan et al., 2002). Among those volatile compounds, sulfur volatiles showed much stronger and stringent odor intensity than other compounds because sulfur compounds had very low odor thresholds (Angelini et al., 1975). Volatiles from lipids played only a small part

Downloaded From Vet-eBooks.com

of the irradiation off-odor (Lee and Ahn, 2003). This indicated that sulfur compounds would be the major volatile components responsible for the characteristic off-odor in irradiated meat, and supported the concept that the changes that occur following irradiation were different from those of warmed-over flavor in the oxidized meat.

6.4.2.2 Mechanism of Off-Odor Production in Irradiated Meat

Ahn (2002) found that side chains of amino acids were susceptible to radiolytic degradation. More than one site of amino acid side chains was susceptible to free radical attack and many volatiles were produced by the secondary chemical reactions after the primary radiolytic degradation of side chains. The majority of volatiles produced by irradiation were sulfur compounds, indicating that sulfur-containing amino acids are among the most susceptible to irradiation (Ahn and Lee, 2002).

The perception of odor from samples containing sulfur volatiles changed greatly depending upon their composition and amounts present in the sample. The sulfur compounds were not only produced by the radiolytic degradation of amino acid side chains (primary reaction), but also by the secondary reactions of the primary sulfur compounds with other compounds around them. The amounts and types of sulfur compounds produced from irradiated sulfur amino acids indicated that methionine is the major amino acid responsible for the production of sulfur volatiles. 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). The volatile profiles and sensory characteristics of amino acids clearly explained why irradiation odor was different from lipid oxidation odor, and why lipid oxidation was responsible for only a small part of the off-odor in irradiated meat (Ahn and Lee, 2002).

The mechanisms of volatile production from meat indicated that the majority of the volatiles were from the radiolytic degradation of amino acid side chains, but deamination and decarboxylation from α-carbon were also involved in the production of volatile compounds (Dogbevi et al., 1999). The Strecker degradation, ketonic decarboxylation, and oxidation–reduction were involved in the production of aldehydes. Each amino acid monomers produced different odor characteristics, but the intensities of odor from all nonsulfur amino acid groups were weak. Cysteine and methionine produced similar odor characteristics to that of the irradiated meat, but the amounts of sulfur volatiles from methionine were far greater than that of the cysteine. This indicated that the contribution of volatiles produced from nonsulfur amino acids was minor, although interactions among volatiles, especially aldehydes with other volatile compounds, can contribute to the off-odor of irradiated meat products significantly. Although this study is done using a model system, the results can be extrapolated to a meat system because about 70% of meat is

Downloaded From Vet-eBooks.com

water, and protein (amino acid) is the major component involved in volatile production in meat by irradiation (Ahn et al., 2002, 2016a,b).

Mottram et al. (2002) reported that the degradation of amino acids by oxidative deamination–decarboxylation via Strecker degradation produced branched-chain aldehydes. 2-methyl butanal, 3-methyl butanal, and 2-methyl propanal are the main Strecker degradation products from isoleucine, leucine, and valine, respectively, by irradiation (Ahn et al., 2016a). Davies (1996) reported that irradiation of N-acetyl amino acids and peptides in the presence of oxygen give high yields of side-chain hydroperoxides, which can be formed on both the backbone (at α-carbon positions) and the side chain. In addition to amino acids, fatty acids are also degraded by irradiation. When triglycerides or fatty acids are irradiated, hydrocarbons are formed from fatty acids through various free-radical reactions. Radiolytic degradation of fatty acid methyl ethers were affected by irradiation dose, irradiation temperature, oxygen pressure, and fatty acid composition (Miyahara et al., 2002).

The release of nonpolar hydrocarbons was not influenced, but polar compounds such as aldehydes, ketones, and alcohols were greatly influenced by water. The volatility of aroma compounds depends on the vapor–liquid partitioning of volatile compounds, which determines the affinity of volatile molecules for each phase (Buttery et al., 1973). The interactions among food components such as carbohydrates, lipids, and proteins (Godshall, 1997), and the physicochemical conditions of foods, which influence conformation of proteins, also affect the release of volatile compounds in foods (Lubbers et al., 1998). Jo and Ahn (1999) reported that the amount of volatiles released from oil emulsion correlated negatively with fat content. This indicated that the relative amounts of volatile compounds released from meat systems could be significantly different from those in the aqueous system (Jo and Ahn, 2000).

6.4.3 Color Change

6.4.3.1 Color Changes in Irradiated Raw and Cooked Meat

The color of meat depends on the concentration and chemical status of heme pigments. Oxygen (O_2), CO, S, or NO can be the sixth ligand of heme pigments and is formed only when the heme iron is in reduced form. Although three common forms of myoglobin exist in different proportions, fresh meat color is imparted mainly by oxymyoglobin and deoxymyoglobin. The color of fresh meat is determined by oxygen partial pressure, oxygen diffusion rate, and oxygen consumption rate at meat surface. Under normal conditions, enzymes use up all oxygen available and generate reducing conditions inside meat block. Thus, the pigments in the middle of a meat block are usually in the reduced form (deoxymyoglobin, purple color) and weakly bind with water molecules or are stabilized by distal histidine of globin. Discoloration in fresh meat is mainly caused by oxidation of myoglobin to metmyoglobin when oxygen partial pressure is low, resulting in an unattractive brown color. The oxidized brown color can be turned into

Downloaded From Vet-eBooks.com

a bright red color under air (blooming or oxygenation) if the meat has strong enough reducing power or purple-red under full-vacuum conditions.

The color changes in irradiated meat vary significantly depending on various factors such as irradiation dose, animal species, muscle type, and packaging type (Luchsinger et al., 1996; Ahn et al., 1998a; Nanke et al., 1999). Millar et al. (1995) found that irradiated chicken breasts had a definite color change from the usual brown or purple to a more vivid pink or red as a result of ionizing irradiation in the oxygen-permeable film. Nam and Ahn (2002c) also reported that irradiation increased redness of both aerobically and vacuum-packaged raw turkey breast. However, the vacuum-packaged meat was redder than the aerobically packaged meat and was stable during storage (Luchsinger et al., 1997; Nanke et al., 1998, 1999). Jo et al. (2000) found a significant increase in redness of cooked pork sausages after irradiation. Irradiation and subsequent storage of pork improved the red color even in pale soft and exudative (PSE) pork, indicating that irradiation can be used to increase the acceptability of low-quality pork (Nam et al., 2002b). During the frozen storage, irradiation increased pink color in both aerobically and vacuum-packaged turkey breast, and the pink color was stable (Nam et al., 2002c). Sensory evaluations of irradiated raw turkey breast meat indicated that sensory panelists preferred the red color of irradiated meats to nonirradiated ones because irradiated meat looked fresh (Lefebvre et al., 1994). However, increased redness is a problem in irradiated light meats such as poultry breast and pork loin if the red color of irradiated meats persists in meat after cooking.

In cooked turkey meat, the increased redness was greater inside than on the surface, and the pink color intensity of the inside was stronger in irradiated meat than the nonirradiated (Nam and Ahn, 2003c). Tappel (1957) noted that when precooked meat was irradiated, the gray-brown hematin pigments were converted to uncharacteristic red pigments, especially when the cooked meat was irradiated in the absence of oxygen. Irradiation of red meat changes the red color to brown or gray under aerobic conditions. Immediately after irradiation, the color of ground beef changed from a bright red to a greenish brown, which would be an unattractive beef color for consumers (Nam and Ahn, 2003a).

6.4.3.2 Mechanism of Color Changes in Irradiated Meat

Irradiation produces ligand-forming compounds that can act as the sixth ligand of myoglobin. Tappel (1956) postulated a bright red color of irradiated fresh meat in an inert atmosphere was oxymyoglobin formed by the reaction between metmyoglobin and hydroxyl radicals. However, it is not logical that the pigment is an oxymyoglobin because the red color formed by irradiation has been produced mainly in anoxic conditions. Millar et al. (1995) postulated that the red/pink color in irradiated meat was due to a ferrous myoglobin derivative such as carboxymyoglobin or nitric oxide myoglobin other than oxymyoglobin. Nam and Ahn (2002a,b) characterized the pink pigment

Downloaded From Vet-eBooks.com

formed in irradiated raw and cooked turkey breast as carbon monoxide-myoglobin (CO-Mb). In cooked meat, both undenatured and denatured heme pigments were involved in heme-complex formations with ligands available under the conditions. 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, 2003d). Considerable amounts of CO were produced by radiolysis of organic components and meat (Furuta et al., 1992; Woods and Pikaev, 1994; Nam and Ahn, 2002a,b). Lee and Ahn (2004) reported that glycine, asparagine, glutamine, pyruvate, glyceraldehyde, α-ketoglutarate, and phospholipids were the primary sources of CO production among meat components by irradiation. They indicated that the production of CO was via the radiolytic degradation of meat components and was closely related to the structure of component molecules. They suggested three essential factors for the pink color formation of light meats by irradiation: production of CO, generation of reducing conditions, and CO-Mb ligand formation that intensified the red color greatly.

Watts et al. (1978) found that fresh meat exposed to low levels of CO gas turned red with the formation of CO-Mb. The decrease of oxidation–reduction potential (ORP) in meat played a significant role in CO-Mb formation because the CO-Mb complex can only be formed when heme pigment is in reduced form. Nam and Ahn (2002a,b) showed that irradiation lowered ORP of both aerobically and vacuum-packaged raw and cooked turkey breast meat because irradiation produces hydrated electrons (aqueous e^-), a powerful reducing agent, from water (Swallow, 1984). However, the ORP in irradiated meat increased rapidly during storage under aerobic conditions while stayed stable under vacuum-packaging conditions. The irradiation-induced red pigments were relatively stable against the increased oxidative environment stress during the storage time (Nam and Ahn, 2003d). The increase in ORP facilitated the conversion of myoglobin from ferrous to ferric form, which reduced the affinity of CO to heme pigments, and thus, reduced pink color intensity in such meat. Also, during the storage of irradiated meat under aerobic conditions, CO-Mb receives continuous challenge by oxygen to form Mb-O_2, and eventually oxidizes to metmyoglobin.

The mechanisms of color change in irradiated red meat are different from those of light meats, and the proposed color changing mechanisms in irradiated beef is as follows: irradiation produces aqueous electrons (e_{aq}^-) and hydrogen radicals that have reducing power from water molecules (Thakur and Singh, 1994). Thus, in the absence of O_2, a reducing environment is established, and all the heme pigments in beef 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 $^{\cdot}H$, respectively (Giddings and Markakis, 1972). Therefore, irradiation under aerobic conditions favors ferric Mb (brown color) but produces ferrous Mb (red color) under vacuum conditions. The amount of heme pigments in beef is about >10 times of the light

Downloaded From Vet-eBooks.com

meats, and the proportion of CO-Mb, the compound responsible for color changes in irradiated light meats, to total heme pigments in irradiated red meat is small. Thus, overall beef color is mainly determined by the status of heme pigments, which is determined by the reducing potential of the meat. Irradiation of meat under vacuum conditions or addition of ascorbic acid to aerobically packaged meat creates reducing environments (Wheeler et al., 1996) and can prevent brown color development in ground beef.

6.4.4 Texture Change

The texture change of meat by irradiation is related to protein denaturation and the reduction of water holding capacity (Kanatt et al., 2015a). Zhu et al. (2004) found that irradiation significantly increased centrifugation loss of water from pork loins, but it was partly reversed during refrigerated storage, which could be due to the hydrolysis of muscle proteins. Yoon (2003) reported that irradiated chicken breasts had more cooking loss and higher shear force than the nonirradiated ones. The mechanism for irradiation-induced water loss could be caused by: (1) the damage to the integrity of membrane structure of muscle fibers and (2) denaturation of muscle proteins, which reduced water holding capacity by irradiation. Significant differences in size of sarcomeres between irradiated and nonirradiated breast muscle fibers were observed. However, the texture attributes of chicken breasts were not influenced by low-dose irradiation (1.0 and 1.8 kGy) (Lewis et al., 2002).

6.4.5 Consumer Acceptance of Irradiated Meat

Consumers easily distinguish odor differences between nonirradiated and irradiated meat. Lynch et al. (1991) reported that a set of the unpleasant odor was produced from irradiated turkey breast fillet and the odor was different from nonirradiated samples. Aerobic packaging reduced irradiation off-aroma of raw meat, and consumers could not detect the aroma difference between nonirradiated and irradiated raw and cooked meat after three days of storage. This happened because S-compounds responsible for irradiation off-odor volatilized during storage under aerobic packaging conditions. Lee and Ahn (2003) 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.

Surveys (AMIF, 1993) showed that most supermarket shoppers believed that irradiated foods pose a health risk. The acceptance of irradiated food was affected by consumers' knowledge about food irradiation. Market simulation studies showed that the consumer acceptance of irradiated meat and poultry increased when the participants received additional information

Downloaded From Vet-eBooks.com

about food irradiation (Hashim et al., 1995). The less knowledgeable the participants were about food irradiation, the higher was their level of concern about the process (Bruhn, 1995). However, the effects of positive and negative information about irradiation on consumer response were different: a favorable description of irradiation increased the willingness to pay, and an unfavorable description decreased willingness to pay (Farkas and Mohacsi-Farkas, 2011). When both positive and negative descriptions about irradiation were provided, however, the negative description dominated. The willingness to pay decreased even though the source of negative information was from a consumer advocacy group and was written in a nonscientific manner (Fox et al., 2002).

6.5 Control of Quality Changes in Meat by Irradiation

6.5.1 Additives

Efforts have been made to minimize the negative quality effect of irradiation using various food additives (O'bryan et al., 2008). Antioxidants added to nonirradiated fresh and further processed meat prevented oxidative rancidity, slowed the development of off-flavors, and improved color stability (Morrissey et al., 1997). Phenolic antioxidants interrupt autoxidation of lipids in irradiated meat either by donating hydrogen atom or quenching free radicals (Hsieh and Kinsella, 1989; Nam and Ahn, 2003b). Dietary antioxidant treatments stabilized lipids in membranes, and reduced the extent of lipid oxidation in meat during storage (Morrissey et al., 1997). However, the antioxidant effects of dietary tocopherol in chicken meat differ among muscles types (Ahn et al., 1997).

Lactic acid improved cooked meat appearance and increased myoglobin denaturation during cooking, but produced a tangy off-flavor. However, acid (citric or ascorbic acid) did not affect the redness of irradiated turkey breast (Nam and Ahn, 2002c). Ascorbic acid incorporated to ground beef at the level of 0.1% (w/w) was very efficient in maintaining redness of irradiated ground beef and the color stabilizing effects of ascorbic acid were more distinct in "long-term-aged" than in "pre-aged" irradiated ground beef (Nam and Ahn, 2003a). Ascorbic acid significantly lowered the ORP values, which helped the heme pigments in ferrous status, and stabilized the color of irradiated ground beef (Nam et al., 2003; Nam and Ahn, 2003a).

The addition of antimicrobial agents had synergistic effects with irradiation in killing microorganisms in meat (Jensen et al., 2003). However, the effect of antimicrobial agents on the texture, color, and sensory properties of meat products was not consistent (Sommers and Fan, 2003; Choi and Chin, 2003). The addition of benzoate increased the content of benzene in the volatiles of irradiated RTE turkey ham and breast rolls, and thus caution is needed to use benzoate salt in products for irradiation (Zhu et al., 2004, 2005).

Downloaded From Vet-eBooks.com

6.5.2 Packaging

Packaging is a major factor influencing color and the amounts and types of volatiles detected in irradiated meat. The greenish brown color was problematic when ground beef was irradiated under aerobic conditions, but anaerobic conditions protected the beef from discoloration (Nam et al., 2004). Vacuum packaging prevented oxidative changes and color fading and retained sulfur volatiles inside the packaging bag during storage, which reduced the odor acceptance of irradiated meat (Nam et al., 2002a). Vacuum packaging is an excellent strategy to inhibit lipid oxidation in meat during storage because oxygen is essential for the progress of lipid oxidation (Ahn et al., 2001). Vacuum-packaged meats have mainly purple deoxymyoglobin if the oxygen partial pressure reaches zero. Failure to remove oxygen completely, however, can result in oxidizing conditions associated with low partial oxygen pressure. The impacts of irradiation on meat color are related to oxygen availability and the amount of free radicals formed during irradiation. Nanke et al. (1999) reported that irradiated meat in aerobic packaging discolored more rapidly than nonirradiated samples during display. In irradiated meat, vacuum packaging was better than aerobic packaging in preventing lipid oxidation and oxidation-dependent volatile production, but increased pink color intensity. Aerobic packaging was more desirable for the irradiated meat color than vacuum packaging if lipid oxidation could be controlled (Ahn et al., 2000b, 2001). An appropriate combination of aerobic and anaerobic packaging conditions was effective in minimizing both off-odor volatiles and lipid oxidation in irradiated raw turkey breast during storage, and it also was effective in reducing the generation of pink color in irradiated meat compared to vacuum packaging alone (Nam and Ahn, 2003c,d). Sulfur compounds, the most critical volatiles for off-odor development in irradiated meat, could easily be eliminated under aerobic conditions (Ahn et al., 2001). Nam and Ahn (2003c,d) found that irradiation and aerobic packaging promoted aldehydes production, especially propanal and hexanal, highly correlated with lipid oxidation in turkey breast and thigh meats. The term "double-packaging" describes a packaging method in which meat pieces are individually packaged in oxygen-permeable bags at first, and then a few of them are vacuum packaged in a larger vacuum bag. After a certain period of storage time, the outer vacuum bag is removed and stored until the last day of storage. Double-packaging was very effective in controlling both lipid oxidation-dependent (aldehydes) and radiolytic off-odor (S-compounds) volatiles. However, double-packaging alone was not enough to reduce the pink color of irradiated raw turkey meat (Nam and Ahn, 2003c,d).

6.5.3 Packaging and Additive Combinations

The addition of antioxidants to irradiated meat was an effective method in complementing problems of double-packaging. The addition of sesamol + tocopherol (S + E) or gallate + tocopherol (G + E) combinations lowered the

Downloaded From Vet-eBooks.com

amount of propanal and total volatiles in double-packaged and irradiated raw turkey meat (Nam and Ahn, 2003c). After 10 days of refrigerated storage, however, volatile profiles of irradiated turkey breast were highly dependent upon antioxidant and packaging conditions: sulfur volatiles were not detected in irradiated aerobically packaged or double-packaged meat. However, aerobically packaged irradiated meat without antioxidants produced large amounts of aldehydes (propanal, hexanal) and 2-butanone during storage. The double-packaged meat had lower lipid oxidation products than aerobically packaged meat, but antioxidant combinations significantly reduced the amounts. Therefore, the double-packaging in combination with antioxidants was effective in reducing sulfur volatiles responsible for the irradiation off-odor without any problem in lipid oxidation (Nam and Ahn, 2003c).

The beneficial effects of double-packaging and antioxidant combinations on volatiles were more clearly shown in irradiated cooked meat (Nam and Ahn, 2003c). Double-packaging in combination with gallate + α-tocopherol (G + E) or S + E significantly reduced the redness of irradiated cooked turkey breast meat, but G + E was more effective than S + E. Double-packaging itself was more effective than vacuum-packaging in reducing sulfur volatiles and lipid oxidation-dependent volatiles compared with aerobic packaging in cooked meat. The total amounts of sulfur volatiles in double-packaged irradiated turkey meat with antioxidants were only about 5%–7% of the irradiated vacuum-packaged cooked meat without antioxidants. Production of most aldehydes in irradiated cooked turkey breast was prevented by using antioxidants and double-packaging combinations. The combined use of double-packaging and ascorbic acid was also very effective in reducing off-odor volatiles and maintaining the bright red color of irradiated ground beef (Nam et al., 2004). Both irradiating under vacuum condition and the added ascorbate was helpful in maintaining low ORP of irradiated beef and caused myoglobin to remain in a reduced form.

6.6 Future Research Needed

Most of the irradiation studies are done with raw meat because irradiation is not permitted for meats treated with additives, further processed, or RTE meat products. Therefore, future studies on irradiation should be focused on flavor, color, and taste changes in further processed and precooked RTE meat products. Although odor and color are important factors for consumer acceptance of irradiated raw meat, the most important quality parameter for cooked meat is taste because if irradiated meat has undesirable taste, consumers will never choose irradiated meat again. Currently, no information on the mechanisms and causes of taste/flavor changes in irradiated cooked

Downloaded From Vet-eBooks.com

meat is available. Therefore, research to elucidate the causes and mechanisms of taste changes in irradiated cooked meat to determine the roles of spices and additives on taste/flavor of irradiated processed meat and to develop methods that can control taste/flavor changes in irradiated further processed meat are needed. The effect of those additives on the microcidal efficiency of irradiation should also be determined.

References

Ahn, D. U. (2002). Production of volatiles from amino acid homopolymers by irradiation. *Journal of Food Science,* **67**(7), 2565–2570.

Ahn, D. U. and Lee, E. J. (2002). Production of off-odor volatiles from liposome-containing amino acid homopolymers by irradiation. *Journal of Food Science,* **67**(7), 2659–2665.

Ahn, D. U., Jo, C. and Olson, D. G. (2000a). Analysis of volatile components and the sensory characteristics of irradiated raw pork. *Meat Science,* **54**, 209–215.

Ahn, D. U., Jo, C., Olson, D. G. and Nam, K. C. (2000b). Quality characteristics of pork patties irradiated and stored in different packaging and storage conditions. *Meat Science,* **56**, 203–209.

Ahn, D. U., Lee, E. J., Feng, X. et al. (2016a). Mechanisms of volatile production from non-sulfur amino acids by irradiation. *Radiation Physics and Chemistry,* **119**, 64–73.

Ahn, D. U., Lee, E. J., Feng, X. et al. (2016b). Mechanisms of volatile production from sulfur-containing amino acids by irradiation. *Radiation Physics and Chemistry,* **119**, 80–84.

Ahn, D. U., Nam, K. C., Du, M. and Jo, C. (2001). Effect of irradiation and packaging conditions after cooking on the formation of cholesterol and lipid oxidation products in meats during storage. *Meat Science,* **57**, 413–418.

Ahn, D. U., Olson, D. G., Jo, C., Chen, X., Wu, C. and Lee, J. I. (1998a). Effect of muscle type, packaging, and irradiation on lipid oxidation, volatile production and color in raw pork patties. *Meat Science,* **49**, 27–39.

Ahn, D. U., Olson, D. G., Lee, J. I., Jo, C., Wu, C. and Chen, X. (1998b). Packaging and irradiation effects on lipid oxidation and volatiles in pork patties. *Journal of Food Science,* **63**(1), 15–19.

Ahn, D. U., Sell, J. L., Jeffery, M. et al. (1997). Dietary vitamin E affects lipid oxidation and total volatiles of irradiated raw turkey meat. *Journal of Food Science,* **62**, 954–959.

Alahakoon, A. U., Jayasena, D. D., Jung, S., Kim, S. H., Kim, H. J. and Jo, C. (2015). Effect of electron beam irradiation and high-pressure treatment combined with citrus peel extract on seasoned chicken breast meat. *Journal of Food Processing and Preservation,* **39**, 2332–2339.

American Meat Institute Foundation (AMIF). (1993). *Consumer Awareness, Knowledge, and Acceptance of Food Irradiation*. Washington, DC: American Meat Institute Foundation.

Angelini, P., Merritt, C., Jr., Mendelshon, J. M. and King, F. J. (1975). Effect of irradiation on volatile constituents of stored haddock flesh. *Journal of Food Science,* **40**, 197–199.

Downloaded From Vet-eBooks.com

Arthur, T. M., Wheeler, T. L., Shackelford, S. D., Bosilevac, J. M., Nou, X. W. and Koohmaraie, M. (2005). Effects of low-dose, low-penetration electron beam irradiation of chilled beef carcass surface cuts on *Escherichia coli* O157: H7 and meat quality. *Journal of Food Protection,* **68**(4), 666–672.

Badr, H. M. (2004). Use of irradiation to control foodborne pathogens and extend the refrigerated market life of rabbit meat. *Meat Science,* **67**(4), 541–548.

Batzer, O. F. and Doty, D. M. (1955). Radiation sterilization, nature of undesirable odors formed by gamma irradiation of beef. *Journal of Agricultural and Food Chemistry,* **3**(1), 64–67.

Bhide, M. R., Paturkar, A. M., Sherikar, A. T. and Waskar, V. S. (2001). Pre-sensitization of microorganisms by acid treatments to low dose gamma irradiation with special reference to *Bacillus cereus*. *Meat Science,* **58**(3), 253–258.

Bruhn, C. M. (1995). Strategies for communicating the fact on food irradiation to consumers. *Journal of Food Protection,* **58**, 213–216.

Buttery, R. G., Guadagni, D. G. and Ling, L. C. (1973). Flavor compounds: Volatilities in vegetable oil and oil-water mixtures: Estimation of odor thresholds. *Journal of Agricultural and Food Chemistry,* **21**, 198–201.

Council for Agricultural Science and Technology (CAST). (1986). Ionizing Energy in Food Processing and Pest Control: I. Wholesomeness of food treated with ionizing energy. In *Task Force Report No.109:50*, Ames, IA.

Chen, C. M., Sebranek, J. G., Dickson, J. S. and Mendonca, A. F. (2004). Combining pediocin with postpackaging irradiation for control of *Listeria monocytogenes* on frankfurters. *Journal of Food Protection,* **67**, 1866–1875.

Choi, S. H. and Chin, K. B. (2003). Evaluation of sodium lactate as a replacement for conventional chemical preservatives in comminuted sausages inoculated with *Listeria monocytogenes. Meat Science,* **65**, 531–537.

Clavero, M. R., Monk, J. D., Beuchat, L. R., Doyle, M. P. and Brackett, R. E. (1994). Inactivation of *Escherichia coli* O157:H7, salmonellae, and *Campylobacter jejuni* in raw ground beef by gamma irradiation. *Applied Environmental Microbiology,* **60**, 2069–2075.

Davies, M. J. (1996). Protein and peptide alkoxyl radicals can give rise to C-terminal decarboxylation and backbone cleavage. *Archives of Biochemistry and Biophysics,* **336**, 163–172.

Diehl, J. F. (1995). *Safety of Irradiated Foods*. 2nd ed. New York, NY: Marcel Dekker, 283–289.

Dogbevi, M. K., Vachon, C. and Lacroix, M. (1999). Physicochemical and microbiological changes in irradiated fresh pork loins. *Meat Science,* **51**, 349–354.

Efiok, B. S. (1996). *Basic Calculations for Biomedical and Biological Analyses*. Gaithersburg, MD: AOAC International, 84–86.

El-Bisi, H. M., Snyder, O. P. and Levin, R. E. (1966). Radiation death kinetics of *C. botulinum* spores at cryogenic temperatures. *In:* Ingram, I. M. and Roberts, T. A. (eds.) *Botulism*. London: Chapman and Hall, 89–107.

Fan, X., Sommers, C. H., Thayer, D. W. and Lehotay, S. J. (2002). Volatile sulfur compounds in irradiated precooked turkey breast analyzed with pulsed flame photometric detection. *Journal of Agricultural and Food Chemistry,* **50**(15), 4257–4261.

Farkas, J. (2006). Irradiation for better foods. *Trends in Food Science & Technology,* **17**, 148–152.

Farkas, J. and Andrassy, E. (1993). Interaction of ionizing radiation and acidulants on the growth of the microflora of a vacuum-packaged chilled meat. *International Journal of Food Microbiology,* **19**, 145–152.

Downloaded From Vet-eBooks.com

Farkas, F. and Mohacsi-Farkas, C. (2011). History and future of food irradiation. *Trends in Food Science and Technology,* **22**, 121–126.

Fox, J. A., Hayes, D. J. and Shogren, J. F. (2002). Consumer preferences for food irradiation: How favorable and unfavorable descriptions affect preferences for irradiated pork in experimental auctions. *Journal of Risk Uncertainty,* **24**(1), 75–95.

Furuta, M., Dohmaru, T., Katayama, T., Toratoni, H. and Takeda, A. (1992). Detection of irradiated frozen meat and poultry using carbon monoxide gas as a probe. *Journal of Agricultural and Food Chemistry,* **40**(7), 1099–1100.

Galan, I., García, M. L. and Selgas, M. D. (2011). Irradiation is useful for manufacturing ready-to-eat cooked meat products enriched with folic acid. *Meat Science,* **87**, 330–335.

Giddings, G. G. and Markakis, P. (1972). Characterization of the red pigments produced from ferrimyoglobin by ionizing radiation. *Journal of Food Science,* **37**, 361–364.

Godshall, M. A. (1997). How carbohydrate influence flavor. *Food Technology,* **51**, 63–67.

Halliwell, B. J. M. and Gutteridge, C. (1989). A consideration of atomic structure and bonding. *In*: Halliwell, B. J. M. and Gutteridge, C. (eds.) *Free Radicals in Biology and Medicine*. 2nd ed. London: Clarendon Press, 508–524.

Hashim, I. B., Resurreccion, A.V.A. and MaWatters, K. H. (1995). Disruptive sensory analysis of irradiated frozen or refrigerated chicken. *Journal of Food Science,* **60**, 664–666.

Hayashi, T. (1991). Comparative effectiveness of gamma rays and electron beams in food irradiation. *In*: Thorne, S. (ed.) *Food Irradiation.* London: Elsevier Applied Science, 169–206.

Hsieh, R. J. and Kinsella, J. E. (1989). Oxidation of polyunsaturated fatty acids: Mechanisms, products, and inhibition with emphasis on fish. *Advances in Food Nutrition Research,* **33**, 233–237.

Huq, T., Vu, K. D., Riedl, B., Bouchard, J. and Lacroix, M. (2015). Synergistic effect of gamma (g)-irradiation and microencapsulated antimicrobials against *Listeria monocytogenes* on ready-to-eat (RTE) meat. *Food Microbiology,* **46**, 507–514.

International Atomic Energy Agency (IAEA). (1991). Facts about food irradiation. *International Consultative Group on Food Irradiation*. Vienna: International Atomic Energy Agency. (accessed 23 December 2015).

Jarrett, R. D. Sr. (1982). Isotope (gamma) radiation sources. *In*: Josephson, E. S. and Peterson, M. S. (eds.) *Preservation of Food by Ionizing Radiation* (Vol. 1). Boca Raton, FL: CRC Press, 137–163.

Javanmard, M., Rokni, N., Bokaie, S. and Shahhosseini, G. (2006). Effects of gamma irradiation and frozen storage on microbial, chemical and sensory quality of chicken meat in Iran. *Food Control,* **17**, 469–473.

Jensen, J. M., Robbins, K. L., Ryan, K. J., Homco-Ryan, C., McKeith, F. K. and Brewer, M. S. (2003). Effects of lactic and acetic acid salts on quality characteristics of enhanced pork during retail display. *Meat Science,* **63**, 501–508.

Jo, C. and Ahn, D. U. (1999). Fat reduces volatiles production in oil emulsion system analyzed by purge-and-trap dynamic headspace/gas chromatography. *Journal of Food Science,* **64**(4), 641–643.

Jo, C. and Ahn, D. U. (2000). Production of volatiles from irradiated oil emulsion systems prepared with amino acids and lipids. *Journal of Food Science,* **65**(4), 612–616.

Jo, C., Jin, S. K. and Ahn, D. U. (2000). Color changes in irradiated cooked pork sausage with different fat sources and packaging during storage. *Meat Science,* **55**, 107–113.

Downloaded From Vet-eBooks.com

Josephson, E. S. and Peterson, M. S. (2000). *Preservation of Food by Ionizing Radiation (II)*. Boca Raton, FL: CRC Press, 102–103.

Kanatt, S. R., Chander, R. and Sharma, A. (2015a). Effect of radiation processing on the quality of chilled meat products. *Meat Science,* **69**, 269–275.

Kanatt, S. R., Chander, R. and Sharma, A. (2015b). Effect of radiation processing on meat tenderization. *Radiation Physics and Chemistry,* **111**, 1–8.

Kanatt, S. R., Chawla, S. P., Chander, R. and Bongirwar, D. R. (2002). Shelf-stable and safe intermediate-moisture meat products using hurdle technology. *Journal of Food Protection,* **65**, 1628–1631.

Kim, A. Y. and Thayer, D. W. (1996). Mechanism by which irradiation increases the sensitivity of *Salmonella typhimurium* ATCC 14028 to heat. *Applied Environmental Microbiology,* **62**, 1759–1763.

Kume, T., Furuta, M., Todoriki, S., Uenoyama, N. and Kobayashi, Y. (2009). Status of food irradiation in the world. *Radiation Physics and Chemistry,* **78**, 222–226.

Kundu, D., Gill, A., Lui, C., Goswami, N. and Holley, R. (2014). Use of low dose e-beam irradiation to reduce *E. coli O157:H7*, non-O157 (VTEC) *E. coli* and *Salmonella* viability on meat surfaces. *Meat Science,* **96**, 413–418.

Kwon, J.H. (2010). Research and application of irradiated foods (Chapter 2). In *Safety and Understanding of Irradiated Food.* Korea Food Safety Research Institute, pp. 1–37.

Lagunas-Solar, M. C. (1995). Radiation processing of food: An overview of scientific principles and current status. *Journal of Food Protection,* **58**, 186–192.

Lambert, A. D., Smith, J. P. and Dodds, K. L. (1992). Physical, chemical, and sensory changes in irradiated fresh pork packaged in modified atmosphere. *Journal of Food Science,* **57**, 1294–1299.

Lee, E. J. and Ahn, D. U. (2003). Production of off-odor volatiles from fatty acids and oils by irradiation. *Journal of Food Science,* **68**(1), 70–75.

Lee, E. J. and Ahn, D. U. (2004). Sources and mechanisms of carbon monoxide production by irradiation. *Journal of Food Science,* **69**(6), c485–c490.

Lefebvre, N., Thibault, C., Charbonneau, R. and Piette, J. P. G. (1994). Improvement of shelf-life and wholesomeness of ground beef by irradiation 2: Chemical analysis and sensory evaluation. *Meat Science,* **36**, 371–380.

Lewis, S. J., Velasquez, A., Cuppett, S. L. and McKee, S. R. (2002). Effect of electron beam irradiation on poultry meat safety and quality. *Poultry Science,* **81**, 896–903.

Lubbers, S., Landy, P. and Voilley, A. (1998). Retention and release of aroma compounds in foods containing proteins. *Food Technology,* **52**, 68–74, 208–214.

Luchsinger, S. E., Kropf, D. H., Garcia-Zepeda, C. et al. (1997). Color and oxidative properties of irradiated ground beef patties. *Journal of Muscle Foods,* **8**(4), 445–464.

Luchsinger, S. E., Kropf, D. H., Garcia-Zepeda, C. M. et al. (1996). Color and oxidative rancidity of gamma and electron beam irradiated boneless pork chops. *Journal of Food Science,* **61**(5), 1000–1005, 1093.

Lynch, J. A., Macfie, H. J. H. and Mead, G. C. (1991). Effect of irradiation and packaging type on sensory quality of chill-stored turkey breast fillets. *International Journal of Food Science and Technology,* **26**, 653–668.

Mendonca, A. F., Romero, M. G., Lihono, M. A., Nannapaneni, R. and Johnson, M. G. (2004). Radiation resistance and virulence of *Listeria monocytogenes* following starvation in physiological saline. *Journal of Food Protection,* **67**, 470–474.

Downloaded From Vet-eBooks.com

Merritt, C., Jr., Angelini, P., Wierbicki, E. and Shuts, G. W. (1975). Chemical changes associated with flavor in irradiated meat. *Journal of Agricultural and Food Chemistry,* **23**, 1037–1043.

Millar, S. J., Moss, B. W., MacDougall, D. B. and Stevenson, M. H. (1995). The effect of ionizing radiation on the CIELAB color co-ordinates of chicken breast meat as measured by different instruments. *International Journal of Food Science and Technology,* **30**, 663–674.

Miyahara, M., Saito, A., Kamimura, T., Nagasawa, T., Ito, H. and Toyoda, M. (2002). Hydrocarbon productions in hexane solutions of fatty acid methyl esters irradiated with gamma rays. *Journal of Health Science,* **48**, 418–426.

Morrissey, P. A., Brandon, S., Buckley, D. J., Sheehy, P. J. A. and Frigg, J. (1997). Tissue content of alpha-tocopherol and oxidative stability of broilers receiving dietary alpha-tocopheryl acetate supplementation for various periods pre-slaughter. *British Poultry Science,* **38**, 84–88.

Mottram, D. S., Wedzicha, B. L. and Dodson, A. T. (2002). Acrylamide is formed in the Maillard reaction. *Nature,* **419**, 448–449.

Nam, K. C. and Ahn, D. U. (2002a). Mechanisms of pink color formation in irradiated precooked turkey breast. *Journal of Food Science,* **67**(2), 600–607.

Nam, K. C., and Ahn, D. U. (2002b). Carbon monoxide-heme pigment complexes are responsible for the pink color in irradiated raw turkey breast meat. *Meat Science,* **60**(1), 25–33.

Nam, K. C. and Ahn, D. U. (2002c). Effect of double-packaging and acid combination on the quality of irradiated raw turkey patties. *Journal of Food Science,* **67**(9), 3252–3257.

Nam, K. C. and Ahn, D. U. (2003a). Effects of ascorbic acid and antioxidants on the color of irradiated beef patties. *Journal of Food Science,* **68**(5), 1686–1690.

Nam, K. C. and Ahn, D. U. (2003b). Use of antioxidants to reduce lipid oxidation and off-odor volatiles of irradiated pork homogenates and patties. *Meat Science,* **63**, 1–8.

Nam, K. C. and Ahn, D. U. (2003c). Use of double-packaging and antioxidant combinations to improve color, lipid oxidation, and volatiles of irradiated raw and cooked turkey breast patties. *Poultry Science,* **82**(5), 850–857.

Nam, K. C. and Ahn, D. U. (2003d). Combination of aerobic and vacuum packaging to control color, lipid oxidation and off-odor volatiles of irradiated raw turkey breast. *Meat Science,* **63**(3), 389–395.

Nam, K. C., Du, M., Jo, C. and Ahn, D. U. (2002a). Quality characteristics of vacuum-packaged, irradiated normal, PSE, and DFD Pork. *Innovative Food Science and Emerging Technology,* **3**(1), 73–79.

Nam, K. C., Hur, S. J., Ismail, H. and Ahn, D. U. (2002b). Lipid oxidation, volatiles, and color changes in irradiated raw turkey breast during frozen storage. *Journal of Food Science,* **67**(6), 2061–2066.

Nam, K. C., Kim, Y. H., Du, M. and Ahn, D. U. (2002c). Off-odor and pink color development in precooked, ionizing-radiated turkey breast during frozen storage. *Poultry Science,* **81**, 269–275.

Nam, K. C., Min, B. R., Lee, S. C., Cordray, J. and Ahn, D. U. (2004). Prevention of pinking, off-odor, and lipid oxidation in irradiated pork loin using double packaging. *Journal of Food Science,* **69**(3), FTC214–219.

Nam, K. C., Min, B. R., Park, K. S., Lee, S. C. and Ahn, D. U. (2003). Effects of ascorbic acid and antioxidants on the lipid oxidation and volatiles of irradiated beef patties. *Journal of Food Science,* **68**(5), 1680–1685.

Downloaded From Vet-eBooks.com

Nanke, K. E., Sebranek, J. G. and Olson, D. G. (1998). Color characteristics of irradiated vacuum-packaged pork, beef, and turkey. *Journal of Food Science,* **63**(6), 1001–1006.

Nanke, K. E., Sebranek, J. G. and Olson, D. G. (1999). Color characteristics of irradiated aerobically packaged pork, beef, and turkey. *Journal of Food Science,* **64**, 272–276.

Nawar, W. W. (1986). Volatiles from food irradiation. *Food Review International,* **2**(1), 45–78.

O'bryan, C. A., Crandall, P. G., Ricke S. C. and Olson, D. G. (2008). Impact of irradiation on the safety and quality of poultry and meat products: A review. *Critical Reviews in Food Science and Nutrition,* **48**, 442–457.

Olson, D. G. (1998). Irradiation of food. *Food Technology,* **52**, 56–62.

Patterson, R. L. and Stevenson, M. H. (1995). Irradiation-induced off-odor in chicken and its possible control. *British Poultry Science,* **36**, 425–441.

Proctor, B. E. and Goldblith, S. A. (1951). Food processing with ionizing radiations. *Food Technology,* **5**(9), 376–380.

Roberts, F. B. (2014). Food irradiation is safe: Half a century of studies. *Radiation Physics and Chemistry,* **105**(12), 78–82.

Satin, M. (1993). *Food Irradiation: A Guidebook*. Lancaster, England. Technomic Publishing.

Satin, M. (2002). Use of irradiation for microbial decontamination of meat: Situation and perspectives. *Meat Science,* **62**, 277–283.

Satterlee, L. D., Wilhelm, M. S. and Barnhart, H. M. (1971). Low dose gamma irradiation of bovine metmyoglobin. *Journal of Food Science,* **36**(3), 549–551.

Shahidi, F. and Pegg, R. B. (1994). *Lipids in Food Flavors. ACS Symposium Series* (Vol. 558). Washington, DC: American Chemical Society, 256–279.

Soladoye, O. P., Jua'rez, M. L., Aalhus, J. L., Shand, P. and Este'vez, M. (2015). Protein oxidation in processed meat: Mechanisms and potential implications on human health. *Comprehensive Reviews in Food Science and Food Safety,* **14**, 106–122.

Sommers, C. H. and Fan, X. (2002). Antioxidant power, lipid oxidation, color, and viability of *Listeria monocytogenes* in beef bologna treated with gamma radiation and containing various levels of glucose. *Journal of Food Protection,* **65**, 1750–1755.

Sommers, C. H. and Fan, X. (2003). Gamma irradiation of fine-emulsion sausage containing sodium diacetate. *Journal of Food Protection,* **66**, 819–824.

Sommers, C. H., Fan, X., Handel, P. A. and Sokorai, K. B. (2003). Effect of citric acid on the radiation resistance of *Listeria monocytogenes* and frankfurter quality factors. *Meat Science,* **63**, 407–415.

Steccheni, M. I., Del Torre, M., Sarais, P. G., Fuochi, F., Tubaro, F. and Ursini, F. (1998). Carnosine increases the radiation resistance of *Aeromonas hydrophila* in minced turkey meat. *Journal of Food Science,* **61**, 979–987.

Swallow, A. J. (1984). Fundamental radiation chemistry of food components. *In:* Bailey, A. J. (ed.) *Recent Advances in the Chemistry of Meat*. London: The Royal Society of Chemistry, Burlington House, 165–177.

Tappel, A. L. (1956). Regeneration and stability of oxymyoglobin in some gamma irradiated meats. *Food Research,* **21**, 650–654.

Tappel, A. L. (1957). The red pigment of precooked irradiated meats. *Food Research,* **22**, 408–412.

Taub, I. A., Karielian, R. A., Halliday, J. W., Walker, J. E., Angeline, P. and Merritt, C. (1979). Factors affecting radiolytic effects of food. *Radiation Physics and Chemistry,* **14**, 639–653.

Downloaded From Vet-eBooks.com

Thakur, B. R. and Singh, R. K. (1994). Food irradiation: Chemistry and applications. *Food Review International,* **10**, 437–473.

Thayer, D. W. and Boyd, G. (1991). Effect of ionizing radiation dose, temperature, and atmosphere on the survival of *Salmonella typhimurium* in sterile mechanically deboned chicken meat. *Poultry Science,* **70**, 381–388.

Thayer, D. W. and Boyd, G. (1992). Gamma ray processing to destroy *Staphylococcus aureus* in mechanically deboned chicken meat. *Journal of Food Science,* **57**, 848–851.

Thayer, D. W. and Boyd, G. (1993). Elimination of *Escherichia coli* O157 in meats by gamma irradiation. *Applied Environmental Microbiology,* **59**, 1030–1034.

Thayer, D. W. and Boyd, G. (1995). Radiation sensitivity of *Listeria monocytogenes* on beef as affected by temperature. *Journal of Food Science,* **60**, 237–240.

United States Department of Agriculture-Food Safety and Inspection Service (USDA-FSIS). (1986). Irradiation of pork for control of *Trichinella spiralis*. *Federal Register,* **51**, 1769–1771.

United States Department of Agriculture-Food Safety and Inspection Service (USDA-FSIS). (1999). Meat and poultry irradiation proposal. *USDA Food Safety and Inspection Service.* Available at http://www.fsis.usda.gov/Oa/background/irradprop.htm?redirecthttp=true (accessed 23 December 2015).

Venugopal, V., Doke, S. N. and Thomas, P. (1999). Radiation processing to improve the quality of fishery products. *Critical Review in Food Science and Nutrition,* **39**(5), 391–440.

Watts, D. A., Wolfe, S. K. and Brown, W. D. (1978). Fate of [^{14}C] carbon monoxide in cooked or stored ground beef samples. *Journal of Agricultural and Food Chemistry,* **26**(1), 210–214.

Wheeler, T. L., Koohmaraie, M. and Shackelford, S. D. (1996). Effect of vitamin C concentration and co-injection with calcium chloride on beef retail display color. *Journal of Animal Science,* **74**(8), 1846–1853.

World Health Organization (WHO). (1981). Wholesomeness of irradiated foods. In *Technical Report Series 659*. World Health Organization, Geneva, Switzerland.

World Health Organization (WHO). (1994). Food irradiation. In *Safety and Nutritional Adequacy of Irradiated Food, 5–13*. World Health Organization, Geneva, Switzerland.

World Health Organization (WHO). (1999). High-dose irradiation: Wholesomeness of food irradiated with doses above 10 kGy: Report of a Join FAO/IAEA/WHO Study Group. World Health Organization, Geneva, Switzerland.

Woods, R. J. and Pikaev, A. K. (1994). Interaction of radiation with matter. Selected topics in radiation chemistry. *In:* Woods, R. J. and Pikaev, A. K. (eds.) *Applied Radiation Chemistry: Radiation Processing*. New York, NY: John Wiley and Sons, 59–89 and 165–210.

Xavier, M. P., Dauber, C., Mussio, P. et al. (2014). Use of mild irradiation doses to control pathogenic bacteria on meat trimmings for production of patties aiming at provoking minimal changes in quality attributes. *Meat Science,* **98**, 383–391.

Yoon, K. S. (2003). Effect of gamma irradiation on the texture and microstructure of chicken breast meat. *Meat Science,* **63**, 273–277.

Zhu, M. J., Mendonca, A., Min, B. et al. (2004). Effects of electron beam irradiation and antimicrobials on the volatiles, color and texture of ready-to-eat turkey breast roll. *Journal of Food Science,* **69**(5), C382–C387.

Zhu, M. J., Mendonca, A., Ismail, H. A., Du, M., Lee, E. J. and Ahn, D. U. (2005). Impact of antimicrobial ingredients and irradiation on the survival of *Listeria monocytogenes* and quality of ready-to-eat turkey ham. *Poultry Science,* **84**, 613–620.

Downloaded From Vet-eBooks.com



Taylor & Francis
Taylor & Francis Group
http://taylorandfrancis.com

Downloaded From Vet-eBooks.com

7

Advances in High Hydrostatic Pressure for Meat and Meat Processing

Sencer Buzrul

CONTENTS

7.1 Introduction 228
7.2 Effect of HHP on the Inactivation of Microorganisms in Meat and Meat Products 230
7.2.1 Inactivation of *Listeria monocytogenes* in Meat and Meat Products under HHP 230
7.2.1.1 Different Types of Survival Curves of *L. monocytogenes* in Meat and Meat Products under HHP 232
7.2.2 Methods Used to Improve Microbial Inactivation during HHP Treatment of Meat and Meat Products 232
7.2.2.1 Temperature 232
7.2.2.2 Additives/Antimicrobials 238
7.2.2.3 Multipulsed HHP Treatment of Meat Products 238
7.2.2.4 Hurdle Concept 239
7.2.2.5 Limitations of the Abovementioned Methods 240
7.3 Effects of HHP on Quality Attributes of Meat and Meat Products 241
7.3.1 Effect of HHP on Texture of Meat and Meat Products 241
7.3.2 Effect of HHP on Lipid Oxidation of Meat and Meat Products 241
7.3.3 Effect of HHP on Color of Meat and Meat Products 242
7.3.4 Other Effects of HHP on Meat and Meat Products 243
7.4 Consumer Acceptance of HHP-Treated Meat and Meat Products 244
7.5 Some Important Results from Recent Studies of HHP on Meat Products 245
7.6 Opportunities for HHP Application to Meat and Meat Products 246
7.7 Limitations of HHP on Meat and Meat Products 247
7.7.1 On the Application of HHP on Fresh Meat 247
References 248

Downloaded From Vet-eBooks.com

7.1 Introduction

The modern consumer requires foods that are safe and nutritious, free from additives, taste good, and, for certain products, have a longer shelf life. There are a number of possible scientific solutions to meet these demands, such as genetic modification and gamma irradiation, but these have met with consumer resistance. High hydrostatic pressure (HHP) processing is one technology that has the potential to fulfill both consumer and scientific requirements. In HHP treatment, pressures up to 900 MPa are used to kill many of the microorganisms found in foods, even at room temperature, without degrading vitamins, flavor, and color molecules in the process (Patterson et al., 2007; Polydera et al., 2005).

Macfarlane and coworkers in Australia, who pioneered the use of pressure on meat, carried out some well-designed studies in the 1970s–1980s. However, the main problem associated with these studies was that the pressure vessel used was only capable of reaching pressures of 150 MPa (Bouton et al., 1977; Macfarlane, 1973, 1985; Macfarlane and Morton, 1978; Macfarlane et al., 1981, 1982, 1986). Nevertheless, these studies were a useful starting point of later studies and also clearly demonstrated that treatment of prerigor meat at these pressures led to marked increases in tenderness (Ma and Ledward, 2013). After these pioneering studies, the effects of pressure on meat and meat products were investigated extensively by many researchers and the use of HHP for meat and meat products has been described in different journal papers (Bajovic et al., 2012; Buckow et al., 2013; Campus, 2010; Cheftel and Culioli, 1997; Hugas et al., 2002; Simonin et al., 2012; Sun and Holley, 2010; Teixeira et al., 2014) as well as in different book chapters (Atsushi et al., 2006; Garriga and Aymerich, 2009; Ikeuchi, 2011; Tokuşoğlu and Vural, 2014).

The Spanish company Espuña pioneered the use of HHP for the pasteurization of meat products. Sliced cooked ham with the label "high pressure pasteurized product remains fresh until eaten" was launched in Spain in 1998 (Grèbol, 2002). Even now, it is being sold and distributed in several supermarket chains in Spain. The product's ham slices are vacuum skin-packed with plastic film interleaves to facilitate the separation of slices by the consumer. It has a refrigerated shelf life of 60 days and it is processed for 10 minutes at 400 MPa (Tonello, 2011). Since then, HHP has become an industrial reality for the meat industry, and HHP-treated meat products are available in different parts of the world—see Table 7.1.

A market research study reported that as of 2013, the global HHP products market has achieved $3 billion. Fruit and vegetable products (35% of all industrial applications) are the largest product market for HHP, followed by meat products (31%), seafood and fish products (14%), and then other products (8%) (Koutchma, 2014).

Downloaded From Vet-eBooks.com

TABLE 7.1

Products, Country, and Year of Introduction of the Technology

Country	Year	Product
Spain	1998	Cooked sliced ham and tapas (pork and poultry cuts)
United States	2001	Cooked sliced ham, pork meat products, and Parma ham
United States	2001	Poultry ready-to-eat (RTE) products
United States	2002	Spicy sliced precooked chicken and beef for fajitas
Spain	2002	Sliced ham, chicken and turkey products, cooked and Serrano ham, and chorizo
Spain	2003	Gross slices of ham, turkey, and chicken
Italy	2003	Parma ham, salami, and mortadella
Japan	2004	Raw ham-like pork ham
Japan	2005	Cooked pork meat products, nitrite free ham, sausages, and bacon
Germany	2005	Smoked German ham: whole, sliced, and diced products
Spain	2006	Cooked ham
Canada	2006	Cured meat products
Canada	2006	RTE meat and vegetable meals
Japan	2007	Ham without nitrite
Greece	2010	Deli meats infused with extra virgin olive oil
Greece	2010	Sliced processed meat: mortadella, ham, and salami
Netherlands	2010	Filet American
Romania	2011	Fermented sausages
United States	2011	Hamburger
United States	2011	Deli meat and fermented meats
United Kingdom	2011	Meat and cheese snack products
United States	N.I.	Poultry strips
United States	N.I.	Oven-roasted chicken
Canada	N.I.	Sliced dry-cured meat and cooked meats

Source: Adapted from Bajovic, B. et al., *Meat Science*, 92, 280–289, 2012; Buzrul, S. and Alpas, H., In: Bhat, R., Alias, A. K. and Paliyath G. [eds.], *Progress in Food Preservation*. Wiley-Blackwell, West Sussex, 2012; Campus, M., *Food Engineering Reviews*, 2, 256–273, 2010; Ikeuchi, Y., In: Kerry, J. P. and Kerry, J. F. [eds.], *Processed Meats—Improving Safety, Nutrition and Quality*, Woodhead Publishing, Cambridge, 2011; and reprinted from *Adapting High Hydrostatic Pressure (HPP) for Food Processing Operations*, Koutchma, T., Copyright (2014), Elsevier.

Some examples of high hydrostatic pressure-treated meat products are available on the market.

Note: N.I., No information was found about the year..

In this chapter, an overview about current and fundamental knowledge of HHP and its impact on meat and meat products is presented and some of the research findings are summarized. Opportunities and limitations of HHP on meat and meat products are discussed, and important results of some recent studies are also given.

Downloaded From Vet-eBooks.com

7.2 Effect of HHP on the Inactivation of Microorganisms in Meat and Meat Products

The main purpose of using HHP to treat meat and meat products is to improve microbial safety. The effectiveness of HHP to inactivate bacterial cells is well documented in various studies. Conditions such as the pressure level, process temperature, and holding time of the HHP treatment of food products depend on the type of microorganisms, the various ingredients, and the dispersion medium in foods (Ikeuchi, 2011). In the meat industry, HHP treatment is also an attractive method that can be used to inactivate various microorganisms without affecting the character and features of at least some of the meat products (Garriga and Aymerich, 2009; Garriga et al., 2004; Jofré et al., 2009b; Shigehisa et al., 1991; Tassou et al., 2007). A summary of some research findings associated with HHP inactivation of microorganisms are listed in Table 7.2.

7.2.1 Inactivation of *Listeria monocytogenes* in Meat and Meat Products under HHP

As a severe foodborne pathogen, *Listeria monocytogenes* requires particular attention during food processing and storage because it is moderately heat-resistant and can grow anaerobically under refrigeration (Teixeira et al., 2014). *L. monocytogenes* is also considered a target microorganism of public health concern in dairy and meat products (Koutchma, 2014).

There are many studies on inactivation of *L. monocytogenes* in meat and meat products. Treatment of sliced Iberian and Serrano hams at 450 MPa for 10 minutes significantly reduced the population of *L. monocytogenes* Scott A, spiked at 6 $\log_{10}$CFU/g. After 60 days at 4°C or 8°C, the counts were 3.24 and 4.70 $\log_{10}$CFU/g in HHP-treated and control samples, respectively, for Iberian and 2.73 and 5.07 log CFU/g for Serrano ham (Morales et al., 2006). The effectiveness of HHP (600 MPa, 31°C, 6 minutes) to reduce the risks associated with *L. monocytogenes* in sliced cooked ham was also reported by Garriga et al. (2004). Aymerich et al. (2005) also reported the effect of a treatment at 400 MPa for 10 minutes at 17°C on *L. monocytogenes* artificially spiked in vacuum-packaged sliced cooked ham. The growth of *L. monocytogenes* was inhibited until 40 days of refrigerated storage at 1°C or 6°C. After that period, and at 6°C, *L. monocytogenes* was able to grow until reaching counts similar to that of nonpressurized samples (about 8 $\log_{10}$CFU/g), while at 1°C, they kept to the low levels achieved after pressurization. The effectiveness of HHP treatments at 600 MPa was evaluated in sliced cooked ham spiked with 4 $\log_{10}$CFU/g of *L. monocytogenes* after three months of storage at 1°C and 6°C (Jofré et al., 2008b). The HHP application reduced the levels of *L. monocytogenes* to below 10 CFU/g. These levels continued until the end of storage at both 1°C and 6°C.

Downloaded From Vet-eBooks.com

TABLE 7.2

Summary of HHP Inactivation of Microorganisms in Meat and Meat Products

Microorganism	Product	Process Conditions[a]	Reduction (log_{10})	Reference
Campylobacter jejuni	Pork slurry	300 MPa, 25°C, 10 min	6.0	Shigehisa et al. (1991)
	Poultry	375 MPa, 25°C, 10 min	6.0	Solomon and Hoover (2004)
	Chicken meat	200 MPa, 25°C, 10 min	2.0	Martinez-Rodrigez and Mackey (2005)
	Chicken slurry	450 MPa, 15°C, 1 min	7.0[b]	Lori et al. (2007)
	Cooked ham, dry-cured ham, marinated beef loin	600 MPa, 31°C, 6 min	3.5[b]	Jofré et al. (2009b)
Escherichia coli O157:H7	Poultry	600 MPa, 20°C, 15 min	3.0	Patterson et al. (1995)
	Raw minced meat	700 MPa, 15°C, 1 min	5.0	Gola et al. (2000)
	Ground beef patties	400 MPa, 12°C, 20 min	2.5	Morales et al. (2008)
	Dry fermented salami	483 MPa, 19°C, 5 min	5.0[b]	Porto-Fett et al. (2010)
Salmonella Typhimurium	Minced meat	400 MPa, 20°C, 2 min	4.4	Escriu and Mor-Mur (2009)
Salmonella Enteritidis	Poultry sausages	500 MPa, 50°C, 10 min	7.2	Yuste et al. (2000b)
Salmonella Enteritidis	Chicken breast fillets	400 MPa, 12°C, 15 min	4.8	Morales et al. (2009)
Salmonella Enterica	Fermented sausages	400 MPa, 17°C, 10 min	2.0	Jofré et al. (2009a)
Staphylococcus aureus	Poultry	600 MPa, 20°C, 15 min	3.0	Patterson et al. (1995)
	Marinated beef loin	600 MPa, 31°C, 6 min	2.5	Jofré et al. (2009b)
	Dry-cured ham	600 MPa, 31°C, 6 min	0.5	Jofré et al. (2009b)
	Cooked ham	600 MPa, 31°C, 6 min	1.1	Jofré et al. (2009b)
Staphylococcus carnosus	Cooked ham	500 MPa, 40°C, 10 min	1.3	Hugas et al. (2002)
Lactobacillus sakei	Cooked ham	500 MPa, 40°C, 10 min	4.0	Hugas et al. (2002)
Pseudomonas flourescens	Minced beef	200 MPa, 20°C, 20 min	5.0	Carlez et al. (1993)
Yersinia enterocolitica	Pork slurry	300 MPa, 25°C, 10 min	6.0	Shigehisa et al. (1991)

Source: From Bajovic, B. et al., *Meat Science*, 92, 280–289, 2012 and Simonin, H. et al., *Comprehensive Reviews in Food Science and Food Safety*, 11, 285–306, 2012.

[a] Temperature given is either initial or process temperature.

[b] Total inactivation.

Downloaded From Vet-eBooks.com

HHP has already satisfied the demands of regulatory agencies such as the US Department of Agriculture/Food Safety Inspection Service as an acceptable method of elimination of *L. monocytogenes* in processed meat products (Campus, 2010; Teixeira et al., 2014; USDA, 2006). A summary of some research findings associated with HHP inactivation of *L. monocytogenes* in meat and meat products are listed in Table 7.3.

7.2.1.1 Different Types of Survival Curves of L. monocytogenes *in Meat and Meat Products under HHP*

There is now enough evidence that a log-linear isothermal survival curve of bacterial cells is an exception rather than a rule and the same can be said about semi-logarithmic survival curves of organisms exposed to HHP. A number of nonlinear models for describing the HHP inactivation of microbial cells such as the (modified) Gompertz equation (Chen and Hoover, 2003; Patterson and Kilpatrick, 1998; Yamamoto et al., 2005), Fermi equation (Donsì et al., 2003), log-logistic equation (Buzrul and Alpas, 2004; Chen and Hoover, 2003; Yamamoto et al., 2005), modified Baranyi model (Koseki and Yamamoto, 2007), and Weibull model (Avsaroglu et al., 2006; Buzrul and Alpas, 2004; Chen and Hoover, 2003; Yamamoto et al., 2005) have been proposed in literature—see Figure 7.1 and Table 7.4. While all these models—in their original and modified forms—could be used to fit survival curves of microbial cells inactivated by HHP, none of them can be considered unique.

It can be observed in such figures that *L. monocytogenes* in foods of animal origin under HHP could have different survival curves (Buzrul, 2014a). Some examples in meat and meat products are shown in Figures 7.2 through 7.5. It should be noted that it is still common to report D and z values of microorganisms; however, these values have no or little meaning if the survival curve of an organism is nonlinear.

7.2.2 Methods Used to Improve Microbial Inactivation during HHP Treatment of Meat and Meat Products

7.2.2.1 Temperature

It is known that increase of the treatment temperature during HHP can be used to improve microbial inactivation. Temperatures between 45°C and 60°C allow the use of lower pressure levels and shorter holding times for pathogen inactivation in meat and meat products compared to application at ambient temperature (Moerman, 2005; Simpson and Gilmour, 1997; Tassou et al., 2008; Yuste et al., 2000a). For example, a 5 $\log_{10}$ reduction of *Staphylococcus aureus* can be achieved in chicken treated at 500 MPa, 50°C,

Downloaded From Vet-eBooks.com

TABLE 7.3

Summary of HHP Inactivation of *Listeria monocytogenes* in Meat and Meat Products

Product	Strain	Initial Count	Process Conditions[a]	Reduction (log_{10})	Reference
Cooked beef mince	NCTC 11994	8.70	375 MPa, 45°C, 5 min	4.30	Simpson and Gilmour (1997)
Cooked chicken mince	–	–	375 MPa, 18°C, 30 min	~ 3.40	–
Raw chicken mince	–	–	375 MPa, 18°C, 25 min	~ 4.60	–
	–	–	–	–	–
Turkey breast meat	Cocktail of five strains (PSU1), PSU2, PSU9, PSU21, ATCC 19115)	7.00	400 MPa, 1°C, 1 min	0.50	Chen (2007)
			400 MPa, 20°C, 1 min	0.10	
			400 MPa, 50°C, 1 min	4.10	
			500 MPa, 1°C, 1 min	1.40	
			500 MPa, 20°C, 1 min	0.90	
			500 MPa, 40°C, 1 min	3.80	
–	–	–	–	–	–
Cooked ham	Cocktail of three strains (CTC1010, CTC1011, CTC1034)	4.00	600 MPa, 10°C, 5 min	~ 3.50	Jofré et al. (2007)
–	–	–	–	–	–
Mortadella	CTC1034	~ 7.00	450 MPa, 15°C, 15 min	>4.00	Hereu et al. (2012)
Cooked ham	–	–	450 MPa, 15°C, 15 min	>5.00	
–	–	–	–	–	–
RTE-sliced ham	Cocktail of five strains (ATCC 7644, NCTC 10890, ATCC 19112, ATCC 19114, ATCC 19115)	5.00	400 MPa, 17°C, 3 min	0.95	Myers et al. (2013)
		–	–	–	–
–	–	–	600 MPa, 17°C, 3 min	4.25	–

[a] Temperature given is either initial or process temperature.

Downloaded From Yes-Books.com

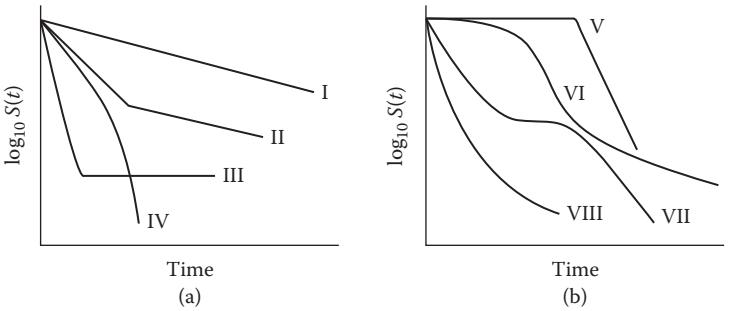


FIGURE 7.1
Commonly observed types of survival curves. (a) Linear (I), biphasic (II), strong (extreme) tailing (III), convex (IV). (b) Shoulder (V), sigmoidal type 1—starting with a convex and ending up with concave (VI), sigmoidal type 2—starting with a concave and ending up with convex (VII), concave (VIII). (Adapted from Xiong, R. et al., *International Journal of Food Microbiology*, 46, 45–55, 1999; Peleg, M., *Critical Reviews in Food Science and Nutrition*, 43, 645-658, 2003; and Geeraerd, A. H. et al., *International Journal of Food Microbiology*, 102, 95–105, 2005.)

15 minutes, whereas a pressure level of approximately 700 MPa would be necessary to obtain the same reduction at 20°C (Patterson and Kilpatrick, 1998). Yuste et al. (2000b) concluded that HHP treatment (500 MPa, 65°C, 5 minutes) could be an effective preservation method to replace heat pasteurization (80°C, 40 minutes) in cooked sausages, achieving the same preserving effect in terms of microbial inactivation and growth inhibition during chilled storage. However, heat-transfer limitations in particulate food products can still prevent their successful pasteurization by HHP treatments at elevated temperatures (Moerman, 2005).

The use of low temperatures (<15°C) during HHP treatment could also be an effective strategy to improve microbial inactivation. A pork-isolated strain of *Pseudomonas fluorescens* was reduced by 5 $\log_{10}$ when treated at 200 MPa, 5°C, 10 minutes, and the bactericidal effect decreased as the temperature increased to 35°C (López-Caballero et al., 2002). Chen (2007) found that *L. monocytogenes* was more resistant to pressure (500 MPa, 1 minute) at temperatures between 10°C and 30°C. When temperatures decreased below 10°C or increased above 30°C, *L. monocytogenes'* susceptibility to pressure increased. This enhanced inactivation effect was more pronounced when meat samples were treated at higher temperatures. In the study by Yuste et al. (2002), HHP treatment at subzero temperatures (450 MPa, –20°C, 15 minutes) was not effective at decreasing the microbial count in poultry meat, probably because samples were treated while frozen and thus presented a lack of plasticity (Simonin et al., 2012).

Downloaded From Vet-eBooks.com

TABLE 7.4

Models Used to Describe Survival Curves of Microorganisms

Model	Equation Number	Equation	Reference
Linear	(1)	$\log_{10} S(t) = -kt$ or $\log_{10} S(t) = -\frac{t}{D}$	Chick (1908)
Biphasic	(2)	$\log_{10} S(t) = \log_{10}\left(fe^{-k_1 t} + (1-f)e^{-k_2 t}\right)$	Cerf (1977)
Biphasic	(3)	If $t \le t_i$ $\log_{10} S(t) = -k_1 t$ If $t > t_i$ $\log_{10} S(t) = -k_1 t_i - k_2 (t - t_i)$	Corradini et al. (2007)
Shoulder	(4)	$\log_{10} S(t) = -\log_{10}\left\{1 + \exp\left[k(t - t_{\min})\right]\right\}$	Peleg (1996)
Shoulder	(5)	If $t \le t_{lag}$ $\log_{10} S(t) = 0$ If $t > t_{lag}$ $\log_{10} S(t) = -k(t - t_{lag})$	Peleg (2000)
Concave	(6)	$\log_{10} S(t) = -bt^n$ $n < 1$	Peleg and Cole (1998)
Convex	(7)	$\log_{10} S(t) = -bt^n$ $n < 1$	Peleg and Cole (1998)
Strong tailing	(8)	$\log_{10} S(t) = -\frac{at}{b + t}$	Peleg (2006)
Sigmoid (start with concave end up with convex)	(9)	$\log_{10} S(t) = -\frac{at}{(1 + bt)(c - t)}$	Peleg (2003)
Sigmoid (start with convex end up with concave)	(10)	$\log_{10} S(t) = A\exp\left[-\exp(kt_i)\right] - A\exp\left[-\exp\left(-k(t - t_i)\right)\right]$	Linton et al. (1995)

Downloaded From: Ebooks.com

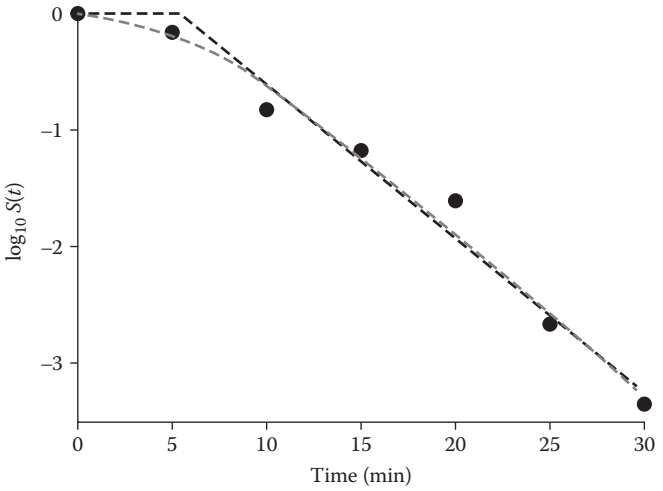


FIGURE 7.2
Survival data (black circles) of *Listeria monocytogenes* NCTC 11994 in cooked chicken mince at 375 MPa, about 18°C fitted with Equations 4 (dashed gray line) and 5 (dashed black line)—see Table 7.4. (From Simpson, R. K. and Gilmour, A., *Food Microbiology*, 14, 567–573, 1997.)

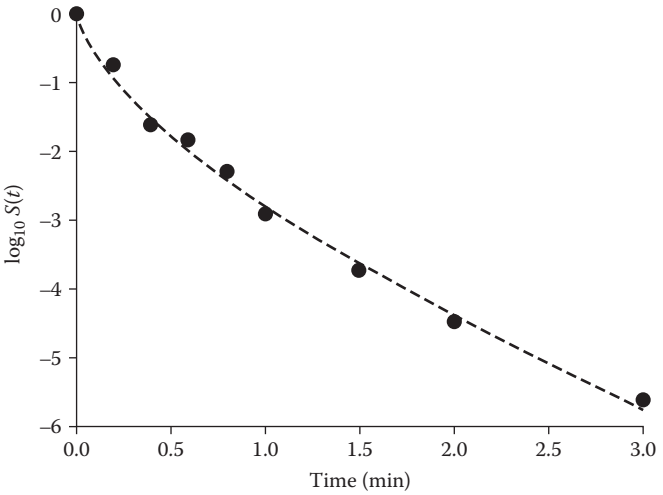


FIGURE 7.3
Survival data (black circles) of five-strain cocktail of *L. monocytogenes* (PSU1, PSU2, PSU9, PSU21, and ATCC 19115) in turkey breast meat at 400 MPa, 50°C fitted with Equation 6 (dashed black line)—see Table 7.4. (From Chen, H., *International Journal of Food Microbiology*, 117, 55–60, 2007.)

Downloaded From Vet-eBooks.com

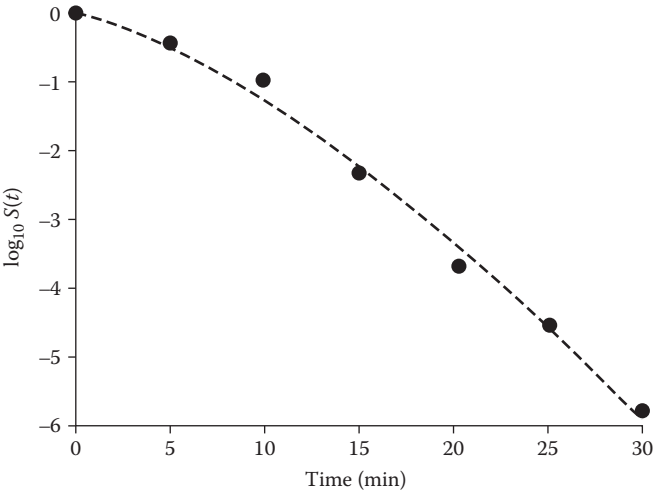


FIGURE 7.4
Survival data (black circles) of *L. monocytogenes* NCTC 11994 in raw chicken mince at 375 MPa, about 18°C fitted with Equation 7 (dashed black line)—see Table 7.4. (From Simpson, R. K. and Gilmour, A., *Food Microbiology*, 14, 567–573, 1997.)

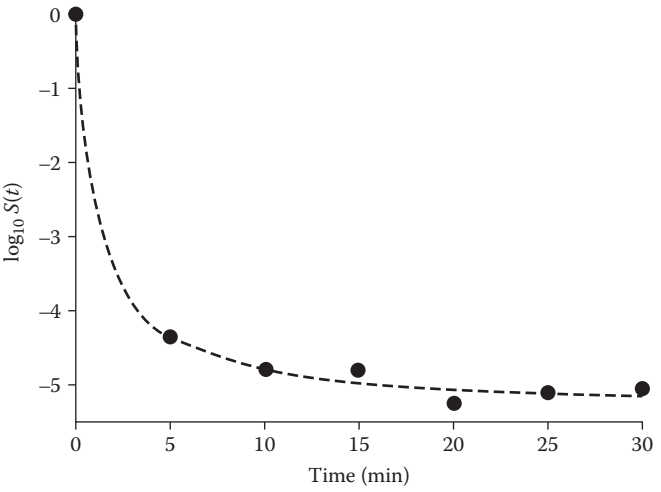


FIGURE 7.5
Survival data (black circles) of *L. monocytogenes* NCTC 11994 in cooked beef mince at 375 MPa, 45°C fitted with Equation 8 (dashed black line)—see Table 7.4. (From Simpson, R. K. and Gilmour, A., *Food Microbiology*, 14, 567–573, 1997.)

Downloaded From Vet-eBooks.com

7.2.2.2 Additives/Antimicrobials

The synergism between bacteriostatic additives and HHP has been reported (Aymerich et al., 2005; Krockel and Muller 2002; Marcos et al., 2008b; Ogihara et al., 2009). Organic acids can be used in meat products to delay the growth of bacteria during storage without negative effects on the sensory quality of the products (Diez et al., 2008). In sliced cooked ham prepared with 1.8% potassium lactate, the growth of *L. monocytogenes* during chilled storage at 6°C was delayed, and the combination of this treatment with HHP treatment (400 MPa, 17°C, 10 minutes) totally inhibited the recovery of *L. monocytogenes* during chilled storage at 6°C (Aymerich et al., 2005). Lactate (1.4%) was also efficient for preventing *L. monocytogenes* growth after a cold-chain break in HHP-treated (400 MPa, 17°C, 10 minutes) cooked ham (Marcos et al., 2008b). In fact, HHP alone decreased the initial count of *L. monocytogenes* from 5 to 2 $\log_{10}$ CFU/g, but it failed to prevent the bacteria from recovering after a cold-chain break arising during the storage.

Among antimicrobials, bacteriocins have also been used in combination with HHP to increase microbial inactivation in meat and meat products (Garriga et al., 2002; Marcos et al., 2008b; Yuste et al., 1998, 2002). Bacteriocins can be either incorporated into the product formulation or applied directly to meat before the HHP treatment. The use of active packaging containing antimicrobials is an additional solution that has been shown to be effective (Jofré et al., 2008a; Marcos et al., 2008a).

Nisin is currently the only bacteriocin to be widely used as a food preservative. Its effectiveness in reducing the *Listeria* risk is low, and the use of potassium lactate has been shown to be more effective for inhibiting cell recovery during chilled storage (Aymerich et al., 2005). Garriga et al. (2002) investigated the behavior of several foodborne bacteria inoculated in a HHP-treated (400 MPa, 17°C, 10 minutes) meat model system with added bacteriocins, enterocins A and B, sakacin K, pediocin AcH, and nisin. Nisin was very efficient for inhibiting the recovery of *Escherichia coli, S. aureus,* and *Leuconostoc carnosum* during subsequent chilled storage; however, it failed to inhibit the recovery of *L. monocytogenes*. The other bacteriocins were effective with *L. monocytogenes,* but not with the other tested bacteria. In HHP-treated (400 MPa, 17°C, 6 minutes) cooked ham, the introduction between slices of alginate films containing enterocin reduced the count of *L. monocytogenes* below the level of detection, whereas either HHP treatment or active packaging alone had no effect on the bacteria count (Marcos et al., 2008a).

7.2.2.3 Multipulsed HHP Treatment of Meat Products

HHP treatment can consist of a single exposure period, that is, compression to target pressure, holding time at the target pressure, and decompression to atmospheric pressure (single-pulsed HHP or spHHP), or the application of multiple exposure periods in which more than one exposure period

Downloaded From Vet-eBooks.com

exists, that is, compression, holding time, and decompression followed by another compression, holding time, and decompression (multipulsed HHP or mpHHP) (Buzrul et al., 2009). MpHHP could be used to lower the peak pressure necessary to inactivate the microorganisms in foods (Pilavtepe-Çelik et al., 2011).

Several authors have studied the potential of using mpHHP processing of meat to improve process lethality. MpHHP (350 or 450 MPa, 2°C, 3 pulses × 5 minutes) applied to mechanically recovered poultry meat showed that the mpHHP treatment was slightly better than the spHHP treatment (350 or 450 MPa, 2°C, 15 minutes) for psychrotrophs, but the mpHHP treatment did not offer better results than the spHHP treatment for mesophiles (Yuste et al., 1998). Similar results were also obtained by Yuste et al. (2001), but in this case, mpHHP was applied as a 60 MPa treatment (moderate pressure) followed by a 450 MPa treatment (high pressure). These authors did not obtain any increase in efficiency in comparison with spHHP at 450 MPa. On the other hand, the use of the mpHHP treatment instead of the spHHP treatment was observed to be more advantageous for the inactivation of *E. coli* O157:H7 in ground beef (Morales et al., 2008) and *Salmonella* Enteritidis in chicken breast fillets especially at higher pressures (Morales et al., 2009).

Morales et al. (2008) and Del Olmo et al. (2010) studied the effect of the spHHP and mpHHP treatments on color and texture of beef patties and chicken breast fillets, respectively. Changes in the color and texture of ground beef caused by spHHP and mpHHP treatments of the same lethality for *E. coli* O157:H7 (20 minutes for spHHP at 400 MPa and 4 pulses × 1 minute for mpHHP at 400 MPa) were similar (Morales et al., 2008). Color parameters (L^*, a^*, and b^*) were significantly higher for both treatments than for vacuum-packaged control fillets. Similarly, the texture of chicken breast fillets was also significantly affected by both treatments (Del Olmo et al., 2010).

7.2.2.4 Hurdle Concept

The hurdle concept is the combination of different strategies, preservation factors, or techniques to produce safe, stable, and high-quality food products. This concept consists of adding several barriers (hurdles) to bacterial life and growth (Simonin et al., 2012). For example, in the study of Yuste et al. (1998), mpHHP treatment (450 MPa, 2°C, 3 pulses × 5 minutes), nisin (200 ppm), and weak acidity (pH 5.42 provided by the addition of glucono delta-lactone) reduced the psychrotrophic and mesophilic bacterial counts to levels below 2 $\log_{10}$ CFU/g in mechanically recovered poultry meat during 1 month of chilled storage. Nisin proved to be more efficient in reducing the indigenous flora of HHP-treated poultry meat when used at a weakly acidic pH (Yuste et al., 1998, 2002). In fermented sausages, the inherent hurdles (low a_w and acidity) combined with HHP (Porto-Fett et al., 2010) were used to reduce *Listeria* and *Salmonella* risks. In this study, fermentation and drying of inoculated salami at 7 $\log_{10}$ CFU/g induced reductions of 1.1–1.3

Downloaded From Vet-eBooks.com

$\log_{10}$ CFU/g for *L. monocytogenes* and 4.2–4.8 $\log_{10}$ CFU/g for *Salmonella sp.* In addition, following pressurization (600 MPa, 1–5 minutes or 483 MPa, 5–12 minutes) *L. monocytogenes* and *Salmonella* were reduced by an additional 1.6 to ≥ 5.0 $\log_{10}$ CFU/g and 1.9–2.4 $\log_{10}$ CFU/g, respectively, compared to their levels after fermentation and drying (Porto-Fett et al., 2010). The use of lactate, HHP, and 1°C refrigeration is a successful combination that could reduce the *Listeria* risk in cooked ham (Aymerich et al., 2005; Marcos et al., 2008b). In fact, in HHP-treated (400 MPa, 17°C, 10 minutes) cooked ham inoculated with *L. monocytogenes*, a recovery of the bacteria was observed during storage at 6°C, but no recovery was observed when the ham was formulated with 1.4% of lactate or when it was stored at 1°C (Aymerich et al., 2005). Storage conditions are thus of great importance for cell recovery after HHP treatment. Generally, the lower the storage temperature, the better is the resulting microbial quality, except for certain specific products, such as fermented sausages, that benefit from storage at ambient temperature (Jofré et al., 2009a).

7.2.2.5 Limitations of the Abovementioned Methods

The use of elevated temperatures could be advantageous to reduce the target pressure for some meat products; however, temperature would have negative impacts on the organoleptic properties of fresh meat. On the other hand, low temperature could be used for fresh meat but this will require accurate control of the temperature in large-scale devices used in the industry (Urrutia et al., 2007).

The use of antimicrobials such as nisin has been shown to have a synergistic effect on bacterial inactivation; however, antimicrobial spectrum of most natural preservative systems is restricted to a narrow group of microorganisms. For instance, nisin and other bacteriocins are effective against only some gram-positive bacteria, but not against gram-negative bacteria. Further, sensitive bacterial strains may develop resistance when exposed to sublethal doses of an antimicrobial. A well-studied example of this is nisin resistance (Mazzotta and Montville, 1999; Mendoza et al., 1999). Finally, protein, fat, or other components in complex foods such as meat may protect target microorganisms, for instance, by adsorbing antimicrobial components. One way to overcome these limitations is to use combinations of two or more biopreservatives with different targets (Zapico et al., 1998) or to combine antimicrobials with other preservation techniques (García-Graells et al., 2000).

Although there is now enough evidence that the mpHHP treatment is an effective way of inactivating microorganisms, there is no commercial application of the mpHHP treatment to date. The most important reason for this is that the mpHHP treatment is a longer application and thus more expensive than the spHHP treatment (Alemán et al., 1998; Buzrul et al., 2009; Buzrul, 2014b; Kingsley et al., 2006; Rivalain et al., 2012). In addition, more compli-

Downloaded From Vet-eBooks.com

cated HHP equipment which can withstand fast compression and decompression rates (to reduce the total duration of the treatment) are likely needed for commercial applications (Buzrul, 2015).

7.3 Effects of HHP on Quality Attributes of Meat and Meat Products

7.3.1 Effect of HHP on Texture of Meat and Meat Products

The effect of HHP on the texture of muscle foods has been investigated since 1973, when Macfarlane (1973) reported the potential use of HHP for pressure-induced tenderization of meat (Campus, 2010). There is agreement among consumers that tenderness is the most important factor of all attributes that characterize the eating quality of meat (Denoyella and Lebihan, 2003; Jung et al., 2000; Sun and Holley, 2010). Many authors reported that HHP treatment (100–200 MPa) for a few minutes can improve meat tenderness when applied to prerigor meat (Bouton et al., 1977; Elgasim and Kennick, 1980; Ohmori et al., 1991).

In postrigor meat, HHP (150–200 MPa) causes tenderization of meat after long exposure times (20–30 minutes) at high temperature values (40°C–80°C) (Bouton et al., 1977; Locker and Wild; 1984; Ma and Ledward, 2004; Macfarlane, 1973; Sikes et al., 2010)

In summary, meat tenderness is dependent on rigor stage, pressure, temperature, and their combination. Pressure values lower than 200 MPa can tenderize prerigor meat, whereas tenderization of postrigor meat with HHP treatment can only be achieved at higher temperatures (Sun and Holley, 2010).

HHP also induces meat protein modifications (gelation, solubilization, and aggregation) differently than heat-induced changes. HHP results in varying effects on meat product texture and water retention, depending mainly on the product's form and composition, the pressure level, and the pressure/temperature combination. Combining HHP and heat can sometimes improve the water retention in meat batters, depending on the batter's composition and the process parameters. HHP can also be used to improve meat binding in restructured meat. Nevertheless, each specific product should be tested individually for the resulting effects of HHP on its quality (Simonin et al., 2012).

7.3.2 Effect of HHP on Lipid Oxidation of Meat and Meat Products

There is enough evidence that HHP treatment of meat and meat products can trigger lipid oxidation, and such oxidation affects eating quality through flavor deterioration (rancidity), color changes, loss of nutritive value, and alterations of textural and functional properties through concomitant pro-

Downloaded From Vet-eBooks.com

tein denaturation (Buckow et al., 2013; Fuentes et al., 2010; Refsgaard et al., 2000; Viljanen et al., 2004).

The effect of HHP on lipid oxidation on pork (Cheah and Ledward, 1995, 1996, 1997), beef (Ma et al., 2007; McArdle et al., 2010), and poultry (Beltran et al., 2004; Bragagnolo et al., 2007; Dissing et al., 1997; Kruk et al., 2011; Orlien et al., 2000; Tuboly et al., 2003) has established that pressure levels between 300 and 600 MPa are critical for inducing lipid oxidation in fresh meat (Bajovic et al., 2012).

In cured meat products, it was reported that presliced dry-cured ham was more susceptible to oxidative reactions and lipid oxidation compared to the control (Fuentes et al., 2010) whereas the oxidative stability of dry-cured ham pressurized at 600 MPa was not affected (Clariana et al., 2011b). As for the color, the effect on lipid oxidation by HHP is more pronounced for fresh meat than for cured meat products (Clariana et al., 2011a).

Addition of natural antioxidants such as rosemary extract to meat and meat products can help to minimize the effect of HHP on lipid oxidation. Addition of a rosemary extract to beef patties completely prevented formation of lipid oxidation products after nine days of chilled storage in both control and samples pressure-treated up to 600 MPa (Robbins et al., 2006). Bragagnolo et al. (2007) demonstrated that addition of rosemary to minced chicken meat samples before HHP treatment (600 MPa, 10°C, 10 minutes) protected against lipid oxidation resulting from HPP when the product was subsequently cooked. Similar findings were observed by Ma et al. (2007) using additions of vitamin E to ground beef.

Lipid oxidation can also be controlled by using metal chelators such as citrate or EDTA (Ikeuchi, 2011). Further possibilities proposed to inhibit lipid oxidation is to limit oxygen availability in the packaging, use of antioxidant active packaging (Bolumar et al., 2011), or use of different antioxidants derived from natural by-products like tomato (Alves et al., 2012).

In conclusion, HHP treatment of fresh and processed meats induces lipid oxidation. Depending on the pressure level, and to a lesser extent, on holding time, lipid oxidation most often occurs during subsequent storage. Moreover, HHP can be as damaging as heat treatment in terms of the oxidation level in cold-stored meat products. Generally, HHP has little effect on lipid oxidation below 300 MPa; however, it can have a significant effect at higher pressure values (Simonin et al., 2012). The possible effects of enhanced lipid oxidation on the eating quality of HHP-treated meats during storage need to be considered and controlled either through appropriate HHP treatments (pressure levels and holding times), packaging, or use of natural antioxidants (Buckow et al., 2013; Tume et al., 2010).

7.3.3 Effect of HHP on Color of Meat and Meat Products

It is well known that HHP influences fresh meat's color. It has often been reported that application of pressure above 200 MPa drastically changes

Downloaded From Vet-eBooks.com

the appearance of red meat within a few minutes of treatment even at low temperatures (Carlez et al., 1993; Jung et al., 2003; Korzeniowski et al., 1999; Ledward, 2000; Tintchev et al., 2010). As pressure is increased, major structural proteins of the muscle became denatured (Cheah and Ledward, 1996) resulting in an opaque appearance of the flesh, which further reduces the visualization of other meat pigments. Typically, the lightness (L* values) of red meat increases significantly after pressure treatments above 250 MPa, whereas the redness (a* values) decreased at 400–500 MPa resulting in a gray-brown meat with a cooked aspect (Carlez et al., 1995; Jung et al., 2003; Tintchev et al., 2010). Studies of beef and minced beef meat showed a decrease of the total myoglobin content at pressures in the range 250–500 MPa, whereas the proportion of the gray metmyoglobin increased at the expense of cherry-red oxymyoglobin at 400–500 MPa (Carlez et al., 1995).

Overall, HHP caused drastic changes in the color of fresh meat and thus made the commercialization of HHP-treated fresh meat difficult (Cheftel and Culioli, 1997), since color is one of the most important attributes of fresh meat that consumers use as a purchasing criterion (Faustman and Cassens, 1990).

The color of cured products is less affected by pressure than is fresh meat's color (Karlowski et al., 2002; Rubio et al., 2007), although significant changes have also been reported. For example, a decrease in redness in dry-cured ham was observed above 200 MPa (Andrés et al., 2004, 2006; Cava et al., 2009). Cava et al. (2009) observed that color differences between HHP-treated (300 MPa, 14°C, 30 minutes) and untreated dry-cured ham and loin disappeared during subsequent storage at 4°C for 90 days. Changes induced by pressure were noticeable after the treatment; however, after 60 and 90 days of storage, differences were no longer found between treated and untreated products. To avoid color changes in cured meat products, cooking before HHP treatment has been recommended (Goutefongea et al., 1995).

In general, the negative impact of HHP on fresh meat and meat products is dependent on different parameters, and not all of them are well-understood as of yet. In general, HHP-induced color changes vary according to the myoglobin content and are more dramatic for fresh red meat than for white meat and cured meat products. Undesired changes can be limited by optimizing the process parameters of HHP treatment such as pressure, time, temperature, curing, oxygen removal, and increased pH. When looking for a reduction of the color changes induced by HHP, one should keep in mind that measures to protect the color quality and stability can result in changed microbial inactivation and thus affect the safety and shelf-life of the final product (Bajovic et al., 2012).

7.3.4 Other Effects of HHP on Meat and Meat Products

It is evident that HHP is capable of inducing various structural changes on muscle proteins of meat and these resulting changes are dependent on the magnitude of the pressures/temperatures and the duration of the treatment

Downloaded From Vet-eBooks.com

(Buckow et al., 2013). The application of HHP on proteins leads to different degrees of protein structure modification. As a general mechanism, the application of HHP induces unfolding of the protein structure and subsequent folding after pressure release. This can lead, depending on the specific protein and conditions applied, to partial or total denaturation and tuning of electrostatic interactions. Based on the principle of Le Chatelier and Braun, reactions with a decrease in volume are favored by pressure (Bajovic et al., 2012).

One of the remarkable effects of HHP on meat is the modification of the actin–myosin complex. Pressure induces structural changes in the main constituent of muscle filaments, which are possibly caused by increased ATPase activity at 30 MPa as well as with an increase of soluble materials from the myofibrils enhanced by pressurization above 150 MPa (Nishiwaki et al., 1996).

Sensory acceptance of HHP-treated meat products depends on color, texture, aroma, and taste modifications induced by the process. Problems of sensory acceptance occur with raw products, mainly because of visible color changes. Cooked and cured products are less modified by pressure, and good sensory acceptance is generally reported despite some changes in aroma and taste profiles (Simonin et al., 2012).

7.4 Consumer Acceptance of HHP-Treated Meat and Meat Products

Although HHP is now a reality in the food industry, there are still many consumers who are not aware of this technology. One way to understand the consumer's awareness/acceptance/interest on HHP technology is to administer surveys.

Personal interviews revealed that HHP was acceptable to the majority of consumers in France and Germany (Butz et al., 2003). An online consumer survey in the United States was implemented by Hicks et al. (2009) to assess the awareness of alternative food-processing technologies, consumer food safety attitudes and knowledge, and the willingness to pay for HHP-treated products. The survey was completed by 1204 adults. Results revealed that while traditional methods—that is, canning, freezing, and microwaving—were all well recognized by over 80% of respondents, only 8% recognized HHP. Trends indicated that an increase in age, education, and income reflected greater food safety knowledge. Given an explanation of HHP and its benefits, 39% of respondents indicated they would be willing to pay an additional cost for such foods, with higher income and education having the most impact (Hicks et al., 2009).

Surveys were conducted by Cardello et al. (2007) to 225 potential consumers of foods processed by innovative and emerging food technologies (HHP, pulsed electric fields, irradiation, ionizing energy, genetic modification)

Downloaded From Vet-eBooks.com

to assess the factors contributing to their interest in using such products. Respondents rated their interest in 49 different food-product concepts that varied in food type, processing or production technology, costs, benefits, risks, endorsing agencies, and product information. HHP has a strong positive influence on consumer interest, compared to irradiation and genetic modification (Cardello et al., 2007).

In summary, consumers are ready to pay extra for new HHP-treated meat products if they have higher quality. It is essential that the quality and safety of the new product are assessed prior to launch in the market (Ikeuchi, 2011). Further study is required for marketing of pressurized food products to provide consumers with clear information about the treatments, its purposes, and gained benefits (Teixeira et al., 2014). Consumer's willingness to pay once they are informed could encourage industry to look more favorably on this technology (Hicks et al., 2009).

7.5 Some Important Results from Recent Studies of HHP on Meat Products

In this section, some results from recent studies are given. A recent work by de Oliveira et al. (2015) showed that the addition of carvacrol (200 ppm) to sliced low-sodium, vacuum-packed turkey breast ham notably improved the preservation effects of HHP (600 MPa, 25°C, 3 minutes) processing. This combined treatment was able to reduce the growth rates of lactic acid and psychrotrophic bacteria, and extend the lag-phase of *L. innocua*. In addition, the triggered lipid oxidation events by HHP may be delayed by the addition of carvacrol. The preservative effects during the shelf life can be potentiated by the presence of natural barriers, and the use of carvacrol represents a promising technology against sublethal injury and cell recovery phenomena (de Oliveira et al., 2015).

Montiel et al. (2015) studied the effects of reuterin, lactoperoxidase, lactoferrin, and HHP (450 MPa, 6°C, 5 minutes) on the inactivation of foodborne pathogens in cooked ham. Individual treatments of reuterin, lactoperoxidase, and HHP diminished the growth of *L. monocytogenes* in cooked ham, but a recovery of growth was observed during storage at 4°C after 35 days. Combined treatments of HHP with reuterin or lactoperoxidase increased synergistically the antimicrobial effect against *L. monocytogenes* and avoided the recovery. Reuterin and lactoperoxidase added individually to cooked ham diminished *S.* Enteritidis and *E. coli* O157:H7 levels during the 35 days of challenge at 4°C and 10°C. Pressure treatments, individually and in combination with antimicrobials, inhibited the growth of these Gram-negative microorganisms during the 35 days of storage. The result showed that lactoperoxidase combined with HHP was the most effective treatment at reducing the growth of pathogens tested. Also, the

Downloaded From Vet-eBooks.com

application of reuterin combined with HHP would be a useful hurdle technology to improve the safety of cooked ham (Montiel et al., 2015).

The results of a recent study by Ros-Polski et al. (2015) indicated that, in general, the use of HHP treatment (0–300 MPa, ambient temperature, 1–3 minutes) in salted (0–2.5 g/100 g) white chicken meat improved the texture of the cooked meat and color of raw meat. Overall, NaCl at lower concentrations along with HHP treatment improved white chicken meat color and texture. This information can be used when developing HHP treatments as a processing alternative to reduce NaCl content while maintaining the physical attributes and ensuring meat functionality and quality (Ros-Polski et al., 2015).

A more recent study by Teixeira et al. (2016) investigated the effect of growth temperature, process temperature, and sodium chloride concentration on the lethality of HHP to a five-strain cocktail of *L. monocytogenes* in cooked ham. Results reveal that cultures grown at 8°C were more resistant than cultures grown at 20°C and 32°C in broth; however, cultures grown at 8°C and 32°C were equally resistant to pressurizations at 5°C and 32°C on ham; whereas cultures grown at 8°C tended to be more sensitive to treatments at -5°C when compared to cultures grown at 32°C. *L. monocytogenes* was more pressure resistant in ham with 3% (w/v) NaCl when compared to ham with 1% (w/v) NaCl.

It is thus remarkable that the modest decrease of the NaCl content of ham from 3% to 1% had a measurable impact on pressure resistance of *L. monocytogenes*. The meat industry currently aims to reduce the salt content of ready-to-eat meat products. The study by Teixeira et al. (2016) indicates that even a modest reduction of the NaCl content can improve food safety by decreasing the pressure resistance of *L. monocytogenes* (Teixeira et al., 2016).

7.6 Opportunities for HHP Application to Meat and Meat Products

HHP is a well-known technology to inactivate pathogenic and spoilage microorganisms in meat products. Commercially applied pressure levels are between 400 and 600 MPa with processing times of three to seven minutes at ambient temperatures which in most cases lead to an inactivation of about four log units of the majority of vegetative pathogenic and spoilage microorganisms < resulting in increased shelf life and improved safety (Bajovic et al., 2012).

The thermo-labile nature of protein meat systems and the described effects of pressure on meat and meat proteins allow the development of novel meat-based products and the usage of HHP as a time- and energy-saving processing step for the meat industry. Functional and rheological properties of meat can be improved and novel meat-based products with decreased salt content

Downloaded From Vet-eBooks.com

or higher nutritional level can be developed (Bajovic et al., 2012). Since HHP has value in the formation of gels from myofibrillar protein at low (0.2 M) salt concentration, HHP may have potential for the development of low-sodium-containing processed meat products. Current technological limitations of 1.7%–2.1% (w/v) NaCl may be overcome by the use of HHP and will enable manufacture of these products with more healthful levels of NaCl (Sun and Holley, 2010).

7.7 Limitations of HHP on Meat and Meat Products

HHP treatment can be used to increase the safety and storage life especially of ready-to-eat meat products but cannot be used to establish the safety of these products. Further studies should be carried out to ensure microbiological safety of ready-to-eat meat products. The hurdle concept could be an effective way to achieve this goal.

Moreover, capital equipment cost can be a problem for some companies. Nevertheless, as the technology improves, it may be possible to reduce the cost of both the equipment and the treatment. An additional problem is that current batch sizes are a restriction for operations with high line speeds (Sun and Holley, 2010).

7.7.1 On the Application of HHP on Fresh Meat

HHP at ambient temperatures increases the shelf life of fresh meat; however, HHP causes several transformations such as color loss and texture changes. The meat becomes more gel-structured and paler, losing the typical appearance of fresh meat (Bajovic et al., 2012). The color changes are similar to those in cooked meat as the actomyosin denatures at about 200 MPa and the myoglobin denatures and/or coprecipitates with other proteins at about 400 MPa. In addition, at pressures ≥400 MPa the unsaturated lipids in the meat become more susceptible to oxidation, probably due to the release of iron from complexes present in meat and/or changes in the lipid membrane (Ma and Ledward, 2013).

Depending on the process parameter applied, HHP-treated meat would possibly not be recognized as fresh meat by consumers (Bajovic et al., 2012). Under European Union (EU) regulation, "fresh meat" has not undergone any preserving process other than chilling, freezing, or quick-freezing, including meat that is vacuum-wrapped or wrapped in a controlled atmosphere (The European Parliament and the Council of the European Union, 2004). Overall, the color change induced by HHP in the meat as well as the current legal definition of fresh meat has drastically limited the use of HHP for fresh meat in the markets (Bajovic et al., 2012). Even if legislation would allow it, it is unlikely that many consumers would be prepared to buy such meat.

Downloaded From Vet-eBooks.com

However, if prerigor meat is subjected to pressures of about 100–150 MPa, below those necessary to cause color changes, it becomes significantly more tender than its untreated counterpart, and this may now be a commercially viable process given the decreasing cost of HHP equipment. When treated at 100–200 MPa, while the temperature is raised from ambient to around 60°C postrigor meat also yields a tender product and this may also be a commercially attractive process to parts of the food industry, for example, those involved in catering (Ma and Ledward, 2013).

Undesired color changes, especially for fresh red meat, can be limited by changing the process parameters such as pressure, time, and temperature as well as by curing, oxygen removal, and the increase of product pH by ingredients (Bajovic et al., 2012).

References

Alemán, G. D., Ting, E. Y., Farkas, D. F., Mordre, S. C., Hawes, A. C. O. and Torres, J. A. (1998). Comparison of static and step-pulsed ultra-high pressure on the microbial stability of fresh cut pineapple. *Journal of the Science of Food and Agriculture*, **76**, 383–388.

Alves, A. B., Bragagnolo, N., da Silva, M. G., Skibsted, L. H. and Orlien, V. (2012). Antioxidant protection of high-pressure processed minced chicken meat by industrial tomato products. *Food and Bioproducts Processing*, **90**, 499–505.

Andrés, A., Adamsen, C., Møller, J., Ruiz, J. and Skibsted, L. (2006). High-pressure treatment of dry-cured Iberian ham. Effect on colour and oxidative stability during chill storage packed in modified atmosphere. *European Food Research and Technology*, **222**, 486–491.

Andrés, A. I., Møller, J. K. S., Adamsen, C. E. and Skibsted, L. H. (2004). High pressure treatment of dry-cured Iberian ham. Effect on radical formation, lipid oxidation and colour. *European Food Research and Technology*, **219**, 205–210.

Atsushi, S., Ken, K., Hiroyuki, T., Tadayuki, N. and Yoshihide, I. (2006). Application of high hydrostatic pressure to meat and meat processing. *In*: Nollet, L. M. L. and Toldrá, F. (eds.) *Advanced Technologies for Meat Processing*. Boca Raton, FL: CRC Press, 193–271.

Avsaroglu, M. D., Buzrul, S., Alpas, H., Akcelik, M. and Bozoglu, F. (2006). Use of the Weibull model for lactococcal bacteriophage inactivation by high hydrostatic pressure. *International Journal of Food Microbiology*, **108**, 78–83.

Aymerich, M. T., Jofré, A., Garriga, M. and Hugas, M. (2005). Inhibition of *Listeria monocytogenes* and *Salmonella* by natural antimicrobials and high hydrostatic pressure in sliced cooked ham. *Journal of Food Protection*, **68**, 173–177.

Bajovic, B., Bolumar, T. and Heinz, V. (2012). Quality considerations with high pressure processing of fresh and value added meat products. *Meat Science*, **92**, 280–289.

Beltran, E., Pla, R., Yuste, J. and Mor-Mur, M. (2004). Use of antioxidants to minimize rancidity in pressurized and cooked chicken slurries. *Meat Science*, **66**, 719–725.

Downloaded From Vet-eBooks.com

Bolumar, T., Andersen, M. L. and Orlien, V. (2011). Antioxidant active packaging for chicken meat processed by high pressure treatment. *Food Chemistry*, **129**, 1406–1412.

Bouton, P. E., Harris, P. V., Macfarlane, J. J. and O'Shea, J. M. (1977). Effect of pressure treatments on the mechanical properties of pre- and post-rigor meat. *Meat Science*, **1**, 307–318.

Bragagnolo, N., Danielsen, B. and Skibsted, L. H. (2007). Rosemary as antioxidant in pressure processed chicken during subsequent cooking as evaluated by electron spin resonance spectroscopy. *Innovative Food Science and Emerging Technologies*, **8**, 24–29.

Buckow, R., Sikes, A. and Tume, R. (2013). Effect of high pressure on physicochemical properties of meat. *Critical Reviews in Food Science and Nutrition*, **53**, 770–786.

Butz, P., Needs, E. C., Baron, A. et al. (2003). Consumer attitudes to high pressure food processing. *Food Agriculture and Environment*, **1**, 30–34.

Buzrul, S. (2014a). A review on describing the survival curves of *Listeria monocytogenes* in foods of animal origin under high hydrostatic pressure. *Kafkas Üniversitesi Veteriner Fakültesi Dergisi*, **20**, 321–327.

Buzrul, S. (2014b). Multi-pulsed high hydrostatic pressure treatment of microorganisms: A review. *Innovative Food Science and Emerging Technologies*, **26**, 1–11.

Buzrul, S. (2015). Multi-pulsed high hydrostatic pressure treatment of foods. *Foods*, **4**, 173–183.

Buzrul, S. and Alpas, H. (2004). Modeling the synergistic effect of high pressure and heat on the inactivation kinetics of *Listeria innocua*: A preliminary study. *FEMS Microbiology Letters*, **238**, 29–36.

Buzrul, S. and Alpas, H. (2012). Treatment of foods using high hydrostatic pressure. *In:* Bhat, R., Alias, A. K. and Paliyath, G. (eds.) *Progress in Food Preservartion*. West Sussex: Wiley-Blackwell, 375–388.

Buzrul, S., Alpas, H., Largeteau, A. and Demazeau, G. (2009). Efficiency of pulse pressure treatment for inactivation of *Escherichia coli* and *Listeria innocua* in whole milk. *European Food Research and Technology*, **229**, 127–131.

Campus, M. (2010). High pressure processing of meat, meat products and seafood. *Food Engineering Reviews*, **2**, 256–273.

Cardello, A. V., Schutz, H. G. and Lesher, L. L. (2007). Consumer perceptions of foods processed by innovative and emerging technologies: A conjoint analytic study. *Innovative Food Science and Emerging Technologies*, **8**, 73–83.

Carlez, A., Rosec, J.-P., Richard, N. and Cheftel, J. C. (1993). High pressure inactivation of *Citrobacter freundii, Pseudomonas fluorescens,* and *Listeria innocua* in inoculated minced beef muscle. *Lebensmittel-Wissenschaft und -Technologie*, **26**, 357–363.

Carlez, A., Veciana-Nogues, T. and Cheftel, J. C. (1995). Changes in colour and myoglobin of minced beef meat due to high pressure processing. *Lebensmittel-Wissenschaft und -Technologie*, **28**, 528–538.

Cava, R., Ladero, L., González, S., Carrasco, A. and Ramírez, M. R. (2009). Effect of pressure and holding time on colour, protein and lipid oxidation of sliced dry-cured Iberian ham and loin during refrigerated storage. *Innovative Food Science and Emerging Technologies*, **10**, 76–81.

Cerf, O. (1997). A review. Tailing of survival curves of bacterial spores. *Journal of Applied Bacteriology*, **42**, 1–19.

Cheah, P. B. and Ledward, D. A. (1995). High-pressure effects on lipid oxidation. *Journal of the American Oil Chemists' Society*, **72**, 1059–1063.

Downloaded From Vet-eBooks.com

Cheah, P. B. and Ledward, D. A. (1996). High pressure effects on lipid oxidation in minced pork. *Meat Science*, **43**, 123–134.

Cheah, P. B. and Ledward, D. A. (1997). Inhibition of metmyoglobin formation in fresh beef by pressure treatment. *Meat Science*, **45**, 411–418.

Cheftel, J. C. and Culioli, J. (1997). Effects of high pressure on meat: A review. *Meat Science*, **46**, 211–236.

Chen, H. (2007). Temperature-assisted pressure inactivation of Listeria monocytogenes in Turkey breast meat. *International Journal of Food Microbiology*, **117**, 55–60.

Chen, H. and Hoover, D. G. (2003). Pressure inactivation kinetics of *Yersinia enterocolitica* ATCC 35669. *International Journal of Food Microbiology*, **87**, 161–171.

Chick, H. (1908). An investigation of the laws of disinfection. *Journal of Hygiene Cambridge*, **8**, 92–158.

Clariana, M., Guerrero, L., Sárraga, C., Díaz, I., Valero, Á. and García-Regueiro, J. A. (2011a). Influence of high pressure application on the nutritional, sensory and microbiological characteristics of sliced skin vacuumpacked dry-cured ham. Effects along the storage period. *Innovative Food Science and Emerging Technologies*, **12**, 456–465.

Clariana, M., Guerrero, L., Sárraga, C. and Garcia-Regueiro, J. A. (2011b). Effects of high pressure application (400 and 900 MPa) and refrigerated storage time on the oxidative stability of sliced skin vacuum packed dry-cured ham. *Meat Science*, **90**, 323–329.

Corradini, M. G., Normand, M. D. and Peleg, M. (2007). Modeling non-isothermal heat inactivation of microorganisms having biphasic isothermal survival curves. *International Journal of Food Microbiology*, **116**, 391–399.

de Oliveira, T. L. C., Leite, B. R. D., Ramos, A. L. S., Ramos, E. M., Piccoli, R. H. and Cristianini, M. (2015). Phenolic carvacrol as a natural additive to improve the preservative effects of high pressure processing of low-sodium sliced vacuum-packed turkey breast ham. *LWT–Food Science and Technology*, **64**, 1297–1308.

Del Olmo, A., Morales, P., Ávila, M., Calzada, J. and Nuñez, M. (2010). Effect of single-cycle and multiple-cycle high-pressure treatments on the colour and texture of chicken breast fillets. *Innovative Food Science and Emerging Technologies*, **11**, 441–444.

Denoyella, C. and Lebihan, E. (2003). Intramuscular variation in beef tenderness. *Meat Science*, **66**, 241–247.

Diez, A. M., Santos, E. M., Jaime, I. and Rovira, J. (2008). Application of organic acid salts and high-pressure treatments to improve the preservation of blood sausage. *Food Microbiology*, **25**, 154–161.

Dissing, J., Bruun-Jensen, L. and Skibsted, L. H. (1997). Effect of high-pressure treatment on lipid oxidation in turkey thigh muscle during chill storage. *Zeitschrift für Lebensmitteluntersuchung und Forschung*, **205**, 11–13.

Donsì, G., Ferrari, G. and Maresca, P. (2003). On the modeling of the inactivation kinetics of *Saccharomyces cerevisiae* by means of combined temperature and high pressure treatments. *Innovative Food Science and Emerging Technologies*, **4**, 35–44.

Elgasim, E.A. and Kennick, W.H. (1980). Effect of pressurization of pre-rigor beef muscles on protein quality. *Journal of Food Science*, **45**, 1122–1124.

Escriu, R. and Mor-Mur, M. (2009). Role of quantity and quality of fat in meat models inoculated with *Listeria innocua* or *Salmonella* Typhimurium treated by high pressure and refrigerated stored. *Food Microbiology*, **26**, 834–840.

Downloaded From Vet-eBooks.com

The European Parliament and the Council of the European Union. (2004). Regulation (EC) No 853/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific hygiene rules for on the hygiene of foodstuffs. *Official Journal of the European Union*, **139**, 1–155.

Faustman, C. and Cassens, R. G. (1990). The biochemical basis for discoloration in fresh meat: A review. *Journal of Muscle Foods*, **1**, 217–243.

Fuentes, V., Ventanas, J., Morcuende, D., Estévez, M. and Ventanas, S. (2010). Lipid and protein oxidation and sensory properties of vacuum packaged dry-cured ham subjected to high hydrostatic pressure. *Meat Science*, **85**, 506–514.

García-Graells, C., Valckx, C. and Michiels, C. W. (2000). Inactivation of *Escherchia coli* and *Listeria innocua* in milk by combined treatment with high hydrostatic pressure and the lactoperoxidase system. *Applied and Environmental Microbiology*, **66**, 4173–4179.

Garriga, M. and Aymerich, T. (2009). Advanced decontamination technologies: High hydrostatic pressure on meat products. *In:* Toldrá, F. (ed.) *Safety of Meat and Processed Meat*. New York, NY: Springer, 183–208.

Garriga, M., Aymerich, M. T., Costa, S., Monfort, J. M. and Hugas, M. (2002). Bactericidal synergism through bacteriocins and high pressure in a meat model system during storage. *Food Microbiology*, **19**, 509–518.

Garriga, M., Grèbol, N., Aymerich, M. T., Monfort, J. M. and Hugas, M. (2004). Microbial inactivation after high-pressure processing at 600 MPa in commercial meat products over its shelf life. *Innovative Food Science and Emerging Technologies*, **5**, 451–457.

Geeraerd, A. H., Valdramidis, V. P. and Van Impe, J. F. (2005). GInaFiT, a freeware tool to assess non-log-linear microbial survivor curves. *International Journal of Food Microbiology*, **102**, 95–105.

Gola, S., Mutti, P., Manganelli, E., Squarcina, N. and Rovere, P. (2000). Behaviour of *E. coli* 0157: H7 strains in model system and in raw meat by HPP: Microbial and technological aspects. *High Pressure Research*, **19**, 91–97.

Goutefongea, R., Rampon, V., Nicolas, J. and Dumont, J. P. (1995). Meat color changes under high pressure treatment. *In Proceeding of the 41st International Congress of Meat Science and Technology, San Antonio, Texas,* 384–385.

Grèbol, N. (2002). Commercial use of high hydrostatic pressure in sliced cooked ham in Spain. *In:* Hayashi, R. (ed.) *Trends in High Pressure Bioscience and Biotechnology, Progress in Biotechnology*, vol. 19. Amsterdam: Elsevier Science, pp. 385–388.

Hereu, A., Dalgaard, P., Garriga, M., Aymerich, T. and Bover-Cid, S. (2012). Modelling the high pressure inactivation kinetics of *Listeria monocytogenes* on RTE cooked meat products. *Innovative Food Science and Emerging Technologies*, **16**, 305–315.

Hicks, D. T., Pivarnik, L. F., McDermott, R., Richard, N., Hoover, D. G. and Kniel, K. E. (2009). Consumer awareness and willingness to pay for high-pressure processing of ready-to-eat food. *Food Science Education Research*, **8**, 32–38.

Hugas, M., Garriga, M. and Monfort, J. M. (2002). New mild technologies in meat processing: High pressure as a model technology. *Meat Science*, **62**, 359–371.

Ikeuchi, Y. (2011). Recent advances in the application of high pressure technology to processed meat products. *In:* Kerry, J. P. and Kerry, J. F. (eds.) *Processed Meats—Improving Safety, Nutrition and Quality*. Cambridge: Woodhead Publishing, 590–609.

Downloaded From Vet-eBooks.com

Jofré, A., Aymerich, T. and Garriga, M. (2008a). Assessment of the effectiveness of antimicrobial packaging combined with high pressure to control *Salmonella* spp. in cooked ham. *Food Control*, **19**, 634–638.

Jofré, A., Aymerich, T. and Garriga, M. (2009a). Improvement of the food safety of low-acid fermented sausages by enterocins A and B and high pressure. *Food Control*, **20**, 179–184.

Jofré, A., Aymerich, T., Grebol, N. and Garriga, M. (2009b). Efficiency of high hydrostatic pressure at 600 MPa against food-borne microorganisms by challenge tests on convenience meat products. *LWT-Food Science and Technology*, **42**, 924–928.

Jofré, A., Garriga, M. and Aymerich, T. (2007). Inhibition of *Listeria monocytogenes* in cooked ham through active packaging with natural antimicrobials and high-pressure processing. *Journal of Food Protection*, **70**, 2498–2502.

Jofré, A., Garriga, M. and Aymerich, T. (2008b). Inhibition of *Salmonella* sp. *Listeria monocytogenes* and *Staphylococcus aureus* in cooked ham by combining antimicrobials, high hydrostatic pressure and refrigeration. *Meat Science*, **78**, 53–59.

Jung, S., de Lamballerie-Anton, M. and Ghoul, M. (2000). Textural changes in bovine meat treated with high pressure. *High Pressure Research*, **19**, 69–74.

Jung, S., Ghoul, M. and de Lamballerie-Anton, M. (2003). Influence of high pressure on the color and microbial quality of beef meat. *Food Science and Technology*, **36**, 625–631.

Karlowski, K., Windyga, B., Fonberg-Broczek, M. et al. (2002). Effects of high pressure treatment on the microbiological quality, texture and colour of vacuum-packed pork meat products. *High Pressure Research*, **22**, 725–732.

Kingsley, D. H., Guan, D., Hoover, D. G. and Chen, H. (2006). Inactivation of hepatitis A virus by high-pressure processing: The role of temperature and pressure oscillation. *Journal of Food Protection*, **69**, 2454–2459.

Korzeniowski, W., Jankowska, B., Kwiatkowska, A. (1999). The effect of high pressure on some technological properties of pork. Electronic *Journal of Polish Agricultural Universities*, **2**, 1–7, #04. Available at http://www.ejpau.media.pl/volume2/issue2/food/art-04.html

Koseki, S. and Yamamoto, K. (2007). A novel approach to predicting microbial inactivation kinetics during high pressure processing. *International Journal of Food Microbiology*, **116**, 275–281.

Koutchma, T. (2014). *Adapting High Hydrostatic Pressure (HPP) for Food Processing Operations*. Burlington, NY: Elsevier.

Krockel, L. and Muller, W. D. (2002). Inactivation of bacteria on vacuum-packaged sliced Bologna-type sausage by high hydrostatic pressures. *Fleischwirtschaft*, **82**, 121–124.

Kruk, Z. A., Yun, H., Rutley, D. L., Lee, E. J., Kim, Y. J. and Jo, C. (2011). The effect of high pressure on microbial population, meat quality and sensory characteristics of chicken breast fillet. *Food Control*, **22**, 6–12.

Ledward, D. A. (2000). Effects of pressure on protein structure. *High Pressure Research*, **19**, 1–10.

Linton, R. H., Carter, W. H., Pierson, M. D. and Hackney, C. R. (1995). Use of modified Gompertz equation to model non-linear survival curves for *Listeria monocytogenes* Scott A. *Journal of Food Protection*, **9**, 946–954.

Locker, R. H. and Wild, D. J. C. (1984). Tenderisation of meat by pressure-heat involves weakening of the gap filaments in the myofibril. *Meat Science*, **10**, 207–233.

Downloaded From Vet-eBooks.com

López-Caballero, M., Carballo, J. and Jiménez-Colmenero, R. (2002). Microbial inactivation in meat products by pressure/temperature processing. *Journal of Food Science*, **67**, 797–801.

Lori, S., Buckow, R., Knorr, D., Heinz, V. and Lehmacheri, A. (2007). Predictive model for inactivation of *Campylobacter* spp. by heat and high hydrostatic pressure. *Journal of Food Protection*, **70**, 2023–2029.

Ma, H. J. and Ledward, D. A. (2004). High pressure/thermal treatment effects on the texture of beef muscle. *Meat Science*, **68**, 347–355.

Ma, H. and Ledward, D. A. (2013). High pressure processing of fresh meat—Is it worth it? *Meat Science*, **95**, 897–903.

Ma, H. J., Ledward, D. A., Zamri, A. I., Frazier, R. A. and Zhou, G. H. (2007). Effects of high pressure/thermal treatment on lipid oxidation in beef and chicken muscle. *Food Chemistry*, **104**, 1575–1579.

Macfarlane, J. J. (1973). Pre-rigor pressurization of muscle: Effects on pH, shear value and taste panel assessment. *Journal of Food Science*, **38**, 294–298.

Macfarlane, J. J. (1985). High pressure technology and meat quality. *In:* Lawrie, R. A. (ed.) *Developments in Meat Science-3*, London: Elsevier Applied Science, 155–184.

Macfarlane, J. J., McKenzie, I. J. and Turner, R. H. (1986). Pressure-heat treatment of meat: Changes in myofibrillar proteins and ultrastructure. *Meat Science*, **17**, 161–176.

Macfarlane, J. J., McKenzie, I. J. and Turner, R. H. (1982). Pressure-induced pH and length changes in muscle. *Meat Science*, **7**, 169–181.

Macfarlane, J. J., McKenzie, I. J., Turner, R. H. and Jones, P. N. (1981). Pressure treatment of meat: Effects on thermal transitions and shear values. *Meat Science*, **5**, 307–317.

Macfarlane, J. J. and Morton, D. J. (1978). Effects of pressure treatment on the ultrastructure of striated muscle. *Meat Science*, **2**, 282–288.

Marcos, B., Aymerich, T., Monfort, J. M. and Garriga, M. (2008a). High-pressure processing and antimicrobial biodegradable packaging to control *Listeria monocytogenes* during storage of cooked ham. *Food Microbiology*, **25**, 177–182.

Marcos, B., Jofré, A., Aymerich, T., Monfort, J. M. and Garriga, M. (2008b). Combined effect of natural antimicrobials and high pressure processing to prevent *Listeria monocytogenes* growth after a cold-chain break during storage of cooked ham. *Food Control*, **19**, 76–81.

Martinez-Rodriguez, A. and Mackey, B. M. (2005). Factors affecting the pressure resistance of some *Campylobacter* species. *Letters in Applied Microbiology*, **41**, 321–326.

Mazzotta, A. S. and Montville, T. J. (1999). Characterization of fatty acid composition, spore germination, and thermal resistance in a nisin-resistant mutant of *Clostridium botulinum* 169B and in the wild-type strain. *Applied and Environmental Microbiology*, **65**, 659–664.

Mendoza, F., Maqueda, M., Galvez, A., Martinez-Bueno, M. and Valdivia. E. (1999). Antilisterial activity of peptide AS-48 and study of changes induced in the cell envelope properties of an AS-48-adapted strain of *Listeria monocytogenes*. *Applied and Environmental Microbiology*, **65**, 618–625.

McArdle, R., Marcos, B., Kerry, J. P. and Mullen, A. (2010). Monitoring the effects of high pressure processing and temperature on selected beef quality attributes. *Meat Science*, **86**, 629–634.

Moerman, F. (2005). High hydrostatic pressure inactivation of vegetative microorganisms, aerobic and anaerobic spores in pork Marengo, a low-acidic particulate food product. *Meat Science*, **69**, 225–232.

Downloaded From Vet-eBooks.com

Montiel, R., Martín-Cabrejas, I. and Medina, M. (2015). Reuterin, lactoperoxidase, lactoferrin and high hydrostatic pressure on the inactivation of food-borne pathogens in cooked ham. *Food Control*, **51**, 122–128.

Morales, P., Calzada, J., Ávila, M. and Nuñez, M. (2008). Inactivation of *Escherichia coli* O157: H7 in ground beef by single-cycle and multiple-cycle high-pressure treatments. *Journal of Food Protection*, **71**, 811–815.

Morales, P., Calzada, J. and Nuñez, M. (2006). Effect of high-pressure treatment on the survival of *Listeria monocytogenes* Scott A in sliced vacuum-packaged Iberian and Serrano cured hams. *Journal of Food Protection*, **69**, 2539–2543.

Morales, P., Calzada, J., Rodríguez, B., de Paz, M. and Nuñez, M. (2009). Inactivation of *Salmonella* Enteritidis in chicken breast fillets by single-cycle and multiple-cycle high pressure treatments. *Foodborne Pathogens and Diseases*, **6**, 577–581.

Myers, K., Cannon, J., Montoya, D., Dickson, J., Lonergan, S. and Sebranek, J. (2013). Effects of high hydrostatic pressure and varying concentrations of sodium nitrite from traditional and vegetable-based sources on the growth of *Listeria monocytogenes* on ready-to-eat (RTE) sliced ham. *Meat Science*, **94**, 69–76.

Nishiwaki, T., Ikeuchi, Y. and Suzuki, A. (1996). Effects of high pressure treatment on Mg-enhanced ATPase activity of rabbit myofibrils. *Meat Science*, **43**, 145–155.

Ogihara, H., Yatuzuka, M., Horie, N., Furukawa, S. and Yamasaki, M. (2009). Synergistic effect of high-hydrostatic-pressure treatment and food additives on the inactivation of *Salmonella enteritidis*. *Food Control*, **20**, 963–966.

Ohmori, T., Shigehisa, T., Taji, S. and Hayashi, R. (1991). Effect of high pressure on the protease activities in meat. *Agricultural and Biological Chemistry*, **55**, 357–361.

Orlien, V., Hansen, E. and Skibsted, L. H. (2000). Lipid oxidation in high-pressure processed chicken breast muscle during chill storage: Critical working pressure in relation to oxidation mechanism. *European Food Research and Technology*, **211**, 99–104.

Patterson, M. F. and Kilpatrick, D. J. (1998). The combined effect of high hydrostatic pressure and mild heat on inactivation of pathogens in milk and poultry. *Journal of Food Protection*, **61**, 432–436.

Patterson, M. F., Linton, M. and Doona, C. J. (2007). Introduction to high pressure processing of foods. *In:* Doona, C. J. and Feeherry, F. E. (eds.) *High Pressure Processing of Foods*. Ames, IA: IFT Press/Blackwell Publishing, pp. 1–14.

Patterson, M. F., Quinn, M., Simpson, R. and Gilmour, A. (1995). Sensitivity of vegetative pathogens to high hydrostatic pressure treatment in phosphate-buffered saline and foods. *Journal of Food Protection*, **58**, 524–529.

Peleg, M. (1996). Evaluation of the Fermi equation as a model of dose-response curves. *Applied Microbiology and Biotechnology*, **46**, 303–306.

Peleg, M. (2000). Microbial survival curves—The reality of flat shoulders and absolute thermal death times. *Food Research International*, **33**, 531–538.

Peleg, M. (2003). Calculation of the nonisothermal inactivation patterns of microbes having sigmoidal isothermal semilogarithmic survival curves. *Critical Reviews in Food Science and Nutrition*, **43**, 645–658.

Peleg, M. (2006). *Advanced Quantitative Microbiology for Food and Biosystems: Models for Predicting Growth and Inactivation*. Boca Raton, FL: CRC Press.

Peleg, M. and Cole, M. B. (1998). Reinterpretation of microbial survival curves. *Critical Reviews in Food Science and Nutrition*, **38**, 353–380.

Pilavtepe-Çelik, M., Buzrul, S., Alpas, H., Largeteau, A. and Demazeau, G. (2011). Multi-pulsed high hydrostatic pressure treatment for inactivation and injury of *Escherichia coli*. *Journal für Verbraucherschutz und Lebensmittelsicherheit*, **6**, 343–348.

Downloaded From Vet-eBooks.com

Polydera, A. C., Stoforos, N. G. and Taoukis, P. S. (2005). Effect of high hydrostatic pressure treatment on post processing antioxidant activity of fresh navel orange juice. *Food Chemistry*, **91**, 495–503.

Porto-Fett, A. C. S., Call, J. E., Shoyer, B. E. et al. (2010). Evaluation of fermentation, drying, and/or high pressure processing on viability of *Listeria monocytogenes*, *Escherichia coli* O157: H7, *Salmonella* spp., and *Trichinella spiralis* in raw pork and Genoa salami. *International Journal of Food Microbiology*, 140, 61–75.

Refsgaard, H. H. F., Brockhoff, P. M. B. and Jensen, B. (2000). Free polyunsaturated fatty acids cause taste deterioration of salmon during frozen storage. *Journal of Agricultural and Food Chemistry*, **48**, 3280–3285.

Rivalain, N., Roquain, J., Boiron, J. M. et al. (2012). High hydrostatic pressure treatment for the inactivation of *Staphylococcus aureus* in human blood plasma. *New Biotechnology*, **29**, 409–414.

Robbins, K. L., Lakshmanan, R., Sebranek, J. G. and Jung, S. (2006). Efficiency of rosemary and BHA/BHT antioxidant to delay lipid and color deterioration of pressurized meat products. *In Proceedings of Annual Meeting and Food Exposition of the Institute of Food Technologists,* Orlando, Florida.

Rubio, B., Martínez, B., García-Cachán, M. D., Rovira, J. and Jaime, I. (2007). Effect of high-pressure preservation on the quality of dry cured beef "Cecina de Leon." *Innovative Food Science and Emerging Technologies*, **8**, 102–110.

Ros-Polski, V., Koutchma, T., Xue, J., Defelice, C. and Balamurugan, S. (2015). Effects of high hydrostatic pressure processing parameters and NaCl concentration on the physical properties, texture and quality of white chicken meat. *Innovative Food Science and Emerging Technologies*, **30**, 31–42.

Shigehisa, T., Ohmori, T., Saito, A., Taji, S. and Hayashi, R. (1991). Effects of high hydrostatic pressure on characteristics of pork slurries and inactivation of microorganisms associated with meat and meat products. *International Journal of Food Microbiology*, **12**, 207–215.

Sikes, A., Tornberg, E. and Tume, R. (2010). A proposed mechanism of tenderising post-rigor beef using high pressure-heat treatment. *Meat Science*, **84**, 390–399.

Simonin, H., Duranton, F. and de Lamballerie, M. (2012). New insights into the high-pressure processing of meat and meat products. *Comprehensive Reviews in Food Science and Food Safety*, **11**, 285–306.

Simpson, R. K. and Gilmour, A. (1997). The resistance of *Listeria monocytogenes* to high hydrostatic pressure in foods. *Food Microbiology*, **14**, 567–573.

Solomon, E. B. and Hoover, D. G. (2004). Inactivation of *Campylobacter jejuni* by high hydrostatic pressure. *Letters in Applied Microbiology*, **38**, 505–509.

Sun, X. D. and Holley, R. A. (2010). High hydrostatic pressure effects on the texture of meat and meat products. *Journal of Food Science*, **75**, R17–R21.

Tassou, C. C., Galiatsatou, P., Samaras, F. J. and Mallidis, C. G. (2007). Inactivation kinetics of a piezotolerant *Staphylococcus aureus* isolated from high-pressure-treated sliced ham by high pressure in buffer and in a ham model system: Evaluation in selective and non-selective medium. *Innovative Food Science and Emerging Technologies*, **8**, 478–484.

Tassou, C. C., Panagou, E. Z., Samaras, F. J., Galiatsatou, P. and Mallidis, C. G. (2008). Temperature-assisted high-hydrostatic-pressure inactivation of *Staphylococcus aureus* in a ham model system: Evaluation in selective and nonselective medium. *Jounal of Applied Microbiology*, **104**, 1764–1773.

Downloaded From Vet-eBooks.com

Teixeira, P., Kolomeytseva, M., Silva, J., Castro, S. M. A. and Saraiva, J. A. (2014). High hydrostatic pressure applied to ready-to-eat meat products. *Frontiers in Food Science and Technology*, **1**, 14–23.

Teixeira, J. S., Maier, M. B., Miller, P., Gänzle, M. G. and McMullen, L. M. (2016). The effect of growth temperature, process temperature, and sodium chloride on the high pressure inactivation of *Listeria monocytogenes* on ham. *European Food Research and Technology*, **242**, 2021–2029.

Tintchev, F., Wackerbarth, H., Kuhlmann, U. et al. (2010). Molecular effects of high-pressure processing on food studied by resonance Raman. *Annals of New York Academy of Science*, **1189**, 34–42.

Tonello, C. (2011). Case studies on high-pressure processing of foods. *In:* Zhang, H. Q., Barbosa-Cánovas, G. V., Balasubramaniam, V. M., Dunne, C. P., Farkas, D. F. and Yuan, J. T. C. (eds.) *Nonthermal Processing Technologies for Food*. Chichester: Wiley-Blackwell, pp. 36–50.

Tokuşoğlu, Ö. and Vural, H. (2014). High-pressure processing for freshness, shelf-life quality of meat products and value-added meat products. *In:* Tokuşoğlu, Ö. and Swanson, B. G (eds.) *Improving Food Quality with Novel Food Processing Technologies*. Boca Raton, FL: CRC Taylor & Francis, pp. 85–110.

Tuboly, E., Lebovics, V. K., Gaál, Ö., Mészáros, L. and Farkas, J. (2003). Microbiological and lipid oxidation studies on mechanically deboned turkey meat treated by high hydrostatic pressure. *Journal of Food Engineering*, **56**, 241–244.

Tume, R. K., Sikes, A. L. and Smith, S. B. (2010). Enriching *M. Sternomandibularis* with alpha-tocopherol by dietary means does not protect against the lipid oxidation caused by high-pressure processing. *Meat Science*, **84**, 66–70.

Urrutia, G., Arabas, J., Autio, K. et al. (2007). SAFE ICE: Low-temperature pressure processing of foods: Safety and quality aspects, process parameters and consumer acceptance. *Journal of Food Engineering*, **83**, 293–315.

USDA, Food Safety and Inspection Service. (2006). Compliance guidelines to control *Listeria monocytogenes* in post-lethality exposed RTE Meat and Poultry Products. Available at http://www.fsis.usda.gov/OPPDE/rdad/FRPubs/97-013F/LM_Rule_Compliance_Guidelines_May_2006.pdf (Accessed 28 February 2016).

Viljanen, K., Kylli, P., Kivikari, R. and Heinonen, M. 2004. Inhibition of protein and lipid oxidation in liposomes by berry phenolics. *Journal of Agricultural and Food Chemistry*, **52**, 7419–7424.

Xiong, R., Xie, G., Edmondson, A. E. and Sheard, M. A. (1999). A mathematical model for bacterial inactivation. *International Journal of Food Microbiology*, **46**, 45–55.

Yamamoto, K., Matsubara, M., Kawasaki, S., Bari, M. L. and Kawamoto, S. (2005). Modeling the pressure inactivation dynamics of *Escherichia coli*. *Brazilian Journal of Medical and Biological Research*, **38**, 1253–1257.

Yuste, J., Mor-Mur, M., Capellas, M., Guamis, B. and Pla, R. (1998). Microbiological quality of mechanically recovered poultry meat treated with high hydrostatic pressure and nisin. *Food Microbiology*, **15**, 407–414.

Yuste, J., Pla, R., Capellas, M. and Mor-Mur, M. (2002). Application of high-pressure processing and nisin to mechanically recovered poultry meat for microbial decontamination. *Food Control*, **13**, 451–455.

Yuste, J., Pla, R., Capellas, M., Ponce, E. and Mor-Mur, M. (2000b). High-pressure processing applied to cooked sausages: Bacterial populations during chilled storage. *Journal of Food Protection*, **63**, 1093–1099.

Downloaded From Vet-eBooks.com

Yuste, J., Pla, R., Capellas, M., Sendra, E., Beltran, E. and Mor-Mur, M. (2001). Oscillatory high pressure processing applied to mechanically recovered poultry meat for bacterial inactivation. *Journal of Food Science*, **66**, 482–484.

Yuste, J., Pla, R. and Mor-Mur, M. (2000a). *Salmonella enteritidis* and aerobic mesophiles in inoculated poultry sausages manufactured with high-pressure processing. *Letters in Applied Microbiology*, **31**, 374–377.

Zapico, P., Medina, M., Gaya, P. and Nuñez M. (1998). Synergistic effect of nisin and the lactoperoxidase system on *Listeria monocytogenes* in skim milk. *International Journal of Food Microbiology*, **40**, 35–42.

Downloaded From Vet-eBooks.com



Taylor & Francis
Taylor & Francis Group
http://taylorandfrancis.com

Downloaded From Vet-eBooks.com

8

Hydrodynamic Pressure Processing to Improve Meat Quality and Safety

Tomás Bolumar and Stefan Toepfl

CONTENTS

8.1 Introduction 259
8.2 HDP or Shock Wave Technology 260
8.2.1 Fundamentals 261
8.2.2 Development of Equipment 263
8.3 HDP for Improving Meat Quality 267
8.3.1 HDP for Meat Tenderization 267
8.3.2 HDP for Modification of Meat Functionality 274
8.3.3 Molecular and Structural Effects of HDP in Meat 276
8.4 HDP for Improving Meat Safety 281
8.5 Cost Analysis of HDP Technology 286
8.5.1 Packaging 286
8.5.2 Equipment 287
8.5.3 Quantifying Meat Benefits 289
8.5.4 Overall Cost Analysis of HDP or Shock Wave Technology for the Meat Industry 289
8.6 Future Considerations 291
8.7 Conclusion 291
Acknowledgments 292
References 292

8.1 Introduction

Meat quality and safety are essential for the meat industry. For fresh meat, tenderness is the most important eating quality trait (Mennecke et al., 2007), and thus, it has great impact on its value and repeated purchase by consumers (Grunert et al., 2004). Consequently, during the years, many interventions have been developed to improve the tenderness of low-value muscles and to ensure the consistency of high-value muscles. Tenderness, however, is a very complex trait and has proved to be very difficult to guarantee in commercial scenarios.

Downloaded From Vet-eBooks.com

Therefore, the development of novel interventions to improve tenderness would greatly contribute to boost the profitability of the meat industry (Bolumar et al., 2013). In addition, meat safety assurance is on top of the list of industry priorities. Food safety is assured in the industry by the application of strict risk management systems that exclude from the food production chain the presence of chemical, physical, or biological materials that are harmful to human health upon consumption. The development of alternative processing methods for microbial inactivation provides the industry with tools to guarantee food safety and can contribute to process optimization and cost reductions.

In recent years, the use of novel food processing technologies, beyond the use of heat treatments, such as high-pressure processing (HPP), pulsed electric fields, and high-power ultrasounds, has emerged due in part to the considerable effort undertaken by the scientific community in the last 20 years. This has led to the development and scale up of different technologies which are nowadays available for testing in different fields and for specific applications regarding food quality and safety (Barbosa-Canovas et al., 2004; Doona et al., 2010). Hydrodynamic pressure processing (HDP) is one of these novel processing technologies that emerged on scientific ground and is now developing for transfer to the industry. Meat tenderization and microbial decontamination using HDP are promising areas to deliver alternative methods to comply with the demands of the future meat industry for high-quality and safe meat (Bolumar et al., 2013).

This chapter begins by describing the fundamental principles to generate a shock wave and presents this together with the engineering development of shock wave equipment. Then, the effects of HDP on meat quality traits such as tenderness and meat functional properties are presented. Following on, the effects of HDP on muscle micro- and ultrastructure are described to shed light on the molecular mechanisms responsible for the observed effects. Later, the potential use of HDP for microbial inactivation is also described and discussed. Throughout the chapter, the HDP method for meat tenderization and microbial inactivation is compared with current and novel practices and methods applied with the same purpose in order to provide the reader and the industry with alternatives and set out the specific context for its use. Finally, a cost–benefit analysis of the technology including processing and packaging costs in relation to benefits on meat quality is outlined as well as directions on how to improve the technology.

8.2 HDP or Shock Wave Technology

HDP differs from HPP. HPP is a processing method in which a product is placed within a vessel filled with a fluid, normally water, and the pressure is transmitted to the product by compressing the surrounding liquid with pumps. In HPP, pressure is applied to the product in a static way as such pressure is transmitted instantaneously and uniformly across the entire product, while in HDP, the pressure is applied in a dynamic way as pressure pulses. The fact is that

Downloaded From Vet-eBooks.com

the application of high pressure to food systems offers possibilities that cannot be achieved by common processing techniques, and thus, it has a high potential for the development of novel and future food applications. Indeed, HPP is the most successful nonthermal technology applied in the food industry so far with more than 200 units operating worldwide (Bolumar et al., 2015). Commercial HPP equipment operates in the pressure range up to 600 MPa. Today, the benefits of HPP on microbial inactivation are well established and HPP-treated food products such as juices, dairy products, sauces, or meat products can be found in the market (Hendrickx and Knorr, 2002). Furthermore, HPP can also be used as a method to modify food structures with positive effects if applied under the right conditions. More information on the use of HPP for structure modification is reviewed in the book chapter by Bolumar et al. (2016). In this chapter, HDP is presented and described. Overall, HDP, like HPP, can be used for structure modification and microbial inactivation although the way that pressure is applied to the system is substantially different as described above.

8.2.1 Fundamentals

HDP or shock wave is the application of high-pressure waves up to 1 GPa in fractions of milliseconds. A shock wave is instantaneously generated and is characterized by the intensity achieved (pressure level) and its propagation along the time course (rise time). The shock wave propagates across the surrounding medium with high energy–momentum at speeds higher than the speed of sound. It travels rapidly through the fluids (water) and any objects which are an acoustical match with water (Mayer et al., 2009; Yang, 2011). Since meat is composed of 75% water the pressure wave crosses the meat and at points where acoustic impedances differ, an energy–momentum transfer occurs, which, in turn, creates mechanical stress that tears the muscle structure. This produces what could be termed a "rupture effect," and as a result, the meat instantaneously softens; an accelerated aging of the meat has also been observed over time (Bolumar et al., 2013).

Shock waves are mechanical pressure pulses in fluids. The generation of a shock wave by electrical discharge occurs by converting electrical energy into mechanical energy under water. A shock wave can be generated by piezoelectric, electromagnetic, electrothermal, or electrodetonative methods (Mayer et al., 2009; Yang, 2011). The highest energy conversion rate can be accomplished by electrothermal and electro-detonative procedures and these methods have been the most applied in food processing research so far due to the higher energy of these shock waves. When the electro-detonative process takes place in a tensioned wired electrode, chemical energy is released by the evaporation and explosion. The explosive expansion generates a shock wave. The range of penetration depth of a shock wave is affected by the propagation loss and the absorption, reflection, and refraction in and on the different materials of the treatment area and surroundings. Due to the propagation loss as a consequence of the increase of the shock wave front, the power density at

Downloaded From Vet-eBooks.com

a point from the origin of the shock wave decreases with the square of the distance. Therefore to increase the energy density in medical devices, focusing of the shock wave is applied which leads to a locally limited workspace. To increase the range of energy release, particularly when used for the treatment of foods, it is essential to obtain sufficient processing volumes with homogenous distribution of treatment intensity. Simultaneously, the focusing of the shock wave energy to the material to be treated permits the reduction of the mechanical load applied to the treatment area.

As previously mentioned, the shock waves commonly used in food processing so far can be generated by a detonation of an explosive or by an electrical discharge of a high voltage between two electrodes under water. The detonation of an explosive and the subsequent generation of a dynamic pressure wave is obvious. But in the case of an electrical discharge, it is noteworthy to briefly describe the underliying mechanism. The application of high energy by use of a capacitor discharge underwater between two electrodes leads to the formation of a plasma channel wherein the medium is ionized. The electric breakdown of liquids is initiated by the application of the high electric field on the electrode followed by rapid propagation and branching of plasma channels (Locke et al., 2006). The operational principle of shock wave equipment based on electrical discharge is outlined in Figure 8.1. A basic shock wave system consists of a high-voltage power supply, a capacitor bank, and a high-voltage/current switch to discharge the stored electric energy across the electrodes. By variations of charging voltage and capacity, the energy per pulse can be varied. The treatment intensity can be further adjusted with the number of pulses applied to the sample.

So far, HDP has mainly been applied for meat tenderization (Solomon et al., 1997, 2006; Claus et al., 2001b; Claus, 2002; Bolumar et al., 2013). HDP can be also applied to foodstuffs for different purposes like the opening of oysters

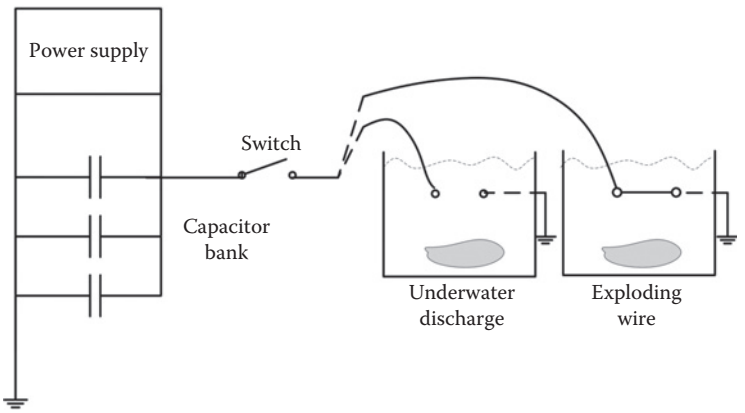


FIGURE 8.1
Operation principle of electrohydraulic shock wave equipment. (Reprinted from *Meat Science*, 92, Bajovic et al., Quality considerations with high pressure processing of fresh and value added meat products, 280–289. Copyright 2012, with permission from Elsevier.)

Downloaded From Vet-eBooks.com

(Paparella and Allen, 1970), the improvement of the extraction of saponin from tomato (Manabe et al., 2011), and the production of functional rice flour (Miyafuji et al., 2011; Shimojima et al., 2011). In general, shock wave technology as a method for structure modification can assist in extraction processes and waste treatment as well as cleaning and fragmentation processes of plant materials. Some work has also been done in the area of microbial inactivation as later is examined for the case of meat (Section 8.3).

8.2.2 Development of Equipment

A historical perspective of the evolution of HDP or shock wave technology is presented in Figure 8.2 that highlights chronologically the major events on the evolution of the engineering development of HDP technology from the 1970s until now. The development of commercial shock wave equipment for meat tenderization is described in the following paragraphs.

The very first rudimentary systems of the use of shock waves or HDP as a method for food structure modification or "food tenderizer" were conceived early in the 1950s (Simjian, 1957) (Figure 8.2). The use of HDP as an alternative method for "food tenderization" has been investigated since the 1970s (Godfrey, 1970), although the first semi-industrial shock wave units were not developed until the 1990s in the United States and those were based on the detonation of explosives (Long, 1993, 1994; Solomon et al., 1997; Figure 8.2). It was not until then that the development of the so-called "Hydrodyne Process" and related equipment allowed an extensive and thorough investigation, mostly conducted by Professor Morse Solomon from the Food Technology and Safety Laboratory (Beltsville, MD) and partners (Figure 8.2). Those works provided clear evidence of muscle tenderization under different conditions and shed valuable information for future experiments and equipment design (Solomon et al., 2006). However, the use of explosives in the food industry raised different safety concerns for operators and the potential contamination of the meat itself with chemical residues from the explosion, and therefore, its transfer to the industry was never accomplished.

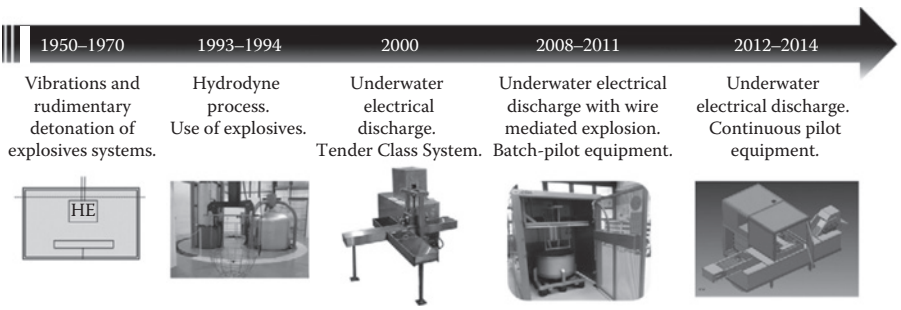


FIGURE 8.2
Historical perspective of shock wave technology for meat tenderization.

Downloaded From Vet-eBooks.com

During the last decade, the second generation of HDP equipment based on electrical discharges underwater rather than the detonation of explosives was conceived (Long, 2000) (Figures 8.1 and 8.2). The use of electrical discharges underwater avoids the problems associated with the use of explosives and allows a continuous treatment process as there is no longer the need for loading/unloading of explosives between shock waves. These systems are favorable as they make the application more feasible in terms of automation and reduction in processing time, and they facilitate the modulation of the shock wave intensity by delivery of gradual electrical intensities and number of pulses per treatment. This shock wave technology generated by electrical discharges underwater led to an attempt at a commercial HDP system intended for meat tenderization, the Tender Class System (TCS), by the American company Hydrodyne Incorporated. The TenderClass (TDC) is shown in Figure 8.3. The TDC system has not been fully applied in the meat industry to the best of our knowledge.

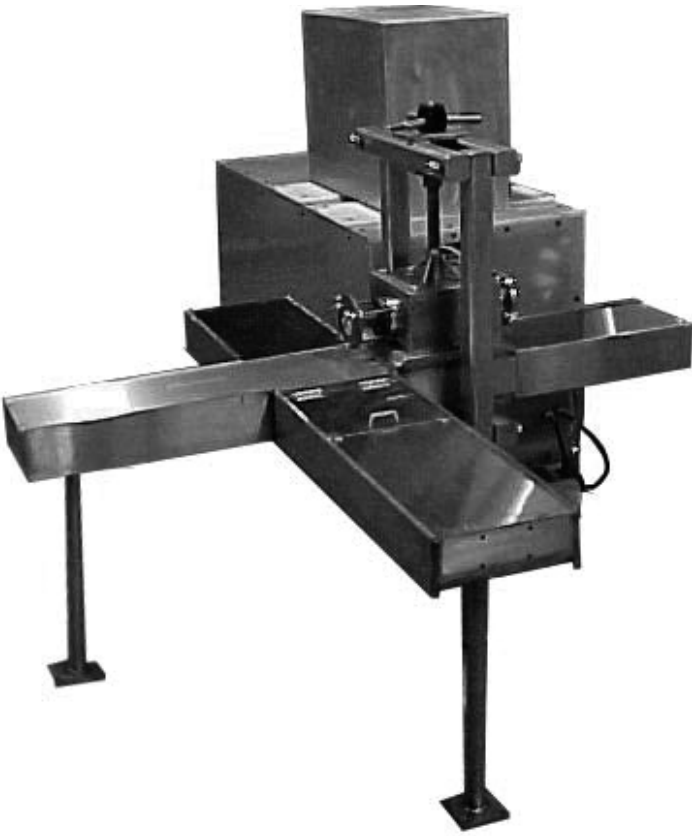


FIGURE 8.3
Photo of the Tender Class System (TCS) unit from Hydrodyne Inc. (Courtesy of Bill McKenna, Hydrodyne Inc. CEO and Professor Jim Claus, University of Wisconsin-Madison).

Downloaded From Vet-eBooks.com

Finally, the construction firstly of a pilot-batch prototype, and then a continuous semi-industrial-scale shock wave prototype for the continuous treatment of meat was undertaken at the German Institute of Food Technologies (DIL) (Figures 8.2, 8.4, and 8.5). During the period from 2008 to 2011 and within a German-funded research project, a pilot-batch prototype for the generation of shock waves intended for meat tenderization by electro-detonative and electrohydraulic underwater discharges was designed and built at the DIL (Heinz et al., 2011). The pilot-batch prototype relied on underwater discharges of electric energy between two electrodes mediated by metal wires (aluminum and copper). A prototype was developed with an average power output of 2 kV and a peak power of 40 kV with a vessel volume of 50 L (Figure 8.4). An effective energy conversion from electrical to mechanical energy was achieved and demonstrated to be effective to tenderize meat. This pilot-batch unit was successfully used to decrease the maturation time of beef cuts from 14 to 7 days (Toepfl and Heinz, 2009).

At a later stage, the development of an upgraded industrial prototype for a continuous shock wave treatment of meat was accomplished within the European-funded project, Shockmeat (http://www.shockmeat.eu/) (Toepfl et al., 2013). A drawing scheme of the main parts of the prototype is shown in Figures 8.5 and 8.6. The shock wave prototype has a conveyor belt for the transport of the meat to the treatment area where an electrically generated

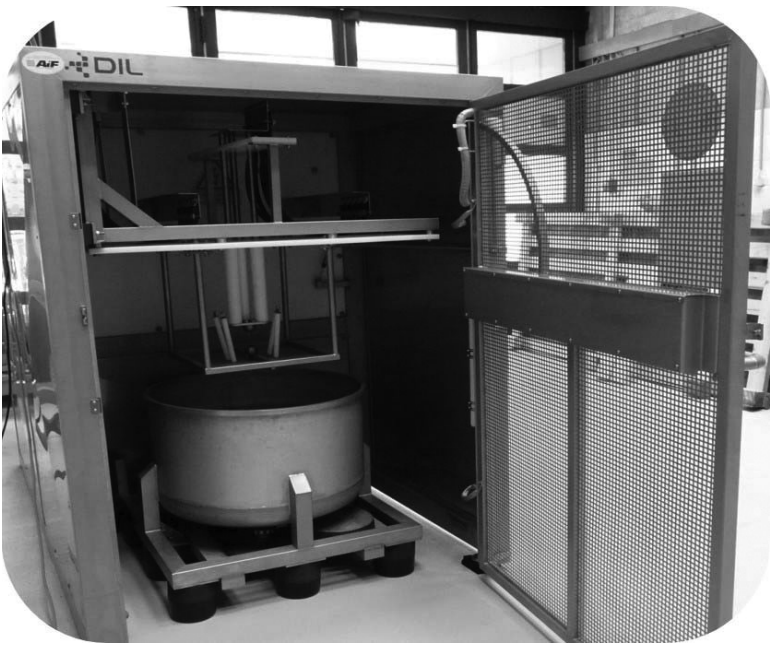


FIGURE 8.4
Shock wave prototype intended for meat tenderization developed within a German-funded research project, AiF-15884 N (2008–2011).

Downloaded From Vet-eBooks.com

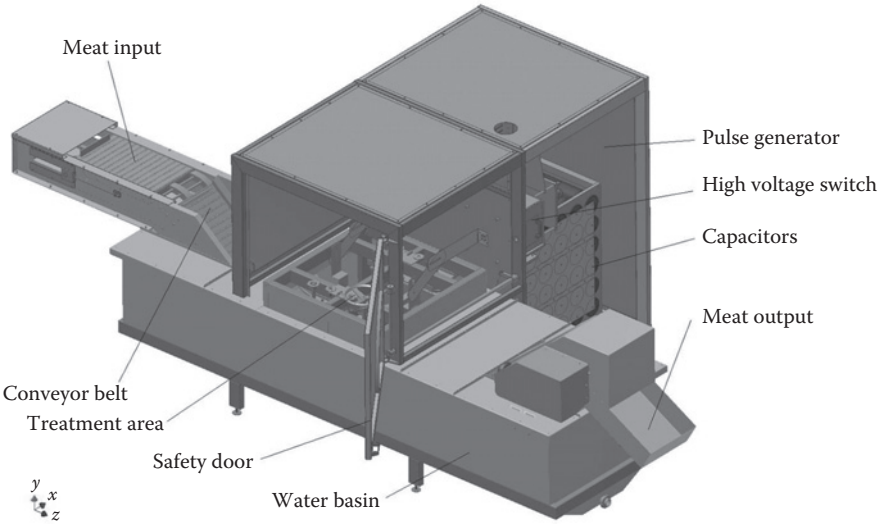


FIGURE 8.5
Main parts of the shock wave prototype intended for meat tenderization developed within the European-funded research project, ShockMeat (2012–2013). (Reprinted from *Meat Science*, 95, Bolumar et al., New developments in shockwave technology intended for meat tenderization: Opportunities and challenges, 931–939. Copyright 2013, with permission from Elsevier.)



FIGURE 8.6
Photo of the shock wave prototype intended for meat tenderization developed within the European-funded research project, ShockMeat (2012–2013).

Downloaded From Vet-eBooks.com

shock wave is applied to the meat. Energy storage by the capacitors, current, and voltage applied determine the shock wave intensity and this is controlled by an automatic programmable interface. The number of pulses applied in the treatment can be set up taking into account the belt speed and the number of pulses applied per traveled distance. These parameters allow for an adjustable and targeted treatment of shock wave intensity and number of pulses. This shock wave prototype showed promising effects on meat tenderization (10%–25% reduction of Warner–Bratzler shear force [WBSF] in different muscles of beef and pork). However, during the project execution, it was also pointed out that in order to benefit from the application of the HPD technology in an industrial environment some improvements were required. These included the development of a stronger and highly resistant packaging material, better interaction between the dynamic forces of the shock wave and the packaging, and a further characterization of the processing settings required for a particular meat (i.e., specific meat primal cut and type of animal) (Bolumar et al., 2013).

Recently, the use of high-efficiency compact sparkers, based on the sparker source system by Schaefer (1998), was demonstrated on a laboratory scale, with favorable changes in tenderness (10%–57% reduction of the shear force) of steaks (2.5 cm thick) and intact beef loins (Bowker et al., 2011) being achieved. Sparkers are electrically driven acoustic sources that produce high-pressure shock waves, similar to explosives. This system is essentially equivalent to the electrohydraulic system described (Figure 8.5) but on a miniaturized scale. While this technique did not damage the packaging during HDP (major drawback of the larger unit, Figure 8.5), the scale-up to industrial standard for this miniaturized HDP unit would be very difficult given the small treatment area and the long processing times required, and as such, it is a laboratory-scale technology at this time.

8.3 HDP for Improving Meat Quality

8.3.1 HDP for Meat Tenderization

Meat tenderness (toughness or resistance to cut) has long been recognized as the most important eating quality trait of fresh meats (Mennecke et al., 2007; Grunert et al., 2004). Basically, meat tenderness depends on three main components: (1) degree of contraction of the muscle sarcomere (sarcomere length), (2) extent of integrity/degradation of structural myofibrillar proteins (proteolysis), and (3) connective tissue content/composition ("background toughness") (Koohmaraie et al., 2002; Sentandreu et al., 2002). Accordingly, different intrinsic tenderness can be listed. These include the extent to which muscle is contracted when it sets into *rigor mortis*, the extent

Downloaded From Vet-eBooks.com

to which certain proteins are broken down postmortem through the action of proteolytic enzymes such as calpains and cathepsins, the ultimate pH, the amount of connective tissue and the extent of cross-linking between peptide chains within collagen molecules, and concentration of intramuscular fat (marbling) in muscle (Purchas, 2004). These determinants are dependent on particular phenotypes and environmental effects (Koohmaraie et al., 2002; Anderson et al., 2012). The relative contribution of each determinant to the ultimate tenderness varies with genetic-related factors such as muscle type and breed, production factors such as grain/pasture feeding and age, preslaughter factors such as glycolytic potential, handling, temperament, and stress, and early postmortem events such as pH and temperature of the carcass' muscles, as well as the duration and temperature at which the product is stored postmortem. Being such a complex quality trait, it is not surprising that tenderness is one of the most difficult meat quality traits to assure for the industry. But consumers are willing to pay more for guaranteed tender meat (Miller et al., 2001).

To improve meat tenderness, different interventions aiming to minimize muscle fiber shortening at the rigor onset and to increase the degree of proteolysis (aging) can be employed. Unfortunately, it is not an easy task to implement them in practice due to the high differences in cattle phenotype which impact on metabolic rates and muscle structure together with unpredictable environmental circumstances, such as weather conditions and animal stress, all impacting meat quality and the further ability for the meat to mature. In addition, aging meat involves processing costs in terms of time, room, and energy (14–21 days in cold storage) and certain cuts do not tenderize well. Alternatively, the "background toughness" associated with collagen could be reduced to improve tenderness. However, there are currently very few treatments that enable the contribution of connective tissue to tenderness to be manipulated (Purslow, 2005, 2014). Cooking method and consumer expectations also influence meat tenderness and its perception and therefore must be considered.

Within the industrial practices of meat processing, tenderization interventions can be classified as biological, chemical, and mechanical.

Biological interventions for meat tenderization are those that rely on muscle biochemistry, and thus, attempt to modulate the molecular events governing the conversion of muscle to meat. Such interventions impose certain conditions that affect the temperature and pH of the carcass muscles, the conditions at the onset of *rigor mortis*, and further reactions such as proteolysis by endogenous enzymes. The temperature at which the carcass reaches rigor and the actuation of endogenous proteolytic enzymes (ratio enzyme activity and their inhibitors) are key determinants for the level of sarcomere shortening and the progression of meat aging, respectively, both having a major impact on meat tenderness. Biological interventions include methods such as optimization of carcass chilling, use of electrical stimulation, prerigor muscle stretching, and traditional aging. Further, detailed information on

Downloaded From Vet-eBooks.com

this area can be obtained by consulting the following references: Troy (1999), Thompson (2002), Thompson et al. (2006), Bowker et al. (2010a), and Bolumar et al. (2013).

Chemical interventions for meat tenderization include the addition of chemical compounds that have different effects on muscle structure as well as of exogenous enzymes that contribute to the proteolysis of muscle proteins. Chemical compounds such as salts, organic acids, and phosphates exert positive effects on meat tenderness when incorporated into meats. Hence, the incorporation of brines with these compounds through immersion, injection, and marination is a well-established method to tenderize meat (Berge et al., 2001). However, there is usually a significant price reduction in comparison to its counterpart, "fresh steaks." The addition of enzymes is another possibility to increase tenderness but the inactivation of the enzyme is problematic as it can render an over-tenderized meat with the development of off-flavors (Sullivan and Calkins, 2010).

Mechanical interventions for meat tenderization disrupt muscle structure with mechanical forces. The simplest mechanical tenderization method is chopping or mincing of meat. However, this is accompanied with a significant reduction in price. Another possibility is to use blade or needle tenderization of cuts. This tenderization method is used for improving tenderness of low-value tough meat cuts. This affects the appearance of the steaks, and can result in potential microbial cross-contamination and color changes in the penetration area. "High pressure processing" has also been investigated as a method for tenderness improvement. The effect of HPP is due to the disruptive nature induced by pressure that causes a dissociation of the myofibrillar proteins (Sun and Holley, 2010). Depending on the pressure, the application of HPP to prerigor meat inhibits the metabolism and the conversion of muscle to meat resulting in higher pH and higher tenderness score (Souza et al., 2011). With postrigor meat, the tenderizing effect of HPP is only observed if pressure is combined with heat (~60°C) (Sikes et al., 2010; Sikes and Tume, 2014), thought to result from the release of cathepsins from disrupted lysosomes.

HDP is another postmortem mechanical intervention for meat tenderization. In general, tenderness improvement ranging from 10% to 70% in different species such as beef, pork, lamb, turkey, and chicken has been documented after application of HDP treatment. Table 8.1 provides an extensive summary of the effects of HDP or shock wave treatment on meat tenderness using different prototypes, treatment intensities, animal species, and muscles during the last 20 years. Red meats, especially beef, have the biggest problems with tenderness. The HDP treatment can be used to reduce tenderness variation within the same muscle as observed along the length of beef strip loins (Spanier et al., 2000), and therefore, HDP can be used to ensure consistency in tenderness. The variation in tenderness improvements with regard to HDP treatment is due to a myriad of factors such as the initial tenderness of the meat, treatment of fresh or frozen/thawed meat, postmortem time of

Downloaded From Vet-eBooks.com

TABLE 8.1

Effect of Hydrodynamic Pressure Processing (HDP) on Meat Tenderness

Species	Primal Cut	Process Conditions	Tenderness Improvement (%)[a]	Reference
Beef	*Longissimus*	Explosives (50, 75, and 100 g), container (208 L), diameter (51 cm), steel plate (2 cm thick), distance (30.5 cm), 60–70 MPa	68	Solomon et al. (1997)
	Semimembranous		59	
	Biceps femoris		52	
	Semitendinosus		55	
	Longissimus	Explosives (100 g), same conditions as experiment above (Solomon et al., 1997)	37	Zuckerman and Solomon (1998)
	Strip loin	Explosives (100 g), same conditions as experiment above (Solomon et al., 1997)	21–40	Spanier et al. (2000)
	Strip loin	Explosives (100 g), same conditions as experiment above (Solomon et al., 1997)	47	Spanier and Romanowski (2000)
	Top rounds	Electrical discharge. Energy setting (3–2), pulses (1–2)	20–28	Claus (2002)
	Biceps femoris	Explosives (105, 200, and 305 g), distance (26.7 cm), pressure (83, 104, and 124 MPa)	10–24	Schilling et al. (2002)
	Rib eye	Explosives (100 g), distance (31 cm)	29	Solomon et al. (2008)
	Strip loin	Explosives (100 g), distance (31 cm)	23	Bowker et al. (2008b)
	Loin	Electrical discharge. Use of a high-efficiency sparker. Distance (3.75–7.5 cm), pulses (5, 10, 40, 80), pressure (6–7 MPa)	20–30	Bowker et al. (2011)
	Silverside	Electrical discharge. Semi-industrial batch prototype manufactured at the German Institute of Food Technologies. Cylindrical steel vessel (1 m of diameter), distance (20 cm), pulses (5, for the silverside, and 1, for the loin)	25	Bolumar et al. (2013)
	Loin (*Longissimus lumborum*)		18	
	Loin (*L. lumborum*)	Electrical discharge. Semi-industrial batch prototype manufactured at the German Institute of Food Technologies. Cylindrical steel vessel (1 m of diameter), distance (20 cm), pulses (5, for the silverside, and 1, for the loin)	18	Bolumar et al. (2014)

(*Continued*)

Downloaded From: Vet-eBooks.com

TABLE 8.1 (*Continued*)

Effect of Hydrodynamic Pressure Processing (HDP) on Meat Tenderness

Species	Primal Cut	Process Conditions	Tenderness Improvement (%)[a]	Reference
Pork	Loin	Explosives (150 g), distance (36.0 cm), pressure (68.9 MPa)	17	Moeller et al. (1999)
	Loin	Electrical discharge. Tender Class System. Energy setting (2) and pulses (2)	29	Claus (2002)
	Topside	Electrical discharge. Cylindrical steel vessel (1 m of diameter), distance (20 cm), pulses (2, for the topside, and 2, for the silverside)	-1	Bolumar et al. (2013)
	Silverside		5	
Lamb	*Longissimus*	Explosives (100 g), same conditions as experiment above (Solomon et al., 1997)	33–67	Solomon et al. (1998)
	Semitendinosus		no response	
Turkey	Breast	Explosives (100 g), container (98 L), diameter (40 cm), steel plate (1.3 cm thick), distance (31.0 cm)	13	Bowker et al. (2010a)
	Breast	Electrical discharge. Hydrodyne pilot plant. Energy setting (72%), pulses (2)	12	Claus et al. (2001a)
Chicken	Breast	Explosives (200 g at 20 cm, 350 g at 23 cm, 275 g at 20 cm, and 350 at 20 cm)	19–28	Meek et al. (2000)
	Breast	Explosives (40 g), Hydrodyne research prototype, stainless steel cylinder (20.3 × 48 cm)	42	Claus et al. (2001b)
	Breast	Electrical discharge. Hydrodyne pilot plant. Energy setting (45%), pulses (2)	22	Claus et al. (2001a)

[a] Reduction of Warner–Bratzler shear force (WBSF) by HDP or shock wave treatment compared to control untreated.
HDP, hydrodynamic pressure processing.

Downloaded From: Vet-Books.com

application (pre- or postrigor), storage duration, and specific conditions of HDP treatment (e.g., configuration of the shock wave container, quantity, placement and shape of the explosives, and position and distance from the point of explosion/sparking) (Claus et al., 2001a,b; Solomon et al., 2004, 2006, 2008, 2011). Generally, the tougher the meat the easier to gain a reduction in cutting force. Also, there are different effects if the meat is treated fresh or frozen. Treatment of fresh meat with HDP treatment gave better results than did frozen meat (Solomon et al., 1997). Furthermore, it was reported that if fresh and frozen meats were treated by HDP, freezing accounted for a 14% reduction of WBSF whereas treatment of fresh meat followed by freezing resulted in a 30% reduction of WBSF (Solomon et al., 2008). Most of the data regarding tenderness improvements in meat have been conducted using different systems/units based on explosives though some experiments have also been conducted using electrical discharges underwater. HDP systems based on electrical discharges, such as the TDC, have been reported to improve tenderness in beef loin and turkey and chicken breasts (Table 8.1). More recently, HDP treatment using a batch-pilot prototype based on electrical discharge underwater manufactured at the DIL was tested on beef silverside. The silverside was treated as an intact primal cut whereas, for the loin, the HDP treatment was applied on steaks 26 mm thick. The WBSF was measured at seven days of storage (simulating commercial life span of the meat from the processor to the supermarket and consumer); an 18% and 25% WBSF reduction was reported, respectively (Bolumar et al., 2013). The same semi-industrial batch prototype was used on steaks of beef loin (*Longissimus lumbarum*) of 26 mm thickness. The WBSF was reduced by 18% in this case (Bolumar et al., 2014).

As well as beef, other species have been subjected to HDP treatments. In pork, despite the effect of HDP treatment on WBSF (17% WBSF reduction), no effect was detected by sensory analysis. Color, drip loss, and microbial counts were not affected in this study (Moeller et al., 1999). Claus (2002) and Bolumar et al. (2013) reported either improved tenderness or minor or no effect, respectively, after HDP treatment of pork meat (Table 8.1). Generally, pork is usually tender *per se* and as such it is more difficult to make it more tender. Lamb has also been the object of study (Table 8.1). Especially the *Callipyge* lamb, which has tougher meat than normal lambs, has been subjected to HDP treatment. A 33%–67% reduction in WBSF for the loin and no response for the eye of round was reported for *callipyge* lamb meat (Solomon et al., 1998). Turkey breast has been reported to show improved tenderness following HDP treatment (13% and 12% reduction of WBSF) (Bowker et al., 2010a; Claus et al., 2001a). However, cooking loss was higher in HDP-treated turkey breast though HDP facilitated early deboning and provided tenderness-enhanced fillets (Claus et al., 2001a). Chicken breast has also been evaluated. HDP treatment reduced WBSF by 20%–40% in different studies (Meek et al., 2000; Claus et al., 2001a,b). Regarding the time of postmortem application, a greater effect was observed if HDP treatment was applied postrigor

Downloaded From Vet-eBooks.com

rather than prerigor. In this sense, HDP treatment of chicken breasts, when applied at 77 minutes postmortem, did not tenderize the meat as did when treatment was applied at 24 hours postmortem (Claus et al., 2001b). In these studies, cooking loss and color were not affected (Claus et al., 2001a,b). The postrigor application is consistent with the disruption of the meat structure as prerigor meat is more flexible and somehow malleable.

During the European-funded project "ShockMeat," a comparison of HDP, or shock wave technology, with other tenderization methods such as low-temperature long-time treatment (LTLT) and brine injection was carried out. The meat treated with the different tenderization methods was instrumentally and sensory analyzed. The tenderness scores assessed by sensory analysis are shown in Figure 8.7. The HDP effect was evaluated in relation to eating quality and improved tenderness. The overall variation between the four treatments could be ascribed to differences in textural properties that clearly differentiated LTLT treatment (63°C for 15 hours) from the other tenderizing technologies ($p < .001$) (tenderness score of 8.3, Figure 8.7). The blade injection with brine to 10% (w/w) and to a final concentration of salt in the product of 0.6% also resulted in a significant improvement in the sensorial tenderness (tenderness score of 7.0, Figure 8.7). The shock wave treatment resulted in a tenderness score of 5.6, 0.6 units higher than the control untreated. Even though this difference obtained using the HDP method seems minimal there is room for more improvements by the efficient interaction between the HDP and the meat and the power of the shock wave in different ways. The use of LTLT treatment is an effective method to produce tender and juicy meat,

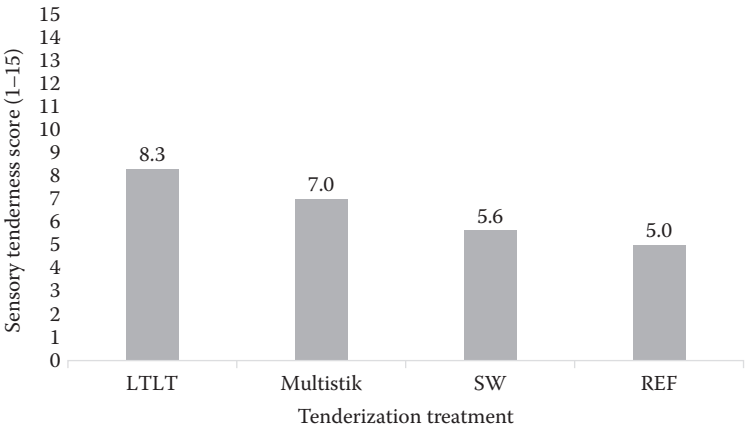


FIGURE 8.7
Sensory tenderness score of beef eye of round after application of different tenderization interventions. LTLT, low-temperature long-time treatment (63°C for 15 hours); Multistik, blade injection of brine (10% w/w, 0.6% salt); SW, shock wave (1 shot, 40 kV); and REF, reference or control (no treatment). Work performed during the European-funded research project, ShockMeat (2012–2013).

Downloaded From Vet-eBooks.com

but in this treatment the meat is cooked, and so the meat cannot be considered "fresh meat" and there would be an associated price reduction. LTLT could be a good technology for ready-to-eat dishes containing meat. In addition, LTLT processing takes 15–20 hours whereas the shock wave treatment is done in seconds. The brine injection is a good method for tenderizing meats and is being widely used in some countries, for instance, for the tenderization of chicken breast with a concomitant enhancement in juiciness. The main problem with the blade injection of whole muscles is the possibility of microbial cross-contamination of the center of the cut, and consequently, the possibility of growth of spoilage and pathogenic bacteria impairing shelf life and in the worst scenario leading to food poisoning. Again the injected meat product could not be considered "fresh meat" and this always brings a significant price reduction of the meat cut. In contrast, HDP does not affect the visual appearance of the meat, and therefore, can be commercialized as "fresh meat" allowing meat processors to obtain the highest prices of the market.

8.3.2 HDP for Modification of Meat Functionality

HDP can change meat structure thereby impacting the functionality of the system. HDP, applied as pressure pulses, can result in pressure-induced structural modifications. These structural modifications are based on the effect of high pressure on cell membranes and the biopolymers present in the system such as proteins. In meat, muscle proteins could be modified whereby the functionality of the proteins is impacted. These modifications could lead to diverse food applications such as the creation of novel textures and improvement of water binding. The modification of food structure using HPP has been recently reviewed (Bolumar et al., 2016). As explained in Section 8.2, HDP is a dynamic application of pressure in comparison to a static application in HPP. Nevertheless, similar effects could be expected though the effect of HDP in the modification of the functionality has been scarcely investigated. The structural changes in foods caused by HPP have been investigated mostly during the last decade (Bolumar et al., 2016). Several examples are described using HPP for structural modification, for instance, an HPP treatment of meat batters that allows a salt reduction in meat products since HP-induced changes can result in meat batters requiring less salt to create a stable physical structure. HP treatment affects the disruption of noncovalent interactions (hydrophobic and electrostatic) followed by a rearrangement of intra- and intermolecular interactions at the interfaces of proteins leading to different degrees of denaturation, disassociation, aggregation, and gelation. HP induces unfolding of proteins and can modify the quaternary and tertiary structure of proteins, whereby protein functionality could be affected. In the literature, few papers deal with the mechanisms involved in the functionality changes occurring in meat and the majority of them use high pressure (HPP). Regarding water-binding capacity, both improvements and no

Downloaded From Vet-eBooks.com

changes have been reported. This suggests that there is much more work to be done to document how HDP or shock wave might be used to improve the yield of processed meats. There seem to be some unraveled interactions between meat, the formulation, and processing that still needs to be sorted. Chan et al. (2011) showed that pressures of 50–100 MPa increased the water-binding capacity of low pH meat (pale, soft, and exudative [PSE] like) and the pressure treatment gave a better gel network formation. Furthermore, they showed that protein solubility was increased in both low and normal pH meat. The authors suggested that HPP (50–100 MPa) could be used as an alternative technology to reduce salt and improve water retention in meat products. Flynn et al. (2014) found that HPP (150 MPa) increased emulsion stability and reduced cooking loss when salt was reduced in breakfast sausages. At 1.5% NaCl, the cooking loss was reduced from 17.6% (0 MPa) to 16.2% (150 MPa). HP-induced benefits on the structure of meat products are due to additional swelling and solubilization of muscle myofibrillar proteins. The modification of protein structure, and so functionality, using HDP has been very scarcely investigated. Increased water retention using HDP treatment has been described in some cases. The U.S. patent 7,485,331 states that the Hydrodyne System improved water binding of sirloin during marination (Long, 2009). The uptake of water during marinating of Hydrodyne-treated sirloin increased from 10% to 14%. In another study, HDP treatment enhanced brine absorption, increased processing yield, and improved texture of marinated turkey breasts without impacting color (L*, a*, and b*) and expressible moisture (Bowker et al., 2010a). Therefore, HDP treatment could be used as a pretreatment prior to marination or injection to increase yield. Marinated samples (15% and 30% brine levels) had lower ($p < .001$) WBSF values and lower ($p < .05$) hardness, cohesiveness, and chewiness values compared with nonmarinated samples (Bowker et al., 2010a). In another study, Bowker et al. (2010b) reported on the influence of HDP and aging on the processing characteristics and final meat quality of moisture-enhanced pork loins. WBSF and expressible moisture decreased ($p < .0001$) with aging from 1 to 8 days, but were not significantly affected by HDP treatment. Again, HDP-treated loins had greater ($p < .05$) brine retention after overnight equilibration and thus a higher ($p < .05$) processing yield than controls. In addition, HDP samples had lower drip loss values than controls. HDP treatment demonstrated the potential to improve the texture and WHC of moisture-enhanced pork loins with minimal impact on the visual appearance of the final product (Bowker et al., 2010b). In contrast, Schillinger et al. (2002) treated beef *biceps femoris* with hydrodynamic shock waves before producing frankfurters (cooked to internal temperature of 71°C). They found that the HDP treatment did not affect the myofibrillar or sarcoplasmic protein solubility, cooking yield, color, textural properties, or gel strength. In this study, it was concluded that beef trims obtained from HDP-treated meat could be used interchangeably with normal beef trims in the production of frankfurters. Therefore, the use of HDP treatment did not provide any advantage in comparison with no

Downloaded From Vet-eBooks.com

pretreatment of the raw meat. It is noteworthy to consider that frankfurters are made out of minced meat of very small particle size, in contrast to marination of whole muscles, which favors protein accessibility, interactions, and functionality. However, only a few studies have been done in this area so far, and thus, more research is needed to better understand the potential of HDP treatment for modification of protein functionality. HDP could be a technological processing aid in the production of meat products, whole muscles, or meat emulsions with reduced salt and phosphate content and enhanced yields and texture.

8.3.3 Molecular and Structural Effects of HDP in Meat

The tenderization effect of HDP has been ascribed to two main mechanisms: (1) physical disruption of muscle structures and (2) enhanced proteolysis of structural muscle proteins. Both effects have been reported by the use of HDP or shock wave (Bolumar et al., 2013). A shock wave travels rapidly through the water and any object which is an acoustical match with water. At points where acoustic impedances differ stress is created, which in turn tears muscle structure. This produces what could be called a "rupture effect" and as a consequence, meat tenderization is favored. Furthermore, the high pressure–momentum reached during HDP treatment could increase cathepsin activity due to their release from lysosomes analogously as has been described after HPP (Ohmori et al., 1992; Homma et al., 1994; Grossi et al., 2012). Muscle cathepsin and peptidase (amino- and dipeptidyl-) activities from control and shock wave-treated beef were not different (Bolumar et al., 2014). This fact can confirm that enzyme activity *per se* is not enhanced but it cannot dismiss the possibility that shock wave facilitates the contact between the structural muscle proteins and the endogenous proteolytic enzymes, which could potentially lead to an accelerated maturation. In the same study, Bolumar et al. (2014) analyzed the protein pattern of sarcoplasmic and myofibrillar proteins at one and seven days of chilling storage by using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). No differences in the protein bands between control and HDP-treated meat for sarcoplasmic and myofibrillar proteins at day 1 or at day 7 could be observed (Figure 8.8). In agreement with our results, other authors did not observe major changes in sarcoplasmic and myofibrillar protein patterns visualized by SDS-PAGE analysis (Bowker et al., 2008a,b). Bowker et al. (2008b) reported some minor changes in sarcoplasmic proteins bands corresponding to 143, 65, 44, 36, and 19 kDa. In this study, changes in sarcoplasmic protein composition were significantly correlated to WBSF ($r = -0.58$ to 0.45). Sarcoplasmic protein solubility decreased with both HDP ($p < .05$) and aging ($p < .0001$). Changes in solubility were significantly correlated to SDS-PAGE band intensities ($r = -0.53$ to 0.60). Data suggested that HDP and aging cause changes to sarcoplasmic proteins, which may be an indicator of proteolysis and tenderization. In another study, Bowker et al. (2008a) reported that HDP treatment

Downloaded From Vet-eBooks.com

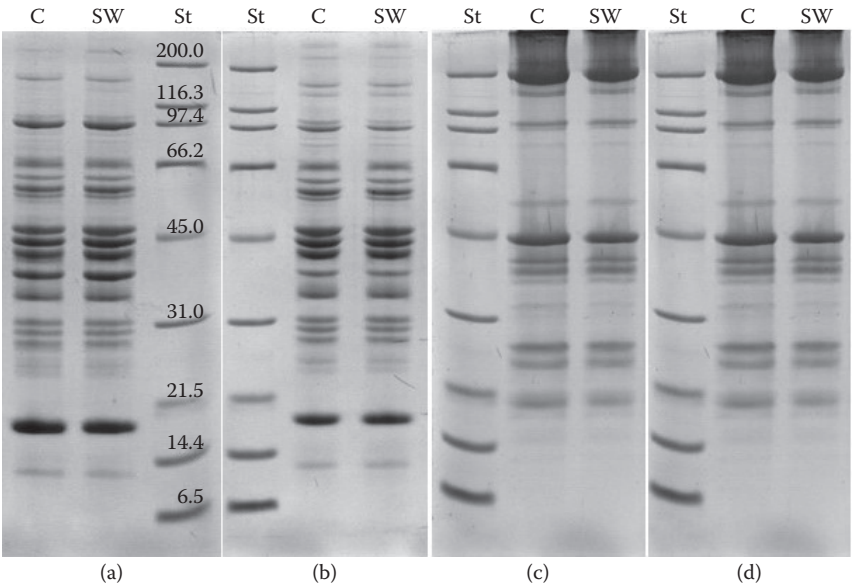


FIGURE 8.8
SDS-PAGE analysis of sarcoplasmic and myofibrillar proteins from control and shock wave-treated beef *Longissimus thoracis* steaks at day 1 and 7 of storage under refrigerated conditions. (a) Sarcoplasmic proteins at day 1, (b) sarcoplasmic proteins at day 7, (c) myofibrillar proteins at day 1, and (d) myofibrillar proteins at day 7. Lane C, control sample; lane SW, shock wave-treated sample; lane St, standard proteins (kDa); SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis. (Reprinted from *Meat Science*, 98, Bolumar et al., Effect of electrohydraulic shockwave treatment on tenderness, muscle cathepsin and peptidase activities and microstructure of beef loin steaks from Holstein young bulls, 759–765. Copyright 2014, with permission from Elsevier.)

influenced troponin T (TnT) degradation as confirmed by using a more sensitive technique than the SDS-PAGE such as Western Blot. They reported that shock wave in combination with aging decreased the intensity of the TnT band and enhanced the accumulation of the 30-kD fragment which arose from the TnT degradation, suggesting that HDP tenderization was caused by both physical disruption of the myofibril apparatus and enhanced postmortem proteolysis (Bowker et al., 2008a). Shock wave and aging decreased the intensity of the TnT band and enhanced the accumulation of the 30-kD TnT degradation product (Bowker et al., 2008b). An accelerated maturation after HDP treatment, enabling a shorter maturation time for the industry, has been described in the literature (Heinz et al., 2011). During aging, the percentage of proteins in the soluble fraction was increased with HDP treatment while the insoluble myofibrillar proteins were reduced. Similar results were seen when the myofibrillar fragmentation index was correlated to tenderness during aging (Spanier and Romanowski, 2000). More recently, changes in the sarcoplasmic protein fraction of beef muscle with aging (0, 5, and 8 days) and HDP treatment were evaluated using capillary electrophoresis (CE)

Downloaded From Vet-eBooks.com

and reversed-phase high-performance liquid chromatography (RP-HPLC). Aging and HDP interactions were not detected using either separation technique. With CE analysis, no HDP effects were observed; however, the relative peak area of eight protein peaks ranging in size from 17 to >200 kDa were influenced by postmortem aging. Separation of proteins with RP-HPLC demonstrated that HDP influenced the relative size of two protein peaks while postmortem aging effects were observed in six peaks. Alterations in the sarcoplasmic protein fractions detected by both CE and RP-HPLC were correlated to WBSF measurements. Overall, the data demonstrate that HDP has minimal effects on sarcoplasmic proteins and that aging-related changes in the water-soluble protein fractions of muscle may be useful as indirect indicators of beef tenderness (Bowker et al., 2012).

Microscopic examinations using transmission electron microscopy (TEM) of HDP meat displayed myofibrillar fragmentations in the region adjacent to the *z*-lines as well as *z*-line fragments attached to the A-band on both sides of the fractures (Zuckerman and Solomon, 1998; Figure 8.9). Also, increased intra-myofibrillar spaces with longitudinal gaps or splits in the myofibril lattice were observed (Zuckerman and Solomon, 1998). Jagged edges at the *z*-line and the thin filaments in HDP-treated meat have also been described by Claus (2002) (Figure 8.9). These scientists suggested that physical tearing rather than proteolysis was behind the tenderization.

Recently, Bolumar et al. (2014) conducted microscopic analysis on HDP-treated meat by using confocal laser scanning microscopy (CLSM), and showed very clearly that HDP causes considerable disruptions of muscle fibers and tissue (Figure 8.10a through c, control vs. d through f, shock wave treated).

By using scanning electron microscopy (SEM), a larger endomysium space, the space between muscle fibers, could also clearly be observed in the HDP meat, especially at day 7 (Figure 8.11, white arrow). Furthermore, the appearance of additional "white networks" (Figure 8.11, within a circle)

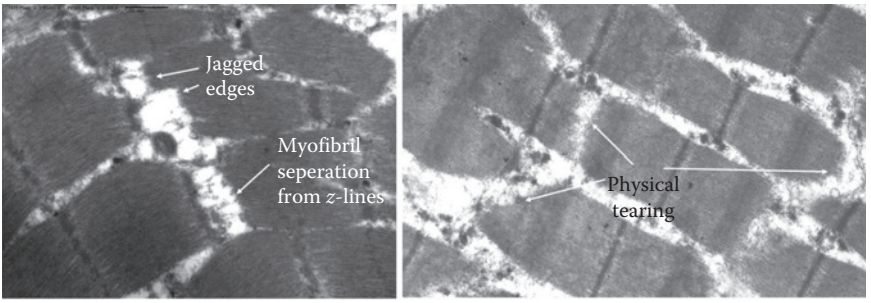


FIGURE 8.9
TEM of shock wave-treated (a) beef and (b) chicken. (a) Early deboned Holstein beef after shock wave processing with TCS (separations at *z*-line) and (b) early deboned chicken after shock wave processing with explosives (physical tearing). TEM, transmission electron microscopy. (Photo courtesy of Professor Jim Claus, University of Wisconsin-Madison.)

Downloaded From Vet-eBooks.com

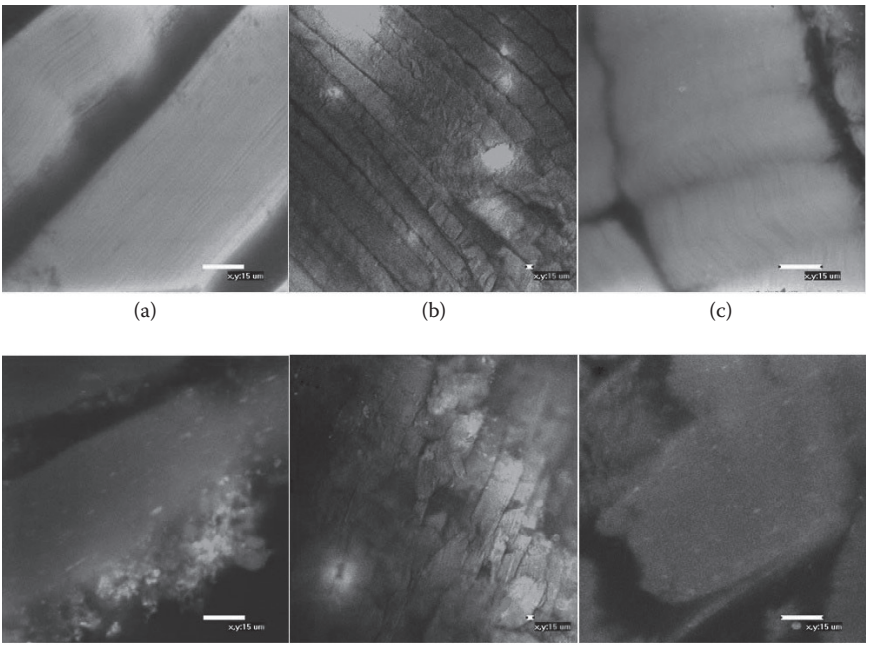


FIGURE 8.10
CLSM images from control and shock wave-treated beef *longissimus thoracis* steaks at days 1 and 7 of storage under refrigerated conditions. (a) Control at day 1, (b) control at day 7, (c) control at day 7, (d) shock wave-treated at day 1, (e) shock wave-treated at day 7, and (f) shock wave-treated at day 7. CLSM, confocal laser scanning microscopy. (Reprinted from *Meat Science*, 98, Bolumar et al., Effect of electrohydraulic shockwave treatment on tenderness, muscle cathepsin and peptidase activities and microstructure of beef loin steaks from Holstein young bulls, 759–765. Copyright 2014, with permission from Elsevier.)

was observed in HDP or shock wave-treated samples (Figure 8.11, comparing images a through c [control] to images d through f [shock wave treated]). This observation may be related to the release of intracellular contents or most likely an alteration of the endomysium collagen fibril network. A wider endomysium space might be presumably due to a displacement within the muscle fibers at the collagen level. This is in agreement with Zuckerman et al. (2013) who, using SEM, reported that HDP treatment disrupted the integrity of the collagen fibril network of the endomysium in both nonaged and aged samples and that WBSF and connective tissue changes were greater in HDP samples in comparison to the control.

Zuckerman et al. (2013) examined the effect of HDP treatment on intramuscular connective tissue. In general, HDP did not cause extensive tearing or perforation of the endomysial connective tissue sheaths surrounding individual muscle fibers. Deformations to the honeycomb structure of the endomysium, however, were observed in the shock wave-treated samples (Figure 8.12). A higher incidence of aggregated fragments of collagen fibrils

Downloaded From Vet-eBooks.com

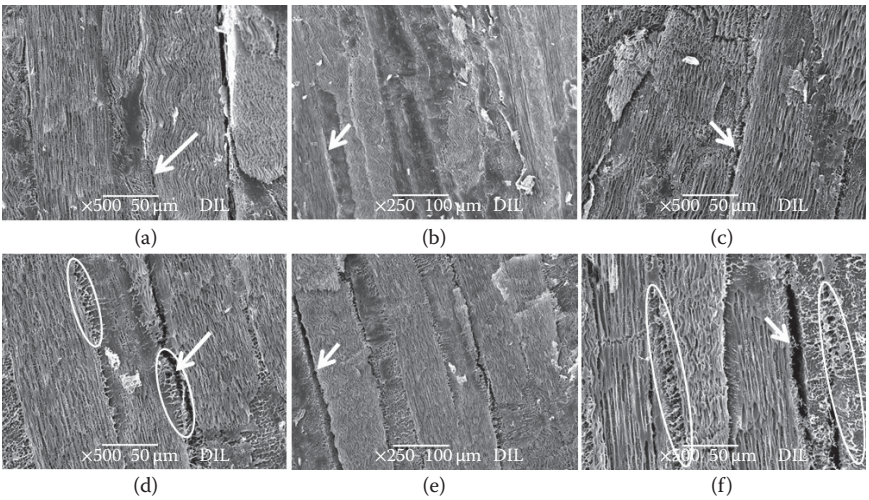


FIGURE 8.11
SEM images from control and shock wave-treated beef *Longissimus thoracis* steaks at day 1 and 7 of storage under refrigerated conditions. (a) Control at day 1, (b) control at day 7, (c) control at day 7, (d) shock wave-treated at day 1, (e) shock wave-treated at day 7, and (f) shock wave-treated at day 7. Arrows point out the separation between muscle fibers (endomysium space), circles point out additional "white networks." SEM, scanning electron microscopy. (Reprinted from *Meat Science*, 98, Bolumar et al., Effect of electrohydraulic shockwave treatment on tenderness, muscle cathepsin and peptidase activities and microstructure of beef loin steaks from Holstein young bulls, 759–765. Copyright 2014, with permission from Elsevier.)

on the surface of the endomysial sheaths was observed in the shock wave-treated samples (Figure 8.12). They suggested that these aggregates on the endomysium are likely parts of collagen fibrils separated from the connective tissue matrix, due to physical weakening of the collagen network. Based on the underlying assumption that physical disruptions to the microstructure of intramuscular connective tissue improve meat tenderness, these data suggested that shock wave or HDP meat tenderization is caused at least partially by alterations to connective tissue in addition to their known impact on the myofibrillar component of muscle (Zuckerman et al., 2013).

Overall, HDP or shock wave treatment has been shown to induce disruptive effects on the *z*-line, the muscle microstructure, and the connective tissue (Zuckerman and Solomon, 1998; Bolumar et al., 2014; Zuckerman et al., 2013). In addition, an enhanced maturation (i.e., a further reduction of WBSF at the same time of maturation) has been observed (Heinz et al., 2011) and evidenced by a faster degradation of the TnT protein and accumulation of the 30-kD protein band (Bowker et al., 2008a). Furthermore, the release of cathepsins from lysosomes by HDP or shock wave treatment has not been demonstrated so far, but if this were so, it could be a good target for the benefit of the technology in order to enhance proteolysis driven by endogenous muscle endo-proteases during meat storage.

Downloaded From Vet-eBooks.com

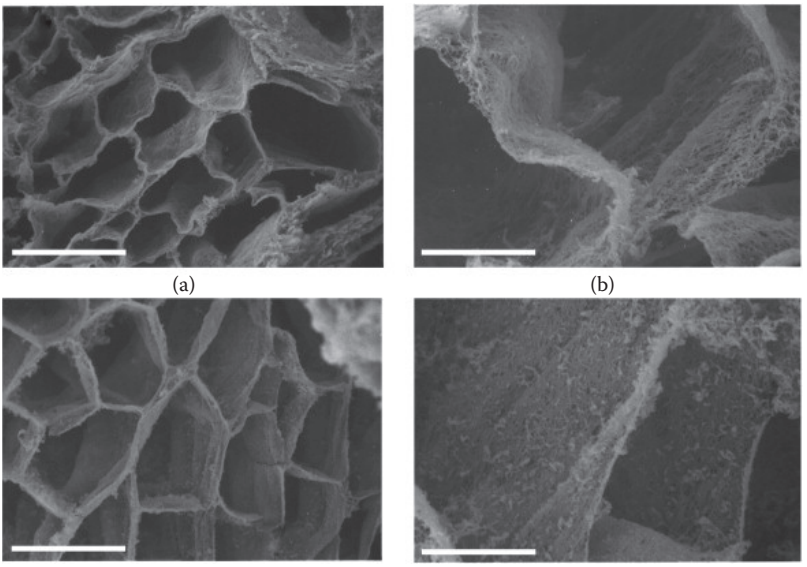


FIGURE 8.12
SEM of intramuscular connective tissue prepared from beef *musculus semimembranosus* following 14 days of aging posttreatment: (a and b) nontreated controls, (c and d) shock wave-treated samples. Scale bars indicate 50 µm (a, c) and 10 µm (b, d). SEM, scanning electron micrographs. (Reprinted from *Meat Science*, 95, Zuckerman et al., Microstructure alterations in beef intramuscular connective tissue caused by hydrodynamic pressure processing, 603–607. Copyright 2013, with permission from Elsevier.)

8.4 HDP for Improving Meat Safety

Food safety is a must for meat processors. The meat industry has to deal with microbial contamination and growth of both spoilage and pathogenic bacteria, which can jeopardize shelf life and cause foodborne illnesses. Thus, the industry needs to manage the biological risks and demands tools to control these microbial hazards. Traditionally, the meat industry has used and still uses chemical substances such as disinfectants and preservatives and heat treatments to control microbial growth. Yet interventions, such as hot water (80°C–96°C) carcass treatment to raise carcass surface temperatures, organic acid (lactic, acetic, and citric) carcass washes of surfaces to reduce the surface pH, steam pasteurization of carcass in a closed chamber, and steam vacuum, can be used as interventions to decontaminate fresh meat (Acuff, 2014). But nowadays, consumers demand nonchemical and mild processing treatments for superior nutritional preservation. These mild preservation technologies ideally should aim at energy saving and be environmentally friendly. Several examples of these mild preservation techniques with good potential in the meat industry are HPP, controlled instantaneous decompression (DIC),

Downloaded From Vet-eBooks.com

oscillating magnetic fields (ohmic heating, dielectric heating, and microwaves), high-intensity pulsed light, x-rays, and electron beams (Hugas et al., 2002). Some of these novel mild technologies have found application as a decontamination and pasteurization method but high costs, legal restrictions, consumer perceptions, and insufficient adaptation to the industrial environment have limited a wider use. HDP treatment can also result in biological inactivation, and as such, can be used as an additional hurdle to microbial growth. The exact mechanisms for the microbial inactivation of HDP are not well understood. Two physical effects must be behind the inactivation: (1) cavitation and (2) exposure to high-pressure conditions. Cavitation is the rapid and violent expansion and collision of microscopic cavities that generates high-energy density particles and microregions of very high temperatures (T) and pressures (P) which may be responsible for inactivation of bacteria (Patel et al., 2012). Also exposure to high pressure can lead to microbial inactivation. Protein denaturation is one of the key mechanisms for microbial inactivation in HPP and irreversible changes in proteins start at a comparative level to that required for inactivation of microorganisms (400–600 MPa) (Bolumar et al., 2015). HDP pressure levels are around 100 MPa though localized higher pressures may occur (Solomon et al., 2006, 2011). Pressures in the range of 100 MPa might cause sublethal injuries which can lead to death and delay of growth more than direct lethal damage/inactivation.

Zuckerman et al. (2002) claimed an efficient microbial inactivation (in log cfu/g) (5–7.5 log reduction) of different microorganisms suspended in a 0.9% NaCl solution by a system based on pulsed high-current underwater discharges. A US patent also claims a microbial inactivation of 90% of surface bacteria populations in meat (Long, 2001). This level of inactivation will greatly increase the shelf life of meat, which is of large economical importance. However, Williams-Campell and Solomon (2002) described a 1.5–2 log reduction in ground beef after HDP treatment, which increased to 4.5 log after 14 days of storage. And no differences were found in coliform bacteria and aerobic plate counts of pork loins (Moeller et al., 1999). Overall, HDP treatment has been investigated as a microbial decontamination technique although contradictory reports coexist, especially when the application is used in real meat systems (Solomon et al., 2006). Table 8.2 summarizes the literature data found in relation to microbial decontamination of meat matrices.

HDP-treated meat products showed a significant reduction in the naturally occurring spoilage microbiota on stew-sized pieces of beef and pork, ground beef, and intact beef muscle (Schilling et al., 2003; Williams-Campbell and Solomon, 2002) though these reductions (in log cfu/g) were low (0.3 and 1–2 log), respectively (Table 8.2). Williams-Campbell and Solomon (2002) investigated the use of HDP as a technology to reduce spoilage microorganisms found in fresh beef. HDP reduced bacteria (1–2 log cfu/g) in retail ground beef and beef roasts (stew-sized pieces of 30–40 g) on day 0 after HDP application. In a shelf-life storage experiment, there was an immediate reduction (1.5 log) following HDP (day 0) and a 4.5-log difference between control

Downloaded From Vet-eBooks.com

TABLE 8.2

Effect of HDP on Bacteria Inactivation in Meat Matrixes

Bacteria	Matrix	Treatment	Inactivation Level (log cfu/g)	Comments	Reference
Aerobic plate counts	Ground beef and beef roasts (pieces of 30–40 g)	HDP (100 g binary explosive), steel shock wave container	1–2	There was an immediate reduction in the cfu (~1.5 log) and a difference after 14 days of storage (4.5 log)	Williams-Campbell and Solomon (2002)
Standard plate count at 35°C	*L. lumborum*	Explosives (100 g), container (98 L), steel plate (1.3 cm thick), distance (30.5 cm)	0.3	HDP improved tenderness of intact loins	Schilling et al. (2003)
Salmonella Typhimurium	Minced chicken (100g)	Explosives (50 g), container (98 L), steel plate (1.3 cm thick), distance (30.5 cm)	0.1–0.25	Minimal reduction	Patel et al. (2006)
Escherichia coli O157:H7	Ground beef (samples of 60 g)	Explosives (100 g), container (54 L), steel plate (1.3 cm thick), distance (30.5 cm)	<1		Podolak et al. (2006)
Listeria monocytogenes and aerobic cells	Frankfurters	Explosives (100 g), container (54 L), steel plate (1.3 cm thick), distance (30.5 cm)	1–2	A synergetic effect combining nisin and HDP treatment allowed a 2 log cfu/g reduction in *L. monocytogenes* after 28 days of storage	Patel et al. (2007)
L. monocytogenes and aerobic cells	Beef frankfurters	Explosives (100 g), container (54 L), steel plate (1.3 cm thick), distance (30.5 cm)	1–2	HDP was combined with antimicrobials such as sodium diacetate and pediocin	Patel et al. (2009)
E. coli O157:H7 *S.* Typhimurium, and *L. monocytogenes*	Beef cubes (2.54 cm)	Explosives (100 g), container (54 L), distance (30.5 cm)	0.5–1		Patel et al. (2012)

Downloaded From Yes-Books.com

samples (9 log) and HDP-treated samples (4.5 log) after 14 days of storage. In this study, the authors concluded that it was possible to reduce spoilage microorganisms found in ground and stew meat pieces, which could extend the shelf life of meat products (Williams-Campbell and Solomon, 2002). The effect of HDP on the survival of *Escherichia coli* O157:H7 in ground beef was investigated by Podolak et al. (2006) (Table 8.2). The ground beef was inoculated with a six strain cocktail of *E. coli* O157:H7 at three different concentrations (10^3, $l0^4$, and $l0^6$ cfu/g). The initial concentrations of *E. coli* were 1.29×10^3, 2.88×10^4, and $2.19 \times l0^6$ cfu/g. After HDP treatment, the populations were reduced ($p < .05$) to 9.12×10^2, 2.40×10^4, and 1.91×10^6 cfu/g, respectively. Although the reduction in *E. coli* populations due to HDP treatment was statistically significant, the practicability of the reduction was marginal (1 log or even less reduction). Therefore, other hurdle parameters should be included along with the HDP treatment for more practical inactivation of *E. coli* in ground beef (Podolak et al., 2006). In this regard, HDP has also been assayed in combination with antimicrobials. Populations of *Listeria monocytogenes* in beef frankfurters were significantly reduced by HPD when used in conjunction with nisin after 28 days of refrigerated storage (Patel et al., 2007). Patel et al. (2006) reported that HDP treatment was unable to kill significant amounts of *Salmonella* in minced chicken (Table 8.2). In another work, HDP treatment significantly reduced (1 log cfu/g) *L. monocytogenes* (Patel et al., 2009). The combination of pediocin and HDP processing showed a synergistic effect with a 2-log reduction for *L. monocytogenes* in frankfurters compared to control samples (Patel et al., 2009). The penetration of surface-inoculated bacteria into the meat due to HDP treatment was reported to be around 200–300 μ in chicken breast and beef steaks (Lorca et al., 2002, 2003). This penetration into the meat does not carry a safety hazard to consumers provided it is cooked properly. A heat treatment when cooking the meat will easily destruct the bacteria at this depth below the surface. The effect of HDP on the inactivation of *E. coli* O157:H7, *Salmonella* Typhimurium, and *L. monocytogenes* attached to beef surface was recently evaluated by Patel et al. (2012) (Table 8.2). The HDP treatment significantly reduced *E. coli* O157:H7 (0.75 log cfu/g), *S.* Typhimurium (1.09 log cfu/g), and *L. monocytogenes* (0.55 log cfu/g) from beef surfaces (Figure 8.13). HDP-induced injury, as determined by plating on nonselective media, revealed that ca. 0.5 log cfu/g of *E. coli* O157:H7 and *S.* Typhimurium cells were injured. Most cell injury occurred at the cell membrane level as observed by TEM (Figure 8.14; Patel et al., 2012).

HDP treatment has also been investigated to inactivate parasites in meat. Gamble et al. (1998) reported that the numbers of *Trichinella spiralis* recovered from infested pork were significantly reduced by treatment with the "Hydrodyne Process" as compared with untreated. However, the infectivity of this parasite when the larvae from Hydrodyne-treated meat were inoculated into mice was not eliminated. HDP treatment has also been assayed in relation to the inactivation of viruses. Sharma et al. (2008) compared the

Downloaded From Vet-eBooks.com

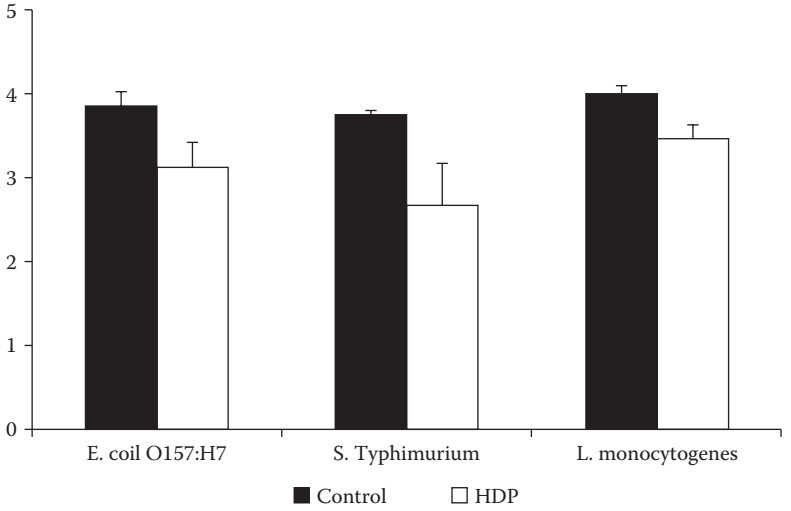


FIGURE 8.13
Effect of HDP on survival of *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella* Typhimurium on beef cubes (log cfu/g). HDP, hydrodynamic pressure processing. (Reprinted from *Innovative Food Science and Emerging Technologies*, 14, Patel et al., Inactivation and injury of pathogens on intact beef treated with hydrodynamic pressure, 38–45. Copyright 2012, with permission from Elsevier.)

inactivation of foodborne viruses by HDP or HPP and found that both technologies significantly reduced foodborne viruses on pork sausages (1–3 log reduction), but complete inactivation was not possible to achieve using these pressure treatments.

Overall, HDP treatment could have some decontamination effect under the right settings. However, notable inactivation levels have not been clearly demonstrated in meat products so far with maximum inactivation level being 1–2 log reduction (Bolumar et al., 2013; Patel et al., 2012). Nevertheless, HDP treatment for microbial decontamination has been scarcely investigated given the few groups with the available equipment to carry out experimental work worldwide, and definitely deserves further scientific attention. Additional hurdles such as addition of bacteriocins (e.g., nisin) and/or organic acids (e.g., lactic acid) could be employed to achieve further reduction of pathogens present in meat surfaces. High pressure-induced injured cells can grow under favorable conditions and may regain their pathogenic potential, and therefore, the population of injured cells should be considered in devising terminal treatment for reducing pathogens on foods. New engineering developments in shock wave equipment could deliver the required technical pressure conditions to achieve significant biological inactivation in the near future so providing an effective microbial decontamination method to the meat industry.

Downloaded From Vet-eBooks.com

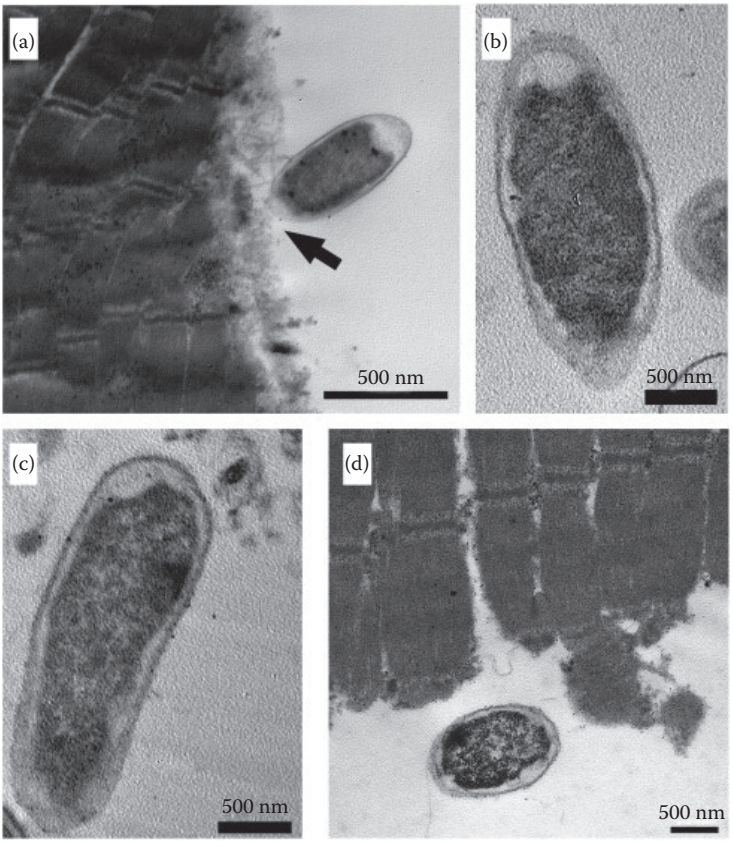


FIGURE 8.14
Transmission electron microscopy (TEM) images of hydrodynamic pressure processing (HDP)-treated *S.* Typhimurium. All cells show pressure-induced injuries in the form of polar (a through c) or peripheral invagination of the cellular membrane. (a) *Salmonella* strongly attached to meat surfaces. Arrow indicates the actual site of physical binding of bacteria to muscular tissue. (Reprinted from *Innovative Food Science and Emerging Technologies*, 14, Patel et al., Inactivation and injury of pathogens on intact beef treated with hydrodynamic pressure, 38–45. Copyright 2012, with permission from Elsevier.)

8.5 Cost Analysis of HDP Technology

A cost analysis considering cost of HDP technology and cost of the extra packaging required for the most recent continuous shock wave prototype developed at the DIL (Figures 8.5 and 8.6) is presented in Table 8.3.

8.5.1 Packaging

Currently, there is no packaging able to withstand the intensity of the shock waves generated during HDP treatment intended for meat tenderization.

Downloaded From Vet-eBooks.com

TABLE 8.3

Cost Analysis of HDP or Shock Wave Technology for Meat Tenderization

		Cost					
		Shock Wave Technology[a]					
Case Study	Throughput (kg/h)	Amortization (€/kg)	Spare Parts (€/kg)	Operative Costs (€/kg)	Subtotal (€/kg)	Extra Packaging (€/kg)	Total (€/kg)
1	500	0.160	0.024	0.041	0.225	0.172	0.397
2	1800	0.050	0.007	0.012	0.069	0.172	0.241
Average	1150	0.105	0.015	0.026	0.147	0.172	0.319

[a] The amortization costs for the shock wave technology are estimated assuming a one shift working scheme (8 h/d), 50% occupancy of the equipment time, and a 5-year period for payback of investment.

Some of the typical damages that occurred in the packaging as a consequence of the power and harshness of the HDP treatment can be observed in Figure 8.15. Figure 8.15a shows a broken bag caused by the fact that the meat did not have enough expansion space within the bag, which led to enormous mechanical pressure on the sealing bonds. Furthermore, the commercial bags showed higher incidence of opened seals (Figure 8.15b). Other damage included small pinholes (primarily started from the inside of the bags) (Figure 8.15c and e) and large holes caused by burning (Figure 8.15d and f). Also, large cracks were seen that damaged the entire bag. Research and development of an HDP-resistant packaging material is one of the key developments required to underpin the HDP technology to the industry. Efforts addressing the development of this type of packaging will require the involvement of packaging materials scientists and most likely commercial packaging companies that can provide up-to-date expertise adapted to the needs of the meat industry in terms of cost and compatibility with an industrial food environment.

8.5.2 Equipment

The cost of the shock wave technology can be estimated by durable costs (i.e., machine depreciation) and operative costs (i.e., spare parts, energy and water consumption, and personnel cost). The prototype throughput can be estimated taking into account the speed of the belt, the number of shots that a meat cut receives, and the size and weight of the meat cut. Depending on the specific treatment (mainly number of pulses applied) and size of meat cut, the throughput (kg/h) will vary (see the following calculation example). The minimum distance that a shock wave can be applied with the shock wave unit built at DIL (Figures 8.5 and 8.6) is 30 mm. This would be the worst case scenario for the productivity of the shock wave unit and corresponds to

Downloaded From Vet-eBooks.com

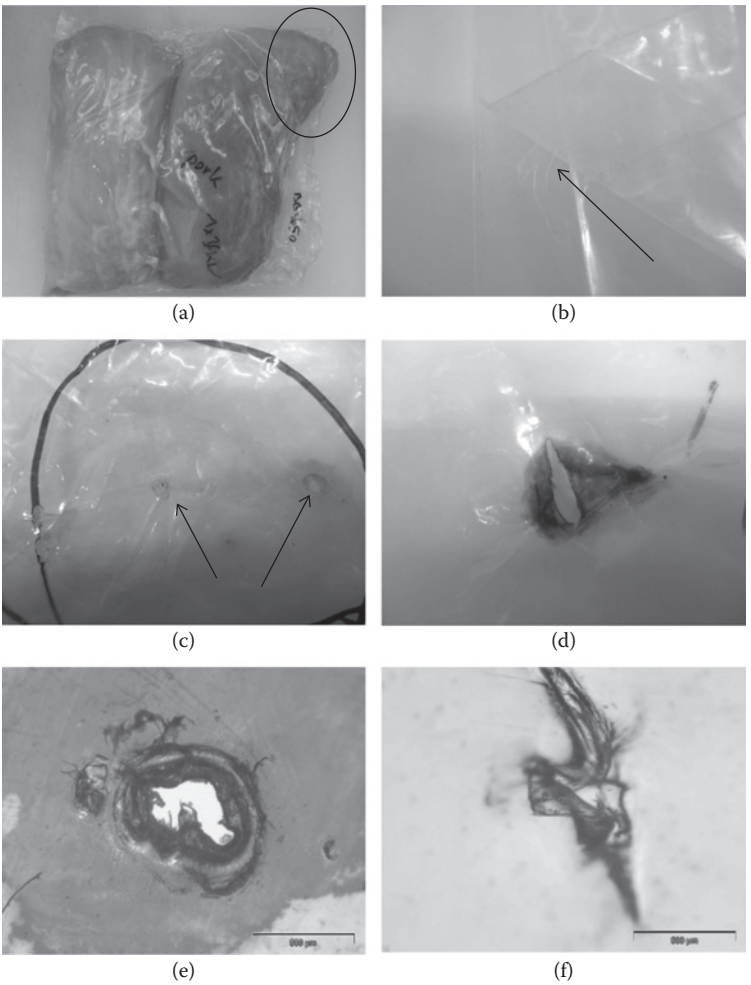


FIGURE 8.15
Typical damages in the packaging material after HDP or shock wave treatment. (a) Breaking of the sealing due to the expansion of the meat, (b) details of an opened sealing, (c) pinholes (leak points) in the packaging (located immersing the bag under water-containing colorants), (d) pinholes as a consequence of burned plastic, and (e and f) microscopy images of a hole generated during HDP treatment. HDP, hydrodynamic pressure processing. Work performed during the European-funded research project, ShockMeat (2012–2013).

the conditions employed in the testing of the prototype. If fewer shots are applied (e.g., every 60 or 90 mm) the productivity will become higher. The time required between shots is 2.5 seconds.

Throughput calculation example:

For a meat cut of 240 mm length and applying a shock wave every 30 mm (240 mm/30 mm = 8) a total of 8 pulses will be applied per meat cut.

Downloaded From Vet-eBooks.com

- For a small meat cut (e.g., beef eye of round) the result is ca. 3 kg × 180 (meat cuts/h) = 540 kg.
- For a big meat cut (e.g., beef top side) the result is ca. 10 kg × 180 (meat cuts/h) = 1800 kg.

Main spare parts to consider are insulators, electrodes, an insulator jacket, reflector, conductive sheet metals, and a high-voltage switch.

8.5.3 Quantifying Meat Benefits

Benefits on meat can be estimated based on improvements in tenderness and so price.

Miller et al. (2001) made a national consumer evaluation on strip loin steaks of known WBSF values, ranging from tough (>5.7 kg) to tender (<3.0 kg). In that study, they assessed the monetary value that consumers place on tenderness by determining the average price that a consumer would pay for a steak in four tenderness categories (toughest, tough, intermediate tenderness, and tender). The prices for these categories would be 12.30, 12.45, 12.94, and 13.53 (US $/kg) (Miller et al., 2001).

8.5.4 Overall Cost Analysis of HDP or Shock Wave Technology for the Meat Industry

The cost of the application of shock wave technology comprises equipment costs (i.e., amortization of investment) and operating costs (i.e., spare parts, energy and water consumption, and personnel cost). The following throughputs of 540–1800 kg/h and a life span of five years have been taken as assumptions for the calculation of operative costs and payback of investment. The amortization cost is based on a one shift working scheme (8 h/d) and 50% occupancy of equipment time. With these assumptions, a total processing cost of 0.069–0.225 €/kg of meat processed is estimated for the respective throughputs and an average cost of 0.147 €/kg of meat is calculated (Table 8.3).

The cost in extra packaging is based on a consumption of 50,000 bags of the optimal material, a highly resistant packaging material, at the standard commercial cost in the market. An extra cost of around 0.172 €/kg of meat was estimated (Table 8.3). However, it must be noted that there is no packaging material that is fully resistant to HDP or shock wave at the moment. Consequently, a reprocessing will have to be considered, which makes implementation more difficult in industrial practices.

The benefit for the meat can be estimated based on the assumption that an improvement in the tenderness perception of the meat with one point within a sensory scale (1–15) may increase the extra value of the meat. This increase of one point in tenderness appreciation in sensorial panel is realistic by using the continuous shock wave prototype developed at the DIL. The translation

Downloaded From Vet-eBooks.com

of the improved tenderness corresponding to one point in a sensory scale (1–15) can be estimated in (€/kg) for different beef meat cuts assuming that this will increase the value of the meat to a certain percentage depending on specific markets (1%–5% added value to price).

Overall, it can be concluded that with the current technological development status of the shock wave technology, only applications in which quality/tenderness improvement will lead to higher benefits than 0.3 €/kg of meat would make the technology profitable. The shock wave technology could be easily integrated in the meat processing industry at the abattoirs after the onset of *rigor mortis* (i.e., boning room) or at the reception of meat processing plants (Figure 8.16). Toll processing is another possible option, where companies offer this service to the meat industry, similarly to what

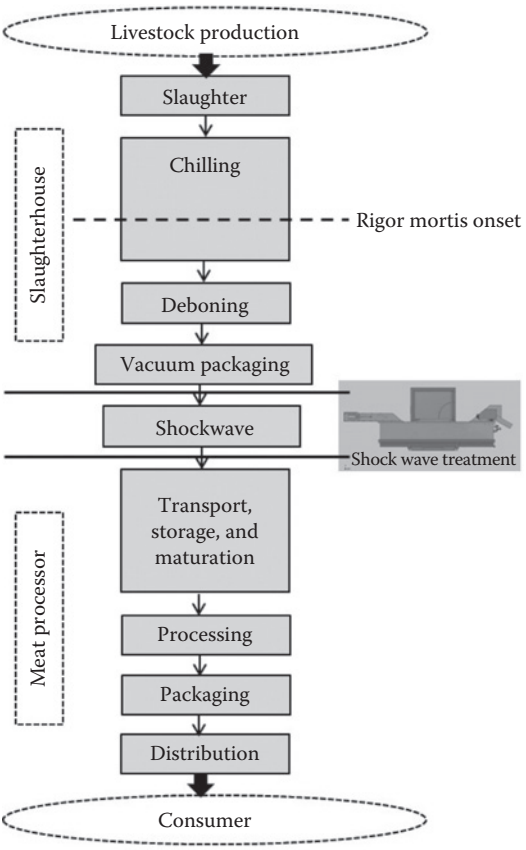


FIGURE 8.16
Meat processing layout with integrated shock wave unit intended for meat tenderization. (Reprinted from *Meat Science*, 95, Bolumar et al., New developments in shockwave technology intended for meat tenderization: Opportunities and challenges, 931–939. Copyright 2013, with permission from Elsevier.)

Downloaded From Vet-eBooks.com

happens with other novel food processing technologies such as HPP, which also has a high initial investment cost, thereby facilitating the use of the technology by the industry.

8.6 Future Considerations

HDP or shock wave technology could be profitable for meat cuts of high commercial value (e.g., loin) or for revalorization of low-value cuts (e.g., brisket) and market niches of premium meats which could absorb the costs of the technology (~0.3 €/kg of meat). Other benefits of the use of HDP would be the attainment of consistent meat quality and improved consumer satisfaction, both resulting in consumer confidence and branding. But these factors are marginal compared to the effect on the net cost €/kg and a prerequisite before the meat industry truly decides to invest in shock wave technology. Other factors of importance to ensure success of the technology are the noise and vibration during operation, which can be avoided by placing the process in a different and isolated room. This is a common practice for some operations in the meat industry where noisy processes such as mechanical deboning of meat and rendering require physical separation to ensure a physical barrier/separation that prevents cross-contamination. Shock wave technology could be further developed to improve throughput and particularly, to create a better interaction between the equipment and the packaged product to minimize the amount of damaged packages, which is a requirement at the moment. Engineering developments seem to be the keystone for the transfer of the technology to the industry. As with many technologies, compliance with the industry requirements in terms of cost and operability will be essential prior to investment and uptake.

8.7 Conclusion

HDP or shock wave technology represents a low-cost and noninvasive intervention for meat tenderization with no negative impact on microbiological and chemical stability (drip loss, color, and flavor). However, commercial application is not yet available because of limitations of suitable packaging materials as well as the need for an efficient shock wave delivery process in a commercial environment. Should these be achieved, HDP would make it possible to obtain remarkable improvements in meat tenderness with a very short treatment time and low processing costs.

Downloaded From Vet-eBooks.com

Acknowledgments

Grant agreement number 2870340 (Development of Shock Wave Technology for Packed Meat) from the European Union's Seventh Framework Program managed by REA (Research Executive Agency, http://ec.europa.eu/research/rea) (FP7/2007–2013) is acknowledged. Critical review of this manuscript by Bo-Anne Rohlik and Ron Tume is acknowledged.

References

Anderson, M. J., Lonergan, S. M., Fedler, C. A., Prusa, K. J., Binning, J. M. and Huff-Lonergan, E. (2012). Profile of biochemical traits influencing tenderness of muscles from the beef round. *Meat Science,* **91**(3), 247–254.

Acuff, G. R. (2014). Microbial contamination, Decontamination of Fresh Meat. In C. Devine and M. Dikeman (Ed.), *Encyclopedia of Meat Sciences, Volume 2* (Vol. 2, pp. 276-279). London, UK: Elsevier Ltd.

Bajovic, B., Bolumar, T. and Heinz, V. (2012). Quality considerations with high pressure processing of fresh and value added meat products. *Meat Science,* **92**(3), 280–289.

Barbosa-Canovas, G. V., Tapia, M. S. and Cano, P. (2004). *Novel Food Processing Technologies*. Boca Raton, FL: CRC Press, Taylor & Francis.

Berge, P., Ertbjerg, P., Larsen, L. M., Astruc, T., Vignon, X. and Møller, A. J. (2001). Tenderization of beef by lactic acid injected at different times post mortem. *Meat Science,* **57**, 347–357.

Bolumar, T., Bindrich, U., Toepfl, S., Toldrá, F. and Heinz, V. (2014). Effect of electro-hydraulic shockwave treatment on tenderness, muscle cathepsin and peptidase activities and microstructure of beef loin steaks from Holstein young bulls. *Meat Science,* **98**, 759–765.

Bolumar, T., Enneking, M., Toepfl, S. and Heinz, V. (2013). New developments in shockwave technology intended for meat tenderization: Opportunities and challenges. A review. *Meat Science,* **95**(4), 931–939.

Bolumar, T., Georget, E. and Mathys, A. (2015). High pressure processing (HPP) of foods and its combination with electron beam processing. *In:* Pillai, S. and Shayanfar, S. (eds.) *Electron Beam Pasteurization and Complementary Food Processing Technologies*. Cambridge: Woodhead Publishing, pp. 127–155.

Bolumar, T., Middendorf, D., Toepfl, S. and Heinz, V. (2016). Structural changes in foods caused by high-pressure processing. *In:* Balasubramaniam, V. M., Barbosa-Canovas, G. and Lelieveld, H. (eds.) *High Pressure Processing of Food—Principles, Technology and Applications*. New York, Springer-Verlag, pp. 509–537.

Bowker, B. C., Callahan, J. A. and Solomon, M. B. (2010a). Effects of hydrodynamic pressure processing on the marination and meat quality of turkey breasts. *Poultry Science,* **89**(8), 1744–1749.

Bowker, B. C., Eastridge, J. S., Paroczay, E. W., Callahan, J. A. and Solomon, M. B. (2010b). Aging/tenderization mechanisms. *In:* Toldrá, F. (ed.) *Handbook of Meat Processing*. Iowa, IA: Wiley-Blackwell, pp. 87–104.

Downloaded From Vet-eBooks.com

Bowker, B. C., Fahrenholz, T. M., Paroczay, E. W., Eastridge, J. S. and Solomon, M. B. (2008a). Effect of hydrodynamic pressure processing and aging on the tenderness and myofibrillar proteins of beef strip loins. *Journal of Muscle Foods,* **19**(1), 74–97.

Bowker, B. C., Fahrenholz, T. M., Paroczay, E. W. and Solomon, M. B. (2008b). Effect of hydrodynamic pressure processing and aging on sarcoplasmic proteins of beef strip loins. *Journal of Muscle Foods,* **19**, 175–193.

Bowker, B. C., Fahrenholz, T. M., Sarnoski, P. J. and Solomon, M. B. (2012). Alterations in the sarcoplasmic protein fraction of beef muscle with postmortem aging and hydrodynamic pressure processing. *Journal of Food Science,* **77**(6), C594–C602.

Bowker, B., Liu, M., Callahan, J. and Solomon, M. (2010b). Effect of hydrodynamic pressure processing on the processing and quality characteristics of moisture-enhanced pork loins. *Journal of Food Science,* **75**(4), S237–S244.

Bowker, B. C., Schaefer, R. B., Grapperhaus, M. J. and Solomon, M. B. (2011). Tenderization of beef loins using a high efficiency sparker. *Innovative Food Science and Emerging Technologies,* **12**(2), 135–141.

Chan, J. T. Y., Omana, D. A. and Betti, M. (2011). Application of high pressure processing to improve the functional properties of pale, soft, and exudative (PSE)-like turkey meat. *Innovative Food Science and Emerging Technologies,* **12**, 216–225.

Claus, J. R. (2002). Shock treatment—Shock waves are an effective tool for tenderizing meat. *Meat and Poultry,* **48**(12), 61–63.

Claus, J. R., Schilling, J. K., Marriott, N. G., Duncan, S. E., Solomon, M. B. and Wang, H. (2001a). Hydrodynamic shockwave tenderization effects using a cylinder processor on early deboned broiler breasts. *Meat Science,* **58**, 287–292.

Claus, J. R., Schilling, J. K., Marriott, N. G., Duncan, S. E., Solomon, M. B. and Wang, H. (2001b). Tenderization of chicken and turkey breasts with electrically produced hydrodynamic shockwaves. *Meat Science,* **58**, 283–286.

Doona, C. J., Kustin, K. and Feeherry, F. E. (eds.) (2010). *Case Studies in Novel Food Processing Technologies. Innovations in Processing, Packaging, and Predictive Modelling*. Cambridge: Woodhead Publishing Series in Food Science, Technology and Nutrition.

Flynn, C., Cruz-Romero, M. C., Troy, D., Mullen, A. M. and Kerry, J. P. (2014). The application of high-pressure treatment in the reduction of salt levels in reduced-phosphate breakfast sausage. *Meat Science,* **96**, 1266–1274.

Gamble, H., Solomon, M. B. and Long, J. (1998). Effects of hydrodynamic pressure on the viability of *Trichinella spiralis* in pork. *Journal of Food Protection,* **61**, 637–639.

Godfrey, C. S. (1970). Apparatus for tenderizing food. *US Patent 3, 492, 688*.

Grossi, A., Gkarane, V., Otte, J. A., Ertbjerg, P. and Orlien, V. (2012). High pressure treatment of brine enhanced pork affects endopeptidase activity, protein solubility, and peptide formation. *Food Chemistry,* **134**(3), 1556–1563.

Grunert, K. G., Bredahl, L. and Brunsø, K. (2004). Consumer perception of meat quality and implications for product development in the meat sector—A review. *Meat Science,* **66**, 259–272.

Heinz, V., Töpfl, S., Schwägele, F. and Münch, S. (2011). AiF-15884 N: Anwendung elektrohydraulischer Stoßwellen zur Desintegration biologischer Gewebe am Beispiel der Zartmachung von Rindfleisch. *In: Forschungskreis der Ernährungsindustrie e. V. (FEI)*, Bonn, Germany.

Hendrickx, M. E. G. and Knorr, D. (2002). *Ultra High Pressure Treatments of Foods. Food Engineering Series*. New York, NY, London: Kluwer Academic Publishers.

Homma, N., Ikeuchi, Y. and Suzuki, A. (1994). Effects of high pressure treatment on the proteolytic enzymes in meat. *Meat Science,* **38**, 219–228.

Downloaded From Vet-eBooks.com

Hugas, M., Garriga, M. and Monfort, J. M. (2002). New mild technologies in meat processing: High pressure as a model technology. *Meat Science,* **62**, 359–371.

Koohmaraie, M., Kent, M. P., Shackelford, S. D., Veiseth, E. and Wheeler, T. L. (2002). Meat tenderness and muscle growth: Is there any relationship? *Meat Science,* **62**, 345–352.

Locke, B. R., Sato, M., Sunka, P., Hoffmann, M. R. and Chang, J.-S. (2006). Electrohydraulic discharge and non-thermal plasma for water treatment. *Industrial and Engineering Chemistry Research,* **45**(3), 882–905.

Long, J. B. (1993). Tenderizing meat. *US Patent 5,273,766*.

Long, J. B. (1994). Apparatus for tenderizing meat. *US Patent 5,328,403.*

Long, J. B. (2000). Treatment of meat by capacitor discharge. *US Patent 6,120,818.*

Long, J. B. (2001). Method for killing bacteria in meat. *US Patent 6,200,615.*

Long, J. B. (2009). Tenderizing of meat. *US Patent 7,485,331*.

Lorca, T. A., Claus, J. R., Eifert, J. D., Marcy, J. E. and Sumner, S. S. (2003). Penetration of surface-inoculated bacteria as a result hydrodynamic shock wave treatment of beef steaks. *Journal of Food Protection,* **65**, 616–620.

Lorca, T. A., Pierson, M. D., Claus, J. R., Eifert, J. D., Marcy, J. E. and Sumner, S. S. (2002). Penetration of surface-inoculated bacteria as a result of electrically-generated hydrodynamic shock wave treatment of boneless skinless chicken breasts. *Journal of Poultry Science,* **82**, 1205–1210.

Manabe, H., Takemoto, A., Maehara, H. et al. (2011). Efficient improved extraction of tomato saponin using shock waves. *Chemical and Pharmaceutical Bulletin,* **59**(11), 1406–1408.

Mayer, D., Dorfmüller, C. and Lachat, M. (2009). Die extrakorporale Stoßwellentherapie als neue Methode zur Stimulation der Heilung komplexer Wunden: Eine Übersicht. *Zeitschrift Fur Wundheilung,* **14**(1), 14–19.

Meek, K. I., Claus, J. R., Duncan, S. E. et al. (2000). Quality and sensory characteristics of selected post-rigor, early deboned broiler breast meat tenderized using hydrodynamic shock waves. *Poultry Science,* **79**, 126–136.

Mennecke, B. E., Townsend, A. M., Hayes, D. J. and Lonergan, S. M. (2007). A study of the factors that influence consumer attitudes toward beef products using the conjoint market analysis tool. *Journal of Animal Science,* **85**, 2639–2659.

Miller, M. F, Carr, M., Ramsey, C. B., Crockett, K. L. and Hoover, L. C. (2001). Consumer thresholds for establishing the value of beef tenderness. *Animal Science,* **79**, 3062–3068.

Miyafuji, Y., Shimojima, K., Tanaka, S. et al. (2011). Development of the pressure vessel for manufacturing the rice-powder using the underwater shock wave. In *ASME Conference Proceedings*, Washington, DC, USA (44540), 53–56.

Moeller, S., Wulf, D., Meeker, D., Ndife, M., Sundararajan, N. and Solomon, M. B. (1999). Impact of the hydrodyne process on tenderness, microbial load, and sensory characteristics of pork longissimus muscle. *Journal of Animal Science,* **77**, 2119–2123.

Ohmori, T., Shigehisa, T., Taji, S. and Hayashi, R. (1992). Biochemical effects of high hydrostatic-pressure on the lysosome and proteases involved in it. *Bioscience Biotechnology and Biochemistry,* **58**, 1285–1288.

Paparella, M. W. and Allen, M. (1970). Gaping oysters by shockwave energy. *Chesapeake Science,* **11**(2), 116–119.

Patel, J. R., Bhagwat, A. A., Sanglay, G. C. and Solomon, M. B. (2006). Rapid detection of *Salmonella* from hydrodynamic pressure-treated poultry using molecular beacon real-time PCR. *Food Microbiology,* **23**, 39–46.

Patel, J., MacArisin, D., Sanglay, G. and Murphy, C. (2012). Inactivation and injury of pathogens on intact beef treated with hydrodynamic pressure. *Innovative Food Science and Emerging Technologies,* **14**, 38–45.

Downloaded From Vet-eBooks.com

Patel, J. R., Sanglay, G. C., Sharma, M. and Solomon, M. B. (2007). Combining antimicrobials and hydrodynamic pressure processing for control of *Listeria Monocytogenes* in frankfurters. *Journal of Muscle Foods,* **18**, 1–18.

Patel, J. R., Sanglay, G. C. and Solomon, M. B. (2009). Control of *Listeria monocytogenes* on frankfurters with antimicrobials and hydrodynamic pressure processing. *Journal of Muscle Foods,* **20**, 227–241.

Podolak, R., Solomon, M. B., Patel, J. R. and Liu, M. N. (2006). Effect of hydrodynamic pressure processing on the survival of *Escherichia coli* O157:H7 in ground beef. *Innovative Food Science and Emerging Technologies,* **7**, 28–31.

Purchas, R. W. (2004). Tenderness measurement. *In: Encyclopedia of Meat Sciences*, vol. 3. Amsterdam: Elsevier, pp. 452–459.

Purslow, P. P. (2005). Intramuscular connective tissue and its role in meat quality. *Meat Science,* **70**, 435–447.

Purslow, P. (2014). New developments on the role of intramuscular connective tissue in meat toughness. *Annual Review of Food Science and Technology,* **5**, 133–153.

Schaefer, R. B. (1998). Sparker source systems. *US Patent 5,841,737*.

Schilling, M. W., Claus, J. R., Marriot, N. G., Solomon, M. B., Eigel, W. N. and Wang, H. (2002). No effect of hydrodynamic shock wave on protein functionality of beef muscle. *Journal of Food Science,* **67**, 335–340.

Schilling, M. W., Marriott, N. G., Wang, H. and Solomon, M. B. (2003). Characteristics of USDA utility cow beef subjected to blade tenderization and hydrodynamic shock-waves. *Journal of Muscle Foods,* **14**, 131–142.

Sentandreu, M. A., Coulis, G. and Ouali, A. (2002). Role of muscle endopeptidases and their inhibitors in meat tenderness. *Trends in Food Science and Technology,* **13**, 398–419.

Sharma, M., Shearer, A. E. H., Hoover, D. G., Liu, M. N., Solomon, M. B. and Kniel, K. E. (2008). Comparison of hydrostatic and hydrodynamic pressure to inactivate foodborne viruses. *Innovative Food Science and Emerging Technologies,* **9**, 418–422.

Shimojima, K., Miyafuji, Y., Tanaka, S. et al. (2011). Study of the suitable pressure vessel for the rice-powder manufacturing using the underwater shock wave. In *ASME Conference Proceedings*, Washington, DC, USA (44540), 49–52.

Sikes, A., Tornberg, E. and Tume, R. (2010). A proposed mechanism of tenderising post-rigor beef using high pressure-heat treatment. *Meat Science,* **84**(3), 390–399.

Sikes, A. L. and Tume, R. K. (2014). Effect of processing temperature on tenderness, colour and yield of beef steaks subjected to high-hydrostatic pressure. *Meat Science,* **97**(2), 244–248.

Simjian, L. G. (1957). Food tenderizer. *US Patent 2,806,246*.

Solomon, M. B., Carpenter, C. E., Snowder, G. D. and Cockett, N. E. (1998). Tenderizing callipyge lamb with the hydrodyne process and electrical stimulation. *Journal of Muscle Foods,* **9**, 305–311.

Solomon, M. B., Liu, M. N., Patel, J. R., Bowker, B. C. and Sharma, M. (2006). Hydrodynamic pressure processing to improve meat quality and safety. *In:* Nollet, L. M. L. and Toldrá, F. (eds.) *Advanced Technologies for Meat Processing.* Boca Raton, FL: CRC Press/Taylor & Francis, pp. 219–244.

Solomon, M. B., Liu, M., Patel, J., Paroczay, E. and Eastridge, J. (2004). Tenderness improvement in fresh and frozen/thawed beef strip loins treated with hydrodynamic pressure processing. *Journal of Animal Science,* **82**, 18–22.

Solomon, M. B., Liu, M. N., Patel, J., Paroczay, E., Eastridge, J. and Coleman, S. W. (2008). Tenderness improvement in fresh or frozen/thawed beef steaks treated with hydrodynamic pressure processing. *Journal of Muscle Foods,* **19**(1), 98–109.

Downloaded From Vet-eBooks.com

Solomon, M. B., Long, J. B. and Eastridge, J. S. (1997). The hydrodyne: A new process to improve beef tenderness. *Journal of Animal Science,* **75**, 1534–1537.

Solomon, M. B., Sharma, M., and Patel, J. R. (2011). Hydrodynamic pressure processing of meat products. *In:* Zhang, H. Q., Barbosa-Canovas, G. V., Balasubramaniam, V.M., Dunne, C. P., Farkas, D. and Yuan, J. T.C. (eds.) *Non-Thermal Processing Technologies for Food*. Brisbane, Milton QLD 4064 Australia, Wiley Blackwell, IFT Press, pp. 98–108.

Souza, C. M., Boler, D. D., Clark, D. L. et al. (2011). The effects of high pressure processing on pork quality, palatability, and further processed products. *Meat Science,* **87**, 419–427.

Spanier, A. M., Berry, B. W. and Solomon, M. B. (2000). Variation in the tenderness of beef strip loins and improvement in tenderness by use of hydrodynamic pressure processing (HDP). *Journal of Muscle Foods,* **11**(3), 183–196.

Spanier, A. M. and Romanowski, R. (2000). A potential index for assessing the tenderness of hydrodynamic pressure (HDP)-treated beef strip loins. *Meat Science,* **56**, 193–202.

Sullivan, G. and Calkins, C. R. (2010). Application of exogenous enzymes to beef muscle of high and low-connective tissue. *Meat Science,* **85**(4), 730–734.

Sun, X. D. and Holley, R. A. (2010). High hydrostatic pressure effects on the texture of meat and meat products. *Journal of Food Science,* **75**(1), R17–R22.

Thompson, J. (2002). Managing meat tenderness. *Meat Science,* **62**, 295–308.

Thompson, J. M., Perry, D., Daly, B., Gardner, G. E., Johnston, D. J. and Pethick, D. W. (2006). Genetic and environmental effects on the muscle structure response post-mortem. *Meat Science,* **74**, 59–65.

Toepfl, S., Bolumar, T. and Heinz. V. (2013). Development of shockwave technology for packed meat. *European Funded Project, FP7 (2007–2013) Under Grant Agreement Number 287034*.

Toepfl, S. and Heinz, V. (2009). New options for targeted product modification. *Fleischwirtschaft International,* **3**, 11–13.

Troy, D. J. (1999). Enhancing the tenderness of beef. Final report. Project Armis No. 3916. The National Food Centre. Research and training for the food industry. Research Report no 11.

Williams-Campbell, A. M. and Solomon, M. B. (2000). New non-thermal postharvest technology to improve food safety: Hydrodynamic pressure processing. In *Proceedings of the International Society for Optical Engineering*, 4206. Boston, MA, 167–173.

Williams-Campbell, A. M. and Solomon, M. B. (2002). Reduction of spoilage microorganisms in fresh beef using hydrodynamic pressure processing. *Journal of Food Protection,* **65**(3), 571–574.

Yang, Y. (2011). *Plasma discharge in water and its application for industrial cooling water treatment*. PhD dissertation, Drexel University, Philadelphia, PA.

Zuckerman, H., Bowker, B. C., Eastridge, J. S. and Solomon, M. B. (2013). Microstructure alterations in beef intramuscular connective tissue caused by hydrodynamic pressure processing. *Meat Science,* **95**(3), 603–607.

Zuckerman, H., Krasik, Y. E. and Felsteiner, J. (2002). Inactivation of microorganisms using pulsed high-current underwater discharges. *Innovative Food Science and Emerging Technologies,* **3**, 329–336.

Zuckerman, H. and Solomon, M. B. (1998). Ultrastructural changes in bovine longissimus muscle caused by Hydrodyne process. *Journal of Muscle Foods,* **9**(4), 419–426.

Downloaded From Vet-eBooks.com

9

Emerging Technologies for the Meat Processing Industry

Kumari Shikha Ojha, Joseph P. Kerry, and Brijesh K. Tiwari

CONTENTS

9.1 Introduction 297
9.2 Emerging Technologies 298
9.2.1 Ultrasound Technology 298
9.2.1.1 Decontamination of Meat 300
9.2.1.2 Meat Tenderization 301
9.2.1.3 Brining 302
9.2.3 Pulsed Electric Field 303
9.2.4 Cold Plasma Technology 304
9.2.5 Light-Based Technologies 308
9.3 Conclusions 312
References 313

9.1 Introduction

Novel technologies, including high-pressure processing, pulsed electric field (PEF), oscillating magnetic fields (OMFs), light-based technologies, high-pressure carbon oxide, ultrasound processing, and microwaves, are currently being developed, optimized, and tested on a variety of food products including meat and meat products. These technologies have shown commercial potential and some of these technologies (e.g., high-pressure processing, see also Chapter 7) have been employed at commercial scale for meat products. Emerging technologies have shown potential to overcome several challenges faced by the meat industry such as improving the process efficiency, new product development, and extension of shelf life while improving the safety profile of meat products. One of the key challenges is related to decontamination of meat surfaces by using heat or chemical-based treatments, which can exert negative effects on meat quality. Low temperature treatment, which includes the application of chemicals, for example, organic acids and chlorine, in a concentration between 1.5% and 2.5% have been reported to lower meat pH and reduce

Downloaded From Vet-eBooks.com

both pathogens and spoilage microorganisms by up to 1–3.5 log units. These chemicals are recommended to be applied either by spraying or dipping meat in water containing chemical disinfectants. These chemicals have been used outside the European Union (EU) for years and have a profound antimicrobial effect. Since these chemicals are not likely to be accepted in the EU, it is highly relevant to search for alternative techniques for meat decontamination. Further, decontamination of meat surfaces using chemicals often relies heavily on potable water usage. The additional impetus of reducing water usage as well as chemical emissions has led to a focus on non-water-based or reduced water-usage-based decontamination technologies. Driven by the need to find more effective decontamination methods, and in light of increasing market and legislative pressures, in the face of highly publicized incidences of foodborne illness, the meat sector is increasingly interested in the development and implementation of new processing technologies. Some of the novel food processing technologies including high-pressure processing have paved a path toward commercialization whereas some novel processing techniques including light-based technologies, ultrasound processing, PEF, and cold plasma are currently being evaluated for meat processing applications. The objective of this chapter is to demonstrate the application of these technologies for meat applications.

9.2 Emerging Technologies

9.2.1 Ultrasound Technology

Ultrasonic waves are sound waves having frequencies above the threshold of hearing for humans and typically divided into three regions of frequency, power ultrasound (20–100 kHz), high-frequency ultrasound (100 kHz–2 MHz), and diagnostic ultrasound (>1 MHz). Ultrasound is an innovative and versatile technology that has its wide range of applications. High-frequency and diagnostic ultrasound is in use for meat carcass evaluation for several decades (Pathak et al., 2011). Ultrasound scanning technology is a noninvasive method to predict carcass characteristics that utilizes ultrasonic waves to generate ultrasonic images, which are employed for visualization of various body parts, with real-time tomographic imaging feasible. Although commercial applications of power ultrasound have been demonstrated in some industries (e.g., chemical, cosmetic, textile, polymer, and petrochemical), it has recently gained interest in the food industry including meat processing. Power ultrasound has been known to influence technological properties and quality of fresh and processed meat. However, limited research studies are available demonstrating the application of ultrasound for various unit operations in meat value chain. The application of ultrasound in meat processing to date includes surface decontamination, tenderization, brining, freezing, thawing, cooking, and cutting of meat (Alarcon-Rojo et al., 2015). Table 9.1 demonstrates the applications of ultrasound for various meat processes.

Downloaded From Vet-eBooks.com

TABLE 9.1

Applications of Ultrasound for Meat Processing

Meat	Condition	Effect of Ultrasound	Reference
Decontamination of meat			
Chicken wing surface	2.5 W/cm^{-2}, 40 kHz, 3 or 6 min	Microorganism reduction Higher reduction with higher time *Escherichia coli* has been found to be more sensitive to ultrasound	Kordowska-Wiater and Stasiak (2011)
Fresh pork jowl	SonoSteam treatment, steam (130°C, 3.5–5 atm) and ultrasound 30–40 kHz for 0.5–2 s	Less skin and surface bacteria	Morild et al. (2011)
Chicken carcasses	SonoSteam treatment, a combination of steam at 90°C–94°C and ultrasound at 30–40 kHz through specially designed nozzles	*Campylobacter* and total count viable reduction	Musavian et al. (2014)
Bovine semitendinosus muscle	Ultrasonic frequency of 40 kHz and an intensity of 11 W/cm^{-2}	Reduced growth of mesophilic, psychrophilic bacteria and total coliforms in beef stored at 4°C	Caraveo et al. (2015)
Tenderization of meat			
Beef (semimembranosus) 24 h postmortem and matured for 24, 48, 72, or 96 h at 2°C	2 W/cm^{-2}, 45 kHz, 2 min	No effect on pH or color. Reduced hardness	Stadnik and Dolatowski (2011)
Hen breast meat 0, 1, 3, or 7 days at 4°C	24 kHz, 12 W/cm^{-2}, 15 s period	Reduced shear force No change in cooking loss	Xiong et al. (2012)
Chicken breast and soybean gels	**20 kHz, 450 W for 0, 3, 6, 9, and 12 min (4 or 2 s pulses)**	More viscoelastic gel, improved Water-and fat-binding capacities and textural properties. Homogeneous fine network microstructures	Zhao et al. (2014)

(*Continued*)

Downloaded From Vet-eBooks.com

TABLE 9.1 (*Continued*)

Applications of Ultrasound for Meat Processing

Meat	Condition	Effect of Ultrasound	Reference
Beef longissimus lumborum muscles	Ultrasonic probe (20 kHz) at power of 100 and 300 W	Simultaneous application of both ultrasound and papain enzyme improved the tenderness of beef	Barekat and Soltanizadeh (2017)
Brining			
Pork longissimus dorsi	40 kHz; 37.5 W/dm^{-3}	Higher salt and water diffusion	Cárcel et al. (2007)
Pork longissimus dorsi	2–4 W/cm^{-2}, 20 kHz	Higher salt diffusion. Diffusion coefficient increases with ultrasound intensity	Siró et al. (2009)
Pork longissimus thoracis and lumborum	0, 40, 56, 72 W/cm^{-2}, 34–40 kHz, 2, 4, 6 h	Reduction of salting time without changes in sensory attributes	McDonnell et al. (2014)
Bovine longissimus dorsi muscles	20 kHz ultrasonic probe at different power levels (150, 200, 250, and 300 W)	Ultrasound intensity could significantly improve the D values of NaCl and water Space between myofibrils increased with increased ultrasound intensity	Kang et al. (2016)

9.2.1.1 Decontamination of Meat

Power ultrasound has the ability to generate physical (micromechanical) and chemical effects resulting in the disruption of cellular structures. The bactericidal effect of ultrasound is largely attributed to intracellular cavitation, which can cause thinning of cell membranes, temperature increase, and production of free radicals (Butz and Tauscher, 2002; Chemat and Khan, 2011). It is proposed that in the compression and rarefaction cycles of ultrasound energy, fluctuating pressures induce formation and collapse of microscopic cavitation bubbles resulting in micromechanical shock waves. These shock waves can disrupt cellular structural and functional components leading to cell lysis (Hoover, 2000). Free radicals, mainly hydroxyl radicals, are produced during the collapse of cavitation bubbles as a result of sonolysis of water. These radicals (e.g., OH^-, H^+<) through a series of reactions, produce hydrogen peroxide (H_2O_2) and other reactive species. Cavitation, production of radicals, and microstreaming can cause thinning of the cell membrane and DNA damage leading to microbial inactivation (Alarcon-Rojo et al., 2015; Earnshaw et al., 1995; Turantaş et al., 2015). The inactivation of various pathogenic and spoilage microorganisms

Downloaded From Vet-eBooks.com

including *Escherichia coli*, *Listeria monocytogenes*, *Salmonella* sp., and *Bacillus* sp. pertinent to meat products is extensively reported in the literature (Piyasena et al., 2003). However, most studies indicate lower antimicrobial efficacy of ultrasound depending upon the conditions employed. Thus, a combination of ultrasound with pressure (manosonication), ultrasound and heat (thermosonication), ultrasound, heat, and pressure (manothermosonication), or the combination of ultrasound with other nonthermal approaches has often been recommended for improving efficacy (Pagán et al., 1999a,b). Kordowska-Wiater and Stasiak (2011) investigated the removal of gram-negative bacteria (*Salmonella anatum, E. coli, Proteus* sp. *and Pseudomonas fluorescens*) from the surface of chicken skin after treatment with ultrasound (40 kHz and 2.5 W/cm^{-2} for 3 or 6 min) in water and in aqueous 1% lactic acid. Sonication in water alone or in lactic acid for 3 min resulted in a 1.0 cfu/cm^{-2} reduction on the skin surface, but longer treatment (6 min) resulted in a reduction of over 1.0 cfu/cm^{-2} in the water samples and 1.5 log cfu/cm^{-2} in the lactic acid-treated samples. Ultrasound treatment in combination with lactic acid may be a suitable method for decontamination of the poultry skin. Herceg et al. (2013) studied the effect of high-intensity ultrasound on the inactivation of suspensions containing *E. coli*, *Staphylococcus aureus*, *Salmonella* sp., *L. monocytogenes*, and *Bacillus cereus* treated with an ultrasound probe operating at 20 kHz and amplitudes of 60, 90, and 120 μm for 3, 6, and 9 min at 20°C, 40°C, and 60°C. Increasing any of these three parameters improved the inactivation rates of bacteria in suspension. The results also showed increased inactivation after prolonged treatment time, especially in combination with high temperature and amplitude level. Ultrasound in combination with steam has shown potential for inactivation of pathogenic and spoilage microorganism. For instance, a study investigated the decontaminating effect of SonoSteam treatment (ultrasound + steam) on broiler carcasses under commercial processing conditions with a rate of 8500 carcasses per hour (Musavian et al., 2014). SonoSteam treatment achieved reductions of approximately 1.0 log for *Campylobacter* and 0.7 log for total viable counts with no significant changes in sensorial properties of chicken carcasses.

9.2.1.2 Meat Tenderization

As tenderness is one of the most important attributes of meat quality, researchers and meat processors have always been looking for efficient tenderization processes. One method of increasing meat tenderness and sensory attributes is by employing ultrasound. Although the application of ultrasonic techniques for meat tenderization was reported as early as the 1970s by Zayas and Orlova, the actual mechanism is still unclear and results are inconsistent in some cases. The tenderization effect of ultrasound is attributed to various mechanisms including physical disruption of muscle tissues through cavitation-induced effects such as high shear, pressure, and temperature; high proteolysis through the release of either cathepsins from lysosomes and/or Ca^{2+} from intracellular

Downloaded From Vet-eBooks.com

stores; and the activation of calpains by Ca^{2+} (Jayasooriya et al., 2004, 2007; Turantaş et al., 2015).

Several studies have focused on the effect of ultrasound on meat tenderization showing positive and no significant effects on meat tenderization (Table 9.1). For example, Dickens et al. (1991) determined the effect of ultrasound (40 kHz, 15 min) on the texture and cooking loss of cooked broiler breast meat and observed that the ultrasound treatment did not affect cooking loss, but improved texture significantly. Dolatowski and Twarda (2004) reported that ultrasound treatment (45 kHz, 2 W/cm^{-2} or 25 kHz, 2 W/cm^{-2}) during the rigor mortis period (up to 24 h postmortem) resulted in improved tenderness and higher water-holding capacity in the *semimembranosus* muscles. On contrary, Got et al. (1999) employed high-frequency (2.4 MHz) ultrasound to treat prerigor meat and observed an initial increase in firmness (compression force) of raw meat compared to untreated or postrigor sonicated meat. After 14 days of aging, this group observed no difference between untreated and prerigor or postrigor ultrasound-treated meat. Ultrasound in combination with other methods is also reported to be effective for improving marination, tenderness, and modifying the functional properties of meat and poultry products (Turantaş et al., 2015). For example, Xiong et al. (2012) reported that contribution of mechanical disruption by ultrasound (24 kHz for 4 min) and endogenous proteolytic enzymes can be a suitable method for improving tenderness and decreasing the cooking loss of chicken meat. Barekat and Soltanizadeh (2017) evaluated the effects of ultrasonic treatment alone, enzyme treatment, and simultaneous application of both ultrasound and papain on the tenderness and certain other physicochemical properties of *Longissimus lumborum* muscles. Results showed that the most proteolytic activity and the highest tenderness value were obtained when the combined treatment was applied at ultrasonic power of 100 W for 20 min. They also concluded that the combination of ultrasound radiation and papain treatment could be employed for the production of tender beef pieces.

9.2.1.3 Brining

Low-frequency ultrasound in the range of 20–100 kHz is known to enhance several mass transfer processes, including brining and curing of meat. It is well recognized that ultrasound allows faster and uniform diffusion of brine solution into the meat tissues. Various physical and chemical phenomena including agitation, vibration, pressure, shock waves, shear forces, microjets, compression and rarefaction, acoustic streaming, cavitation, and radical formation are responsible for the ultrasonic effect. The main driving force for enhancing salt diffusion is acoustic cavitation. It has been proved that power ultrasound treatment can directly increase the diffusion coefficient, which can shorten the curing time. Cárcel et al. (2003) immersed sliced pork in saturated brine solution at 2°C for 45 min with different agitation. It was reported that the sodium chloride and water content in the pork samples were higher

Downloaded From Vet-eBooks.com

with than without ultrasound treatment. Siró et al. (2009) also revealed that ultrasonic brining significantly improved salt diffusion in meat, in comparison with static brining. McDonnell et al. (2014) reported that it took only 2 h to finish curing of pork ham, achieving 2.25% NaCl content, using ultrasound treatment while it needed about 4 h for the control treatment. Some authors pointed out that the mass transfer was not affected until intensity thresholds were reached. Cárcel et al. (2007) reported the thresholds as 39 and 51 W/cm^{-2}. But Siró et al. (2009) exhibited the mass transfer enhancement effect of ultrasound (20 kHz) at ultrasound intensity as low as 2–4 W/cm^{-2}. They also reported an exponential increase of the diffusion coefficient with the ultrasound intensity. Recently, Kang et al. (2016) evaluated the effects of ultrasound intensity and salt concentration of brining for accelerating brine transfer into beef. The results of the diffusion coefficient study showed that ultrasound intensity could significantly improve the diffusion coefficient values of NaCl and water with best results at an ultrasound intensity of 20.96 W/cm^{-2}.

9.2.3 Pulsed Electric Field

High-intensity PEF treatment is a nonthermal technology, which has demonstrated the ability to inactivate microorganisms, decrease the activity of enzymes, and extend the shelf life of food products without significantly affecting the sensorial and nutritional attributes (Barba et al., 2015; Toepfl et al., 2014). This technology involves the application of short pulses (often <1 s) of high-intensity electric field (typically 20–80 kV/cm^{-1}) at frequencies of up to 1000 Hz to foods placed between two electrodes in batch or continuous system (Mosqueda-Melgar et al., 2008). PEF technology has demonstrated several promising applications and commercial interest for liquid foods; however, significant challenges have been encountered for solid foods including meat, hence it has no current commercial application in the meat industry to date. Although there are limited studies on the effect of PEF on meat, there are several possible concepts for the application of PEF in meat and meat products that focus on the permeabilization of cell membranes leading to microbial inactivation and disruption of the cell structure, which can be exploited for accelerating mass transfer (e.g., extraction, curing, and drying). Apart from these, PEF can induce changes in the structure and texture of meat, potentially improving its functional properties such as tenderness, color, and water-holding capacity hence aiding in the development of new products (Arroyo and Lyng, 2017; Faridnia et al., 2015).

Studies in recent years have shown that PEF treatment can enhance meat tenderness and quality attributes of beef muscles (Faridnia et al., 2015; Suwandy et al., 2015a,b). For example, Faridnia et al. (2014) reported that tenderness and color stability of beef *longissimus thoracis* muscles are not affected by PEF (0.2–0.6 kV/cm^{-1}, 1–50 Hz, 20 μs) treatments and a subsequent vacuum aging. Faridnia et al. (2015) evaluated the effect of freezing as a pretreatment prior to PEF treatment (1.4 kV/cm^{-1}, pulse width 20 μs, frequency

Downloaded From Vet-eBooks.com

50 Hz, and total specific energy 250 kJ/kg) on the quality traits of *semitendinosus* beef muscle. They observed that the combined freezing–thawing and PEF resulted in improved tenderness indicated by reduced shear force, while no differences were found for the fresh PEF-treated samples. Ma et al. (2016) studied the effects of chilling and freezing prior to PEF treatment on the volatile profile and sensory attributes of different cooked lamb muscles (i.e., shoulder, rib, and loin). Lamb samples were treated at electric field strength of 1–1.4 kV/cm^{-1}, specific energy of 88–109 kJ/kg^{-1}, frequency of 90 Hz, pulse width of 20 μs and pulse number of 964. The results showed that prolonged storage time and frozen-thawed pretreatment led to significant increases in volatile compounds due to lipid and protein oxidation. PEF also resulted in significant changes of volatile compounds in different meat cuts. Temporal dominance of sensations showed that both storage and PEF treatment affected the temporal flavor of meaty and oxidized flavor attributes. Particularly, a longer storage period was associated with oxidized flavor, while PEF-treated samples were associated with browned, juicy, livery, and meaty flavor attributes. Effects of PEF and OMF combination technology on the extension of supercooled state within chicken breasts were investigated by Mok et al. (2017). They observed that the PEF + OMF supercooling was highly effective in the maintenance of original meat qualities without significant physical damages or chemical changes, which opens up the possibility of advanced designing of innovative food freezers for long-term preservation of "fresh-like" foods at subzero temperature.

9.2.4 Cold Plasma Technology

Cold plasma technology (CPT) has shown promise as a decontamination tool on inert food-contact surfaces, foods, and abiotic surfaces (Klockow and Keener, 2009; Misra et al., 2011; Niemira, 2012). CPT has numerous potential applications for the food industry including the disinfection of food surfaces including meat, poultry, and fresh produce and dry ingredients (Fernandez et al., 2013; Noriega et al., 2011; Ziuzina et al., 2014). This technology has also been successfully applied for surface decontamination of packaging material and for improving functional properties of polymeric materials (Güleç et al., 2006; Lee et al., 2015; Ozdemir et al., 1999; Pankaj et al., 2014). The antimicrobial efficacy of CPT is related to the plasma source, the power level, the gas mixture used in plasma generation, and duration of exposure. The inactivation effect of CPT on pathogenic and spoilage microbes pertinent to a range of foods has been demonstrated extensively. Cold plasma provides inactivation effects through the generation of various reactive species which are lethal to microbial cells. Reactive oxygen and nitrogen species are the main species responsible for antimicrobial effects with strong oxidative damages to cellular components. One of the major mechanisms of action for CPT is the chemical effects observed through the formation of reactive gas species (ROS). Gas plasma offers a very effective route to surface disinfection and

Downloaded From Vet-eBooks.com

decontamination, benefiting from many of the highly reactive ROS such as OH radicals, singlet and triplet oxygen, and superoxide. Most of these reactive species interact with water leading to the formation of OH^- which are deleterious to the microbial cells by causing up to 90% of DNA damage and oxidation of cellular membranes and other cell components (Liao et al., 2017; Thirumdas et al., 2015). Most of these reactive (gas) species revert back to original (gas) form within minutes after treatment due to their short half-life. There are two mechanisms responsible for microbial inactivation: (1) electrostatic disruption and (2) oxidation or damage of membrane or cellular components. In most of the cases, plasma treatment causes disintegration of microbial cells and induces pitting on microbial cells as shown in Figures 9.1 and 9.2.

Plasma technologies offer a range of generation methods (e.g., dielectric barrier discharge, microwave plasma, pen-type plasma or plasma-treated

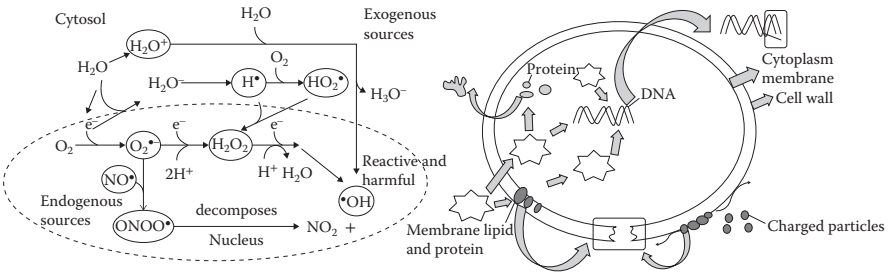


FIGURE 9.1
Dissociation of water molecule into reactive species and microbial inactivation mechanisms of cold plasma technology in biological and physical aspects.

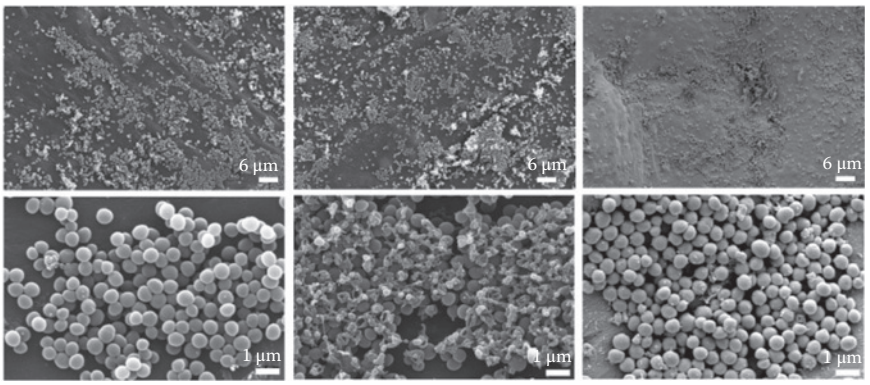


FIGURE 9.2
Scanning electron microscopic pictures of *Staphylococcus aureus* ATCC 12600 on the beef jerky. (a) Before plasma treatment. (b) After plasma treatment for 5 min. (c) After heat treatment to 70°C for 5 min. (From Kim, J.-S. et al., *Innovative Food Science and Emerging Technologies*, **22**, 124–130, 2014.)

Downloaded From Vet-eBooks.com

water) and method of product exposure (e.g., in-package, surface treatment), which can be applied to a range of meat products. Decontamination of fresh pork using microwave plasma (2.45 GHz, 1.2 kW) has shown to maintain microbial load in the range of 2–3 log cfu/g during storage at 5ºC for 20 days with some changes in color (Fröhling et al., 2012). Similar microbial load reductions for *E. coli* and *L. monocytogenes* have been reported for dielectric barrier plasma treatment of pork loin with input gases of He alone and He + O_2 (Kim et al., 2013). Cold plasma driven by radio frequency using argon as a feed gas has shown a significant reduction of 3–4 log cfu/g in *S. aureus* on the surface of beef jerky after 10 min of treatment time whereas similar log reductions were observed after 2 min on the surface of polystyrene with no significant change observed in the fatty acid composition, color, and shear force of the beef jerky samples (Kim et al., 2014). Table 9.2 shows some recent examples of plasma-treated meat products demonstrating application of CPT for the meat industry.

One of the key advantages of the cold plasma technique compared to others is the fact that it can cause microbial inactivation within a package. The effect of in-pack plasma in conjunction with modified atmospheric packaging (MAP) used for common meat products on the inactivation of *E. coli*, *L. monocytogenes*, and *S. aureus* associated with meat products was investigated by Han et al. (2016). They observed a maximum reduction of 1.5 log using a high-oxygen atmosphere, while using air and high nitrogen atmospheres yielded lower antimicrobial efficacy. In-package decontamination of ready-to-eat meat products sealed in linear-low-density polyethylene bags with 30% O_2 was reported to reduce microbial load in the range of 0.8 ± 0.4 to 1.6 ± 0.5 log cfu/g depending on power and treatment time with significant increase in thiobarbituric acid reactive substances (TBARS) and loss of redness value by 70% after a 14-day storage period (Rød et al., 2012). A study carried out by Wang et al. (2016) showed that no significant differences were observed in mesophiles, psychrophiles, and pseudomonas counts for plasma-treated (80 kV for 180 s) chicken fillets packed in food trays containing air and control, whereas a significant reduction in spoilage microbes were observed for plasma-treated chicken fillets packed in modified atmosphere (MA) gas (O_2:CO_2:N_2 = 65:30:5).

Studies have shown that the cold plasma technique can be employed for decontamination of meat surfaces, which poses significant food safety challenges. Numerous reactive chemical species formed during plasma depending on the gas composition. These short-lived reactive species not only have antimicrobial effects but may also cause oxidation of macromolecules leading to alteration of sensory and nutritional properties. CPT has shown to cause lipid oxidation in plasma-treated bresaola (Rød et al., 2012), raw pork, and beef (Jayasena et al., 2015). Generally, high plasma power, treatment time, storage conditions posttreatments, and lipid content of meat are some key factors which govern the rate of lipid oxidation in meat products. Oxidation of lipids is mainly attributed to the formation of highly reactive

Downloaded From Vet-eBooks.com

TABLE 9.2

Application of Cold Plasma Technology for Meat Processing

Application	Processing Conditions	Salient Findings	References
Microbial inactivation of beef jerky	Dielectric barrier discharge using a bipolar square waveform voltage at 15 kHz; ambient air; treatment time: 0–10 min	2–3 log cfu/g reduction; no significant changes in the metmyoglobin content, shear force, and myofibrillar fragmentation index; some changes in sensorial properties of plasma-treated beef jerky samples	Yong et al. (2017)
Shelf life of chicken fillets	Plasma treatment (80 kV for 3 min) in air or modified atmosphere gas (O_2:CO_2:N_2 = 65:30:5) and stored at 4°C after treatment	Improved microbiological shelf life of plasma-treated samples in modified atmosphere No significant differences in the surface L* value regardless of the treatment	Wang et al. (2016)
Nitrate reduction in emulsion-type sausages	Plasma-treated water	No significant differences in total aerobic count, color, peroxide value, and sensory qualities between control (sodium nitrate) and plasma-treated water for the manufacture of emulsion-type sausage	Jung et al. (2015)
Pork loins	Dielectric barrier discharge plasma (3 kV, 30 kHz bipolar square wave) with input gases He and He + O_2 Treatment time up to 10 min	He + O_2 showing higher efficiency of microbial reduction when compared to He alone Higher inactivation with longer treatment time for *Listeria monocytogenes* and *Escherichia coli*; higher TBARS values for He + O_2; significant differences in sensory quality	Kim et al. (2013)

TBARS, thiobarbituric acid reactive substances.

Downloaded From Vet-eBooks.com

oxidative species formed during plasma treatment. CPT has shown that the use of nitrates can be avoided by the application of plasma-treated water in the manufacture of emulsion-type sausages cured with plasma-treated water. Sausages prepared using plasma-treated water as a nitrite source in curing process or synthetic sodium nitrite at a concentration of nitrite ion 70 mg/kg did not show any noticeable effects on the total aerobic bacterial counts, color, and peroxide values of sausages compared with those with sodium nitrite throughout 28 days of storage at 4°C. Further, sausage with added plasma-treated water had lower concentrations of residual nitrite compared to sodium nitrite during the storage period (Jung et al., 2015). Mutagenicity using the Ames test and immune toxicity studies with 8-week-old female mice of emulsion-type sausage cured with plasma-treated water showed negative results thus demonstrating that the plasma-treated water can be a suitable replacement for sodium nitrite for meat products (Kim et al., 2016).

Studies have shown that plasma technologies can improve microbial safety profile of meat and can extend microbiological shelf life of meat products. However, higher power and treatment times required to enhance microbial load reduction in meat products may cause undesirable changes in nutritional and eating quality of meat. These undesirable changes are mainly associated with lipid oxidation, changes in color, and development of odor. Limited studies have demonstrated the effect of plasma on nutritional and sensorial quality of plasma-treated meat products. Further studies are required to establish the commercial viability of CPT for surface decontamination or meat product applications.

9.2.5 Light-Based Technologies

Light-based technologies are reported and described with several synonyms including pulsed light (PL), high-intensity pulsed light (HIPL), intense light pulse (ILP), pulsed white light (PWL), and pulsed ultraviolet light (P-UV). In some cases, these are also described and reported based on the wavelength employed within the electromagnetic spectrum. Apart from physicochemical properties of the food, the efficacy of light-based technologies strongly depends on various factors including wavelength, pulsing, treatment duration, and energy (fluence, J/cm^{-2}) as shown in Figure 9.3 (Heinrich et al., 2016). Within the food industry, ultraviolet (UV) light wavelengths in the range of 10–400 nm have been shown effective against various pathogenic and spoilage microorganisms pertinent to food. UV light can be classified in three different regions depending on the wavelength or penetration power: UV-A, UV-B, and UV-C range from 315–400 nm, 280–315 nm, and 200–280 nm, respectively. Among these classifications, UV-C is very effective for inactivation of various pathogenic and spoilage microorganisms. PL (combined visible and UV photons at energies up to 50 J/cm^{-2}) has a broad spectrum of wavelength in the range of 170–1000 nm. Compared to UV light, PL has higher intensity. Infrared

Downloaded From Vet-eBooks.com

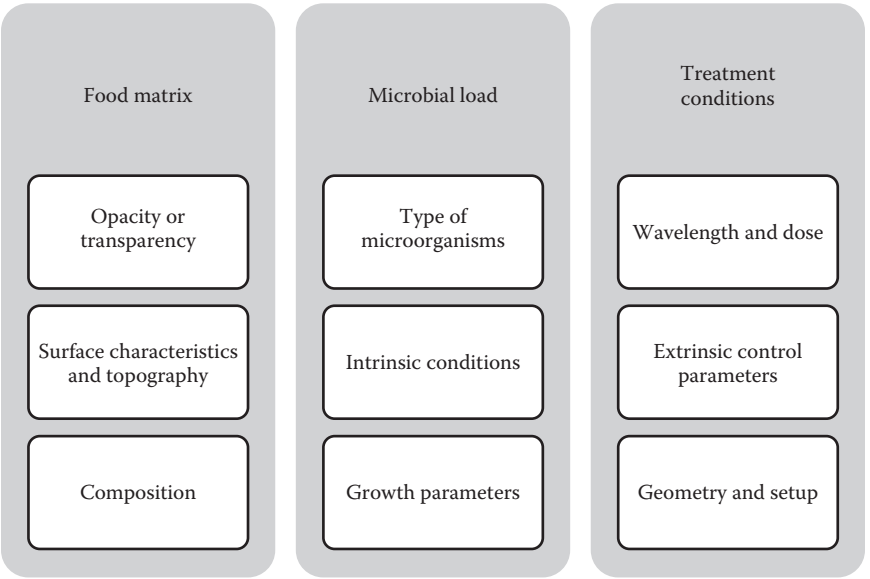


FIGURE 9.3
Various factors affecting efficacy of light-based technologies. Light-based technologies have been reported for surface decontamination of fresh-cut meat surfaces as well as ready-to-eatmeat products (see Table 9.3).

radiations have wavelengths in range of 750 nm to 1 mm, which can be classified in three spectral regions based on wavelength, namely near infrared (NIR, 0.75–1.4 μm), mid-infrared (MIR, 1.4–3.0 μm), and far-infrared (FIR, 3–1000 μm), respectively.

Light-based technologies including UV light, PL, and infrared irradiations are nonthermal with mild thermal character. These techniques find applications in meat processing for improving the safety profile of meat and ready-to-eat meat products. Light-based technologies are of particular interest for decontamination of meat products and surfaces as they do not require potable water to achieve desired microbial safety. One of the key advantages of light-based technologies is that they can be easily applied to the processing line after thermal treatment, for example, after slicing of cooked ham where post-cooking contamination can be eliminated prior to packaging. Compared to other novel technologies, the application of light-based technologies seems to be legally placed in better position at least in the United States because of its approval in the production, processing, and handling of food and food-contact surfaces for the control of surface microorganisms, provided that the treatment uses a xenon lamp with emission wavelengths between 200 and 1000 nm, with a pulse width not exceeding 2 ms, and the cumulative level of treatment does not exceed 12 J/cm^{-2} (21 CFR 179.41, issued by the Food and Drug Administration, US FDA, 2005) (Rajkovic et al., 2010a). Whereas, in the EU,

Downloaded From Vet-eBooks.com

according to EC 258/97, novel technologies require evidence to prove that new technologies do not alter nutritional composition of treated foods. Research carried out to date shows minimal or no changes in nutritional composition of food treated with light-based technologies.

UV light in the range of 200–280 nm (UV-C) has been used primarily for decontamination of food surfaces and causes microbial inactivation that is mainly due to photochemical, photothermal, or photophysical effects, which include chemical modification of proteins and DNA. The formation of 6,4-photoproducts and cyclobutane pyrimidine dimers in their DNA, alteration of cellular components, and preventing cells from replicating are key mechanisms associated with microbial inactivation (Anderson et al., 2000; Krisko and Radman, 2010; Sommers et al., 2017). UV-C light has been tested to improve the microbial safety and quality of chicken meat (Sommers et al., 2017), frog meat (Silva et al., 2015), sliced fermented salami (Rajkovic et al., 2017), and ready-to-eat cured meat products (Ganan et al., 2013) against pathogenic microorganisms including *L. monocytogenes*, *Salmonella Typhimurium*, and *S. aureus*. Significant reductions of up to 1.5 and 1.8 log cfu/cm^{-2} for *L. monocytogenes* and *S. Typhimurium* with a fluence of 11.9 J/cm^{-2} can be obtained for ready-to-eat meat products (Ganan et al., 2013). A systematic review was carried out on the sensory quality of various types of meat and meat products treated by light-based technologies, including PL, PWL, and pulsed UV light (Rajkovic et al., 2010b). They concluded that sensory quality strongly depends on animal species, type of meat product, and dose applied. Odor followed color is one of the most significant sensory parameters that are affected by light-based technologies. Light-based technologies have less impact on sensory quality of dry-cured meat compared to cooked meat products while fermented meat products are the least influenced.

TABLE 9.3

Application of Light-Based Technologies for Meat Processing

Light-Based Technologies	Application	Conditions	Salient Findings	Reference
PL	Fermented salami slices	Fluence of 3–15 J/cm^{-2} Treatment followed by MAP 80% CO_2/20% N_2	*Listeria monocytogenes, Escherichia coli* 0157:H7, *Salmonella Typhimurium*, and *Staphylococcus aureus* for 2.24, 2.29, 2.25, and 2.12 log cfu/g on the surface of dry fermented salami No significant increase in lipid oxidation except for samples treated at 15 J/cm^{-2}	Rajkovic et al. (2017)

(*Continued*)

Downloaded From Vet-eBooks.com

TABLE 9.3 (*Continued*)

Application of Light-Based Technologies for Meat Processing

Light-Based Technologies	Application	Conditions	Salient Findings	Reference
PL treatment (30% UV light [12% UV-C, 10% UV-B, and 8% UV-A], 30% infrared radiation, and 40% visible light)	Ready-to-eat dry-cured meat products (*salchichón* and loin	Fluence rate of 0.7, 2.1, 4.2, 8.4, and 11.9 J/cm^{-2}	Maximum log reductions between 1.5 and 1.8 cfu/cm^{-2} obtained for both microorganisms at fluence rate of 11.9 J/cm^{-2} No significant changes in sensorial properties	Ganan et al. (2013)
UV-C intensities (0.65, 1.04, and 1.68 mW/s/cm^{-2})	Bullfrog back meat	Packed bullfrog's back meat was then exposed to low (0.65 mW/s/cm^{-2}), medium (1.04 mW/s/cm^{-2}), and high (1.68 mW/s/cm^{-2}) UV-C intensities over different time intervals (60, 100, and 140 s)	A reduction of 3 log cfu/g for all treatments compared to control	Silva et al. (2015)
Pilot-scale pulsed UV light system with two pairs of pulsed UV lamps	Whole chicken	0.25 J/cm^{-2} per pulse; 30, 45, 60, 90, 120, and 180 s; distance: 5 cm; 3 Hz	0.87–1.43 log (*E. coli*) Temperature increase up to 44.1°C; discolorations at high energy doses	Keklik et al. (2011)
PL (200–1100 nm)	Pork	3, 10, or 30 J/cm^{-2}; distance 3 cm; 1 Hz/4°C; 15 days	0.96 log (raw pork) and 0.99 log (roast pork) Temperature increase by 11°C–12°C, dose-dependent color changes of pork; dose-dependent lipid peroxidation of salmon and pork at high fluencies	Nicorescu et al. (2014)

(*Continued*)

Downloaded From Vet-eBooks.com

TABLE 9.3 (*Continued*)

Application of Light-Based Technologies for Meat Processing

Light-Based Technologies	Application	Conditions	Salient Findings	Reference
ILPs in range of UV to IR	Beef/pork, chicken/turkey and deer/rabbit/kangaroo	1 and 5 pulses; 3.4 J/cm^{-2} per pulse; 0.5 Hz; distance: 6 and 10 cm; discharge voltage: 3 kV	Dose and type of meat-dependent sensory changes; impact on odor of all meat; significant sensory deviation of turkey; color unaffected in chicken and rabbit; dose-dependent discoloration of pork and turkey meat	Tomašević (2015)
PL treatment (30% UV light [12% UV-C, 10% UV-B, and 8% UV-A], 30% infrared radiation, and 40% visible light)	Cooked ham Bologna	0.7, 2.1, 4.2, 8.4 J/cm^{-2}; through PA/PE/VA plastic bags/aerobic and vacuum conditions, 4°C, up to 54 days	*L. monocytogenes* 0.75–1.78 log (cooked ham) 0.41–1.11 log (bologna) Low levels of lipid oxidation and slight discoloration directly after PL exposure; no impact on sensory attributes of cooked ham, negative impact on sensory properties of bologna at high fluencies, shelf-life extension of cooked ham by 30 days under vacuum packaging	Hierro et al. (2011)

MAP, modified atmospheric packaging; PL, pulsed light; UV, ultraviolet.

9.3 Conclusions

Various new technologies have been advocated for potential application across the meat production chain for food safety intervention and meat product development. Various emerging technologies discussed in this chapter demonstrates the potential to replace, at least partly, the traditional embraced techniques for meat processing and preservation, as the industry seeks to become more environmentally and economically sustainable. Apart from decontamination, novel technologies can also play an important role in various operations including drying, cooking, curing, freezing, and packaging with an aim to improve the process efficiency, quality, and nutritional profile of meat products.

Downloaded From Vet-eBooks.com

This chapter identifies a group of selected technologies that have shown significant potential for improving shelf life and technological properties of meat that has emerged during the past few years. These technologies represent a rapid, efficient, and reliable alternative to improve the quality of meat, but it also has the potential to develop new products with a unique functionality. Finally, it is worth noting that while many innovative food processing techniques have shown good potential for improving the nutritive quality of processed meat, a significant proportion has not been scaled up for new food applications.

The exact mechanisms of emerging technologies discussed in this chapter for meat give only a limited amount of information. There are numerous, complex, and perplexing mechanisms that are proposed. A better understanding of the complex physicochemical mechanisms of action and their effects on the technological and functional properties of meat products would also contribute to reinforce the commercial viability of these technologies for the meat industry.

References

Alarcon-Rojo, A., Janacua, H., Rodriguez, J., Paniwnyk, L. and Mason, T. (2015). Power ultrasound in meat processing. *Meat Science,* **107**, 86–93.

Anderson, J. G., Rowan, N. J., MacGregor, S. J., Fouracre, R. A. and Farish, O. (2000). Inactivation of food-borne enteropathogenic bacteria and spoilage fungi using pulsed-light. *IEEE Transactions on Plasma Science,* **28**(1), 83–88.

Arroyo, C. and Lyng, J. G. (2017). Electroprocessing of meat and meat products. *In:* Cummins, E. J. and Lyng, J. G. (eds.) *Emerging Technologies in Meat Processing: Production, Processing and Technology*. Wiley Blackwell, pp. 103–130.

Barba, F. J., Parniakov, O., Pereira, S. A. et al. (2015). Current applications and new opportunities for the use of pulsed electric fields in food science and industry. *Food Research International,* **77**, 773–798.

Barekat, S. and Soltanizadeh, N. (2017). Improvement of meat tenderness by simultaneous application of high-intensity ultrasonic radiation and papain treatment. *Innovative Food Science and Emerging Technologies,* **39**, 223–229.

Butz, P. and Tauscher, B. (2002). Emerging technologies: Chemical aspects. *Food Research International,* **35**(2), 279–284.

Caraveo, O., Alarcon-Rojo, A. D., Renteria, A., Santellano, E. and Paniwnyk, L. (2015). Physicochemical and microbiological characteristics of beef treated with high-intensity ultrasound and stored at 4°C. *Journal of the Science of Food and Agriculture,* **95**(12), 2487–2493.

Cárcel, J., Benedito, J., Bon, J. and Mulet, A. (2007). High intensity ultrasound effects on meat brining. *Meat Science,* **76**(4), 611–619.

Cárcel, J., Benedito, J., Mulet, A. and Riera, E. (2003). Mass transfer effects during meat ultrasonic brining. In *Proceedings of World Congress on Ultrasonics WCU*. Paris, France, 431–434.

Chemat, F. and Khan, M. K. (2011). Applications of ultrasound in food technology: Processing, preservation and extraction. *Ultrasonics Sonochemistry,* **18**(4), 813–835.

Downloaded From Vet-eBooks.com

Dickens, J., Lyon, C. and Wilson, R. (1991). Effect of ultrasonic radiation on some physical characteristics of broiler breast muscle and cooked meat. *Poultry Science,* **70**(2), 389–396.

Dolatowski, Z. J. and Twarda, J. (2004). Einfluss von Ultraschall auf das Wasserbindungsvermögen von Rindfleisch. *Fleischwirtschaft,* **84**(12), 95–99.

Earnshaw, R., Appleyard, J. and Hurst, R. (1995). Understanding physical inactivation processes: Combined preservation opportunities using heat, ultrasound and pressure. *International Journal of Food Microbiology,* **28**(2), 197–219.

Faridnia, F., Bekhit, A. E. D. A., Niven, B. and Oey, I. (2014). Impact of pulsed electric fields and post-mortem vacuum ageing on beef longissimus thoracis muscles. *International Journal of Food Science and Technology,* **49**(11), 2339–2347.

Faridnia, F., Ma, Q. L., Bremer, P. J., Burritt, D. J., Hamid, N. and Oey, I. (2015). Effect of freezing as pre-treatment prior to pulsed electric field processing on quality traits of beef muscles. *Innovative Food Science and Emerging Technologies,* **29**, 31–40.

Fernandez, A., Noriega, E. and Thompson, A. (2013). Inactivation of *Salmonella enterica* serovar Typhimurium on fresh produce by cold atmospheric gas plasma technology. *Food Microbiology,* **33**(1), 24–29.

Fröhling, A., Durek, J., Schnabel, U., Ehlbeck, J., Bolling, J. and Schlüter, O. (2012). Indirect plasma treatment of fresh pork: Decontamination efficiency and effects on quality attributes. *Innovative Food Science and Emerging Technologies,* **16**, 381–390.

Ganan, M., Hierro, E., Hospital, X. F., Barroso, E. and Fernández, M. (2013). Use of pulsed light to increase the safety of ready-to-eat cured meat products. *Food Control,* **32**(2), 512–517.

Got, F., Culioli, J., Berge, P. et al. (1999). Effects of high-intensity high-frequency ultrasound on ageing rate, ultrastructure and some physico-chemical properties of beef. *Meat Science,* **51**(1), 35–42.

Güleç, H. A., SarIoglu, K. and Mutlu, M. (2006). Modification of food contacting surfaces by plasma polymerisation technique. Part I: Determination of hydrophilicity, hydrophobicity and surface free energy by contact angle method. *Journal of Food Engineering,* **75**(2), 187–195.

Han, L., Ziuzina, D., Heslin, C. et al. (2016). Controlling microbial safety challenges of meat using high voltage atmospheric cold plasma. *Frontiers in Microbiology*, *7*, 977.

Heinrich, V., Zunabovic, M., Varzakas, T., Bergmair, J. and Kneifel, W. (2016). Pulsed light treatment of different food types with a special focus on meat: A critical review. *Critical Reviews in Food Science and Nutrition,* **56**(4), 591–613.

Herceg, Z., Markov, K., Sobota Šalamon, B., Režek Jambrak, A., Vukušić, T. and Kaliterna, J. (2013). Effect of high intensity ultrasound treatment on the growth of food spoilage bacteria. *Food Technology and Biotechnology,* **51**(3), 352–359.

Hierro, E., Barroso, E., De la Hoz, L., Ordóñez, J. A., Manzano, S. and Fernández, M. (2011). Efficacy of pulsed light for shelf-life extension and inactivation of *Listeria monocytogenes* on ready-to-eat cooked meat products. *Innovative Food Science and Emerging Technologies,* **12**(3), 275–281.

Hoover, D. G. (2000). Ultrasound. *Journal of Food Science,* **65**(s8), 93–95.

Jayasena, D. D., Kim, H. J., Yong, H. I. et al. (2015). Flexible thin-layer dielectric barrier discharge plasma treatment of pork butt and beef loin: Effects on pathogen inactivation and meat-quality attributes. *Food Microbiology,* **46**, 51–57.

Jayasooriya, S., Bhandari, B., Torley, P. and D'arcy, B. (2004). Effect of high power ultrasound waves on properties of meat: A review. *International Journal of Food Properties,* **7**(2), 301–319.

Downloaded From Vet-eBooks.com

Jayasooriya, S. D., Torley, P., D'arcy, B. R. and Bhandari, B. R. (2007). Effect of high power ultrasound and ageing on the physical properties of bovine Semitendinosus and Longissimus muscles. *Meat Science,* **75**(4), 628–639.

Jung, S., Kim, H. J., Park, S. et al. (2015). The use of atmospheric pressure plasma-treated water as a source of nitrite for emulsion-type sausage. *Meat Science,* **108**, 132–137.

Kang, D.-C., Wang, A.-R., Zhou, G.-H., Zhang, W.-G., Xu, S.-M. and Guo, G.-P. (2016). Power ultrasonic on mass transport of beef: Effects of ultrasound intensity and NaCl concentration. *Innovative Food Science and Emerging Technologies,* **35**, 36–44.

Keklik, N., Demirci, A. and Bock, R. (2011). Surface decontamination of whole chicken carcasses using a pilot-scale pulsed UV light system. *Transact ASABE,* **54**, 993–1000.

Kim, J.-S., Lee, E.-J., Choi, E. H. and Kim, Y.-J. (2014). Inactivation of *Staphylococcus aureus* on the beef jerky by radio-frequency atmospheric pressure plasma discharge treatment. *Innovative Food Science and Emerging Technologies,* **22**, 124–130.

Kim, H. J., Sung, N. Y., Yong, H. I. et al. (2016). Mutagenicity and immune toxicity of emulsion-type sausage cured with plasma-treated water. *Korean Journal for Food Science of Animal Resources,* **36**(4), 494–498.

Kim, H.-J., Yong, H. I., Park, S., Choe, W. and Jo, C. (2013). Effects of dielectric barrier discharge plasma on pathogen inactivation and the physicochemical and sensory characteristics of pork loin. *Current Applied Physics,* **13**(7), 1420–1425.

Klockow, P. A. and Keener, K. M. (2009). Safety and quality assessment of packaged spinach treated with a novel ozone-generation system. *LWT-Food Science and Technology,* **42**(6), 1047–1053.

Kordowska-Wiater, M. and Stasiak, D. M. (2011). Effect of ultrasound on survival of gram-negative bacteria on chicken skin surface. *Bull Vet Inst Pulawy,* **55**, 207–210.

Krisko, A. and Radman, M. (2010). Protein damage and death by radiation in *Escherichia coli* and *Deinococcus radiodurans*. *Proceedings of the National Academy of Sciences,* **107**(32), 14373–14377.

Lee, T., Puligundla, P. and Mok, C. (2015). Inactivation of foodborne pathogens on the surfaces of different packaging materials using low-pressure air plasma. *Food Control,* **51**, 149–155.

Liao, X., Liu, D., Xiang, Q. et al. (2017). Inactivation mechanisms of non-thermal plasma on microbes: A review. *Food Control,* **75**, 83–91.

Ma, Q., Hamid, N., Oey, I. et al. (2016). Effect of chilled and freezing pre-treatments prior to pulsed electric field processing on volatile profile and sensory attributes of cooked lamb meats. *Innovative Food Science and Emerging Technologies,* **37**(Part C), 359–374.

McDonnell, C. K., Lyng, J. G., Arimi, J. M. and Allen, P. (2014). The acceleration of pork curing by power ultrasound: A pilot-scale production. *Innovative Food Science and Emerging Technologies,* **26**, 191–198.

Misra, N., Tiwari, B., Raghavarao, K. and Cullen, P. J. (2011). Nonthermal plasma inactivation of food-borne pathogens. *Food Engineering Reviews,* **3**(3–4), 159–170.

Mok, J. H., Her, J. -Y., Kang, T., Hoptowit, R. and Jun, S. (2017). Effects of pulsed electric field (PEF) and oscillating magnetic field (OMF) combination technology on the extension of supercooling for chicken breasts. *Journal of Food Engineering,* **196**, 27–35.

Morild, R. K., Christiansen, P., Sørensen, A. H., Nonboe, U. and Aabo, S. (2011). Inactivation of pathogens on pork by steam-ultrasound treatment. *Journal of Food Protection,* **74**(5), 769–775.

Downloaded From Vet-eBooks.com

Mosqueda-Melgar, J., Elez-Martinez, P., Raybaudi-Massilia, R. M., and Martin-Belloso, O. (2008). Effects of pulsed electric fields on pathogenic microorganisms of major concern in fluid foods: A review. *Critical Reviews in Food Science and Nutrition,* **48**(8), 747–759.

Musavian, H. S., Krebs, N. H., Nonboe, U., Corry, J. E. and Purnell, G. (2014). Combined steam and ultrasound treatment of broilers at slaughter: A promising intervention to significantly reduce numbers of naturally occurring campylobacters on carcasses. *International Journal of Food Microbiology,* **176**, 23–28.

Nicorescu, I., Nguyen, B., Chevalier, S. and Orange, N. (2014). Effects of pulsed light on the organoleptic properties and shelf-life extension of pork and salmon. *Food Control,* **44**, 138–145.

Niemira, B. A. (2012). Cold plasma decontamination of foods. *Annual Review of Food Science and Technology,* **3**, 125–142.

Noriega, E., Shama, G., Laca, A., Díaz, M. and Kong, M. G. (2011). Cold atmospheric gas plasma disinfection of chicken meat and chicken skin contaminated with *Listeria innocua*. *Food Microbiology,* **28**(7), 1293–1300.

Ozdemir, M., Yurteri, C. U. and Sadikoglu, H. (1999). Physical polymer surface modification methods and applications in food packaging polymers. *Critical Reviews in Food Science and Nutrition,* **39**(5), 457–477.

Pagán, R., Manas, P., Alvarez, I. and Condón, S. (1999a). Resistance of *Listeria monocytogenes* to ultrasonic waves under pressure at sublethal (manosonication) and lethal (manothermosonication) temperatures. *Food Microbiology,* **16**(2), 139–148.

Pagán, R., Manas, P., Raso, J. and Condón, S. (1999b). Bacterial resistance to ultrasonic waves under pressure at nonlethal (manosonication) and lethal (manothermosonication) temperatures. *Applied and Environmental Microbiology,* **65**(1), 297–300.

Pankaj, S. K., Bueno-Ferrer, C., Misra, N. et al. (2014). Applications of cold plasma technology in food packaging. *Trends in Food Science and Technology,* **35**(1), 5–17.

Pathak, V., Singh, V. and Sanjay, Y. (2011). Ultrasound as a modern tool for carcass evaluation and meat processing: A review. *International Journal of Meat Science,* **1**(2), 83–92.

Piyasena, P., Mohareb, E. and McKellar, R. (2003). Inactivation of microbes using ultrasound: A review. *International Journal of Food Microbiology,* **87**(3), 207–216.

Rajkovic, A., Smigic, N. and Devlieghere, F. (2010a). Contemporary strategies in combating microbial contamination in food chain. *International Journal of Food Microbiology,* **141**(Supplement 1), S29–S42.

Rajkovic, A., Tomasevic, I., De Meulenaer, B. and Devlieghere, F. (2017). The effect of pulsed UV light on *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella Typhimurium*, *Staphylococcus aureus*, and staphylococcal enterotoxin A on sliced fermented salami and its chemical quality. *Food Control,* **73**(Part B), 829–837.

Rajkovic, A., Tomasevic, I., Smigic, N., Uyttendaele, M., Radovanovic, R. and Devlieghere, F. (2010b). Pulsed UV light as an intervention strategy against *Listeria monocytogenes* and *Escherichia coli* O157:H7 on the surface of a meat slicing knife. *Journal of Food Engineering*, **100**(3), 446–451.

Rød, S. K., Hansen, F., Leipold, F. and Knøchel, S. (2012). Cold atmospheric pressure plasma treatment of ready-to-eat meat: Inactivation of *Listeria innocua* and changes in product quality. *Food Microbiology,* **30**(1), 233–238.

Silva, H., Costa, M., Frasao, B. et al. (2015). Efficacy of ultraviolet-C light to eliminate *Staphylococcus aureus* on precooked shredded bullfrog back meat. *Journal of Food Safety,* **35**(3), 318–323.

Downloaded From Vet-eBooks.com

Siró, I., Vén, C., Balla, C., Jónás, G., Zeke, I. and Friedrich, L. (2009). Application of an ultrasonic assisted curing technique for improving the diffusion of sodium chloride in porcine meat. *Journal of Food Engineering,* **91**(2), 353–362.

Sommers, C., Sheen, S., Scullen, O. J. and Mackay, W. (2017). Inactivation of *Staphylococcus saprophyticus* in chicken meat and purge using thermal processing, high pressure processing, gamma radiation, and ultraviolet light (254 nm). *Food Control,* **75**, 78–82.

Stadnik, J. and Dolatowski, Z. J. (2011). Influence of sonication on Warner-Bratzler shear force, colour and myoglobin of beef (m. semimembranosus). *European Food Research and Technology,* **233**(4), 553.

Suwandy, V., Carne, A., van de Ven, R., Bekhit, A. E.-D. A. and Hopkins, D. L. (2015a). Effect of pulsed electric field on the proteolysis of cold boned beef M. Longissimus lumborum and M. Semimembranosus. *Meat Science,* **100**, 222–226.

Suwandy, V., Carne, A., van de Ven, R., Bekhit, A. E.-D. A. and Hopkins, D. L. (2015b). Effect of pulsed electric field treatment on hot-boned muscles of different potential tenderness. *Meat Science,* **105**, 25–31.

Thirumdas, R., Sarangapani, C. and Annapure, U. S. (2015). Cold plasma: A novel non-thermal technology for food processing. *Food Biophysics,* **10**(1), 1–11.

Toepfl, S., Siemer, C., Saldaña-Navarro, G. and Heinz, V. (2014). Overview of pulsed electric fields processing for food. *In:* Sun, D.-W. (ed.) *Emerging Technologies for Food Processing* (2nd Ed). San Diego, CA: Academic Press, pp. 93–114.

Tomašević, I. (2015). The effect of intense light pulses on the sensory quality and instrumental color of meat from different animal breeds. *Biotechnology in Animal Husbandry,* **31**(2), 273–281.

Turantaş, F., Kılıç, G. B. and Kılıç, B. (2015). Ultrasound in the meat industry: General applications and decontamination efficiency. *International Journal of Food Microbiology,* **198**, 59–69.

Wang, J. M., Zhuang, H., Hinton, A. and Zhang, J. H. (2016). Influence of in-package cold plasma treatment on microbiological shelf life and appearance of fresh chicken breast fillets. *Food Microbiology,* **60**, 142–146.

Xiong, G., Zhang, L., Zhang, W. and Wu, J. (2012). Influence of ultrasound and proteolytic enzyme inhibitors on muscle degradation, tenderness, and cooking loss of hens during aging. *Czech Journal of Food Sciences,* **30**(3), 195–205.

Yong, H. I., Lee, H., Park, S. et al. (2017). Flexible thin-layer plasma inactivation of bacteria and mold survival in beef jerky packaging and its effects on the meat's physicochemical properties. *Meat Science,* **123**, 151–156.

Zayas, J. and Orlova, T. (1970). The application of ultrasonic vibrations for tenderisation of meat. *Izvestia vysshykh unhebnykh zavedeniy,* **4**, 54–56.

Zhao, Y.-Y., Wang, P., Zou, Y.-F. et al. (2014). Effect of pre-emulsification of plant lipid treated by pulsed ultrasound on the functional properties of chicken breast myofibrillar protein composite gel. *Food Research International,* **58**, 98–104.

Ziuzina, D., Patil, S., Cullen, P. J., Keener, K. and Bourke, P. (2014). Atmospheric cold plasma inactivation of *Escherichia coli*, *Salmonella enterica* serovar Typhimurium and *Listeria monocytogenes* inoculated on fresh produce. *Food Microbiology,* **42**, 109–116.

Downloaded From Vet-eBooks.com



Taylor & Francis
Taylor & Francis Group
http://taylorandfrancis.com

Downloaded From Vet-eBooks.com

10

Reduction of Contaminant Content in Processed Meats

Peter Šimko

CONTENTS

10.1 Introduction 320
10.2 Polycyclic Aromatic Hydrocarbons 320
10.2.1 Characterization of Polycyclic Aromatic Hydrocarbons 320
10.2.2 Formation of PAHs during Meat Processing 321
10.2.2.1 Smoking 321
10.2.2.2 Grilling 324
10.2.3 Effect of PAHs on Living Organisms 325
10.2.4 Legislative Aspects and International Normalization of PAHs in Smoked Meat and Liquid Smoke Flavors 326
10.2.5 Occurrence of PAHs in Smoked Meats 328
10.2.6 Reduction of PAH Concentrations in LSFs 329
10.2.6.1 Characterization of Physicochemical Processes Taking Place between Liquid Media and LDPE 335
10.2.7 Reduction of PAH Content in Processed Meats 339
10.2.7.1 Reduction of PAH Content during Smoking 339
10.2.7.2 Reduction of PAH Content by Their Photodecomposition 340
10.2.7.3 Reduction of PAH Content by Cooking of Smoked Sausages 342
10.2.7.4 Reduction of PAH Content in Roasted Duck by Packaging into LDPE 344
10.2.7.5 Reduction of PAH Content in Smoked Sausages by Packaging into LDPE 344
10.3 General Conclusions 348
Acknowledgment 349
References 349

Downloaded From Vet-eBooks.com

10.1 Introduction

Meat is an important source for protein including essential amino acids, fat, minerals, vitamins, and other functional nutrients (Biesalski, 2005), and therefore it has been consumed by man to ensure his fundamental nutritional needs, which—finally—has led to the development of modern mankind. There is no doubt that the occasional surplus of meat obtained from hunted animals had become in the past a base for development of simple preservation procedures (such as drying, smoking, salting, fermentation, etc.), which made it possible for mankind to survive unfavorable times.

Although up-to-date meat processing procedures are based on current scientific and professional knowledge, the contamination of processed meat products is still a serious problem due to the fact that some compounds, especially those highly harmful to human health, are being formed permanently in the meat matrix during its processing. Therefore, there is a strong interest to find prevention procedures which could lead to minimization of contaminant formation during meat processing and storage, or look for and apply procedures to eliminate formed contaminants from final products, respectively. Thus, this chapter deals with modern procedures which decrease contaminant content in processed meats to ensure that human exposure to these compounds is as low as possible.

10.2 Polycyclic Aromatic Hydrocarbons

10.2.1 Characterization of Polycyclic Aromatic Hydrocarbons

Polycyclic aromatic hydrocarbons (PAHs) are organic compounds containing only hydrogen and carbon elements in a molecule, consisting of two or more condensed aromatic rings linked together, either *cata*annellated (linearly or angularly) or *peri*condensed. *Cata*condensed PAHs are alternant systems containing only six-membered rings and closed shell systems, with all bonding orbitals occupied by two electrons. The entire group of *cata*-condensed PAHs can be further divided into branched and nonbranched systems. Branched systems are thermodynamically more stable and chemically less reactive than nonbranched systems of the same size. Conversely, *peri*condensed PAHs are either closed shell systems or neutral free orbitals, in which at least one electron is in a nonbonding orbital. Free radicals of this type are stable only if the systems have an odd number of carbon atoms. In addition, *peri*condensed PAHs can be further divided into alternant and nonalternant, depending on the presence of five- or six-membered rings in the molecule (Guillén, 1994). PAHs have a strong lipophilic character and therefore they have a tendency to migrate into adipose tissue or nonpolar

Downloaded From Vet-eBooks.com

parts of meat products (Šimko et al., 1993). In general, the stability of PAHs depends on environmental conditions and they are, in general, relatively stable in the dark. However, light catalyzes their decomposition by oxidation reactions, which happens especially in ultraviolet (UV) light conditions; therefore photodecomposition is a main route of PAH degradation in the environment (Skláršová et al., 2012), when numerous intermediate oxy- and hydroxy compounds are being formed (Bednáriková et al., 2011).

10.2.2 Formation of PAHs during Meat Processing

10.2.2.1 Smoking

In general, smoke is a polydisperse mixture of liquid and solid components with a diameter of 0.08–0.15 μm in gaseous phase of air, carbon oxide, carbon dioxide, water vapor, methane, and other gases. Smoke has a variable composition that depends on various conditions such as procedure and temperature of smoke generation, origin and composition of wood, water content in wood, etc. (Sikorski, 2004). So far, up to 1100 various chemical compounds have been identified and published in literature (Wilms, 2000). The smoking treatment itself is based on successive deposition of compounds such as phenol derivatives, carbonyls, organic acids, and their esters, lactones, pyrazines, pyrols, and furan derivatives on the food surface with their subsequent migration into the food bulk (Maga, 1987). Smoke is generated during thermal combustion of wood, consisting roughly of 50% cellulose, 25% hemicellulose, and 25% lignin, with limited access to oxygen. The thermal combustion of these wood components proceeds at 180°C–300°C, 260°C–350°C, and 300°C–500°C, respectively. However, the decomposition of the wood (as a whole) proceeds also at temperatures reaching up to 900°C and in large excess of oxygen even at 1200°C. The smoke produced at 650°C–700°C is richest in components able to impart desirable organoleptic properties to the treated products. The temperature of generation of smoke can be decreased by increasing the humidity of woods (Tóth, and Potthast, 1984; Šimko, 2005).

Apart from the compounds mentioned above, many other compounds are also formed during smoke generation. One of the most important groups which is harmful to human health is PAHs. These are being formed during the thermal decomposition of wood, especially with limited access of oxygen in the range of 500°C–900°C (Bartle, 1991).

A mechanism of PAH formation based on reactions with stable and radical species, including single-ring aromatics, other PAHs, and acetylene, followed by the nucleation or inception of small soot particles with subsequent soot growth by coagulation and mass addition from gas phase species, and carbonization of the particulate materials, is generally accepted (Richter and Howard, 2000). A rough scheme of PAH formation during thermal combustion of organic matter is shown in Figure 10.1. During the formation of single aromatic rings, propargyl (C_3H_3) and cyclopentadienyl (C_5H_5) radicals

Downloaded From Vet-eBooks.com

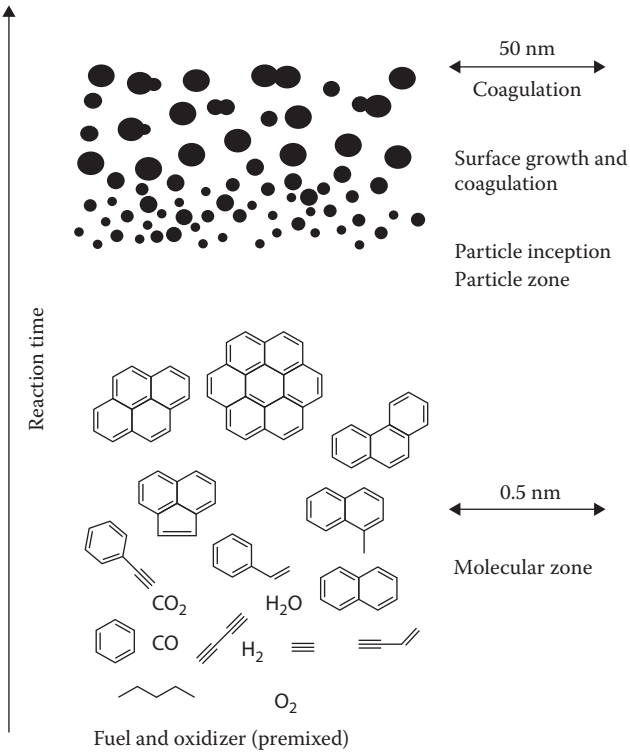


FIGURE 10.1
Simplified scheme of PAH formation during thermal combustion of organic matter. (Reprinted from *Prog. Energy Combust. Sci.*, 26, Richter, H. and Howard, J. B., Formation of polycyclic aromatic hydrocarbons and their growth to soot—A review of chemical reaction pathways, 565–608. Copyright 2000 with permission from Elsevier.)

play a significant role, as follows from Figures 10.2 and 10.3 (McEnally et al., 2006). The temperature of smoke generation plays a decisive role because the amount of PAHs contained in smoke increases linearly with the temperature of smoke generation in the range of 400°C–1000°C (Tóth, and Blaas, 1972). Apart from the formation itself, the temperature also affects structure and number of PAH homologues. For example, the number of PAHs presented in smoked fish can reach the value of 100 various compounds (Grimmer and Böhnke, 1975).

To achieve a rich deep-brown coloring on the surface and very strong aroma profile formation of smoked meat products, the time of smoking is considerably prolonged (Šimko, 2011). Such products are frequently termed as "black-smoked" or "farmhouse smoked," respectively. However, these products also have higher contents of PAHs (Šimko et al., 1991; Roda et al., 1999; Wilms, 2000). "Wild" or direct smoking under uncontrolled technological conditions, or at nonexisting legislative measures, which is typical,

Downloaded From Vet-eBooks.com

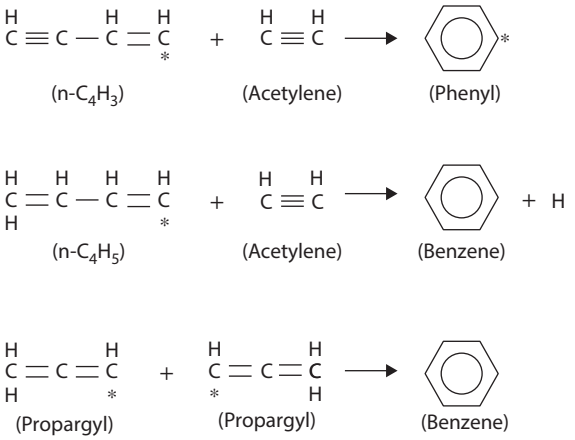


FIGURE 10.2
Schematic diagrams of three important reactions that form single-ring aromatic hydrocarbons from aliphatic hydrocarbons. (Reprinted from *Progress in Energy and Combustion Science*, 32, McEnally, C. S. et al., Studies of aromatic hydrocarbon formation mechanisms in flames: Progress towards closing the fuel gap, 247–294. Copyright 2006 with permission from Elsevier.)

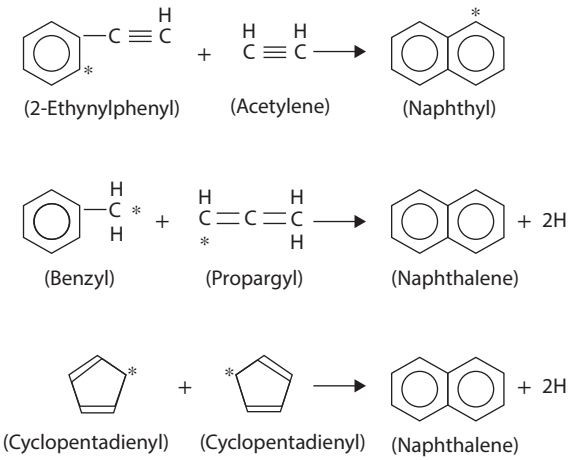


FIGURE 10.3
Schematic diagrams of three important reactions that form two-ring aromatic hydrocarbons from single-ring aromatic and aliphatic hydrocarbons. (Reprinted from *Progress in Energy and Combustion Science*, 32, McEnally, C. S. et al., Studies of aromatic hydrocarbon formation mechanisms in flames: Progress towards closing the fuel gap, 247–294. Copyright 2006 with permission from Elsevier.)

especially, for households and developing countries, leads to enormous PAH contents in smoked foods (Afolabi et al., 1983; Alonge, 1987, 1988; Wretling et al., 2010; Fasano et al., 2016).

Downloaded From Vet-eBooks.com

A relatively new alternative to traditional smoking is aromatization of foods by liquid smoke flavorings (LSFs). It was in the late 1800s when Kansas pharmacist Ernest H. Wright developed and patented a liquid smoke flavor prepared from smoke condensate. More recently, the development of aqueous smoke condensates, also known as "liquid smoke," has resulted in the widespread use of LSFs in many food products to reach the same smoke organoleptic profiles. Today, LSFs are being added to a great variety of products including all forms of meat products (Emmerson, 2011). A main advantage of LSF application lies in the fact that LSFs contaminate meat products far less in comparison to meat products aromatized by traditional procedures because PAHs are removed effectively from smoke condensates during LSF production and therefore real contamination is frequently bellow detection limits (Šimko et al., 1992).

10.2.2.2 Grilling

A formation of PAHs during charcoal broiling of beef steaks was observed for the first time by Lijinsky and Shubik (1964) when average benzo[*a*]pyrene (BaP) content was found at the level of 8 μg/kg. Later, Lijinsky and Ross (1967) revealed that PAHs could be formed during the pyrolysis of the fat that drips down on the hot coals. Larsson et al. (1983) grilled frankfurters over various heat sources such as log fire, log fire embers, cone fire, charcoal fire, electric oven, and frying pan. They found that grilling over log fire resulted in extremely high PAH levels, up to 212 μg/kg BaP. When the grilling was carried out over the embers, the average level of BaP was only 7.7 μg/kg. Relatively high PAHs levels (an average of 17.6 μg of BaP μg/kg) were found in frankfurters grilled over smoldering spruce or pine cones. The BaP levels in charcoal-grilled frankfurters did not exceed 1 μg/kg, whereas charcoal-grilled whole meat samples contained 2.3–6.1 μg/kg probably due to the fact that the fat in frankfurters is not "free, but locked" in an oil/water type of emulsion system, and therefore release of fat in comparison to muscle meat is less possible (Šimko and Knežo, 1992). The lowest formation of PAH was observed during application of electric oven and frying pan. Application of indirect sources of heat as an effective tool for minimization of PAH content has also been confirmed by Farhadian et al. (2011) using two different types of treatments, preheating (steam and microwave) and wrapping (aluminum and banana leaf) of the meat samples prior to charcoal grilling. Apart from fat, there are also other precursors of PAHs such as ergosterol (Cai et al., 2008) and steroid hormones (androsterone, cholesterol, estrone, and estradiol) which are prone to decompose in the thermal range 300°C–900°C and provide substantial amounts of PAHs (Christy et al., 2011). A possible method of PAH formation from cholesterol is shown in Figure 10.4.

Downloaded From Vet-eBooks.com

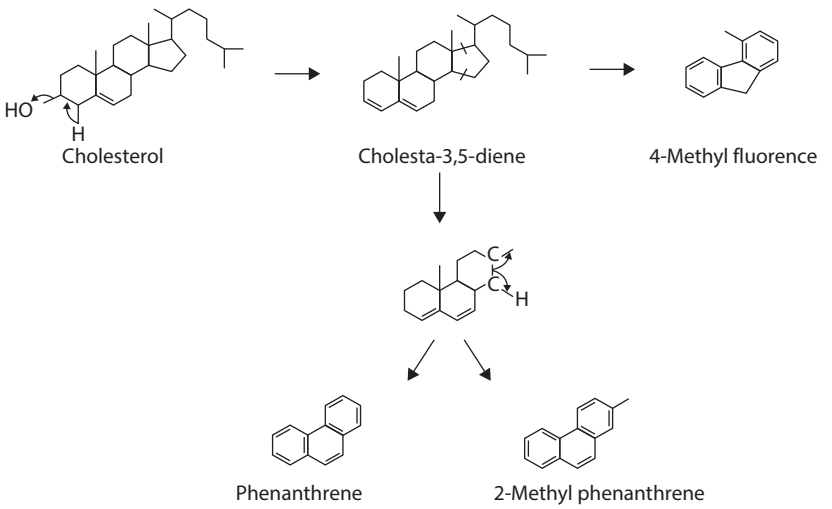


FIGURE 10.4
The pathways of formation from cholesterol to phenanthrene, methylphenanthrene, fluorine, and methylfluorene. (Reprinted from *Food Chemistry*, 124, Christy, A. A. et al., Pyrolytic formation of polyaromatic hydrocarbons from steroid hormones, 1466–1472. Copyright 2011 with permission from Elsevier.)

10.2.3 Effect of PAHs on Living Organisms

The fact that chemicals could cause cancer arose from the observations of Percival Pott of St. Bartholomew's Hospital in London in 1775 when he had noted high incidence of cancer of the scrotum among chimney sweepers who often had to climb up inside chimneys to sweep the soot down. Although he deduced correctly that the soot was responsible for the cancer, in this time, it was not possible to find out the compounds responsible for such serious tissue damage. In 1920, Japanese workers discovered that painting extracts of soot onto the skin of mice caused tumors of the skin. In 1929, the first pure chemical carcinogenic compound—dibenzo[a,h]pyrene (DBa,hP)—was isolated from soot extracts at Chester Beatty Research Institute by Kennaway. In 1953, Doll, on the basis of wide epidemiological and statistical analysis, proved that cigarette smoking was a prime cause of lung cancer. Careful analysis of the smoke and tar obtained from cigarettes showed that it contained many carcinogenic PAHs from which BaP was assessed as the most dangerous compound (Šimko, 2002). According to current knowledge, some PAHs are able to interact in organisms with enzymes (such as aryl hydrocarbon hydroxylases) to form PAH dihydrodiol derivates. These reactive products (so-called "bay region" dihydrodiol epoxides) are believed to be the ultimate carcinogens that are able to form covalently bounded adducts with proteins and nucleic acids. In general, DNA adducts are thought to initiate cell mutation which results

Downloaded From Vet-eBooks.com

in malignancy (Bartle, 1991). The direct mutagenic potential of 14 PAHs and PAH-containing fractions isolated from smoked and charcoal broiled samples was studied toward strains TA 98 and TA 100 using the Ames test. The most potential mutagenicity was observed on PAH fractions isolated from smoked fish, treated before smoking with nitrites in an acid solution (Kangsadalampai et al., 1997). While various compounds have different carcinogenic effects to living organisms, there have been attempts to express objectively the real risk of PAH exposition using so called toxic equivalency factors (Nisbet and La Goy, 1992). On the basis of data from more than 800 epidemiological studies that investigated the association of cancer with consumption of red meat or processed meat (processed means cured, fermented, and smoked), in many countries from several continents, with diverse ethnicities and diets, it was stated that consumption of 50 g processed meat per day can increase risk of colorectal cancer incidence by 18%. Therefore, the Working Group of International Agency for Research on Cancer (IARC) classified processed meat as "carcinogenic to humans" and added it to group 1, which includes dangerous compounds such as aflatoxins, benzene, asbestos, vinyl chloride, etc. (Bouvard et al., 2015).

10.2.4 Legislative Aspects and International Normalization of PAHs in Smoked Meat and Liquid Smoke Flavors

With regard to the information regarding the harmful effects of PAHs on living organisms, some European countries (e.g., Germany, Slovak Republic, Czech Republic) have enacted maximum limits of these compounds in smoked meat products at different levels in the past. To simplify such problems associated with the variability of PAH occurrence, BaP had been accepted, in general, as the indicator of total PAH presence in smoked foods, even in spite of the fact that BaP constitutes only between 1% and 20% of the total carcinogenic PAHs (Andelman and Suess, 1970). To unify approach to assessment of PAHs in food products in the European Union (EC,2002), the European Commission (EC) adopted the limitation of BaP content at a level of 5 μg/kg in smoked meats, smoked meat products, muscle meat of smoked fish, and smoked fishery products (Commission Regulation, 1881/2006/EC). Apart from this, the EC has also adopted either Directive 2005/10/EC laying down the sampling methods and the methods of analysis for the official control of BaP levels in foodstuffs (Commission Directive, 2005/10/EC), or Recommendation 2005/108/EC on the further investigation into the levels of PAHs in certain foods such as benzo[*a*]anthracene (BaA), benzo[*b*]fluoranthene (BbF), benzo[*j*]fluoranthene (BjF), benzo[*k*]fluoranthene (BkF), benzo[*g,h,i*]perylene (Bg,h,iP), chrysene (Chr), BaP, cyclopenta[*c,d*] pyrene (Cc,dP), DahA, dibenzo[*a,e*]pyrene (DBa,eP), DBa,hP, dibenzo[*a,i*] pyrene (DBa,iP), dibenzo[*a,l*]pyrene (DBa,lP), indeno[1,2,3-*cd*]pyrene (IcdP), and 5-methylchrysene (Commission Recommendation, 2005/108/EC). Moreover, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) has

Downloaded From Vet-eBooks.com

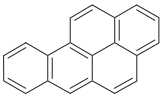
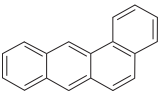
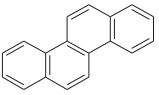
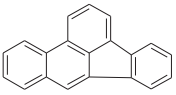
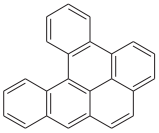
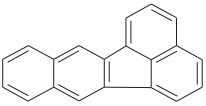
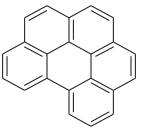
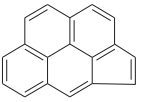
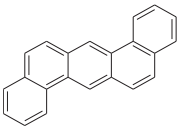
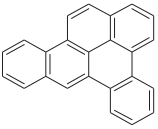
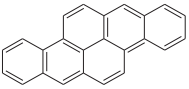
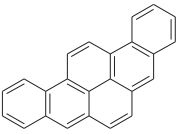
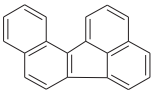
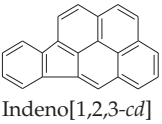
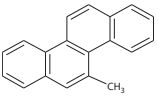
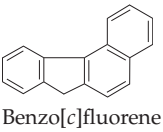
defined another compound benzo[*c*]fluorene (BcF), which should also be monitored with regard to its effects to living organisms (Report of the Joint FAO/WHO Expert Commission on Food Additives, 2005). Structural formulas of these compounds are shown in Table 10.1. Later, Commission Directive 2005/10/EC was replaced by Commission Regulation (EC) No 333/2007 and this document has been amended by Commission Regulation (EC) No 836/2011 (Suranová et al., 2015).

On the basis of a comprehensive survey of PAH presence in foods, the European Food Safety Authority (2008) proposed—apart from BaP—also to include other reference compounds, such as BaA, Chr, and BbF. Consequently, either maximum content of BaP or a sum of all four compounds called "PAH4" were established by the European Commission's Regulation No. 835/2011a, b for smoked, heat-treated meat and meat products. Limits relating to processed meat are shown in Table 10.2.

Concerning LSFs, the EC has adopted Regulation 2065/2003 relating to the production of smoke flavorings intended to be used for food flavoring (Commission Regulation, 2065/2003/EC). This regulation has limited the maximum acceptable concentrations of BaP at 10 µg/kg and BaA at 20 µg/kg in LSFs. Following in this course, the EC recently established the EU list of

TABLE 10.1

Structural Formulas of PAHs Listed in the Recommendation of EC No. 2005/108/EC and Benzo[*c*]Fluorene set by JECFA

 Benzo[*a*]pyrene	 Benzo[*a*]anthracene	 Chrysene	 Benzo[*b*]fluoranthene
 Dibenzo[*a,l*]pyrene	 Benzo[*k*]fluoranthene	 Benzo[*g,h,i*]peryléne	 Cyklopenta[*c,d*] pyréne
 Dibenzo[*a,h*] antracéne	 Dibenzo[*a,e*]pyrene	 Dibenzo[*a,h*]pyrene	 Dibenzo[*a,i*]pyrene
 Benzo[*j*]fluoranthene	 Indeno[1,2,3-*cd*] fluoranthene	 5-methylchrysene	 Benzo[*c*]fluorene

Downloaded From Vet-eBooks.com

TABLE 10.2

Maximum Allowed Levels of PAHs, Set by Commission Regulation (EU) No. 835/2011 for Meat Products

	Maximum Levels (μg/kg)	
Foodstuffs	**Benzo[*a*]pyrene**	**Sum of benzo[*a*]pyrene, benzo[*a*]anthracene, benzo[*b*] fluoranthene, and chrysene[a] (ΣPAH4)**
Smoked meat and meat products	2.0	12.0
Muscle meat of smoked fish and smoked fishery products. The maximum level for smoked crustaceans applies to muscle meat from appendages and abdomen. In case of smoked crabs and crab-like crustaceans (*Brachyura* and *Anomura*), it applies to muscle meat from appendages.	2.0	12.0
Smoked sprats and canned smoked sprats[b] (*Sprattus sprattus*); bivalve molluscs (fresh, chilled, or frozen); and heat-treated meat and meat products[c] sold to the final consumer	5.0	30.0
Bivalve molluscs (smoked)	6.0	35.0

[a] Lower bound concentrations are calculated on the assumption that all the values of the four substances below the limit of quantification are zero.

[b] For the canned product the analysis shall be carried out on the whole content of the can.

[c] Meat and meat products that have undergone a heat treatment potentially resulting in formation of PAH, that is, only grilling and barbecuing.

authorized smoke flavoring primary products for use as such in or on foods and/or for the production of derived smoke flavorings (Commission Implementing Regulation, 1321/2013). Finally, directive 88/388/EEC has limited the maximum residual levels of BaP at 0.03 μg/kg in foodstuffs flavored by LSF (Commission Directive, 88/388/EC).

For international trade purposes, the Joint Expert Committee for Food Additives and Contaminants of the FAO and WHO has adopted a specification that tolerates the concentration in liquid smoke flavors at the levels of 10 μg/kg for BaP and 20 μg/kg for BaA, respectively (Report of the Joint FAO/WHO Expert Commission, 1987).

10.2.5 Occurrence of PAHs in Smoked Meats

In general, smoked meat products are believed to be the food products with the highest content of PAHs, especially in cases of production via traditional procedures, when a food is put near the fireplace for several days and directly exposed (Djinovic et al., 2008; Lorenzo et al., 2010; Škaljac

Downloaded From Vet-eBooks.com

et al., 2014; Zelinkova and Wenzl, 2015). This fact documents data summarized in Table 10.3 showing some findings of PAH4 content in meat products smoked under "intensive" conditions (Purcaro et al., 2013). As follows from Table 10.3, some data are really alarming and confirm clearly the relationship between the direct way of hot smoking and PAH4 content in smoked products as well as an urgent need to find new effective procedures for lowering of PAH content in these food products.

10.2.6 Reduction of PAH Concentrations in LSFs

As mentioned above, LSFs have far lower PAH concentrations and therefore contamination of aromatized meat products could be negligible due to the fact that real PAH contents are well under legislative limits. However, any presence of hazardous/carcinogenic compounds in foods and food additives should never be acceptable while there are available procedures which are able even to remove traces of PAHs. From this point of view, production of LSFs "free of PAHs" is really an attractive challenge for chemical and food scientists to protect humans entirely against exposure to these compounds.

The first study dealing with the possibility of elimination of PAHs from LSFs was carried out by Šimko and Bruncková (1993). In their experiments, liquid smoke flavor was spiked with six PAH compounds, pyrene (Py), BaA, dibenzo[*a,c*]anthracene (DBa,cA), benzo[*e*]pyrene (BeP), BaP, and dibenzo[*a,h*] anthracene DBa,hA at a level of total concentration 45.6 μg/kg and filled into receptacles made of low-density polyethylene (LDPE). As follows from high-performance liquid chromatography (HPLC) records of LDPE and LSF immediately after spiking (Figure 10.5), LDPE was free of PAHs. However, after 14 days of the experiment, PAHs in LSF were "lost" and, on the contrary, they were found in LDPE (Figure 10.6). This process was very effective, because during the experiment total concentrations of PAHs in LSFs dropped by two orders (Figure 10.7) and reached values of 0.73 μg/kg, which was practically under limits of detection. Authors concluded that the elimination was brought about by sorption of PAHs on the LDPE surface due to their similar, nonpolar character. Later, Šimko et al. (1994) tried to reveal the mechanism of this process, and therefore they allowed interaction of PAHs contained in LSFs with LDPE receptacles of cylindrical shapes. Because they observed the same courses of PAH elimination, they made an assumption that this process could be dominantly directed by diffusion.

As the LSFs were not mixed during the experiment, it was a given assumption that the factor limiting the rate of PAH removal from LSFs is the diffusion in the liquid media. Therefore, the authors applied Fick's laws dealing with transportation of mass in a media using the relationship derived for diffusion in a cylinder by Cranck (1976):

Downloaded From Vet-eBooks.com

TABLE 10.3

Some Findings of PAH Occurrence in Smoked Meat Products

	Content (μg/kg)										Source
	BaA		Chr		BbF		BaP		ΣPAH4		
Sample/Number of Samples	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.
Smoked ham/16 smoked directly	<0.3	76.5	<0.3	71.4[a]	<0.3	23.9	<0.3	36.9	<0.3	208.7	Wretling et al. (2010)
Smoked bacon/5 smoked directly	1.9	28.9	1.5	33.4[a]	0.3	10.2	0.4	15.9	<0.3	88.4	
Smoked elk and reindeer meat/1 smoked directly	11.7		13.0[a]		4.5		6.6		35.8		
Smoked meat/3	1.51	2.37	1.56	1.64	0.07	0.15	0.36	0.63	3.5	4.79	Roseiro et al. (2012)
Smoked blood sausages/3	0.88	3.28	0.61	4.94	0.03	0.23	0.32	0.39	1.84	8.84	
Smoked frankfurter-type sausages/10	0.82	1.69	0.74	1.57[a]	0.27	0.61	0.32	0.80	2.06	4.67	Hitzel et al. (2013)
Smoked mini-salamis/9	0.91	2.06	0.93	1.79[a]	0.27	0.62	0.23	0.60	2.34	5.07	
Smoked pork breast/18	0.06	9.60	0.10	11.24	<0.05	4.28	<0.05	2.99	0.16	28.10	Rozentale et al. (2015)
Smoked pork/14	0.05	10.60	0.10	11.07	<0.05	4.60	<0.05	6.03	0.15	27.25	
Smoked pork speck/10	0.13	12.23	0.25	14.50	0.11	4.29	<0.05	3.62	0.48	34.65	
Smoked sausages/21	0.08	14.21	0.13	11.19	<0.05	4.09	<0.05	4.17	0.22	33.66	
Crackers or small sausages/10	0.19	13.13	0.26	12.79	0.13	4.03	0.05	4.63	0.63	34.58	
Spanish smoked meat product "chorizo"/12	ND		ND		ND		0.9	3.21	0.9	3.21	Ledesma et al. (2015)
Smoked garlic pork sausages "chorizo"/8 smoked directly	11	522	21	649	3.2	178	3.1	98	38.3	1387.3	Fasano et al. (2016)

Source: Reprinted from *Food Chemistry*, 201, Semanová, J. et al., Elimination of polycyclic aromatic hydrocarbons from smoked sausages by migration into polyethylene packaging, 1–6. Copyright 2016 with permission from Elsevier.

[a] The sum of Chr and triphenylene.

ND, not determined.

Downloaded From: Vet-eBooks.com

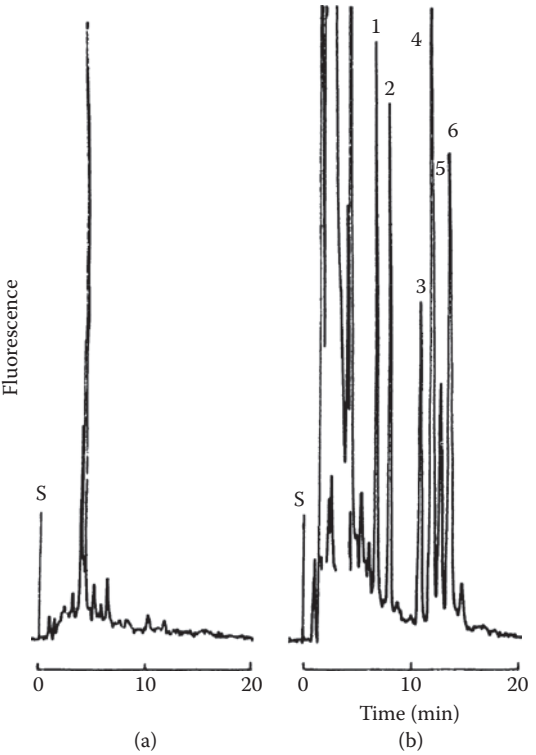


FIGURE 10.5
Chromatograms of PAH analysis in LDPE (a) and LSFs (b) before storage experiment. 1, Pyrene; 2, benzo[*a*]anthracene (BaA); 3, dibenzo[*a,c*]anthracene (DB(a,c)A); 4, benzo[*e*]pyrene (BeP); 5, benzo[*a*]pyrene (BaP); 6, dibenzo[*a,h*]pyrene (DB(a,h)A). (Reprinted from Šimko, P. and Bruncková, B. Lowering of concentration of polycyclic aromatic hydrocarbons in a liquid smoke flavour by sorption into polyethylene packaging. *Food Additives and Contaminants*, 10, (1993):257–263. With permission. Copyright Taylor and Francis © 1993.)

$$\frac{n_t}{n_\infty} = 1 - \sum_{n=1}^{\infty} \frac{4}{a^2 \alpha_n^2} \exp[-D\alpha_n^2 t], \tag{10.1}$$

where n_t is the amount of diffused substance, which left the LSF as a consequence of the diffusion into the LDPE at time t, and n_∞ is the amount of substance, which corresponds to infinite time. D is the diffusion coefficient, a is the radius of the cylinder, and α_n are the roots of the equation:

$$J_0(a \cdot \alpha_n) = 0 \tag{10.2}$$

where J_0 is the zero-order first-class Bessel function.

However, in the experiment, the amount of substance sorbed into the LDPE was not measured, but rather the amount left in the cylindrical liquid

Downloaded From Vet-eBooks.com

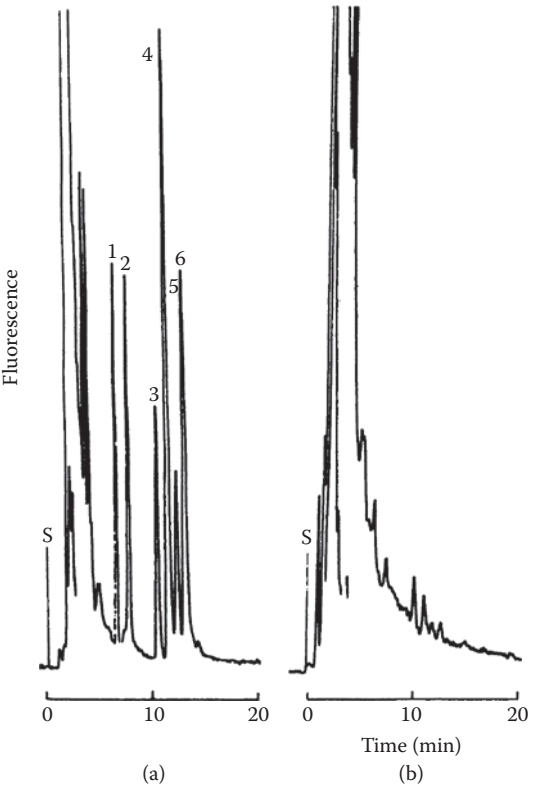


FIGURE 10.6
Chromatograms of PAH analysis in LDPE (a) and LSFs (b) after storage experiment. 1, Py; 2, BaA; 3, DB(a,c)A; 4,BeP; 5, BaP; 6, DB(a,h)A. The chromatogram in (b) was obtained at two times more detector sensitivity that that in (a). (Reprinted from Šimko, P. and Bruncková, B. Lowering of concentration of polycyclic aromatic hydrocarbons in a liquid smoke flavour by sorption into polyethylene packaging. *Food Additives and Contaminants*, 10, (1993): 257–263. With permission. Copyright Taylor and Francis © 1993.)

bulk—LSF. Therefore, after recalculation of the amount of substance, the concentration, Equation 10.1, could be modified to

$$c_t = c_0 \sum_{n=1}^{\infty} \frac{4}{a^2 \alpha_n^2} \exp[-D\alpha_n^2 t], \tag{10.3}$$

where c_0 and c_t are concentrations at time $t = 0$ and t.

Diffusion coefficients of PAHs (Table 10.4) were obtained by the nonlinear least squares method by minimizing the sum of squares of differences between the concentrations of PAHs measured experimentally (A rows of Table 10.5) and those calculated by Equation 10.3 (B rows of Table 10.5). The

Downloaded From Vet-eBooks.com

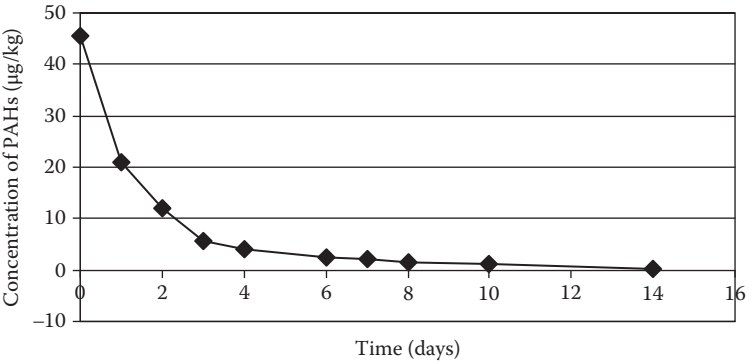


FIGURE 10.7
Reduction of PAH concentration in LSFs stored in LDPE receptacles during 14 days of storage. (Reprinted from Šimko, P. and Bruncková, B. Lowering of concentration of polycyclic aromatic hydrocarbons in a liquid smoke flavour by sorption into polyethylene packaging. *Food Additives and Contaminants*, 10, (1993): 257–263. With permission. Copyright Taylor and Francis © 1993).

TABLE 10.4

Values of Diffusion Coefficients Calculated by Equation 10.3 for Individual and Sum of PAHs in LSFs

Compound	Values of Diffusion Coefficients $D \times 10^2$ (m²/h)
Py	8.7
BaA	4.6
DB(a,c)A	7.4
BeP	5.8
BaP	7.2
DB(a,h)A	7.6
Total	6.3

Source: Reprinted with permission from *Food Chemistry*, 50, Šimko, P. et al., Kinetics of polycyclic aromatic hydrocarbons sorption from liquid smoke flavor into low density polyethylene packaging, 65–68. Copyright 1994 with permission from Elsevier.

minimization was carried out by the simplex method (Nelder and Mead, 1965). Minimized parameters were c_0 and D. The parameter c_0 was minimized because the error of determination of this concentration in trace analysis is usually relatively high, and might affect all values of concentrations calculated. In calculations, the first 20 terms of Equation 10.3 were taken into account. The values α_n were taken from tables (Abramowitz and Stegun, 1964). The agreement between experimental and calculated values of PAH concentrations was very good as can be seen from Table 10.5 when

Downloaded From Vet-eBooks.com

TABLE 10.5

Changes in Concentrations of PAHs (μg/kg) in LSFs Followed during the Experiment

Compound		Time of Storage (h)										
		0	**27**	**68**	**92**	**98**	**116**	**121.5**	**141**	**145.5**	**164**	**σ**
Py	A	14.8	2.45	0.65	0.58	0.50	0.50	0.29	0.29	0.25	ND	0.33
	B	14.7	2.70	0.36	0.11	0.08	0.03	0.03	0.01	0.01	0.00	
BaA	A	26.8	8.23	3.40	1.54	1.79	1.43	1.03	0.99	0.75	ND	0.47
	B	26.5	9.12	3.09	1.64	1.41	0.88	0.74	0.46	0.41	0.24	
DB(a,c)A	A	17.8	3.55	0.78	0.67	0.47	0.43	0.25	0.16	0.13	ND	0.25
	B	17.8	3.80	0.64	0.23	0.17	0.08	0.06	0.03	0.02	0.01	
BeP	A	11.0	3.25	0.63	0.42	0.30	0.30	0.13	0.08	0.07	ND	0.09
	B	11.0	3.16	0.82	0.38	0.31	0.17	0.14	0.08	0.07	0.04	
BaP	A	10.2	2.25	0.44	0.32	0.26	0.21	0.16	0.08	0.07	ND	0.10
	B	10.1	2.34	0.44	0.17	0.13	0.06	0.05	0.02	0.02	0.01	
DB(a,h)A	A	10.7	2.20	0.43	0.31	0.24	0.23	0.10	0.06	0.06	ND	0.10
	B	10.6	2.29	0.39	0.14	0.11	0.05	0.04	0.02	0.01	0.01	
Total	A	91.1	21.9	6.33	3.83	3.54	3.11	1.96	1.67	1.32	ND	1.43
	B	90.7	24.0	5.56	2.37	1.91	1.03	0.80	0.41	0.35	0.18	

Source: Reprinted with permission from *Food Chemistry,* 50, Šimko, P. et al., Kinetics of polycyclic aromatic hydrocarbons sorption from liquid smoke flavor into low density polyethylene packaging, 65–68. Copyright 1994 with permission from Elsevier.

ND, not detectable.

A Rows, experimentally obtained data; B Rows, values obtained by Equation 10.3.

TABLE 10.6

Values of Diffusion Coefficients Calculated by Equation 10.3 for Individual and Sum of PAHs in LSFs

	Total Amount of PAHs (ng) in	
Time of Experiment (h)	**LSF**	**LDPE**
0	1274.7	ND
27	295.3	884.1
68	88.6	1067.4
92	53.6	1055.7
98	49.6	1114.8
116	43.5	1095.7
121.5	27.4	1122.6
141	23.3	1138.8
145.5	18.5	1163.8
164	ND	1187.3

Source: Reprinted from Šimko P. et al. Kinetics of polycyclic aromatic hydrocarbons sorption from liquid smoke flavour into low density polyethylene packaging. *Food Chemistry,* 50, (1994):65–68. With permission. Copyright Elsevier © 1994.

ND, not detectable.

Downloaded From Vet-eBooks.com

comparing rows A and B for the same compound, and it was also demonstrated by the low values of standard deviations (σ) in the right-hand column of Table 10.5. By numerical solution of Equation 10.3 for known values of the diffusion coefficients D and given initial concentration c_0, it is possible to calculate the time at which PAH concentration decreases to a required value c_t. This means that, by Equation 10.3, it is possible to predict the time necessary for lowering PAH concentrations to an acceptable level. For example, if initial concentration c_0 is equal to 10.2 μg of BaP/kg of LSF, it can be calculated that the acceptable concentration, c_t = 9.90 μg/kg, accepted by JECFA (Report of the Joint FAO/WHO Expert Commission on Food Additives, 1987) would be reached within 51 minutes of the experiment. The evidence about sorption processes of PAHs onto LDPE is shown in Table 10.6, where the total amount of PAHs in both LSF and LDPE is compared. As follows from this table, the amount of PAHs "lost" in LSF during the experiment was possible to recover later from the LDPE.

10.2.6.1 Characterization of Physicochemical Processes Taking Place between Liquid Media and LDPE

By the theory of physical chemistry, the sorption of nonelectrolytes at the solid–solution interface may be viewed in terms of two somewhat different physical pictures. The first is that adsorption is essentially confined to a monolayer next to the solid surface, with the implication that succeeding layers are virtually normal bulk solution. The second picture is that of an interfacial layer or region, multimolecular in depth, over which a more slowly decaying interaction potential with the solid is present. From this point of view, adsorption from solution corresponds to a partition between a bulk and an interfacial phase (Adamson, 1990). To find definitively the mechanism of PAH removal from liquid media, water was spiked with three different PAHs and filled into a diffusion cell made of stainless steel. One of the walls was replaced by an LDPE sheet made up of five layers of LDPE films. The depth of PAH migration into this sheet was followed for 143 hours, using high-performance liquid chromatography with selective fluorimetric detection after extraction of PAHs from individual peeled-off films. As can be seen from Table 10.7, the PAH concentrations in water started to decrease immediately after beginning the experiment.

As follows from Table 10.8, the presence of PAHs in the first layer of the PE sheet was possible to determine already after 0.75 hours. With extension of the experiment time, the presence of PAHs was determined also in further layers, and after 69 hours, PAHs were determined in all layers of the PE sheet. The total view of the dependence of PAH migration into bulk polymer upon time is shown in Figure 10.8.

The solution of the second Fick's law for the diffusion of PAHs in polymer placed in the diffusion cell (Cranck, 1976; Moisan, 1985) gives the next equation:

Downloaded From Vet-eBooks.com

TABLE 10.7

Decrease in PAH Concentration (μg/kg) in Water at Various Time Intervals

Time (h)	Fl	Py	BaP
0.00	1350	537	191
0.75	1348	536	190
1.50	1344	534	190
2.50	1337	531	189
3.00	1324	525	188
69.0	1153	455	169
143.0	1054	417	165

Source: Reprinted from *Food Chemistry*, 64, Šimko, P. et al., Removal of polycyclic aromatic hydrocarbons from water by migration into polyethylene, 157–161. Copyright 1999 with permission from Elsevier.

TABLE 10.8

Concentration of PAHs (μg/kg) in Individual Layers of PE Sheet at Various Time Intervals

Time (hr)	Compound	Layer No. 1	Layer No. 2	Layer No. 3	Layer No. 4	Layer No. 5
0.00	Fl	ND	ND	ND	ND	ND
	Py	ND	ND	ND	ND	ND
	BaP	ND	ND	ND	ND	ND
0.75	Fl	523	ND	ND	ND	ND
	Py	244	ND	ND	ND	ND
	BaP	86	ND	ND	ND	ND
	Fl	647	43	ND	ND	ND
1.50	Py	268	25	ND	ND	ND
	BaP	98	11	ND	ND	ND
	Fl	894	57	39	ND	ND
2.50	Py	395	26	15	ND	ND
	BaP	146	6	3	ND	ND
3.00	Fl	1087	101	77	62	ND
	Py	458	42	22	14	ND
	BaP	193	16	10	7	ND
69.00	Fl	6614	1861	319	86	57
	Py	2275	632	122	31	22
	BaP	1242	129	27	17	5
143.00	Fl	7709	2154	1493	459	182
	Py	4009	1060	626	142	79
	BaP	2083	376	109	42	27

Source: Reprinted from *Food Chemistry*, 64, Šimko, P. et al., Removal of polycyclic aromatic hydrocarbons from water by migration into polyethylene, 157–161. Copyright 1999 with permission from Elsevier.

ND, not detectable.

Downloaded From Vet-eBooks.com

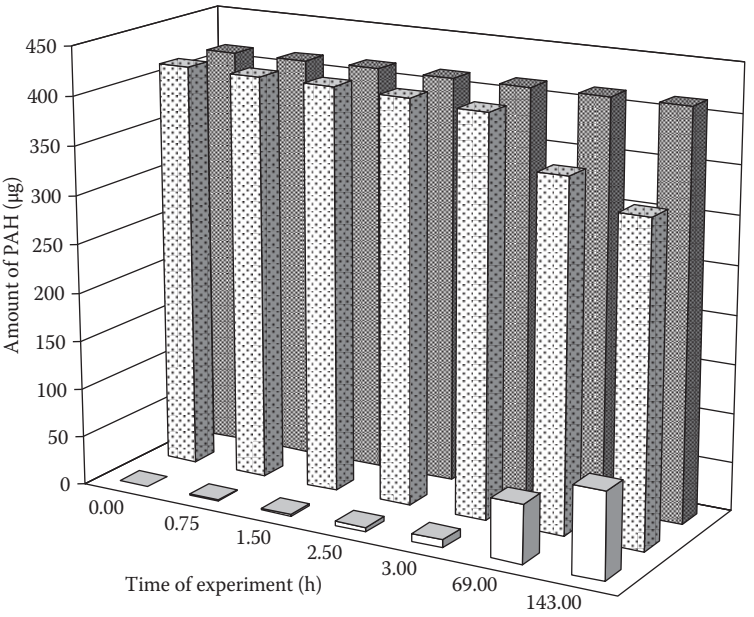


FIGURE 10.8
Amounts of PAHs in liquid media before the experiment—▩, amounts of PAHs in liquid media during the experiment—▨, and amounts of PAHs in LDPE during the experiment—□. (Reprinted from *Food Chemistry,* 64, P. et al., Removal of polycyclic aromatic hydrocarbons from water by migration into polyethylene, 157–161. Copyright 1999 with permission from Elsevier.)

$$c = S.\text{erfc}\left(\frac{x}{2\sqrt{Dt}}\right) \tag{10.4}$$

where c is the concentration of PAHs in the polymer at time t and at the distance x, D is the diffusion coefficient, and S is the solubility of PAHs in the polymer. The amount of diffusant in the region $x–h$ to x is given by Equation 10.5:

$$Q = A\int_{x-h}^{x} c(x,t)\,\mathrm{d}x \tag{10.5}$$

where A is the area and h is the thickness of the polymer layer. It can be derived from Equations 10.4 and 10.5 that the amount of PAHs in the nth polymer layer expressed as a weight of PAHs per weight of polymer, w, is

$$w = S\int_{n-1}^{n} \text{erfc}\left(\frac{hn}{2\sqrt{Dt}}\right)\mathrm{d}n \tag{10.6}$$

where n is the number of the layer.

Downloaded From Vet-eBooks.com

Therefore, the experimental data have been treated using Equation 10.6. The solubilities of individual PAHs in LDPE and the related diffusion coefficients have been obtained from the comparison of the theoretical values given by Equation 10.6 and the experimental values listed in Table 10.9 using the nonlinear least-squares method. The minimization of the sum of squares between theoretical and experimental values has been done by the simplex method. Integration in Equation 10.6 has been performed using the Simpson formula. For example, the courses of experimentally obtained and calculated values are shown in Figures 10.9 and 10.10 for 69 or 143 hours of experiment, respectively. The relationships between measured and calculated values are fairly good. As can be seen from the values of standard deviations in Table 10.9, the agreement between experimental and calculated values is fairly good. The solubilities and diffusion coefficients decrease in the order Fluoranthene (Fl)>Py>BaP,

TABLE 10.9

Solubilities and Diffusion Coefficients of Individual PAHs in LDPE and the Standard Deviation of Calculated Values

Compound	S (μg/kg)	$D \times 10^{10}$ (cm²/s)	σ (μg/kg)
Fl	10,430	7.05	310
Py	5030	5.47	190
BaP	3360	2.53	80

Source: Reprinted from *Food Chemistry,* 64, Šimko, P. et al., Removal of polycyclic aromatic hydrocarbons from water by migration into polyethylene, 157–161. Copyright 1999 with permission from Elsevier.

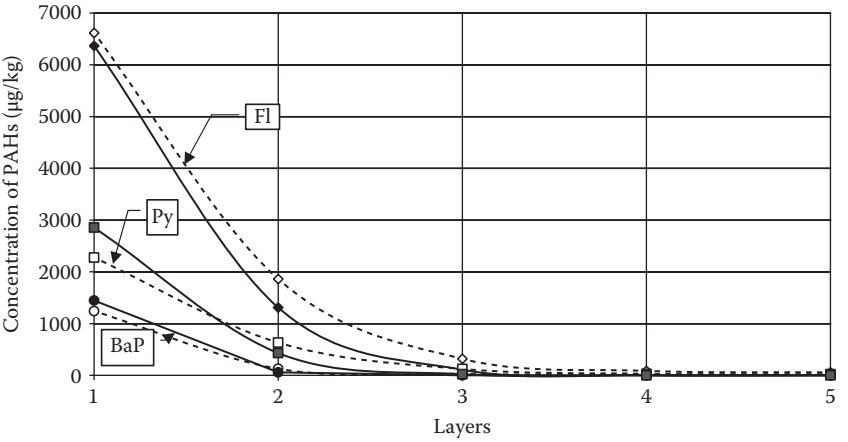


FIGURE 10.9
Dependence of PAHs concentration on the depth of migration in 69 hours of the experiment. - - - measured values, — calculated values. (Reprinted with permission from *Food Chemistry,* 64, Šimko, P. et al., Removal of polycyclic aromatic hydrocarbons from water by migration into polyethylene, 157–161. Copyright 1999 with permission from Elsevier.)

Downloaded From Vet-eBooks.com

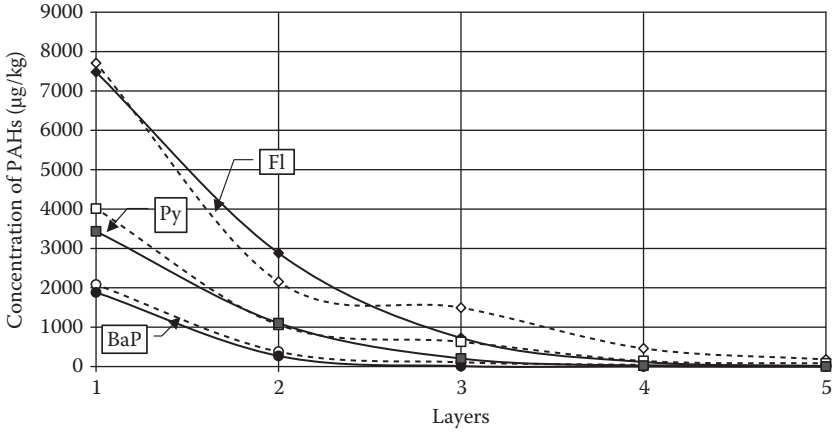


FIGURE 10.10
Dependence of PAH concentration on the depth of migration in 143 hours of the experiment. - - - measured values, — calculated values. (Reprinted from *Food Chemistry,* 64, Šimko, P. et al., Removal of polycyclic aromatic hydrocarbons from water by migration into polyethylene, 157–161. Copyright 1999 with permission from Elsevier.)

which is due to the increasing molar mass of the compounds. These calculated values of diffusion coefficients are more precise as the same values were only estimated for the same material in the previous work (Šimko et al., 1995).

On the basis of the results obtained, it can be concluded that PAHs after leaving the liquid media are primarily adsorbed on the polyethylene surface, with following migration into the bulk polymer, while the transportation of PAHs through the LDPE bulk can be described satisfactorily by Fickian laws of diffusion. This finding has also explained why this process is so effective—it is due to the existence of a permanently unbalanced state between PAH concentrations in the liquid and solid phase, where PAHs migrate continuously into the LDPE bulk; therefore the unbalanced state on the interfacial phase is permanently renewing. It is clear that this process will stop after a balance state between both phases are reached. Later, these findings were confirmed also by Guillén et al. (2000), when they observed reduction of PAH concentration in LSFs stored in LDPE flasks.

10.2.7 Reduction of PAH Content in Processed Meats

In principal, reduction of PAH content in smoked meat products can be realized at any production stage of these products. To keep formal categorization of reduction procedures, they could be applied during smoking, immediately after smoking, and by additional treatment or packaging into LDPE, respectively.

10.2.7.1 Reduction of PAH Content during Smoking

In general, PAH content in smoked meat products is directly proportional to smoking time (Djinovic et al., 2008; Ledesma et al., 2014) and the selec-

Downloaded From Vet-eBooks.com

tion of the mode also has considerable influence on the contents of PAHs, especially in hot smoked meat products. Therefore, the highest PAH contents were detected in sausages smoked with smoldering (intensive) smoke (Wretling et al., 2010; Pöhlmann et al., 2013a). So, shortening of smoking time and lowering of temperature of smoke generation have considerable effects on the final PAH content. Also, the use of synthetic casings instead of natural ones prevents the penetration of PAHs inside meat products smoked by any method due to the diameters of casing pores and molecules of PAHs (Ledesma et al., 2015, 2016). For example, the selection of a cellulose-peelable casing is an effective tool for reducing the PAH content in hot smoked sausages as a high percentage of the PAHs (BaP—77%; PAH4—61%) remains in the peelable casing and does not migrate to the inside of meat products (Pöhlmann et al., 2013b).

10.2.7.2 Reduction of PAH Content by Their Photodecomposition

To obtain data regarding possible effects of light on BaP content in hot smoked meat products, an Atlantic herring (*Clupea harengus*) was treated with hot smoke at a temperature of 82°C in a plant smoke house for 50 minutes (Šimko, 1991). After finishing the smoking, BaP content was determined immediately. Next, the samples were hung in the laboratory at unlimited access to oxygen and daylight at 18°C, and the BaP content was determined after 1, 2, 3, 4, 6, and 7 days of exposition. During this time, the BaP content was lowered from 0.58 to 0.12 µg/kg, as follows from Figure 10.11. Simultaneously, BaP methanolic solution was transferred into far UV silica cells and, after evaporation of the solvent, BaP at an amount of 1 µg

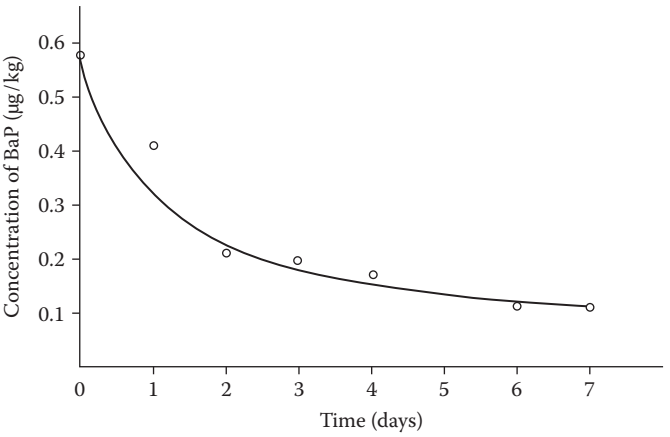


FIGURE 10.11
Dependence of BaP content upon time in smoked fish. (Reprinted from *Food Chemistry,* 40, Šimko, P. Changes of benzo(a)pyrene contents in smoked fish during storage, 293–300. Copyright 1991 with permission from Elsevier.)

Downloaded From Vet-eBooks.com

was exposed to the same conditions as used for the smoked fish. The contents of the cells were analyzed for amount of BaP at the same time intervals. As found, BaP content was affected by light, especially at the initial time of exposition, when all BaP molecules were localized just on the fish surface. But, the process of BaP light decomposition was not the only physicochemical process to take place in the fish. On analyzing surface (skin) and internal layers (meat) at the beginning and at the end of the experiment, it was found that a part of BaP molecules diffused into the fish bulk. So, while BaP content in fish skin decreased from 10.6 to 1.3 μg/kg, BaP in the bulk increased from 0 to 0.1 μg/kg. It was found that BaP degradation in silica cells could be described, in general, by a reaction of zero order, which is typical for photolytic degradations; this means that the amount of decomposed BaP was proportionally equal to the time of light exposition. On the other hand, the diffusion inside of fish bulk brought about the protection of BaP against environmental (decomposition) factors and the stabilization of its residual content in the fish. This course is typical for a reaction of first order, and it is clear that two different physicochemical processes took place there—light decomposition and diffusion—independently of each other. It is clear that extent of BaP content decrease can be affected by the intensity of light, and finally, also by antioxidant activity of phenol derivatives and other antioxidants present in the food matrix. Evidence about decomposition effects on PAHs was given by Sklaršová et al. (2012), who studied decomposition of BaP at two different light wavelengths, 254 and 365 nm, in a nonpolar medium at concentrations of 50, 100, and 150 μg/L. At chosen time intervals, BaP concentration was measured by HPLC using fluorescence detection. Comparing rate constants k and half-lives $\tau_{1/2}$, it was found that decomposition at 365 nm was 15.3 times faster in comparison with the decomposition at 254 nm. The decompositions obeyed the first-order kinetics. Addition of food antioxidants, 2,6-di-*tert*-butyl-4-methylphenol (BHT) and *o*-methoxyphenol (guaiacol), had considerable effect as both accelerated the rate of BaP decomposition—BHT by 1.17 times and guaiacol almost 1.45 times, which is really surprising. While these compounds are currently present in smoke, this observation could explain accelerated photodecomposition of PAHs localized on the surface of processed meats. These findings may represent a basis for a new approach to decrease PAH content in foods, where their presence is inevitable due to the applied production technology (Ledesma et al., 2016). To derive the kinetic equation and eliminate errors associated with nonisothermal heating procedures, BaP dissolved in glyceryl trioctanoate was heated in a glass reaction vessel within the temperature range 297.95–361.85 K with a heating rate of 1 K/min and simultaneously exposed to UV light at wavelength $\lambda = 365$ nm at radiation power 20 mW/cm^2. During the experiment, the BaP concentration decreased from 62.2 to 19.8 μg/L. From experimental results, the parameters characterizing the nonisothermal kinetics of the BaP photooxidation equation have been obtained (Equation 10.7):

Downloaded From Vet-eBooks.com

$$c = c_o \exp\left[-\frac{aT_r\vartheta^{m+1}}{\beta(m+1)}\right] \tag{10.7}$$

where T_r is a reference temperature, c_o and c are the concentrations of BaP in the vessel at the temperatures T_o and T, ϑ is reduced temperature, and a and m are adjustable parameters. The parameters were tested at two isothermal conditions (290.16 and 323.26 K) to verify the suitability of the derived parameters. Comparing calculated and measured data of half-lives of BaP decrease at mentioned temperatures, it was found that calculated half-lives are in good accordance with those experimentally obtained values, where relative standard deviations at 290.16 K were 17.0% and 5.4% at 323.26 K, respectively. The kinetic parameters enable the calculation of the rate constant for any temperature in the isothermal regime and make possible modeling, in general, the kinetics of PAH photooxidation without a deeper insight into their mechanisms.

10.2.7.3 Reduction of PAH Content by Cooking of Smoked Sausages

Sausages belong to a group of typical meat products consumed in considerable quantities over the world. They are commonly made from pork—boneless hams, shoulders, and slab bacon. The proper amounts of each are cut and ground through a 7 mm plate of a meat grinder. The mixture is then combined with seasoning ingredients (NaCl, black and red pepper, garlic, etc.), and stuffed into natural casings. The linked sausages are smoked using hot smoke for seven hours. The sausages are consumed directly, or after cooking in boiling water. To follow the effect of cooking on BaP in sausages, a sausage was analyzed for BaP content, fat content, and dry weight prior to cooking (Šimko et al., 1993). The BaP content was determined not only in whole sausages, but also in sausages in casing and peel-off film, separately; also BaP content in the cooked-out fat remaining in water after cooking was determined. The sausage was cooked in water at the boiling point, taken off at set time intervals, and analyzed. As follows from the experiment, cooking of sausage could be an effective tool for lowering BaP content, because the BaP content was lowered from 4.8 μg/kg to a final value of 1.9 μg/kg. The maximum drop in BaP content was observed during the first 20 minutes of cooking, after which it remained at a constant level. The decrease of BaP content during cooking was directly proportional to the amount of cooked-out fat released from sausages during cooking. Global changes of BaP and fat on time of cooking are shown in Figure 10.12. Although the BaP content found in the sausage casing corresponded to a level of 86 μg/kg, in real terms this represented only 21% of the total BaP content in the sausage; this means that 78% of the

Downloaded From Vet-eBooks.com

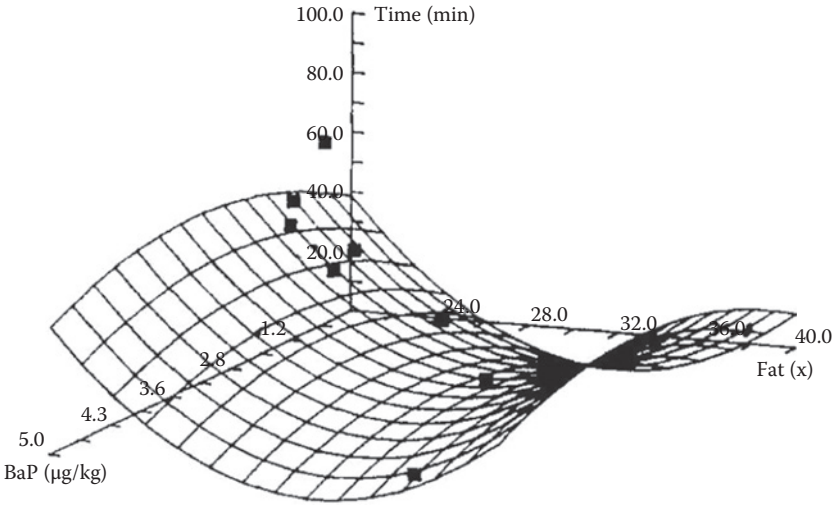


FIGURE 10.12
Three-dimensional diagram of the dependence of BaP content on the fat content and the time of cooking. (Reprinted from *Meat Science*, 34, Šimko, P. Influence of cooking on benzo[*a*]pyrene content in smoked sausages, 301–309. Copyright 1993 with permission from Elsevier.)

total amount of BaP had diffused into the sausage bulk before cooking. Evidence about diffusion of BaP and its high affinity to nonpolar parts, that is, its nonhomogenous distribution in the sausage, was also proven by determination of BaP content in cooked-out fat, when BaP content at the level of 7.7 µg/kg was determined. To the contrary, with regard to the finding that BaP content can be influenced by cooking, frankfurters were also treated by cooking and BaP and fat content was monitored. In comparison to the sausages, it was found that BaP in frankfurters was not affected by cooking at all. Comparing data and production technologies, it was found that although sausages and frankfurters are visually quite similar, they are very different due to their methods of production. While production of sausages was finished immediately after smoking, the frankfurters were still cooked in water steam. For this reason, the BaP content did not change in the frankfurters during additional cooking, because the "free" fat had already been removed from them during their technological production. So, while sausages represented a "crude" mixture of ground pork meat and bacon, frankfurters represented the typical fine cut homogeneous emulsified system, where the "residual" fat was "locked" in three-dimensional structures of denaturated proteins and therefore immobilized. Because the fat content had been stabilized already in the frankfurters during production, it was not further changed considerably during cooking. Then, with regard to the high affinity of BaP to nonpolar parts, its content was also not

Downloaded From Vet-eBooks.com

influenced by additional cooking and it remained at a constant level (Šimko and Knežo, 1992).

10.2.7.4 Reduction of PAH Content in Roasted Duck by Packaging into LDPE

Chen and Chen (2005) roasted whole duck samples at 225°C for 60 minutes, and skin samples were peeled off, analyzed for BaA, BbF, and BaP contents, and then packed in LDPE pouches under vacuum to ensure as much as possible contact of the skin with LDPE. After 24 hours storage at ambient temperature, BaA, BbF, and BaP contents were determined again and compared with the original content. As the authors found, all three contents were changed, and the most effective reduction was observed for BaP, as original content was reduced by 73% (from 3.5 to 0.9 μg/kg), while BbF was reduced by 54% (from 3.69 to 1.69 μg/kg), and BaA by 8% (from 143 to 130 μg/kg), respectively. As the authors concluded, this reduction was brought about by migration of PAHs from meat into the LDPE package and this process of elimination is believed to be a very promising method for elevation of food safety.

10.2.7.5 Reduction of PAH Content in Smoked Sausages by Packaging into LDPE

Smoked sausages were divided into two groups—the first group was packed immediately after smoking into the LDPE using vacuum packaging technology, while the second one was packed 12 hours after finishing the smoking. Also, unpacked sausages were left at the same conditions for comparison purposes. All samples were protected against daylight to prevent light decomposition of PAHs and stored at 15°C. Sausages were sampled at 0, 15, 30, 45, 60, 90, 120, 150, and 180 minutes. As follows from the experimental data in Table 10.10 (A columns, in time = 0), sausages contained relatively high PAH4. However, this content is in close accordance with the findings of other studies (shown in Table 10.3) and it is clear that it is brought about by the mode of smoking, because values of PAH4 content in nonsmoked sausages were under the limit of detection. As follows from the A columns in Table 10.10, PAH4 content started to decrease immediately after packaging into the LDPE. Because this decrease reached an equilibrium state in which PAH4 content in infinite time (c_∞) was not equal to zero, this decrease can be described by kinetic Equation 10.8 derived for cylindrical shape of a food product by Šimko et al. (2004):

$$c_t = c_\infty + (c_0 - c_\infty)\sum_{n=1}^{\infty}\frac{4}{a^2\alpha_n^2}\exp[-D\alpha_n^2 t] \tag{10.8}$$

where c_0, c_t, and c_∞ are the initial concentration, concentration at time t, and equilibrium concentration, a is the radius of the cylinder, α_n were taken from

Downloaded From Vet-eBooks.com

TABLE 10.10

Changes in PAH4 Content in Smoked Sausage during Storage in LDPE Packaging

	BaA			Chr			BbF			BaP		
Time	A (μg/kg)	EMU (%)	B (μg/kg)	A (μg/kg)	EMU (%)	B (μg/kg)	A (μg/kg)	EMU (%)	B (μg/kg)	A (μg/kg)	EMU (%)	B (μg/kg)
0	11.5	8.1	11.4	9.4	6.9	9.1	5.3	4.9	5.3	3.9	7.9	3.9
15	4.3	7.5	5.3	4.7	9.8	5.9	1.7	6.9	1.7	1.4	7.6	1.5
30	4.2	13.1	3.91	5.4	7.2	4.9	2.4	5.2	1.7	1.4	9.2	1.3
45	4.2	8.2	3.4	4.8	12.1	4.2	1.9	8.8	1.7	1.7	8.8	1.3
60	5.2	8.2	3.2	3.4	12.2	3.7	1.8	8.9	1.7	1.5	6.6	1.3
90	3.3	10.9	3.1	3.1	9.9	2.9	2.5	12.9	1.7	1.4	9.9	1.3
120	2.1	8.9	3.1	2.9	11.5	2.5	1.4	10.0	1.7	0.9	11.2	1.3
150	2.8	7.5	3.1	2.2	11.5	2.3	1.4	11.0	1.7	1.0	12.1	1.3
180	2.1	12.9	3.1	1.9	15.7	2.1	0.6	14.0	1.7	1.1	12.8	1.3

Source: Reprinted from *Food Chemistry*, 201, Semanová, J. et al., Elimination of polycyclic aromatic hydrocarbons from smoked sausages by migration into polyethylene packaging, 1–6. Copyright 2016 with permission from Elsevier.

A columns, measured values; EMU, expanded measurement uncertainty ($k = 2$) of measured values listed in A columns; B columns, values calculated using Equation 10.8.

tables (Abramowitz and Stegun, 1964), D is the diffusion coefficient, and t is the time of LDPE–sausage interaction.

Values of diffusion coefficients were calculated by the nonlinear least squares method by minimizing the sum of squares in differences between the PAH4 content measured experimentally and those calculated by Equation 10.8. The minimization was carried out by the simplex method (Nelder and Mead, 1965). Minimized parameters were c_∞ , c_0 - c_∞ , and D, and these values are shown in Table 10.11. In calculations, the first 20 terms of Equation 10.8 were taken into account. Experimental data were used for calculation of D and successively D values were used for calculation for PAH4 content. For comparison purposes, c_t was calculated at the same time as the experimental data. The agreement between experimental and calculated values is fairly good as follows from Table 10.9—columns A and B—which confirms diffusion of PAH4 from smoked sausages into LDPE bulk as a dominant physicochemical migration process of mass transfer between these media. A measure of the distribution of PAH4 between LDPE and smoked sausages is the distribution coefficient β calculated for a time of experiment equal to 180 minutes, given by the ratio (Equation 10.9)

$$\beta = \frac{c_0 - c_\infty}{c_\infty} \tag{10.9}$$

In general, the higher is the value of β, the greater is the part of PAH4 eliminated from smoked sausages and adsorbed onto LDPE. The values of β are

Downloaded From Vet-eBooks.com

TABLE 10.11

Values of Minimized Parameters and Diffusion and Distribution Coefficients

Compound	c_∞	$c_0 - c_\infty$	Diffusion Coefficients D (cm^2/h)	Distribution Coefficients β_{180}
BaA	3.1	8.2	2.5×10^{-2}	2.6
Chr	1.9	7.1	6.8×10^{-3}	3.7
BbF	1.7	3.6	6.4×10^{-1}	2.1
BaP	1.3	2.6	6.1×10^{-2}	2.0

Source: Reprinted from *Food Chemistry*, 201, Semanová, J. et al., Elimination of polycyclic aromatic hydrocarbons from smoked sausages by migration into polyethylene packaging, 1–6. Copyright 2016 with permission from Elsevier.

also listed in Table 10.11. As follows from these values, PAH4 was extensively adsorbed onto LDPE and these values are, for example, higher by one order in comparison to β values for polyethylene terephtalate (PET) packaging (Šimko et al., 2004) which proves better suitability of LDPE for PAH elimination purposes in comparison to PET. Experimental data listed in Table 10.10 show that this procedure is a very effective tool for PAH4 elimination, when the total PAH4 content was lowered by 81%, while reduction of individual compound content varied between 72% and 89%. A measure of diffusion is given—apart from other factors—also by D values for given media. As follows from Table 10.12, there is a great difference in D values for BaP between liquid media (LSFs) and solid media (LDPE). As follows from the table, diffusion of PAH4 in smoked sausages has migration properties closer to liquid than to solid media, and therefore the process of PAH4 elimination will be very effective. Moreover, this phenomenon could be expected also in other similar food matrices such as bacon, salami, etc. Another important finding is that the values of D are transferable to other geometry of smoked products, and therefore they could be used for variously shaped smoked meat products. The advantage of application of the kinetic equation (Equation 10.8) is based on the fact that by numerical solution of the equation for known values of D and given c_o , it is possible to calculate the time at which PAH4 and/or individual compound content decreases to a required value c_t set by legislation. For example, in this experiment, the legislative limit (set by Commission Regulation No. 835/2011) of 12 μg/kg for PAH4 was reached in 28.57 minutes, and acceptable content of 11.9 μg/kg in 29.32 minutes, as also follows from Figure 10.13. Similarly, the legislative limit of 2 μg/kg for BaP was reached in 10.41 minutes, and acceptable content of 1.9 μg/kg in 11.20 minutes. All these changes are in sharp contrast to nonpacked smoked sausages, since the PAH4 content in them remained at a constant level during the whole experiment, as shown in Figure 10.13 by (▲) points (Semanová et al., 2016). A time gap also has a substantial effect on the measure of PAH content

Downloaded From Vet-eBooks.com

TABLE 10.12

Values of Diffusion Coefficients for BaP in Various Media

Medium	D (cm^2/h)
Liquid smoke flavor (Simko et al. 1994)	7.2×10^2
Low-density polyethylene (Simko et al. 1999)	6.9×10^{-14}
Smoked sausage	6.1×10^{-2}

Sources: Reprinted from *Food Chemistry*, 201, Semanová, J. et al., Elimination of polycyclic aromatic hydrocarbons from smoked sausages by migration into polyethylene packaging, 1–6. Copyright 2016 with permission from Elsevier. Reprinted from *Food Chemistry,* 50, Šimko, P. et al., Kinetics of polycyclic aromatic hydrocarbons sorption from liquid smoke flavor into low density polyethylene packaging, 65–68. Copyright 1994 with permission from Elsevier. Reprinted from *Food Chemistry,* 64, Šimko, P. et al., Removal of polycyclic aromatic hydrocarbons from water by migration into polyethylene, 157–161. Copyright 1999 with permission from Elsevier.

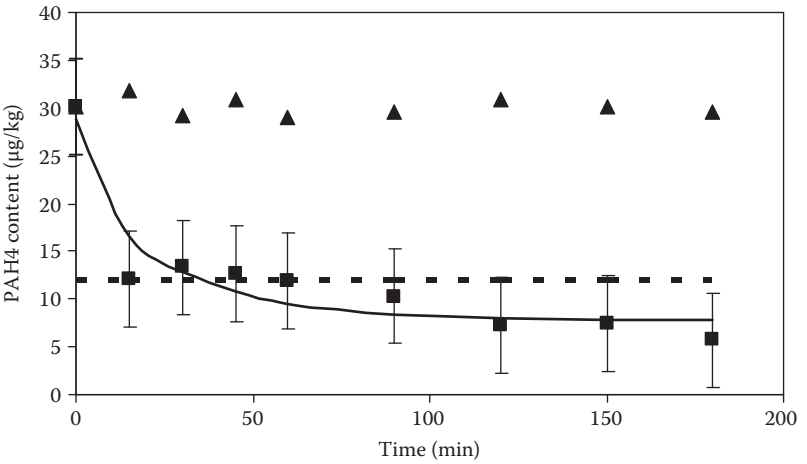


FIGURE 10.13

Total changes in PAH4 content in nonpacked smoked sausages (▲) and smoked sausages packed into polyethylene (■). Legislative limit PAH4 content equal to 12 µg/kg is marked by dashed line, dependence of PAH4 content upon time calculated by kinetic Equation 10.8 is marked by the black curve. Variability of experimental data is marked by error bars. (Reprinted from *Food Chemistry*, 201, Semanová, J. et al., Elimination of polycyclic aromatic hydrocarbons from smoked sausages by migration into polyethylene packaging, 1–6. Copyright 2016 with permission from Elsevier.)

reduction between finishing the smoking and the package operation itself. As follows from Table 10.13, there is a great difference between distribution coefficients measured in sausages packed immediately after smoking, and after a 12-hour delay. This fact could be brought about by migration of PAHs into meat product bulk and therefore extension of the method of migration,

Downloaded From Vet-eBooks.com

TABLE 10.13

Values of Distribution Coefficients Measured in 120 Minutes of Storage Experiment in Sausages Packed into LDPE Immediately (β_0) and after 12-Hour Delay before Packaging (β_{12})

Compound	Distribution Coefficients β_0	Distribution Coefficients β_{12}
BaA	2.65	2.62
Chr	2.30	0.56
BbF	2.82	2.68
BaP	3.24	2.89
Average	2.75	1.85

Source: Data ß$_0$ are reprinted from Food Chemistry, 201, Semanova, L. et al., Elimination of polycyclic aromatic hydrocarbons from smoked sausages by migration into polyethylene packaging, 1 – 6, Copyright (2016) with permission from Elsevier.
Data ß$_{12}$ are so far unpublished data..

which affects, of course, the time of PAH content reduction. Also, incorporation of PAHs into liphophilic structures under the surface of meat products could bring about their immobilization, which would affect global measure of PAH removal from these foods.

10.3 General Conclusions

On the basis of the data and information mentioned above, the following partial conclusions could be postulated:

1. PAHs are permanent contaminants formed during thermal decomposition of organic matter such as wood, cholesterol, or other food components.
2. PAHs have substantial carcinogenic effects on living organisms, and therefore their content is limited by EU legislation.
3. PAH content may be limited by technological procedures such as duration of thermal or smoke treatment, temperature of smoke generation, application of packaging materials protecting migration of PAHs from surface into meat bulk, and photodecomposition of PAHs deposited on the surface of meat products.
4. Apart from the mentioned procedures, PAHs can be also eliminated by migration into a suitable plastic package.
5. Migration of PAHs into a plastic package—dominantly LDPE—is driven by nonequal chemical potentials in package/meat systems, expressed by PAH contents, or concentrations, respectively.

Downloaded From Vet-eBooks.com

6. From the point of view of physical chemistry, the elimination is a combined process composed of adsorption (as the prime process) followed by diffusion (as the second process).
7. The process of PAH migration into LDPE is most effective when smoking has just finished. A time delay before packaging would bring about a decrease of the process due to migration of PAHs into meat products' bulk and their eventual incorporation and immobilization in liphophilic structures.
8. Combination of both these processes offers the unique possibility to eliminate these compounds from the food matrix and elevate food safety in foods, where another process of elimination is impossible, or banned by legislation.

Acknowledgment

This contribution is the result of a project funded by the Slovak Scientific Grant Agency VEGA No. 1/0487/16.

References

Abramowitz, M. and Stegun, I. A. (1964). *Handbook of Mathematical Functions*. Washington, DC: National Bureau of Standards.

Adamson, A. W. (1990). *Physical Chemistry of Surfaces*. New York, NY: John Wiley & Sons.

Afolabi, A. O., Adesulu, E. A. and Oke, O. L. (1983). Polynuclear aromatic hydrocarbons in some Nigerian preserved freshwater fish species. *Journal of Agricultural and Food Chemistry*, **31**, 1083–1090.

Alonge, D. O. (1988). Carcinogenic polycyclic aromatic hydrocarbons (PAH) determined in Nigerian kundi (smoke-dried meat). *Journal of the Science of Food and Agriculture*, **43**, 167–172.

Alonge, D. O. (1987). Factors affecting the quality of smoke-dried meats in Nigeria. *Acta Aliment*, **16**, 263–270.

Andelman, J. B. and Suess, M. J. (1970). PAH in the water environment. *Bulletin WHO*, **43**, 479–508.

Bartle, K. D. (1991). Analysis and occurrence of polycyclic aromatic hydrocarbons in food. *In:* Creaser, C. and Purchase, R. (eds.) *Food Contaminants, Sources and Surveillance*. Cambridge: The Royal Society of Chemistry, 41–60.

Bednáriková, A., Skláršová, B., Kolek, E. and Šimko, P. (2011). New rapid HPLC method for separation and determination of benzo[*a*]pyrene hydroxyderivatives. *Polycyclic Aromatic Compounds*, **31**, 350–369.

Biesalski, H. K. (2005). Meat as a component of a healthy diet—Are there any risks or benefits if meat is avoided in the diet? *Meat Science*, **70**, 509–524.

Bouvard, V., Loomis, D., Guyton, K. Z. et al. (2015). Carcinogenicity of consumption of red and processed meat. *The Lancet Oncology.* doi:10.1016/S1470-2045(15)00444-1.

Downloaded From Vet-eBooks.com

Cai, J., Wang, S. and Su, Q. (2008). Analysis of polycyclic aromatic hydrocarbons formed during off-line pyrolysis of ergosterol by GC–MS–SIM. *Chromatographia,* **68**, 357–363.

Cranck, J. (1976). *The Mathematics of Diffusion*. Oxford: Clarendon Press, pp. 69–81.

Emmerson, E. P. (2011). Improving the sensory and nutritional quality of smoked meat products. *In:* Kerry, J. P. and Kerry, J. F. (eds.) *Processed meats: Improving Safety, Nutrition and Quality*. Cambridge: Woodhead Publishing, pp. 527–545.

Cai, J., Wang, S. and Su, Q. (2008). Analysis of polycyclic aromatic hydrocarbons formed during off-line pyrolysis of ergosterol by GC–MS–SIM. *Chromatographia,* **68**, 357–363.

Chen, J. and Chen, S. (2005). Removal of polycyclic aromatic hydrocarbons by low density polyethylene from liquid model and roasted meat. *Food Chemistry,* **90**, 461–469.

Christy, A. A., Lian, M. I. and Francis, G. W. (2011). Pyrolytic formation of polyaromatic hydrocarbons from steroid hormones. *Food Chemistry,* **124**, 1466–1472.

Commission Directive 88/388/EEC on the approximation of the laws of the Member States relating to flavorings for use in foodstuffs and to source materials for their production. *Official Journal of the European Union*, L0388, 1991:1–10. Available at http://ec.europa.eu/food/fs/sfp/addit_flavor/flav09_en.pdf (accessed 5 February 2016).

Commission Directive 2005/10/EC laying down the sampling methods and the methods of analysis for the official control of the levels of benzo(a)pyrene in foodstuffs. *Official Journal of the European Union*, L34, 2 February 2005:15–20. Available at http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex%3A32005R0208 (accessed 5 February 2016).

Commission Recommendation 2005/108/EC of February 4, 2005 on the further investigation into the levels of polycyclic aromatic hydrocarbons in certain foods. *Official Journal of the European Union*, L34, 8 February 2003:43–45. Available at http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2005:034:0043:0045:EN:PDF (accessed 5 February 2016).

Commission Regulation (EC) 1881/2006/EC setting maximum levels for certain contaminants in foodstuffs. *Official Journal of the European Union*, L364, 20 December 2006:5–24. Available at http://eur-lex.europa.eu/LexUriServ/ LexUriServ.do?uri=OJ:L:2006:364:0005:0024:EN:PDF (accessed 5 February 2016).

Commission Regulation (EC) No 333/2007 laying down the methods of sampling and analysis for the official control of the levels of lead, cadmium, mercury, inorganic tin, 3-MCPD and benzo(a)pyrene in foodstuffs. *Official Journal of the European Union*, L88, 29 March 2007:29–38. Available at http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2007:088:0029:0038:EN:PDF (accessed 5 February 2016).

Commission Regulation (EC) 835/2011a amending Regulation (EC) No 1881/2006 as regards maximum levels for polycyclic aromatic hydrocarbons in foodstuffs. *Official Journal of the European Union*, L215, 19 August 2011:4–8. Available at http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2011:215:0004:0008:EN:PDF (accessed 5 February 2016).

Commission Regulation EC No 836/2011b amending Regulation (EC) No 333/2007 laying down the methods of sampling and analysis for the official control of the levels of lead, cadmium, mercury, inorganic tin, 3-MCPD and benzo(*a*)pyrene in

Downloaded From Vet-eBooks.com

foodstuffs. *Official Journal of the European Union*, L215, 20 August 2011:9–16. Available at http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2011:215:0009:0016:EN:PDF (accessed 5 February 2016).

Commission Implementing Regulation (EU) No 1321/2013 of 10 December 2013 establishing the Union list of authorised smoke flavouring primary products for use as such in or on foods and/or for the production of derived smoke flavourings. Available at http://eur-lex.europa.eu/legal-content/EN/TXT/?uriŁOJ:L:2013:333:TOC. (accessed 5 February 2016).

Djinovic, J., Popovic, A. and Jira, W. (2008). Polycyclic aromatic hydrocarbons (PAHs) in different types of smoked meat products from Serbia. *Meat Science,* **80**, 449–456.

European Commission, Health and Consumer Protection Directorate—General, Scientific Committee on Food. (2002). Opinion of the Scientific Committee on Food on the risk to human health of polycyclic aromatic hydrocarbons in food. SCF/CS/CNTM/PAH/29 Final.

European Food Safety Authority. (2008). Scientific opinion of the panel on contaminants in the food chain on a request from the European Commission on polycyclic aromatic hydrocarbons in food. *EFSA Journal,* **724**, 1–114.

Farhadian, A., Jinap, S. Hanifah, H. N. and Zaidul, I. S. (2011). Effects of meat preheating and wrapping on the levels of polycyclic aromatic hydrocarbons in charcoal-grilled meat. *Food Chemistry,* **124**, 141–146.

Fasano, E., Yebra-Pimentel, I., Martínez-Carballo, E. and Simal-Gandara, J. (2016). Profiling, distribution and levels of carcinogenic polycyclic aromatic hydrocarbons in traditional smoked plant and animal foods. *Food Control,* **59**, 581–90.

Grimmer, G. and Böhnke, H. (1975). Polycyclic aromatic hydrocarbon profile analysis of high protein foods, oils and fats by gas chromatography. *Journal Association of Official Analytical Chemists,* **58**, 725–733.

Hitzel, A., Pöhlmann, M., Schwägele, F., Speer, K. and Jira, W. (2013). Polycyclic aromatic hydrocarbons (PAH) and phenolic substances in meat products smoked with different types of wood and smoking spices. *Food Chemistry,* **139**, 955–62.

Guillén, M. D. (1994). Polycyclic aromatic compounds: Extraction and determination in food. *Food Additives and Contaminants,* **11**, 669–684.

Guillén, M. D., Sopelana, P. and Partearroyo, M. A. (2000). Polycyclic aromatic hydrocarbons in liquid smoke flavorings obtained from different types of wood. Effect of storage in polyethylene flasks on their concentrations. *Journal of Agricultural and Food Chemistry,* **48**, 5083–5087.

Kangsadalampai, K., Butryee, C. and Manoonphol, K. (1997). Direct mutagenicity of the polycyclic aromatic hydrocarbon-containing fraction of smoked and charcoal-broiled foods treated with nitrite in acid solution. *Food Chemical Toxicology,* **35**, 213–218.

Larsson, B. K., Sahlberg, G. P., Eriksson, A. T. and Busk, L. A. (1983). Polycyclic aromatic hydrocarbons in grilled food. *Journal of Agricultural and Food Chemistry,* **31**, 867–873.

Ledesma, E., Rendueles, M. and Díaz, M. (2014). Benzo(a)pyrene penetration on a smoked meat product during smoking time. *Food Additives and Contaminants, Part A,* **1**, 1688–1698.

Ledesma, E., Rendueles, M. and Díaz, M. (2015). Spanish smoked meat products: Benzo(a)pyrene (BaP) contamination and moisture. *Journal of Food Composition and Analysis,* **37**, 87–94.

Downloaded From Vet-eBooks.com

Ledesma, E., Rendueles, M. and Díaz, M. (2016). Contamination of meat products during smoking by polycyclic aromatic hydrocarbons: Processes and prevention. *Food Control,* **60**, 64–87.

Lijinsky, W. and Shubik, P. (1964). Benzo[a]pyrene and other polynuclear hydrocarbons in charcoal-broiled meat. *Science,* **88**, 145–153.

Lijinsky, W. and Ross, A. E. (1967). Production of carcinogenic polynuclear hydrocarbons in the cooking of food. *Food and Cosmetics Toxicology,* **5**, 343–347.

Lorenzo, J. M., Purriños, L., Fontán, M. C. G. and Franco, D. (2010). Polycyclic aromatic hydrocarbons (PAHs) in two Spanish traditional smoked sausage varieties: "Androlla" and "Botillo". *Meat Science,* **86**, 660–664.

Maga, J. A. (1987). The flavor chemistry of wood smoke. *Food Reviews International*, **3**, 139–183.

McEnally, C. S., Pfefferle, L. D., Atakan, B. and Kohse-Höinghaus, K. (2006). Studies of aromatic hydrocarbon formation mechanisms in flames: Progress towards closing the fuel gap. *Progress in Energy and Combustion Science,* **32**, 247–294.

Moisan, J. Y. (1985). Effects of oxygen permeation and stabiliser migration on polymer degradation. *In:* Comyn J. (ed.) *Polymer Permeability*. Oxford: Elsevier.

Nelder, J. A. and Mead, R. A. (1965). A simplex method for function minimization. *The Computer Journal,* **7**, 308–313.

Nisbet, I. C. T. and La Goy, P. K. (1992). Toxic equivalency factors (TEFS) for polycyclic aromatic hydrocarbons. *Regulatory Toxicology and Pharmacology,* **16**, 290–300.

Pöhlmann, M., Hitzel, A., Schwägele, F., Speer, K. and Jira, W. (2013a). Polycyclic aromatic hydrocarbons (PAH) and phenolic substances in smoked Frankfurter-type sausages depending on type of casing and fat content. *Food Control,* **31**, 136–144.

Pöhlmann, M., Hitzel, A., Schwägele, F., Speer, K. and Jira, W. (2013b). Influence of different smoke generation methods on the contents of polycyclic aromatic hydrocarbons (PAH) and phenolic substances in Frankfurter-type sausages. *Food Control,* **34**, 347–355.

Purcaro, G., Moret, S. and Conte, L. S. (2013). Overview on polycyclic aromatic hydrocarbons: Occurrence, legislation and innovative determination in foods. *Talanta,* **105**, 292–305.

Regulation (EC) 2065/2003 of the European Parliament and of the Council on smoke flavourings used or intended for use in or on foods. *Official Journal of the European Union*, L309, 26 November 2003:1–8. Available at http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2003:309:0001:0008:EN:PDF (accessed on 5 February 2016).

Report of the Joint FAO/WHO Expert Commission on Food Additives. 1987. 31:20–22. [Online]. Available at http://whqlibdoc.who.int/trs/WHO_TRS_759.pdf (accessed 2 February 2016).

Report of the Joint FAO/WHO Expert Commission on Food Additives. 2005. JECFA/64/SC: 32–38. [Online]. Available at ftp://ftp.fao.org/es/esn/jecfa/jecfa64_summary.pdf (accessed 2 February 2016).

Richter, H. and Howard, J. B. (2000). Formation of polycyclic aromatic hydrocarbons and their growth to soot—A review of chemical reaction pathways. *Progress in Energy and Combustion Science,* **26**, 565–608.

Roseiro, L. C., Gomes, A., Patarata, L. and Santos, C. (2012). Comparative survey of PAHs incidence in Portuguese traditional meat and blood sausages. *Food and Chemical Toxicology,* **50**, 1891–96.

Downloaded From Vet-eBooks.com

Roda, A., Simoni, P., Ferri, E., et al. (1999). Determination of PAHs in various smoked meat products and different samples by enzyme immunoassay. *Journal of the Science of Food and Agriculture,* **79**, 58–62.

Rozentale, I., Stumpe-Viksna, I., Začs, D., Siksna, I., Melngaile, A. and Bartkevičs, V. (2015). Assessment of dietary exposure to polycyclic aromatic hydrocarbons from smoked meat products produced in Latvia. *Food Control,* **54**, 16–22.

Semanová, J., Skláršová, B., Šimon, P. and Šimko, P. (2016). Elimination of polycyclic aromatic hydrocarbons from smoked sausages by migration into polyethylene packaging. *Food Chemistry,* **201**, 1–6.

Sikorski, Z. E. (2004). Traditional smoking. *In:* Jensen, W. K, Devine, C. and Dikeman M. (eds.) *Encyclopedia of Meat Sciences,* London, Elsevier, pp. 1265–1272.

Skláršová, B., Šimon, P., Kolek, E., Šimko, P. and Bednáriková, A. (2012). Non-isothermal kinetics of benzo[a]pyrene photooxidation in glyceryl trioctanoate. *Polycyclic Aromatic Compounds*, **32**, 580–588.

Suranová, M., Semanová, J., Skláršová, B. and Šimko, P. (2015). Application of accelerated solvent extraction for simultaneous isolation and pre-cleaning up procedure during determination of polycyclic aromatic hydrocarbons in smoked meat products. *Food Analytical Methods,* **8**, 1014–1020.

Šimko, P. (1991). Changes of benzo(a)pyrene contents in smoked fish during storage. *Food Chemistry,* **40**, 293–300.

Šimko, P., Petrík, J. and Karovičová, J. (1992). Determination of benzo(a)pyrene in liquid smoke preparations by high pressure liquid chromatography and confirmation by gas chromatography-mass spectrometry. *Acta Alimentaria,* **21**, 107–114.

Šimko, P. and Knežo, J. (1992). Influence of cooking on benzo(a)pyrene content in frankfurters. *Nahrung*, **36**, 208–209.

Šimko, P., Gergely, Š., Karovičová, J., Drdák, M. and Knežo, J. (1993). Influence of cooking on benzo[*a*]pyrene content in smoked sausages. *Meat Science,* **34**, 301–309.

Šimko, P. (2011). Heat and processing generated contaminants in processed meats. *In:* Kerry, J. P. and Kerry, J. F. (eds.) *Processed Meats: Improving Safety, Nutrition and Quality*. Cambridge: Woodhead Publishing, pp. 478–507.

Šimko, P., Gombita, M. and Karovičová, J. (1991). Determination and occurrence of benzo[a]pyrene in smoked meat products. *Nahrung,* **35**, 103–104.

Šimko, P. and Bruncková, B. (1993). Lowering of concentration of polycyclic aromatic hydrocarbons in a liquid smoke flavour by sorption into polyethylene packaging. *Food Additives and Contaminants,* **10**, 257–263.

Šimko, P., Šimon, P., Khunová, V., Karovičová, J. and Drdák, M. (1994). Kinetics of polycyclic aromatic hydrocarbons sorption from liquid smoke flavour into low density polyethylene packaging. *Food Chemistry,* **50**, 65–68.

Šimko, P., Khunová, V., Šimon, P. and Hrubá, M. (1995). Kinetics of sunflower oil contamination with polycyclic aromatic hydrocarbons from contaminated recycled low density polyethylene film. *International Journal of Food Science and Technology,* **30**, 807–812.

Šimko, P., Šimon, P. and Khunová, V. (1999). Removal of polycyclic aromatic hydrocarbons from water by migration into polyethylene. *Food Chemistry,* **64**, 157–61.

Šimko, P. (2002). Determination of polycyclic aromatic hydrocarbons in smoked meat products and smoke flavouring food additives. *Journal of Chromatography B,* **77**, 3–18.

Downloaded From Vet-eBooks.com

Šimko, P., Šimon, P. and Belajová, E. (2004). Lowering of concentration of polycyclic aromatic hydrocarbons in liquid media by sorption into polyethylene terephthalate—A model study. *European Food Research and Technology,* **219**, 273–276.

Šimko, P. (2005). Factors affecting elimination of polycyclic aromatic hydrocarbons in smoked meat foods and liquid smoke flavorings. *Molecular Nutrition and Food Research,* **49**, 637–647.

Škaljac, S., Petrović, L., Tasić, T. et al. (2014). Influence of smoking in traditional and industrial conditions on polycyclic aromatic hydrocarbons content in dry fermented sausages (Petrovská klobása) from Serbia. *Food Control,* **40**, 12–18.

Tóth, L. and Blaas, W. (1972). Einfluss der Räeuchertechnologie auf den Gehalt von geraeucherten Fleischwaren an cancerogenen Kohlenwasserstoffen. II. Einfluss der Glimmtemperatur des Holzes sowie der Kuehlung, Waesche und Filtration des Räeucherrauches. *Fleischwirtschaft*, **52**, 1419–1422.

Tóth, L. and Potthast, K. (1984). Chemical aspects of the smoking of meat and meat products, *In:* Chichester, C. O., Mrak, E. M. K. and Schweigert, B. S. (eds.) *Advances in Food Research*. New York, NY: Academic Press, pp. 87–158.

Wilms, M. (2000). The developing of modern smokehouses—Ecological and economical aspects. *Fleischwirtsch Int,* **80**, 4–5.

Wretling, A., Eriksson, A., Eskhult, G. A. and Larsson, B. (2010). Polycyclic aromatic hydrocarbons (PAHs) in Swedish smoked meat and fish. *Journal of Food Composition and Analysis,* **23**, 264–72.

Zelinkova, Z. and Wenzl, T. (2015). The occurrence of 16 EPA PAHs in food—A review. *Polycyclic Aromatic Compounds,* **35**, 248–84.

Downloaded From Vet-eBooks.com

11

Nutrigenomics in Food-Producing Animals

Werner G. Bergen

CONTENTS

11.1 Introduction 355
11.2 Application of Genomics to Current Issues in Animal Agriculture ... 356
11.3 Reductionism to Global Gene Expression—Overview 357
11.4 Global and Targeting Differential Gene Expression Studies in Farm Animals 358
11.5 Experimental Approaches to Nutrigenomics Research 360
11.6 Long-Chain Fatty Acids as Nutrigenomic Effectors 360
11.7 Conjugated Linoleic Acids as Regulators of Fatty Acid Synthesis ... 361
11.8 The Role of High-Energy Diets in Promoting IMF Deposition in Finishing Cattle 364
11.9 Glucose versus Acetate as Precursors for IMF 364
11.10 Conclusions and Future Trends 366
References 366

11.1 Introduction

It has been well established that foodstuffs contain three classes of macromolecules (carbohydrates, lipids, and proteins) which supply energy (carbohydrates and lipids) and protein building blocks for animals to function and maintain themselves. As our knowledge of the effect of many individual compounds/nutrients in foodstuffs increased (this statement does not include vitamins), it was recognized that there were other possible contributions of such compounds on specific aspects of regulation of metabolism. Thus, beyond function in cellular components (as plasma membranes, enzymes, glycogen, etc.), some of these compounds appeared to have "bioactive/pathway controlling properties" which may be used in a nutraceutical sense to combat health issues as well as promote animal production and the quality and characteristics of muscle foods. Many of these bioactive nutrients interact/bind with transcription factors (TFs) to regulate major metabolic pathways to regulate metabolism at the transcriptomic/functional genomics level; this is referred to as nutrigenomics. These potential, other putative functions of food compo-

Downloaded From Vet-eBooks.com

nents have created great excitement particularly in the realm of human nutrition and health. There may also be opportunities for regulatory manipulations by dietary components during animal production. In this chapter, issues of nutrigenomics in animal nutrition and production will be explored. Two different/divided concepts have been formulated when relating dietary nutrients content to animal metabolism. These two aspects of nutrient/gene function relationships have been coined nutrigenetics, the concept that a given nutrition regime has been formulated to satisfy all nutrients requirement based on genetic potential, and nutrigenomics, the concept that a feed component may affect physiological function by altering gene expression either directly or indirectly in animals (Joffe and Houghton, 2016).

11.2 Application of Genomics to Current Issues in Animal Agriculture

Improving the efficiency of animal production and optimizing the quality of the animal products have been a long-term goal of the livestock industry. To varying degrees, the definitions of quality of muscle foods have differed over the years. In the past, animal fat was considered a positive attribute of meat products, but today a lean product with just enough fat to optimize flavor and the eating experience is preferred. Such goals have been actualized with various production and animal selection practices. What stands out is that contemporary meat animals (at least in North America) can be characterized as lean and heavier muscled while carrying much less fat than their predecessors. In the last 50 plus years, serious efforts have been made to select for lean animals by traditional means, which resulted in today's animals. These genetic selections based on body composition changes have implications in nutrition, management, and harvesting techniques for these animals, and the further handling of the animal products. While there has been considerable success in increasing lean (protein) gains with much lower whole-body depot fat gains (subcutaneous and visceral fat) in animal production, such emphasis on leanness also has a negative effect on the small adipose depots within skeletal muscle (intramuscular fat [IMF]). These fat depots are associated with organoleptic properties of the muscle foods. In addition, tenderness is a highly valued property of meat (especially beef). Thus, currently animal production procedures are geared toward maximizing lean (protein) gains, counterbalanced by nutritional regimes that will also encourage more depot fat and IMF deposition.

There is interest by some producers (consumers) to make available increased amounts of so-called "healthy" meat products, in particular grass-fed beef (Ruminants). Grass is a relatively low source of dietary energy and animals will not develop IMF except when animals are fed for a long time period. Such a long time span greatly lowers production efficiency (high

Downloaded From Vet-eBooks.com

maintenance energy input) and greatly increases the carbon footprint of such practices. Animals selected for high efficiency of production often do not get adequate dietary energy in such a feeding system and their genetic potential (nutrigenetics) for lean growth is not met. In many cases, tenderness and organoleptic properties are undesirable in muscle foods arising from such production regimes. Clearly, the so-called health benefits are difficult to discern other than that grass-fed beef is a low-fat food product. To date, no one has identified any major change in carcass components that may be construed to provide "additional" health benefits. To date, gene expression works with pasture finished beef have not uncovered putative enzymatic pathways, TFs, or compositional factors that would increase the health value of such products (Burnett, 2014).

Questions about molecular regulation of IMF deposition (marbling), fat deposition in general, lean growth, overall energetic efficiency, and beneficial factors in animal products are all amenable to targeted or genome-wide gene expression/association studies (Williams et al., 2016). Such data, however, are descriptive; further application-type research based on such nutrigenomics data must be done to actualize any benefits. A number of excellent reviews about the role of nutrigenomics in animal production and animal products development have recently been published (Bionaz et al., 2015; Loor et al., 2015; Picard et al., 2015). In addition, some selected publications emphasizing other functional genomics techniques related to food animals are Graungnard et al. (2009), Graungnard et al. (2010), Komolka et al. (2014), Loor et al. (2013), Guo et al. (2014), Lancaster et al. (2014), Roberts et al. (2015), and Loor et al. (2015). This literature has been steadily growing over the last decade.

11.3 Reductionism to Global Gene Expression—Overview

The hallmark of much of biochemistry and physiology research has been and still is reductionism. Here, a biological process has been observed and then a causative delineation is accomplished by isolating organs, then tissue slices, perfusions, cells, and then cell fractionation into nuclei, mitochondria, endoplasmic reticulum, and cytosol. Activities of relevant enzymes are measured in the various fractions and these data were interpreted by relating to whole cells, organs, and whole body function. To attempt a more metabolic interactive holistic approach, reductionist data are utilized in so-called modeling systems concerning animal nutrition and metabolism for interpretation and application (Baldwin et al., 1987; France et al., 1987; Johnson et al., 2012; Tedeschi et al., 2013).

Contemporary experimental approaches utilize high-throughput technologies in studying genomes (DNA sequences, single-nucleotide polymorphism [SNP]), genome function or transcriptomics (messenger RNA

Downloaded From Vet-eBooks.com

[mRNA]), protein synthesis, folding, and function or proteomics along with assessments of metabolic profiles in body and tissue fluids or metabolomics. When focusing strictly on lipid metabolites, this is often referred to as lipidomics. All these procedures can lead to a tremendous accumulation of raw data which have to be calculated, sorted, and adjusted for inherent methodological errors, statistical evaluation, and finally pathway-network analyses. The results can then be matched to biological processes and interpreted in line with the experimental design and hypotheses. Traditional reductionist-based life science researchers have had little difficulty engaging in modern analytical procedures and equipment. What is often lacking is the ability to boil down the plethora of data into useful terms; this requires the application of bioinformatics. Hence, omics research teams typically include bioformaticists. While totally comprehensive analysis of the genome, functional genome, proteome, and metabolome for a given situation in animal production is very useful, nevertheless the work is also very expensive.

All too often people have confidence in equipment, but the proper initial isolation of biological substances (particularly nucleic acids) and purity are an overwhelmingly important starting point for many "omic" studies. The most elaborate downstream equipment and capacity cannot obtain useful data with poor-quality samples. Unfortunately, some researchers unwittingly fail to recognize this pitfall.

11.4 Global and Targeting Differential Gene Expression Studies in Farm Animals

The scope of functional genomic assessments has included organs such as liver, skeletal muscle, adipose tissue, mammary tissue, connective tissue and blood, heart, cancer tissue, and gut epithelium along with the whole tract microbiome. In the food animal production area (but not disease related), principal tissues have been muscle, adipose of various depots, liver, gastrointestinal tract epithelium, and the gut microbiome. Much of the work with the gut microbiome has centered on metagenomics of organism diversity ranging from archaea, bacteroides, and firmicutes to prevotella (Kim et al., 2014; McCann et al., 2014) but this will not be considered in this chapter.

Early work in differential gene expression (DE) utilized complementary DNA (cDNA) probes and Northern analysis of mRNA. In the biomedical field, where the focus was rodent biology, an huge inventory of such probes were developed as the GenBank sequence data for individual genes started to grow; however, many of these rodent-based probes could not be used without further development as probes for homologous genes in cattle or pigs. This development process was aided immensely as GenBank submissions increased for cattle, pigs, and other farm animals.

Downloaded From Vet-eBooks.com

In contemporary times, animal species involved with food production have been sequenced to various degrees of completeness; however, sequence data on many promoters and structural genes are generally available. In early, gene expression work only a limited number of genes were subjected to DE analysis (Reiter et al., 2007; Halsey et al., 2011), but as real-time (quantitative) reverse transcriptase-polymerase chain reaction (RT-PCR) evolved as a main tool for DE, the number of target genes studied in single experiments has greatly expanded (Graugnard et al., 2010; Key et al., 2013; Perkins et al., 2014a,b).

When assessing DE for a set of target genes representing different metabolic pathways (such as peroxisome proliferator-activated receptor alpha [PPARα] for fatty acid catabolism and peroxisome proliferator-activated receptor gamma [PPARγ] for adipogenesis) (Reiter et al., 2007) was done using Northerns or PCR, biological interpretation of results could be readily based on basic understanding of biochemical pathways and accumulated DE data for such genes. The application of microarrays and more recently RNA deep sequencing has enabled researchers to simultaneously assess DE for thousands of genes within a given sample. Except for noting how many genes were up- or downregulated and checking on DE of specific individual genes, a full assessment of such results can only be completed using bioinformatics computer programs and tools (e.g., Ayuso et al., 2015) including Ingenuity Pathway Analysis (IPA), enriched gene ontology (GO)-terms analysis, structural variant analysis, and regulatory TFs analysis. Before any statistical and system biology analyses can be made, however, genes must be evaluated according to a minimum mean group expression threshold, a set level of fold-change DE (such as 1.5, 2, or greater), and an application of appropriate false discovery rates values. The application of these statistical concepts and pathway analysis of data will not be further discussed here. These interpretive workups of the massive data can then be assessed as networks from IPA and enriched biological function related to an overall criterion, such as structural genes, within pathway genes (glycolysis, fat lipolysis), and so forth. In addition, a new wrinkle has emerged in that gene expression can be attenuated at the posttranscriptional level by gene silencing. This is achieved by mRNA degradation or interference at the translation level by microRNA. Present in the genome are DNA sequences for specific microRNA which regulate expression of a given gene (Roberts 2015b; Svoboda et al., 2016). Thus, differential microRNA analysis adds an additional dimension to the interpretation of deep RNA sequencing analyses. Unfortunately, pathway analysis may predict differential expression of genes for a given pathway based on up- or downregulation of a given gene. Ultimately, the function of a pathway is dependent, however, on the pathway's rate-limiting enzyme, thermodynamic considerations, and substrate concentration. Thus, a downstream gene may demonstrate wide DE fluctuations with various treatments but it may be unlikely that this will have any effect on the biochemical function of a pathway.

Downloaded From Vet-eBooks.com

11.5 Experimental Approaches to Nutrigenomics Research

Experimental approaches in vivo can vary depending on the hypotheses to be assessed. The most frequent experimental treatments would be a DE response study to some food source, specific nutrients/molecules, and varying intakes of a given diet. Such experiments can be completed in a fairly short term and samples taken for appropriate "omics" analyses. While often not complicated from the actual execution standpoint, time course studies (e.g., neonatal, nursing, and growth finishing phases) with agricultural animals that require slaughter need extensive animal resources for the overall validly and analysis, and such studies are therefore expensive. Other studies will track genetic progress at the genome level for some trait over generations as, for example, selection for residual feed intake, marbling propensity, rate of growth, protein deposition, and fattening. These types of studies require the maintenance of large herds or flocks through many generations. Finally, cell culture studies may also be useful for DE work with a whole host of potential treatments and incubation protocols.

Functional genomics and transcriptomic research laboratory protocols require optimal sampling procedures and the isolation of high-quality RNA. When conducting genome-wide association studies, genotyping and SNP analysis of good quality DNA is also required. Unfortunately, RNA is much less robust than DNA and always subject to possible degradation during isolation from tissues and cell cultures.

11.6 Long-Chain Fatty Acids as Nutrigenomic Effectors

Long-chain fatty acids are highly hydrophobic molecules involved in numerous structural and dynamic cell functions, including serving as a highly concentrated form of metabolic energy, cell membrane composition and fluidity, functioning as regulatory ligands, and controlling gene expression (Jump et al., 2013, 2015). It is the latter area where dietary long-chain fatty acids may display nutrigenomic properties. In particular, highly unsaturated omega-3 fatty acids (HUFAs) have been shown to attenuate hepatic gene transcription and lipogenesis. A number of TFs are involved in this role of transcriptional regulation by polyunsaturated fatty acids (PUFAs) in the liver including SREBP-1c, PPARγ, and PPARα. HUFAs/PUFAs may affect transcription directly by binding to TFs or indirectly via binding to other TFs and coactivators (e.g., Ou et al., 2001; DeBose-Boyd et al., 2001; Jump et al., 2013; Teran-Garcia et al., 2002). In humans, development of nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH) parallels with the development of obesity and type 2 diabetes. Hence, ώ-3

Downloaded From Vet-eBooks.com

HUFAs (eicosapentaenoic acid [EPA] 20:5 ώ 3; docosahexaenoic acid [DHA] 22:6 ώ-3) have been tested to specifically ameliorate pathologic lipid accumulation in mouse fatty liver models. Dietary DHA showed a much stronger response in attenuating liver lipid accumulation than EPA (Jump et al., 2013). Other similar studies have had similar results and emerging translation issues into human medicine include how much HUFA must be administered to be effective in combatting NAFLD. A proposed mechanism of phytochemical (botanicals; ώ-3 PUFA) related nutrigenomics effects in animals can be found elsewhere (Luo et al., 2016).

In pigs and cattle, the primary sites of lipogenesis are the adipose tissues (Bergen and Mersmann, 2005). To evaluate nutrigenomics effects on de novo fatty acid synthesis by PUFA/HUFA in pigs, studies were conducted to monitor effects of long-chain PUFA on de novo fatty acid synthesis in porcine adipose tissues. Preliminary studies in my laboratory showed that in porcine adipose, as in rodent liver, ώ-3 HUFA (from fish oil) and to some extent ώ-6 PUFA will lower expression of some genes involved in de novo fatty acid synthesis. Once again, the issue of optimal dosage is an important factor in translational application of this work. Flaxseed (linseed oil) and canola oil are sources of 18-carbon ώ-3 fatty acids (linolenic acid). Over the years, some work has been done with these ώ-3 PUFA sources to evaluate their influence on fat deposition and marbling characteristics in pigs. The hypothesis was that ώ-3 PUFAs will depress overall body fat accretion, but these PUFAs as dietary fatty acids will also be channeled to IMF. This would presumably result in leaner pork with increased ώ-3 fatty acid content. On balance, to date such experiments have not yet demonstrated that feeding of ώ-3 fatty acids attenuate fat synthesis and change the fatty acid profile in IMF of pigs. Application of this possible nutrigenomic effect of plant ώ-3 fatty acid sources to attenuate fat deposition and possibly enhance marbling in commercial pork production is minimal.

11.7 Conjugated Linoleic Acids as Regulators of Fatty Acid Synthesis

As far back as the 1800s, a relationship between high-grain diets and butter fat production in lactating was observed. In the last third of the twentieth century, it was noted that there may be a molecule(s) of lipid nature in ruminant animal products (meat, milk, and cheese) that would have antiobesity and cardioprotective effects in animals (Wang and Jones, 2004; Shen and McIntosh, 2016). A team of workers led by Professor M. W. Pariza (University of Wisconsin-Madison, WI) identified these lipid molecules as conjugated linoleic acids (CLAs) (Ha et al., 1987; Pariza et al., 2001). CLAs are a group of unsaturated positional and geometric isomers of linoleic acids (cis, cis

Downloaded From Vet-eBooks.com

nonconjugated 18:2) that initially were touted to be a novel nutrigenomic molecule useful for the treatment of obesity and associated maladies. Since beef and dairy products were a source of CLA, strategies were implemented to advance consumption of these food products based on the presence of bioactive components. Further research was initiated to somehow increase CLA concentrations in ruminant animal products and it was speculated that bison provided muscle foods with higher CLA content than beef. After a number of clinical intervention trials, it was soon noted that potential daily consumption of endogenous CLA from beef or milk products (100–200 mg) was too low to have desired antiobesity effects. About 3 g daily of CLA as a 50–50 mixture of c9, t11 and t10, c12 CLA (active isomer) is usually needed for notable antiobesity effects in humans. Highly concentrated CLA supplements are now available for human use but their overall effectiveness is still not fully clear. From a mechanistic perspective, the t10, c12 CLA isomer reduces adiposity by enhancing lipolysis, fat oxidation, and depressing de novo fatty acid synthesis (Shen and McIntosh, 2016).

Milk fat depression (MFD) was a generally recognized issue when lactating cows are fed diets high in readily fermentable carbohydrates. While initially workers tried to relate MFD to shifts in the acetate/propionate molar ratio in rumen fermentation, the role of biohydrogenation of PUFA (linoleic acid) from high-energy feeds was later related to MFD. Then it was observed that 18:2 hydrogenation resulted in incomplete saturation of all 18:2. Thus, rumen trans-linoleic acid conjugated intermediates of c9, t11 and t10, c12 arose which could be further reduced to trans 11 C 18:1 and trans 10 C 18:1, both of which can be reduced to stearic acid. All these intermediates were found in milk and adipose fat of ruminants. The 18:1 trans-fatty acids could also be reconverted to c9 t11 and t10 18:1 CLA by tissue stearoyl-CoA desaturase. In any case, it has now been well established that t10 c12 is the active CLA in MFD (inhibition of mammary de novo fatty acid synthesis), decreasing adipose de novo fatty acid synthesis in nonruminants and nonlactating cows, but increasing de novo fatty acid synthesis in adipose of lactating cows fed an MFD diet (Bauman and Griinari, 2003; Bauman et al., 2011).

The potential of CLA in enhancing the quality and composition of nonruminant food animals has been studied over the last 25 years. It was shown when fed to pigs, CLA reduced back fat, increased marbling scores, and increased IMF by increasing IMF adipocyte size rather than number (Barnes et al., 2012). Administration of CLA to steers or bovine adipocytes in culture lowered adipose lipid deposition (Smith et al., 2009). Interestingly in lactating dairy cows, t10, c12 CLA inhibits mammary de novo lipogenesis but adipose tissue lipogenesis is actually increased (Thomas and Moore, 1953; Harvatine et al., 2009; Bauman et al., 2011; Bergen, 2009).

As indicated above, many bioactive molecules bind to TFs, which can then turn on/off given metabolic pathways through activating transcription of the TF target genes. Workers have strategically explored the nutrigenomics potential of molecules in animal production by targeting functionally

Downloaded From Vet-eBooks.com

related TF as related to animal production. The current ongoing discovery research in gene expression responses to multiple variables in animal production systems (related to nutrient intake, environmental constraints, management, and husbandry procedures) has identified some key TFs that could affect efficiency of animal foods production and quality. Research indicated that nutrigenomics-based DE findings in this regard tend to revolve around fat synthesis and oxidation with TF such as PPARα, PPARγ, SREBP-1c, and PPCoactivator 1 α (PGC1α) mostly implicated in adipose and muscle tissues (Wahli and Michalik, 2012). The involvement of TF regulating protein synthesis has been much more difficult to identify as this metabolic process is to a large extent under translational or posttranscriptional control. Thus, progress in finding other nutrigenomic molecules that will affect muscle protein synthesis and degradation has been slow. The issue is that such molecules (often amino acids, cAMP-stimulating agents, or beta-adrenergic agonists) either involve substrate level of amino acids effects or interactions with receptors which could affect both transcription and translation, respectively. These molecules in this context could at best be called "quasi-nutrigenomic"; however, they may affect gene expression. The beta-adrenergic agonists attenuate lipogenesis primarily via transcriptomic regulation while enhancing lipolysis and fatty acid oxidation via regulation of both gene expression and posttranscriptional processes. Other work has also shown that beta-agonists enhance myofibrillar synthesis (increase in alpha actin and myosin mRNA) and attenuate muscle protein degradation in beef cattle and pigs (Skjaerlund et al., 1993) by pre- and posttranslational regulation. Unfortunately, more often than not the extent of marbling is also lowered by beta agonists thereby affecting the organoleptic properties of beef and pork. The amino acid leucine has a nutrigenomics-like effect on translation processes (Columbus et al., 2013). Leucine stimulates the target of rapamycin (mTOR) system whereby initiation factors and their binding proteins (eIF2 and eIf4 family) are activated to support enhanced protein synthesis; however, these actions are not mediated via DE. Numerous studies have shown that leucine administration will increase myofibrillar protein synthesis under conditions where increased translation can occur (Columbus et al., 2013). Extra leucine appears to have a beneficial effect in neonates, but broad application of this concept to increasing animal protein production has been limited.

A relatively new paradigm in digestive physiology is that the digested nutrients (amino acids, sugars, and fatty acids) may have direct effects on the overall integrity of the mucosal epithelial cells. Work first with the rumen microbiome and then the lower gut microbiome showed that the anaerobic fermentations of feed and undigested foods, respectively, may produce bioactive molecules that may be critical for the well-being of gut epithelial cells. For example, the short-chain fatty acid (SCFA) butyrate arising from colonic fermentation has been implicated in the attenuation of colonic cell cancer. Colon cancer is characterized by rapid proliferation of colonocytes; this proliferation may be modified by butyric acid. Since the extent of butyrate

Downloaded From Vet-eBooks.com

production can be influenced by undigested food/prebiotics there has been great interest in understanding a putative role of butyric acid on colon cancer and health. Similarly, the pregastric fermentation in the rumen produces butyric acid (and other molecules) and the butyrate may possess beneficial effects on the rumen epithelium (Minuti et al., 2015). Ruminal biohydrogenation of PUFAs and CLAs has already been described previously in this chapter. To what degree nutrigenomic-like actions of gastrointestinal microbiome fermentations will effect animal production is not clear, but this area is receiving increased research attention.

11.8 The Role of High-Energy Diets in Promoting IMF Deposition in Finishing Cattle

Beef cattle consuming relatively low-net-energy feeds such as most grasses and forages during the finishing phase show a slow expression of finish and marbling; animals must be fed for extended periods to achieve finish and adequate marbling on such dietary regimes. To this end, after the growing and often backgrounding phase, feeder cattle are placed on high-energy, readily fermentable carbohydrate diets (such as starch in corn) and a desirable finish and acceptable marbling in beef is achieved after a few months of consuming the finishing diet. It has been shown that fat deposition becomes the principal anabolic process once animals have reached their lean carcass peak. This switch in priority of energy nutrient utilization (or nutrient partitioning) to continue fat deposition as lean tissue deposition slows significantly (Bergen, 1974; Prior and Scott, 1980; Smith and Crouse, 1984; Smith et al., 2009; Smith and Johnson, 2016) has also been related to an increased rate of marbling at that point.

The population of IMF adipocytes is very dynamic, ranging from preadipocytes to fully mature adipocytes. This is also accompanied in shifts in DE of adipogenic (differentiation) and lipogenic genes (fat synthesis) as IMF adipocytes accumulate. Pethick et al. (2004) noted that the fractional rate of IMF fat accretion is similar to that of the major depot fat sites.

11.9 Glucose versus Acetate as Precursors for IMF

The primary precursor for fatty acid synthesis is acetic acid in ruminants (cattle) and glucose in nonruminants (pigs, rodents). The rumen fermentation of all but high-starch-containing feeds produces principally acetic acid, but production of the two other SCFA is also prevalent

Downloaded From Vet-eBooks.com

(propionic acid and butyric acid). Consuming diets high in readily fermentable carbohydrates (starch from *zea maize*), as typical in finishing diets for cattle in the United States, produces a higher molar percent of propionic acid of the total SCFA production. Propionic acid can be channeled directly into hepatic gluconeogenesis since it can enter the anapleoretic portion of the citric acid cycle via succinate. While the present consensus is that in major adipose depots (subcutaneous, visceral, pelvic, kidney, and IMF), acetate is the principal substrate for de novo fatty acid synthesis in ruminants (Bergen and Mersmann, 2005), there is some evidence that in the IMF adipocytes of high-energy diet fed steers, glucose (from propionic acid) may be the primary carbon source for de novo fatty acid synthesis possibly accounting for a rapid rise in intensity of marbling in the finishing phase. These conclusions were based on in vitro incubations of various lipogenic substrates with ruminant liver and adipose slices and with in vivo infusions of radiolabeled acetate and glucose (Prior and Scott, 1980; Smith and Crouse, 1984). It should be noted here that in ruminants as compared to rodents, de novo fatty acid synthesis is minimal in liver (Bergen and Mersman, 2005). Acetic acid as acetyl-CoA is a precursor of de novo fatty acid synthesis in the cytosol and available directly from the rumen fermentation; however, glucose by itself is not a direct substrate for lipogenesis, and there appears to little real biochemical advantage to glucose as a lipogenic substrate in ruminants. Glucose must proceed through glycolysis and then early reactions in the mitochondrial tricarboxylic acid (TCA) cycle to obtain citrate from which then AcCoA arises in the cytosol upon citrate cleavage. A benefit of glucose may be related to stimulation of glucose transporters and the possibility of enhanced nicotinamide adenine dinucleotide phosphate (NADPH+H) production for reducing fatty acid carbon skeletons in IMF adipocytes. Further, acetic acid can never be converted to glucose and since glycerol (arising from glyceroneogenesis) is essential for the synthesis of triglycerides (triacylglycerols), the potential role of glucose in IMF lipogenesis may be significant. In this sense, propionic acid can be similar to a nutrigenomic molecule in that when present in adequate concentrations, this SCFA is not only an energy substrate for ruminant tissues, but also redirects regulation of lipogenesis in ruminant adipose tissues.

Questions about lipogenic precursors in ruminant for de novo fatty acid synthesis have, however, not been fully answered. Recently, workers (Nayananjalie et al., 2015) established a precocious fat deposition model by feeding weaned steer calves high-energy diets, followed by growing and finishing regimes. This experimental model was then utilized to assess differential incorporation of glucose and acetate into subcutaneous, intramuscular, and visceral palmitic acid. To determine differential utilization of lipogenic substrates, these finished cattle were infused with either ($^{2}H_{3}$) acetic acid or (U-$^{13}C_{6}$) glucose. Results showed that irrespective of location of the fat depot, fractional synthesis rates of palmitic acid from acetate were about 13-fold higher than rates observed for glucose. This recent study clearly indicates

Downloaded From Vet-eBooks.com

that acetate is the highly preferred precursor for de novo fatty acid synthesis for all fat depots in ruminants.

11.10 Conclusions and Future Trends

Our understanding of the impacts of nutrigenomics on production, health, and disease of food animals is just emerging. This research field is emerging as an ongoing culmination of application of all the genomics, transcriptomics, and bioinformatics to animal systems. Potential downstream benefits of nutrigenomics research have largely not yet impacted animal agriculture and discovery of many hitherto unknown nutrient–genome interactions are anticipated in the future. In this chapter, a number of topics in nutrigenomics that currently represent this rapidly advancing field of knowledge are described. Potential nutrigenomic effects of amino acids have not been discussed in detail as we cannot readily separate amino acids' role as protein building blocks, impacts on hormonal status and gene expression. From an era of technological innovations and new tools in agriculture (global positioning system [GPS], use of computer technology, minimum tillage, more efficient planting, and harvesting equipment and animal housing) we are now engaged in the discovery and application of biological innovations beyond traditional selection and propagation techniques to secure an adequate, wholesome food supply for an ever-expanding human population.

References

Ayuso, M., Fernández, A., Núñez, Y. et al. (2015). Comparative analysis of muscle transcriptome between pig genotypes identifies genes and regulatory mechanisms associated to growth, fatness and metabolism. *PLOS ONE*, **22**, 10:e0145162. doi:10.1371/journal.pone.0145162.

Baldwin, R. L., France, J., Beever, D. E. et al. (1987). Metabolism of the lactating dairy cow. III. Properties of mechanistic models suitable for evaluation of energetic relationships and factors involved in the partition of nutrients. *Journal of Dairy Research*, **54**, 133–145.

Barnes, K. M., Winslow, N. R., Shelton, A. G. et al. (2012). Effect of dietary conjugated linoleic acid on marbling and intramuscular adipocytes in pork. *Journal of Animal Science*, **90**, 1142–1149.

Bauman, D. E. and Griinari, J. M. (2003). Nutritional regulation of milk fat synthesis. *Annual Review of Nutrition*, **23**, 203–227.

Bauman, D. E., Harvatine, K. J. and Lock, A. L. (2011). Nutrigenomics, rumen-derived bioactive fatty acids, and the regulation of milk fat synthesis. *Annual Review of Nutrition*, **31**, 299–319.

Downloaded From Vet-eBooks.com

Bergen, W. G. (1974). Protein synthesis in animal models. *Journal of Animal Science*, **38**, 1079–1091.

Bergen, W. G. (2009). Milk-fat depression and lipid repartitioning in lactating dairy cows. *Journal of Nutrition*, **139**, 826–827.

Bergen, W. G. and Mersmann, H. J. (2005). Comparative aspects of lipid metabolism: Impact on contemporary research and use of animal models. *Journal of Nutrition*, **135**, 2499–2502.

Bionaz, M., Osorio, J. and Loor, J. J. (2015). Nutrigenomics in dairy cows: Nutrients, transcription factors, and techniques. *Journal of Animal Science*, 93:5531–5553.

Burnett, D. D. (2014). Targeted gene expression profiling in beef cattle under finishing conditions in the south eastern United States: Identification of regulatory factors involved in metabolic efficiency, energy partitioning, and intramuscular fat deposition. PhD dissertation, Auburn University, Alabama.

Columbus, D. A., Fiorotto, M. L. and Davis, T. A. (2013). Leucine is a major regulator of muscle protein synthesis in neonatal pigs. *Amino Acids*, **47**, 259–270.

DeBose-Boyd, R. A., Ou, J., Goldstein, J. L. et al. (2001). Expression of sterol regulatory element-binding protein 1c (SREBP-1c) mRNA in rat hepatoma cells requires endogenous LXR ligands. *Proceedings of the National Academy of Sciences USA*, **98**, 1477–1482.

France, J., Gill, M., Thornly, J. H. et al. (1987). A model of nutrient utilization and body composition in beef cattle. *Animal Production*, **44**, 371–385.

Graugnard, D. E., Berger, L. L., Faulkner, D. B. et al. (2010). High-starch diets induce precocious adipogenic gene network up-regulation in longissimus lumborum of early-weaned Angus cattle. *British Journal of Nutrition*, **103**, 953–963.

Guo, B., Kongsuwan, K., Greenwood, P. L. et al. (2014). A gene expression estimator of intramuscular fat percentage for use in both cattle and sheep. *Journal of Animal Science and Biotechnology*, **5**, 35.

Ha, Y. L., Grimm, N. K. and Pariza, M. W. (1987). Anticarcinogens from fried ground beef: Heat-altered derivatives of linoleic acid. *Carcinogenesis*, **12**, 1881–1887.

Halsey, C. H. C., Weber, P. S., Reiter, S. S. et al. (2011). The effect of ractopamine hydrochloride on gene expression in adipose tissues of finishing pigs. *Journal of Animal Science*, **89**, 1011–1019.

Harvatine, K. J., Perfield, J. W. and Bauman, D. E. (2009). Expression of enzymes and key regulators of lipid synthesis is upregulated in adipose tissue during CLA-induced milk fat depression in dairy cows. *Journal of Nutrition*, **139**, 849–854.

Joffe, Y. T. and Houghton, C. A. (2016). A novel approach to the nutrigenetics and nutrigenomics of obesity and weight management. *Current Oncology Reports*, **18**, 43–50.

Johnson, I. R., France, J., Thornley, J. H. et al. (2012). A generic model of growth, energy metabolism, and body composition for cattle and sheep. *Journal of Animal Science*, **90**, 4741–4751.

Jump, D. B., Depner, C. M., Tripathy, S. et al. (2015). Potential for dietary ω-3 fatty acids to prevent nonalcoholic fatty liver disease and reduce the risk of primary liver cancer. *Advances in Nutrition*, **6**, 694–702.

Jump, D. B., Tripathy, S. and Depner, C. M. (2013). Fatty acid-regulated transcription factors in the liver. *Annual Review of Nutrition*, **33**, 249–269.

Key, C. N., Perkins, S. D., Bratcher, C. L. et al. (2013). Grain feeding coordinately alters expression patterns of transcription factor and metabolic genes in subcutaneous adipose tissue of crossbred heifers. *Journal of Animal Science*, **91**, 2616–2627.

Downloaded From Vet-eBooks.com

Kim, M., Wang, L. and Morrison, M. (2014). Development of a phylogenetic microarray for comprehensive analysis of ruminal bacterial communities. *Journal of Applied Microbiology*, **117**, 949–960.

Komolka, K., Albrecht, E., Wimmers, K. et al. (2014). Molecular heterogeneities of adipose depots—Potential effects on adipose-muscle cross-talk in humans, mice and farm animals. *Journal of Genomics*, **2**, 31–44.

Lancaster, P. A., Sharman, E. D., Horn, G. V. et al. (2014). Effect of rate of weight gain of steers during the stocker phase. III. Gene expression of adipose tissues and skeletal muscle in growing-finishing beef cattle. *Journal of Animal Science*, **92**, 1462–1472.

Loor, J. J., Bionaz, M. and Drackley, J. K. (2013). Systems physiology in dairy cattle: Nutritional genomics and beyond. *Annual Review of Animal Biosciences*, **1**, 365–392.

Loor, J. J., Vailati-Riboni, M., McCann, J. C. et al. (2015). Lactation symposium: Nutrigenomics in livestock: Systems biology meets nutrition. *Journal of Animal Science*, **93**, 5554–5574.

Luo, T., Miranda-Garcia, O., Adamson, A. et al. (2016). Consumption of walnuts in combination with other whole foods produces physiologic, metabolic, and gene expression changes in obese C57BL/6J high-fat-fed male mice. *Journal of Nutrition*, **146**, 1641–1650.

McCann, J. C., Wickersham, T. A., Loor, J. J. et al. (2014). High-throughput methods redefine the rumen microbiome and its relationship with nutrition and metabolism. *Bioinformatics and Biology Insights*, **8**, 109–125.

Minuti, A., Palladino, A., Khan, M. J. et al. (2015). Abundance of ruminal bacteria, epithelial gene expression, and systemic biomarkers of metabolism and inflammation are altered during the peripartal period in dairy cows. *Journal of Dairy Science*, **98**, 8940–8951.

Nayananjalie, W. A., Wiles, T. R., Gerrard, D. E., McCann, M. A., Hanigan, M. D. (2015). Acetate and glucose incorporation into subcutaneous, intramuscular, and visceral fat of finishing steers. *Journal of Animal Science*, **93**, 2451–2459.

Ou, J., Tu, H., Shan, B. et al. (2001). Unsaturated fatty acids inhibit transcription of the sterol regulatory element-binding protein-1c (SREBP-1c) gene by antagonizing ligand-dependent activation of the LXR. *Proceedings of the National Academy of Sciences USA*, **98**, 6027–6032.

Pariza, M. W., Park, Y. and Cook, M. E. (2001). The biologically active isomers of conjugated linoleic acid. *Progress in Lipid Research*, **40**, 283–298.

Perkins, S. D., Key, C. N., Garrett, C. F. et al. (2014a). Residual feed intake studies in Angus-sired cattle reveal a potential role for hypothalamic gene expression in regulating feed efficiency. *Journal of Animal Science*, **92**, 549–560.

Perkins, S. D., Key, C. N., Marvin, M. N. et al. (2014b). Effect of residual feed intake on hypothalamic gene expression and meat quality in Angus-sired cattle grown during the hot season. *Journal of Animal Science*, **92**, 1451–1461.

Pethick, D. W., Harper, G. S. and Oddy, V. H. (2004). Growth, development and nutritional manipulation of marbling in cattle: A review. *Australian Journal of Experimental Agriculture,* **44**, 705–715.

Picard, B., Lebret, B., Cassar-Malek, I. et al. (2015). Recent advances in omic technologies for meat quality management. *Meat Science*, **109**, 18–26.

Downloaded From Vet-eBooks.com

Prior, R. L. and Scott, R. A. (1980). Effects of intravenous infusions of glucose, lactate, propionate or acetate on the induction of lipogenesis in bovine adipose tissue. *Journal of Nutrition*, **110**, 2011–2019.

Reiter, S. S., Halsey, C. H. C., Stronach, B. N., Bartosh, J. L., Owsley, W. F., Bergen, W. G. (2007). Lipid metabolism related gene-expression profiling in liver, skeletal muscle a adipose tissue in crossbred Duroc and Pietrain pigs. *Comparative Biochemistry and Physiology Part D*, **2**, 200–2007.

Roberts, S. L., Lancaster, P. A., DeSilva, U., Horn, G. W., Krehbiel, C. R. (2015a). Coordinated gene expression between skeletal muscle and intramuscular adipose tissue in growing beef cattle. *Journal of Animal Science*, **93**, 4302–4311.

Roberts, T. C. (2015b). The micro-RNA machinery. *Advances in Experimental Medicine and Biology*, **887**, 15–30.

Shen, W. and McIntosh, M. K. (2016). Nutrient regulation: Conjugated linoleic acid's inflammatory and browning properties in adipose tissue. *Annual Review of Nutrition*, **36**, 183–210.

Skjaerlund, D. M., Grant, A. L. and Helferich, W. G. (1993). Determination of the relative abundance of skeletal muscle alpha actin mRNA in muscle of livestock species. *Journal of Animal Science*, **71**, 393–399.

Smith, S. B. and Crouse, J. D. (1984). Relative contributions of acetate, lactate and glucose to lipogenesis in bovine intramuscular and subcutaneous adipose tissue. *Journal of Nutrition*, **114**, 792–800.

Smith, S. B. and Johnson, B. J. (2016). Marbling of cattle to maximize the deposition of intramuscular adipose. *Journal of Animal Science*, **94**(eE-Suppl 5), 374.

Smith, S. B., Kawachi, H. and Choi, C. B. (2009). Cellular regulation of bovine intramuscular adipose development and composition. *Journal of Animal Science*, **87**(14 Suppl), E72–E82.

Svobodova, E., Kubikova, J. and Svoboda, P. (2016). Production of small RNAs by mammalian Dicer. *Pflugers Arch Review*, **468**: 1089–1101.

Tedeschi, L. O., Fox, D. G. and Kononoff, P. J. (2013). A dynamic model to predict fat and protein fluxes and dry matter intake associated with body reserve changes in cattle. *Journal of Dairy Science*, **96**, 2448–2463.

Teran-Garcia, M., Rufo, C., Nakamura, M. T., Osborne, T. F., Clarke, S. D. (2002). NF-Y involvement in the polyunsaturated fat inhibition of fatty acid synthase gene transcription. *Biochemical and Biophysical Research Communications*, **290**, 1295–1299.

Thomas, J.W. and Moore, R.A. (1953). Thyroprotein feeding to dairy cows during successive lactations. *Journal of Dairy Science*, 36, 657–672.

Wahli, W. and Michalik, L. (2012). PPARs at the crossroads of lipid signaling and inflammation. *Trends Endocrinology Metabolism*, **32**, 351–363.

Wang, Y. W. and Jones, P. J. (2004). Conjugated linoleic acid and obesity control: Efficacy and mechanisms. *International Journal of Obesity and Related Metabolic Disorders*, **28**, 941–955.

Williams, E. G, Wu, Y. J., Pooja, J. et al. (2016). Systems proteomics of liver mitochondria function. *Science*, **352**, 1292.

Downloaded From Vet-eBooks.com



Taylor & Francis
Taylor & Francis Group
http://taylorandfrancis.com

Downloaded From Vet-eBooks.com

12

Bioactive Properties of Peptides Generated from Meat Proteins

Keizo Arihara and Yusuke Komiya

CONTENTS

12.1 Introduction 371
12.2 Generation of Peptides from Meat Proteins 372
12.2.1 Gastrointestinal Proteolysis 373
12.2.2 Aging of Meats 373
12.2.3 Fermentation of Meats 374
12.2.4 Enzymatic Treatment 374
12.3 Bioactivities of Meat Protein-Derived Peptides 375
12.3.1 ACE Inhibitory/Antihypertensive Peptides 375
12.3.2 Antioxidative Peptides 378
12.3.3 Opioid Peptides 379
12.3.4 Immunomodulatory Peptides 380
12.3.5 Hypocholesterolemic Peptides 381
12.3.6 Antithrombotic Peptides 381
12.3.7 Antimicrobial Peptides 382
12.3.8 Prebiotic Peptides 382
12.3.9 Bioactive Peptides from By-Products 383
12.3.10 Peptides with Sensory Properties 385
12.4 Peptides and the Maillard Reaction 385
12.4.1 The Maillard Reaction 385
12.4.2 Maillard Reaction and Meat 386
12.4.3 Bioactivities of MRPs from Peptides 387
12.4.4 Bioactivities of Volatile MRPs from Peptides 388
12.5 Conclusions 389
References 391

12.1 Introduction

Functional foods are foods with additional functions related to health promotion or disease prevention by functional ingredients (Arihara, 2006a, 2014; Bagchi and Nair, 2017). Peptides generated from food proteins are

Downloaded From Vet-eBooks.com

representative functional food ingredients (Aleixandre and Miguel, 2012; Bowman, 2015). Although the activities of these peptides are latent in parent food proteins, they are released by enzymatic treatments. Mellander (1950) first reported that bioactive peptides were generated from food proteins. He suggested that casein-derived phosphorylated peptides enhanced vitamin D-independent bone calcification in rachitic infants. Since then, information on bioactivities of peptides generated from food proteins has steadily accumulated, and numerous peptides exhibiting various activities have been discovered (Bowman, 2015; Hettiarachchy, 2012; Owusu-Apenten, 2010). Such peptides include antihypertensive, opioid, immunostimulating, antimicrobial, antithrombotic, hypocholesterolemic, and antioxidative peptides. It goes without saying that these peptides can be generated from meat proteins. There is a great possibility of utilizing such components for developing novel functional meat products and food ingredients (Arihara, 2006b, 2014; Pathera et al., 2017). In this chapter, generation of peptides from meat proteins and potential benefits of such peptides are discussed. Maillard reaction products (MRPs) generated from meat protein-derived peptides are also focused.

12.2 Generation of Peptides from Meat Proteins

Although most proteins contain bioactive sequences, those sequences are inactive or incomplete within the parent proteins. Only via proteolytic digestion are active peptide fragments released from native proteins. Once such peptides are liberated, they can act as regulatory compounds. Along with gastrointestinal digestion, there are several ways in which peptides are generated from meat proteins (Figure 12.1).

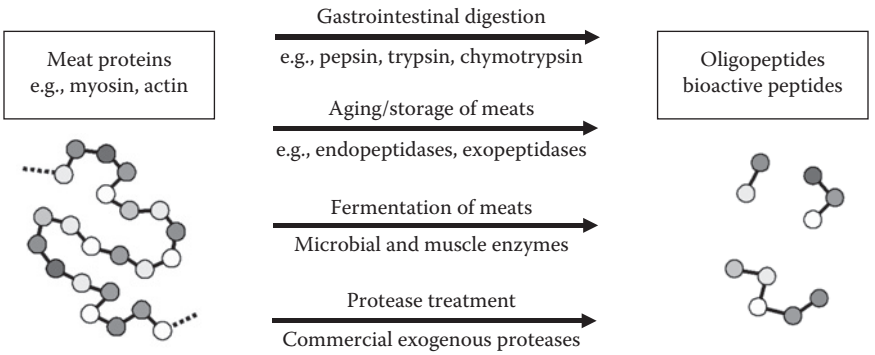


FIGURE 12.1
Generation of peptides from meat proteins.

Downloaded From Vet-eBooks.com

12.2.1 Gastrointestinal Proteolysis

During gastrointestinal proteolysis, bioactive peptides are liberated from food proteins by various digestive enzymes (Pihlanto and Korhonen, 2003). Ingested proteins are attacked first by pepsin in the stomach. They are further hydrolyzed by trypsin, chymotrypsin, elastase, and carboxypeptidase in the small intestine. Katayama et al. (2003b) reported that angiotensin I-converting enzyme (ACE) inhibitory activity was generated from meat proteins (i.e., myosin, actin, tropomyosin, and troponin) by pancreatic protease treatment. ACE inhibitory (antihypertensive) peptides generated from pork meat were found during *in vitro* gastrointestinal digestion (Escudero et al., 2010, 2012). Terashima et al. (2010) identified ACE inhibitory peptides from the hydrolysate of boneless chicken leg meat digested with artificial gastric juice. Recently, Simonetti et al. (2016) reported that *in vitro* gastrointestinal digestion enhanced the biological activity of pig meat. Sayd et al. (2016) identified and quantified peptides released from cooked meat during *in vitro* gastric and intestinal digestion.

12.2.2 Aging of Meats

The content of peptides in meat increases during postmortem aging. Nishimura et al. (1988) reported changes in oligopeptide levels occurring during the storage of beef, pork, and chicken. Oligopeptides increased in all meats during storage. For example, the content of peptide in pork increased from 2.40 mg at day 1 to 3.05 mg/g meat at day 6 (53% increment). Mikami et al. (1995) reported that peptide contents of beef varied widely, ranging from 0.69 to 1.44 mg and from 2.64 to 4.65 mg/g meat 2 and 21 days after slaughter, respectively.

During aging or storage, meat proteins are hydrolyzed by muscle endogenous proteases (Etherington, 1984; Koohmaraie, 1994). Initially, endopeptidases (e.g., calpains and cathepsins) hydrolyze muscle proteins and generate polypeptides. Then such polypeptides are further hydrolyzed by exopeptidases and dipeptyl peptidases. Such enzymatic hydrolysis contributes to improvement of sensory properties of meat (texture, taste, and flavor). In addition, peptides with bioactivities can be generated in meat during postmortem aging. Bauchart et al. (2006) examined the peptide generation in fresh beef after 14 days of ripening and after cooking. In their study, the content of several presumable bioactive peptides was greatly increased. Arihara and Ohata (2015) identified ACE inhibitory (antihypertensive) peptides (i.e., Gly-Pro-Leu-Lys, Ile-Pro-Ile-Lys, Ile-Pro) in aged pork. Also, they showed an increase in ACE inhibitory activity of beef during cold storage (Arihara and Ohata, 2017). Recently, Fu et al. (2017) investigated endogenous release of bioactive (e.g., ACE inhibitory) peptides in beef during aging. Findings provided insights on development of healthy beef through postmortem aging.

Downloaded From Vet-eBooks.com

12.2.3 Fermentation of Meats

Bioactive compounds including peptides are produced during fermentation processes of foods. In most fermented foods, proteolytic changes occur and various peptides are generated from food proteins (Hernández-Ledesma et al., 2004; Hayes and García-Vaquero, 2016). It has been reported that fermented dairy products (e.g., cheese and yoghurt) contain bioactive peptides, such as ACE inhibitory and antioxidative peptides (Bütikofer et al., 2008; Gagnaire et al., 2001; Gobbetti et al., 2007; Gomez-Ruiz et al., 2002; Saito et al., 2000). These peptides are generated by proteolytic activities of starter microorganisms (i.e., lactic acid bacteria and mold).

Proteolytic degradations that occur during fermentation of raw sausages and dry-cured ham have been studied extensively, since components generated from meat proteins are critical for the development of sensory properties of fermented meat products (Toldrá and Flores, 1998; Ordonez et al., 1998; Toldrá, 2014). Although both muscle endogenous and microbial proteolytic enzymes are involved in fermentation of meat products, the former enzymes contribute greatly to meat protein degradation. During fermentation of meat products, primarily, proteins are degraded into peptides by endogenous enzymes (cathepsins B, D, H, and L). Although calpains play a central role in the conditioning of meat, they do not exhibit activity at the end of the salting step (Sarraga et al., 1989). Since most bacteria (e.g., lactobacilli) found in fermented meat products have only weak proteolytic activity, the degradation of proteins (e.g., myosin and actin) is not greatly affected by the bacteria (Hierro et al., 1999). However, lactic acid bacteria influence protein degradation by causing reduction in pH, which results in increased activity of muscle proteases (Kato et al., 1994).

During fermentation of sausages, the content of peptides and amino acids reaches about 1% dry matter of products (Dainty and Blom, 1995). Sentandreu et al. (2003) and Mora et al. (2009) identified small peptides in Spanish dry-cured ham. As described later in this chapter, they identified many bioactive peptides generated in dry-cured ham. López et al. (2015) characterized proteolysis and low-molecular-weight peptides of commercial Argentinean fermented sausages by applying a peptidomic approach. This study represents a first peptidomic approach for fermented sausages, thereby providing a baseline to define key peptides acting as potential biomarkers.

12.2.4 Enzymatic Treatment

The last, but most efficient, method of peptide preparation from food proteins is by utilizing commercial exogenous proteases. Proteases from animal, plant, and microbial sources have been applied to food protein solution for the industrial scale production of bioactive peptides (Agyei and Danquah, 2011; Young and Mine, 2009). Utilization of various commercial proteases is one approach for producing bioactive peptides from food proteins.

Downloaded From Vet-eBooks.com

Many bioactive peptides have been experimentally generated in this way (Korhonen and Pihlanto, 2003; Pihlanto-Leppala, 2001; Pihlanto and Korhonen, 2003). Single proteinases from animal, plant, and microbial sources and combinations of them have been used for the digestion of food proteins (Yoshikawa, 1996). As described later, several proteases have been utilized for the generation of bioactive peptides from meat proteins.

In the meat industry, proteolytic enzymes have been used for meat tenderization. The most commonly used enzymes for meat tenderization are the plant enzymes papain, bromelain, and ficin (Dransfield and Etherington, 1981). In meat treated with enzymatic tenderization, peptides having bioactivities could be generated. On the other hand, effects of commercial proteases on protein breakdown and sensory characteristics of dry fermented sausages have been investigated (Diaz et al., 1997; Bruna et al., 2000). Such treatment would also generate bioactive peptides in meat products. Mirdhayati et al. (2015) prepared hydrolysates of goat meat proteins by using sequential digestion of commercial endoproteinase and protease complex. Since they successfully obtained hydrolysates with ACE inhibitory and antihypertensive activities, they are trying to develop a functional ingredient utilizing a meat protein hydrolysate. More recently, Ryder et al. (2016) evaluated commercially available food-grade microbial protease preparations for their ability to produce bioactive peptides by hydrolyzing meat myofibrillar and connective tissue protein extracts.

12.3 Bioactivities of Meat Protein-Derived Peptides

Since the first discovery of bioactive peptides generated from food proteins (Mellander, 1950), information about such peptides has accumulated (Bowman, 2015; Wu et al., 2015). Various bioactive peptides have been found in hydrolysates of food proteins (Figure 12.2). Such peptides have been also identified in hydrolysates of meat proteins and in meat products (Arihara, 2006b; Arihara and Ohata, 2017; Lafarga and Hayes, 2014; Mora and Toldrá, 2014; Ryan et al., 2011; Udenigwe and Howard, 2013; Wu et al., 2015).

12.3.1 ACE Inhibitory/Antihypertensive Peptides

The most extensively studied bioactive peptide generated from food proteins is the ACE inhibitory peptide (Aleixandre and Miguel, 2012; He et al., 2013; Meisel et al., 2005; Norris and FitzGerald, 2015). ACE inhibitory peptides lower blood pressure through inhibition of ACE in the body. ACE is a dipeptidyl carboxypeptidase, which is widely distributed in mammalian bodies, predominantly as a membrane-bound ectoenzyme in vascular endothelial cells. ACE plays a critical role in the regulation of blood pressure in

Downloaded From Vet-eBooks.com

the renin angiotensin system (Figure 12.3). ACE converts the inactive decapeptide angiotensin I into the potent vasoconstricting octapeptide angiotensin II, resulting in an increase in blood pressure. ACE also inactivates the antihypertensive vasodilator bradykinin. Therefore, by inhibiting the catalytic action of ACE, the elevation of blood pressure can be suppressed (Li et al., 2004). Oshima et al. (1979) first found ACE inhibitory peptides derived from food proteins in the hydrolysate of gelatin. Thereafter, these peptides have been identified in the hydrolysates of various proteins (Aleixandre and Miguel, 2012; He et al., 2013; Norris and FitzGerald, 2015). To date, many ACE-I inhibitory peptides have been isolated from hydrolysates of muscle (meat) proteins and have been reviewed (Ahhmed and Muguruma, 2010; Di Bernardini et al., 2012; Vercruysse et al., 2005; Wu et al., 2015). Meat proteins seem to be good sources of ACE inhibitory peptides (Gu et al., 2011).

Fujita et al. (2000) isolated ACE inhibitory peptides (Leu-Lys-Ala, Leu-Lys-Pro, Leu-Ala-Pro, Phe-Gln-Lys-Pro-Lys-Arg, Ile-Val-Gly-Arg-Arg-Arg-His-

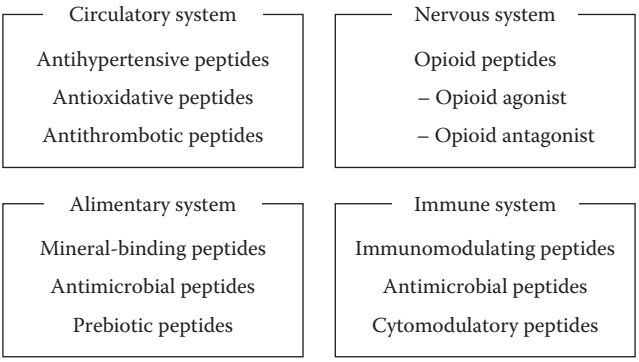


FIGURE 12.2
Representative bioactive peptides generated from food proteins.

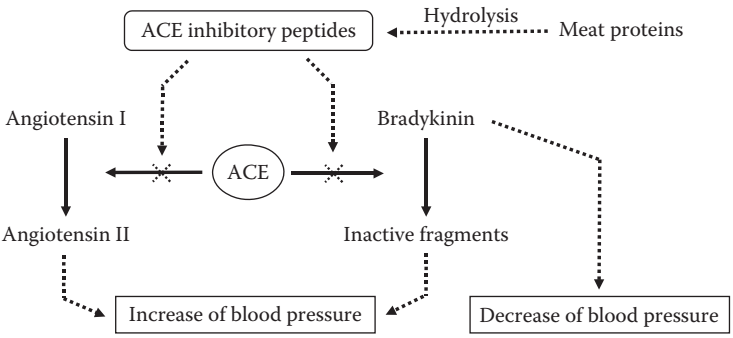


FIGURE 12.3
Blood pressure regulation by angiotensin I-converting enzyme (ACE) and ACE inhibitory peptides generated from meat proteins.

Downloaded From Vet-eBooks.com

Gln-Gly, Phe-Lys-Gly-Arg-Tyr-Tyr-Pro, Ile-Lys-Trp) generated from chicken muscle proteins by thermolysin treatment. Arihara et al. (2001) reported ACE inhibitory peptides in enzymatic hydrolysates of porcine skeletal muscle proteins. Among the digests of porcine muscle proteins, thermolysin digest showed the most potent inhibitory activity. Two ACE inhibitory peptides (Met-Asn-Pro-Pro-Lys and Ile-Thr-Thr-Asn-Pro), which are found in the sequence of myosin heavy chain, were purified from thermolysin digest of myosin. These peptides showed antihypertensive activity when administered orally to spontaneously hypertensive rats (Nakashima et al., 2002). Katayama et al. (2003a,b, 2004) utilized porcine skeletal muscle and respective muscle proteins for proteolytic digestion in a series of studies. All enzymatic hydrolysates of water-soluble protein extracted from pork loin, myosin B, myosin, actin, and tropomyosin, which were prepared by eight kinds of proteases, showed ACE inhibitory activities, and the pepsin-treated hydrolysate showed the highest activity (Katayama et al., 2003a). They isolated a corresponding peptide (Arg-Met-Leu-Gly-Gln-Thr-Pro-Thr-Lys) from hydrolyzed porcine troponin C with pepsin (Katayama et al., 2003b). They also investigated the inhibitory profile of this noncompetitive peptide (Katayama et al., 2004). ACE inhibitory activity and antihypertensive effect were also generated from pork muscle by fermentation with lactic acid bacteria (Arihara et al., 2004). Octapeptide (Val-Phe-Pro-Met-Asn-Pro-Pro-Lys) with ACE inhibitory activity was identified from the fermented pork meat.

Saiga et al. (2003a) reported antihypertensive activity of *Aspergillus* protease-treated chicken muscle extract in spontaneously hypertensive rats. Furthermore, they isolated four ACE inhibitory peptides from the hydrolysate. Among the four peptides, three peptides possessed a common sequence, Gly-X-X-Gly-X-X-Gly-X-X, which is homologous with that of collagen. The peptide Gly-Phe-Hyp-Gly-Thr-Hyp-Gly-Leu-Hyp-Gly-Phe showed the strongest inhibitory activity. Jang and Lee (2005) assayed ACE inhibitory activities of several enzymatic hydrolysates of sarcoplasmic protein extracts from beef rump. An ACE inhibitory peptide (Val-Leu-Ala-Gln-Tyr-Lys) purified from the hydrolysate with the highest ACE inhibitory activity was prepared by using the combination of thermolysin and proteinase A. Sentandreu and Toldrá (2007) identified seven ACE inhibitory peptides from porcine skeletal muscle treated with dipeptidyl peptidase. These included the peptides Arg-Pro, Lys-Ala, Ala-Ala, Gly-Pro, Ala-Arg, Gly-Arg, and Arg-Arg. Di Bernardini et al. (2012) studied ACE inhibitory and antioxidant activities of hydrolysates of bovine brisket sarcoplasmic proteins produced by papain. Similarities between the amino acid sequences of the peptides identified in their study and previously identified antioxidant and ACE inhibitory peptides detailed in the BIOPEP database were outlined.

During fermentation, bioactive peptides are also generated from muscle proteins in meat products. As a source of bioactive peptides including ACE inhibitory (antihypertensive) peptides, Spanish dry-cured ham has been extensively studied by a group of Spanish researchers (Escudero et al., 2013a,

Downloaded From Vet-eBooks.com

2014; Gallego et al., 2014, 2015, 2016; Mora et al., 2014, 2015, 2016). Their great efforts will make traditional cured ham reborn as novel functional meat products. Dellafiora et al. (2015) utilized a hybrid *in silico/in vitro* approach for the identification of ACE inhibitory peptides from Parma dry-cured ham.

Castellano et al. (2013) investigated ACE inhibitory peptides derived from the hydrolysates of sarcoplasmic and myofibrillar porcine proteins by the action of *Lactobacillus sakei* and *Lactobacillus curvatus*. Their results suggested that meat-borne *Lactobacillus* were able to generate peptides with ACE inhibitory activity, highlighting their potential to be used in the development of functional fermented products. Ferranti et al. (2014) identified many ACE inhibitory and antioxidative peptides from cured ham and fermented sausages.

ACE inhibitory peptides from various sources, including meat proteins, have been shown to have antihypertensive effects by oral administration in animal experiments using spontaneously hypertensive rats (Fujita and Yoshikawa, 1999; Fujita et al., 2000; Nakashima et al., 2002). However, the inhibitory potencies of peptides do not always correlate with their antihypertensive effects. Some peptides with potent ACE inhibitory activities *in vitro* are inactive by oral administration. Regarding the ACE inhibitory peptides derived from food proteins, Li et al. (2004) reviewed the phenomenon between inhibitory activity and antihypertensive effect and the structure–activity relationships.

Although many ACE inhibitory peptides with diverse sequence properties have been isolated from food proteins, their structure–activity relationships have not been fully clarified (Li et al., 2004). However, binding of ACE inhibitory peptides to an ACE seemed to be influenced by the C-terminal tripeptide sequence of peptides (Ondetti and Cushman, 1982). Also, studies have demonstrated that the C-terminal amino acid residue of peptides is critical for their potential. It has been reported that many ACE inhibitory peptides with potent activity have Trp, Phe, Tyr, or Pro at their C-terminus (Maruyama et al., 1987; Miyoshi et al., 1991; Cheung et al., 1980).

12.3.2 Antioxidative Peptides

Neutralization and reduced release of free radicals occur through the ingestion of diet. This beneficial action of food is attributed to the antioxidant potency of various compounds, such as vitamin C and E, and polyphenols. Meat contains endogenous peptides with antioxidative activities such as carnosine (β-alanyl-L-histidine) and anserine (β-alanyl-1-methyl-L-histidine) (Arihara, 2014). In addition to these endogenous peptides, antioxidative peptides can be generated from meat proteins by enzymatic hydrolysis (Liu et al., 2016; Stadnik and Kęska, 2015).

Antioxidative peptides have been identified from various food proteins, such as soybean, milk, eggs, and meat (Bernardini et al., 2011; Kim et al., 2012; Liu et al., 2016). Saiga et al. (2003b) identified five antioxidative peptides in the enzymatic hydrolysate of porcine muscle proteins. These peptides contain

Downloaded From Vet-eBooks.com

acidic amino acids, Asp or Glu, in their sequences. Although basic amino acids, such as His and Lys, showed strong antioxidative activity (Chen et al., 1996), acidic peptides as well as basic peptides possess antioxidative activity.

Arihara et al. (2011) investigated antioxidative activities of enzymatic hydrolysates of porcine muscle actomyosin. Three antioxidative peptides were isolated from a papain-treated hydrolysate of actomyosin and were sequenced as Asp-Leu-Tyr-Ala, Ser-Leu-Tyr-Ala, and Val-Trp. Each of these peptides had an antifatigue effect when orally administered to mice in an experiment using a treadmill. 2,2-diphenyl-1-picrylhydrazyl (DPPH) activities and superoxide ion extinguishing ability were identified for peptide extracts isolated from Spanish dry-cured ham (Escudero et al., 2012, 2013b). Recently, Xing et al. (2016) identified antioxidative peptides from dry-cured Xuanwei ham. Asp-Leu-Glu-Glu seemed to be one of the main peptides that plays a key role in the antioxidant activity. Ohata et al. (2016) paid attention to antioxidant activities of fermented meat sauce, which was prepared by mixing ground pork, Koji mold, and salt, and fermenting for 24 weeks. The tripeptide Gln-Tyr-Pro with an extremely high antioxidant activity against the OH-radical was identified.

12.3.3 Opioid Peptides

Opioid peptides are defined as peptides that have an affinity for an opioid receptor as well as opiate-like effects (Pihlanto and Korhonen, 2003). These peptides influence the nerve system and gastrointestinal functions. Typical opioid peptides are endorphins, enkephalin, and prodynorphin. These opioid peptides have the same N-terminal sequence, Tyr-Gly-Gly-Phe. Opioid peptides exert their activity by binding to intestinal-specific receptors of the target cell. The individual receptors are responsible for specific physiological effects (i.e., emotional behavior, suppression of intestinal motility, sedation, and food intake).

Several opioid peptides derived from food proteins have been reported. The *N*-terminal sequence of most of these peptides is Tyr-X-Phe or Tyr-X1-X2-Phe. The *N*-terminal tyrosine residue and the presence of an aromatic amino acid at the third or fourth position form a critical structure that fits with the binding site of opioid receptors (Pihlanto-Leppälä, 2001). Casomorphins, the first identified group of opioid peptides derived from food proteins, are generated from milk casein by enzymatic hydrolysis (Brantl et al., 1979). Generation of casomorphins from casein during gastrointestinal digestion has been demonstrated (Meisel, 1986). Also, proteolysis of milk whey proteins (α-lactalbumin and β-lactoglobulin) with digestive enzymes produced opioid peptides with sequences Tyr-Gly-Leu-Phe and Tyr-Leu-Leu-Phe, which were named α-lactorphin and β-lactorphin (Chiba and Yoshikawa, 1986).

Subcutaneous administration of α-lactorphin dose-dependently lowered systolic and diastolic blood pressures in spontaneously hypertensive rats as well as in normotensive rats (Nurminen et al., 2000). The antihypertensive

Downloaded From Vet-eBooks.com

mechanism of α-lactorphin is not by ACE inhibition but rather appears to be due to interaction with opioid receptors. This provides further evidence for the multifunctional role of bioactive peptides. Thus, mechanisms other than ACE inhibition may be involved in the antihypertensive actions of various peptides. Opioid peptides were also found in hydrolysates of wheat gluten (exorphins) (Fukudome and Yoshikawa, 1992, 1993) and blood hemoglobin (hemorphons) (Nyberg et al., 1997; Zhao et al., 1997).

There has been no report on the generation of opioid peptides from meat proteins. However, possible opioid sequences, such as Tyr-X-Phe or Tyr-X1-X2-Phe, are found in the sequences of muscle proteins (e.g., myosin heavy chain). Therefore, it should be possible to find opioid peptides in meat proteins by proteolytic treatment. Bovine blood hemoglobin is regarded as a minor component in meat and meat products. In some meat products, such as blood sausage, hemoglobin is a major component of the product. Investigation of hemoglobin peptic hydrolysate revealed the presence of biologically active peptides with affinity for opioid receptors (Nyberg et al., 1997; Zhao et al., 1997). These peptides were named hemorphins (VV-hemorphin 7, Val-Val-Tyr-Pro-Trp-Thr-Gln-Arg-Phe; LVV-hemorphin-7, Leu-Val-Val-Tyr-Pro-Trp-Thr-Gln-Arg-Phe). Hemorphins were first isolated from enzymatically treated bovine blood. Later, these peptides were found in the brain, plasma, and cerebrospinal fluid. When proteolytically degraded, hemoglobin gives rise to active peptides that are probably involved in regulatory functions *in vivo* during pain, physical effort, inflammation, and blood pressure variation. Also, a study on quantification of hemorphins in Alzheimer's disease brains suggests that these peptides could be candidates for pharmacological prevention of this disease (Poljak et al., 2004). Another study has demonstrated that valorphin (Val-Val-Tyr-Pro-Trp-Thr-Gln), a fragment of hemorphins, suppressed the proliferation of tumor cells (Blishchenko et al., 2002).

12.3.4 Immunomodulatory Peptides

Immunomodulatory peptides affect both the immune system and cell proliferation responses. It has been reported that several hydrolysates of milk caseins stimulate the immune system. Many studies have shown that immunomodulatory sequences exist within food proteins, especially milk proteins. Although several hydrolysates of milk caseins stimulate the immune system, their modulatory effects are dependent on the proteolytic systems employed. Peptides generated from milk caseins by pancreatin or trypsin inhibited the proliferative responses of murine splenic lymphocytes and Peyer's patch cells (Otani and Hata, 1995). However, digests generated by pepsin or chymotrypsin did not show such activity. Peptides derived from pepsin-trypsin hydrolysis of caseins strongly suppressed mitogen-induced proliferation of peripheral blood mononuclear cells (Kayser and Meisel, 1996). Casein-derived peptides sequenced as Val-Glu-Pro-Ile-Pro-Asn and Gly-Leu-Tyr enhanced phagocytosis of human and murine macrophages

Downloaded From Vet-eBooks.com

and prevented *Klebsiella pneumoniae* infection in mice (Migliore-Samour et al., 1989; Fiat et al., 1993).

Immunomodulating peptides have also been discovered in enzymatic hydrolysates of proteins from various foods, such as eggs (Watanabe et al., 1998), soybean (Yoshikawa et al., 1993), and rice (Matsubayashi and Sakagami, 1999). Although meat protein-derived immunomodulating peptides have not been reported, Kęska and Stadnik (2016) suggested that pork myofibrillar proteins are a promising source of various bioactive peptides based on *in silico* analysis.

12.3.5 Hypocholesterolemic Peptides

Dietary proteins (e.g., milk and soy proteins) have been shown to lower serum cholesterol level (Nagaoka et al., 1992). However, until recently, the mechanism of the hypocholesterolemic activity induced by food proteins was unclear. Based on the hypothesis that hypocholesterolemic peptides derived from food protein exist and influence serum cholesterol level (Sugano et al., 1990), Nagaoka et al. (2001) attempted to clarify the mechanism of the hypocholesterolemic action of milk β-lactoglobulin. A hypocholesterolemic peptide (Ile-Ile-Ala-Glu-Lys) was found in the enzymatic hydrolyzates of bovine milk β-lactoglobulin (Nagaoka, 2012). This peptide has a strong effect on serum cholesterol level, and the hypocholesterolemic activity of the peptide was greater than that of the drug β-sitosterol in rats. Nagaoka et al. (2001) speculated that the peptide reduces the micellar solubility of cholesterol and inhibits cholesterol absorption. However, the mechanisms of interaction between hypocholesterolemic peptides and cholesterol micelle of cholesterol are still unknown. The peptide is speculated to reduce the micellar solubility of cholesterol and inhibit cholesterol absorption.

Morimatsu et al. (1996) examined the effects of a papain-hydrolyzed pork muscle diet on the plasma and liver cholesterol levels in rats. Plasma and liver cholesterol concentrations were significantly lower in rats fed with the hydrolyzed pork. However, the amino acid sequence of pork peptide was not determined.

12.3.6 Antithrombotic Peptides

Venous thromboembolism is related either to trauma, long immobilization, or abnormalities in blood coagulation. Based on the knowledge that the mechanism of blood clotting is similar to that of milk clotting, milk protein–derived antithrombotic peptides have been studied (Fiat et al., 1993; Jollès and Henschen, 1982). Milk clotting occurs by the interaction of casein with a coagulating enzyme. Similarly, blood clotting occurs by the interaction of fibrinogen with thrombin. The dodecapeptide of human fibrinogen and the undecapeptide (Met-Ala-Ile-Pro-Pro-Lys-Lys-Asn-Gln-Asp-Lys) of bovine κ-casein are both structurally and functionally similar. This κ-casein-derived

Downloaded From Vet-eBooks.com

undecapeptide (casoplatelin) inhibited adenosine diphosphate (ADP)-induced platelet aggregation and combined with the fibrinogen receptor of blood platelets (Jollès et al., 1986). Also, tryptic fragments of casoplatelin-inhibited platelet aggregation (Bouhallab et al., 1992).

Shimizu et al. (2009) isolated an antithrombotic peptide fraction from pork muscle hydrolyzed with papain. The peptide fraction with mean molecular weight of 2500 showed antithrombotic activity *in vivo* after oral administration to mice. The fraction with mean molecular weight of 2517 further purified showed antithrombotic activity after oral administration. Further studies are required to identify the active peptide and to reveal the mechanism of the antithrombotic effect.

12.3.7 Antimicrobial Peptides

Antimicrobial peptides have been isolated from milk and egg proteins. Casecidin, generated from milk casein by chymosin digestion, exhibits activity against *Staphylococcus aureus*, *Sarcina*, *Bacillus subtilis*, *Diplococcus pneumoniae*, and *Streptococcus pyogenes* (Lahov and Regelson, 1996). Isracidin is another casein-derived antimicrobial peptide. It protected mice against infection with *S. aureus* and *Candida albicans* (Lahov and Regelson, 1996). Antimicrobial peptides generated by proteolytic digestion of milk lactoferrin have been studied most extensively (Tomita et al., 1994). The hydrolysate exhibits broad antibacterial activity against both gram-positive and gram-negative bacteria. Several lactoferrin-derived antimicrobial peptides named lactoferricins (e.g., lactoferricin B) have been isolated (Shin et al., 1998). A hen egg ovotransferrin-derived antimicrobial peptide (OTAP-92) that is active against *S. aureus* and *Escherichia coli* has been isolated (Ibrahim et al., 2000).

Spanish dry-cured ham has a particular sensory quality that makes it a distinctive food. Its high salt content and reduced water activity contribute to its shelf stability. However, postprocessing actions carried out for the commercialization of these products such as slicing may increase the risk of development of pathogenic microorganisms such as *Listeria monocytogenes*. A peptidomic strategy with mass spectrometry techniques was directed to naturally generated peptides showing antilisterial activity in Spanish dry-cured ham (Castellano et al., 2016). Ten identified peptides inhibited the growth of *L. monocytogenes*. Of these peptides, Arg-His-Gly-Tyr-Met was the most effective, showing a minimum inhibitory concentration (MIC) value of 6.25 mM. This is the first report of the generation of an antimicrobial peptide from meat proteins.

12.3.8 Prebiotic Peptides

Several substances are known to enhance the activity of probiotic bacteria, such as intestinal *Lactobacillus* and *Bifidobacterium*. Such substances are called prebiotics (Gibson, 2004). Oligosaccharides are representative prebiotic substances. In addition to oligosaccharides, the presence of prebiotic peptides

Downloaded From Vet-eBooks.com

has been suggested. Many studies have shown that hydrolysates of milk proteins exhibited stimulation of the growth of lactic acid bacteria and bifidobacteria (Brody, 2000). However, the main growth factors have been estimated to be the sugar moieties (e.g., *N*-acetylglucosamine and glucosamine) of glycosylated peptides (Idota et al., 1994). Nonglycosylated peptides derived from proteins have recently been identified. Liepke et al. (2002) first reported nonglycosylated peptides that selectively stimulate the growth of bifidobacteria. These peptides (5584 and 5801 Da) were isolated from pepsin-treated human milk and identified as lactoferrin fragments. Based on structural characteristics, a small peptide named prebiotic lactoferrin-derived peptide-I (PRELP-I; Cys-Ala-Val-Gly-Gly-Cys-Ile-Ala-Leu) was designed and characterized.

Arihara et al. found that the hydrolysate of porcine skeletal muscle actomyosin digested by papain enhanced the growth of *Bifidobacterium* strains in media (Arihara et al., 2011; Arihara and Ohata, 2011a). One of the corresponding prebiotic peptides was purified from the hydrolysate and identified as a tripeptide (Glu-Leu-Met). Although Glu-Leu-Met showed growth-promoting activity of *Bifidobacterium bifidum*, neither the dipeptides (Glu-Leu, Leu-Met) nor amino acids (Glu, Leu, Met), which are parts of the tripeptides, showed growth-promoting activity. Therefore, the sequence of the tripeptide is critical for its growth-promoting activity. Glu-Leu-Met promoted the growth of 11 strains of 26 *Bifidobacterium* strains in skim milk.

12.3.9 Bioactive Peptides from By-Products

Animal by-products include entire bodies or parts of animals, or products of animal origin, and other products obtained from animals (Lafarga and Hayes, 2014; Toldrá et al., 2012). They are generally recognized as carcasses, skins, bones, meat trimmings, blood, fatty tissues, horns, feet, hoofs, or internal organs. Most parts of animal by-products are discarded as waste or used for low-value products. Since meat by-products (e.g., trimmings and mechanically recovered meat, collagen, and blood) are rich in proteins, bioactive peptides can be generated from them by enzymatic treatments (Arihara and Ohata, 2017; Bernardini et al., 2011; Mora and Toldrá, 2014; Toldrá et al., 2012; Lafarga and Hayes, 2014).

Blood is the most common and first by-product of the meat industry (Parés et al., 2011). Large volumes of blood from domestic animals (e.g., cattle and pigs) are obtained in industrial slaughterhouses. Since blood contains proteins in rich amounts, it seems to be a good source of bioactive peptides (Bah et al., 2013). Antihypertensive, antioxidative, antimicrobial, and opioid peptides have been found in hydrolysates of blood proteins, such as hemoglobin and plasma (Chang et al., 2007). As such peptides, ACE-inhibitory peptides Gly-Phe-Pro-Thr-Thr-Lys-Thr-Tyr-Phe-Pro-His-Phe and Val-Val-Tyr-Pro-Trp-Thr, which are corresponding to the sequences of porcine hemoglobin, are identified (Yu et al., 2006). As described earlier, biologically active peptides with affinity for the opioid receptor were found in the hemoglobin peptic hydrolysate (Nyberg et al., 1997; Zhao et al., 1997).

Downloaded From Vet-eBooks.com

Several antimicrobial peptides have been found in the hydrolysates of hemoglobin. Fogaca et al. (1999) identified the first antimicrobial peptide from bovine hemoglobin. This peptide (FLSFPTTKTYFPHFDLSHGSAAQVKGHGAK) was active against *Micrococcus luteus*. Froidevaux et al. (2001) found the peptide from the hydrolysate of bovine α-hemoglobin. The peptide (VLSAADKGNVKAAWGKVGGHAAE) was also active against *M. luteus*. Furthermore, the peptide (VTLASHLPSDFTPAVHASLDKFLANVSTVL) obtained from bovine hemoglobin showed activity against *M. luteus*, *Listeria innocua*, *E. coli*, and *Salmonella enteritidis* (Daoud et al., 2005). Also, Nedjar-Arroume et al. (2006) identified four peptides from bovine hemoglobin. These peptides were active against *M. luteus*, *L. innocua*, *E. coli*, and *S. enteritidis*. VNFKLLSHSLLVTLASHL was purified from the hydrolysate of bovine α-hemoglobin and showed antimicrobial activity against *E. coli*, *S. aureus*, and *C. albicans* (Hu et al., 2011). Recently, Przybylski et al. (2016) identified a small antimicrobial peptide (Thr-Ser-Tyr-Arg, fragment of hemoglobin) from pepsin-treated bovine cruor.

Collagen is the most abundant protein in mammals (Mora and Toldrá, 2014). The most common motifs in the amino acid sequence of collagen are Gly-Pro-X and Gly-X-Hyp, where X can be any amino acid other than Gly, Pro, or Hyp. Since collagen lacks certain essential amino acids, it has less nutritional value than most other food proteins (Gomez-Guillen et al., 2011). Saiga et al. (2003a) reported antihypertensive activity of *Aspergillus* protease-treated chicken muscle extract in spontaneously hypertensive rats. Furthermore, they isolated four ACE inhibitory peptides from the hydrolysate. Three of those four peptides possessed a common sequence, Gly-X-X-Gly-X-X-Gly-X-X, which is homologous with that of collagen. Banerjee and Shanthi (2012) identified ACE inhibitory peptides generated from bovine Achilles tendon collagen hydrolyzed with bacterial collagenase. The identified peptides were AKGANGAPGIAGAPGFPGARGPSGPQGPSGPP and PAGNPGADGQPGAKGANGAP. Kim et al. (2001) hydrolyzed bovine skin, rich in collagen, with pronase E. Three antioxidative peptides were purified from pronase E hydrolysate. The peptide Gly-Pro-Hyp-Gly-Pro-Hyp-Gly-Pro-Hyp-Gly showed the highest activity with the thiobarbituric acid reactive substances (TBARSs) method. Li et al. (2007) hydrolyzed porcine skin collagen with different protease treatments and found four antioxidative peptides. Their sequences were identified as Gln-Gly-Ala-Arg, Leu-Gln-Gly-Met, Leu-Gln-Gly-Met-Hyp, and Hyp-Cys.

Fermentation of by-product from meat processing has been used to generate bioactive compounds (Hayes and García-Vaquero, 2016). Goat placenta has long been used in Chinese medicine and is recognized as rich in bioactive components (Chakraborty and Bhattacharyya, 2005). Hou et al. (2014) assayed peptides generated in goat placenta by fermentation. They identified that the DPPH scavenging activity of goat placenta peptides reached 85.43% at a concentration of 1.4 μg/mL.

Downloaded From Vet-eBooks.com

12.3.10 Peptides with Sensory Properties

Apart from bioactivities, food protein–derived peptides contribute significantly to sensory properties of foods (Pihlanto and Korhonen, 2003; Gonzalez de Llano and Polo Sanchez, 2003; Nishimura and Kato, 1988; Temussi, 2012). In foods with protein-hydrolysis processes, such as fermentation and aging, generation of flavor peptides is an important event. Peptides that have a savory flavor are known to be generated from food proteins. Some savory peptides (Glu-Asp-Glu, Asp-Glu-Ser, Ser-Glu-Glu) were generated in fish muscle protein hydrolysates (Noguchi et al., 1975). An octapeptide with delicious taste (Lys-Gly-Asp-Glu-Glu-Ser-Leu-Ala) was isolated from beef treated with papain (Yamazaki and Maekawa, 1980). Later, this peptide was called "beef meaty peptide" or "savory taste-enhancing peptide" (Hau et al., 1997). Henriksen and Stahnke (1997) fractionated low-molecular-weight water-soluble compounds extracted from dried sausages. Fractions containing smaller peptides enhanced savory taste impressions.

Umami taste-enhancing peptides (Glu-Glu, Glu-Val, Ala-Asp-Glu, Ala-Glu-Asp, Asp-Glu-Glu, and Ser-Pro-Glu) were found in chicken protein hydrolysate (Maehashi et al., 1999). Okumura et al. (2004) investigated sourness-suppressing peptides generated in cooked pork loins. They obtained a fraction that exhibited sourness-suppressing and umami-enhancing effects from an extract prepared from vacuum-cooked pork loins. Three peptides purified from this fraction had a common sequence (Ala-Pro-Pro-Pro-Pro-Ala-Glu-Val-His-Glu-Val). A synthetic peptide having this common sequence exhibited suppression of sour taste. Also, peptides containing this sequence were generated during postmortem aging (Kitamura et al., 2005).

12.4 Peptides and the Maillard Reaction

12.4.1 The Maillard Reaction

Numerous chemical reactions occur in foods during manufacture, storage, and cooking and they change the color, aroma, taste, and nutritional value of foods. The Maillard reaction is a critical chemical reaction in such changes (Losso, 2016; Shahidi et al., 2014). This reaction occurs between amines (e.g., amino acids, peptides, and proteins) and carbonyl compounds, especially reducing sugars. Since food protein–derived peptides are widely utilized in many processed foods, the changes of peptides by the Maillard reaction in foods deserve concern and research (Arihara et al., 2017).

The Maillard reaction occurs in several stages (Figure 12.4). The initial stage of the reaction involves a Schiff base formation between the free amino and carbonyl group (amino-carbonyl reaction). This is followed by the rearrangement of the Schiff base to an Amadori compound. The intermediate stage

Downloaded From Vet-eBooks.com

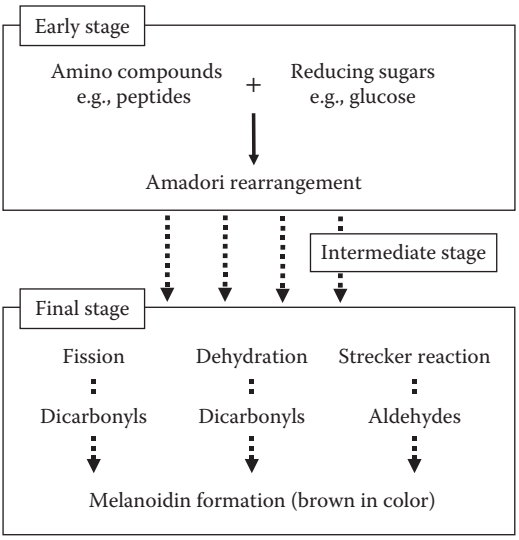


FIGURE 12.4
Outline of the Maillard reaction of amino compounds and reducing sugars.

starts from the Amadori compounds. They react further by several pathways, such as enolization, dehydration, aldol condensations, and Strecker degradation, to generate numerous compounds. Melanoidins, which are the final products of the reaction, are nitrogen-containing polymeric substances that decompose with difficulty (Hayase, 1996). Melanoidins demonstrate physiologically positive effects because of unique partial structures in the molecules. Melanoidins have a strong scavenging activity against active oxygen species, such as hydroxyl radicals, hydrogen peroxides, and superoxides. Also, they show antimicrobial activity and the ability to chelate different minerals and to promote the growth of beneficial bacteria (Morales et al., 2012).

12.4.2 Maillard Reaction and Meat

Meat and meat products are closely related with the Maillard reaction. Meat and meat products are very complex systems with numerous compounds and generally cooked before eating. Although raw meat has little or no aroma and only a blood-like taste, each type of cooked meat has a characteristic flavor (Shahidi et al., 2014). More than 1000 compounds have been identified in the aroma profiles of cooked meat products and the Maillard reaction has a critical role in generating such compounds (Pegg and Shahidi, 2014). Free amino groups of amino acids or peptides in meat can react with reducing sugars in the presence of heat. MRPs generated in cooked meat include many important classes of meat flavor compounds such as pyrazines, oxazoles, thiophenes, thiazoles, and other heterocyclic compounds. Sulfur compounds are especially important for the characteristic aroma of meat.

Downloaded From Vet-eBooks.com

Cheng et al. (2011) explored the MRPs of enzymatic hydrolysates (peptides) prepared from beef proteins for the relationship between flavor products and reactants in model system. The majority of peptides participating in the Maillard reaction were reported to have a molecular weight from 1000 to 2000 Da. Furans and furanones were the major products from the degradation of carbohydrates. Pyrazines were produced from the reaction of peptides and xylose. Thiazole, thiophene, and thiols were generated from the degradation of thiamin. The Maillard reaction of peptides is also a critical phenomenon in meat products without heat treatment (Jayasena et al., 2013; Ventanas et al., 1992; Weerawatanakorn et al., 2015). Hams from the Iberian breed are processed by the traditional dry-curing procedure and proteolysis and autooxidation occur during ripening/drying. As a result, peptides, nitrogen, and carbonyl compounds are accumulated. In the next step, a rapid increase of amino acid nitrogen is observed, and aldehydes decrease notably. This change seems to be due to condensations between carbonyls and free amino acids. While hams are kept in the cellar, the overall increase of amino acid nitrogen is in contrast to the slow rate of accumulation of some individual amino acids. This is presumably due to degradation to volatile derivatives, where Maillard reactions could be involved.

12.4.3 Bioactivities of MRPs from Peptides

MRPs show some biological activities, such as antioxidative activity, antibacterial activity, and ACE inhibitory activity (Somoza, 2005; Van Lancker et al., 2011). Several studies have focused on the heating effect on the functional properties of MRPs generated from vegetable protein hydrolysate (Lertittikula et al., 2007; Li et al., 2009). However, the published information about animal proteins (e.g., meat proteins) involved in the generation about flavor compounds in the Maillard reaction is still limited.

Arihara et al. have been trying to develop peptide-based functional food ingredients by the Maillard reaction (Arihara et al., 2017). Since collagen is an abundant protein in animal by-products, attention has been given to developing functional materials from collagen (Arihara et al., 2013, 2017). Collagen contains approximately 33% glycine, which is a nonessential amino acid, thus it has been considered of low nutritional value. However, glycine is a good source for the Maillard reaction with reducing sugars. Also, collagen does not contain asparagine, which is a major amino acid in potatoes and cereals. Acrylamide, which is found in a range of fried and oven-cooked foods, is a known human neurotoxin and has been classified as probably carcinogenic in humans. The main formation pathway of acrylamide in foods is linked to the Maillard reaction and, in particular, the amino acid asparagine (Mottram et al., 2002; Stadler et al., 2002; Blank, 2005). Therefore, proteins without asparagine such as collagen are appropriate candidates for the process of the Maillard reaction.

Downloaded From Vet-eBooks.com

Collagen was heated to transfer to gelatin and digested by commercial proteases (Arihara et al., 2017). Such collagen-derived peptides were added with reducing sugars (e.g., xylose) and a pH adjuster (e.g., sodium carbonate). Since the Maillard reaction prefers the alkaline condition, a pH adjuster was added in the reaction solution. The solution was further heated at 90°C for the Maillard reaction. The activities by using superoxide ion and DPPH radical were increased significantly by heat treatment (the Maillard reaction). Oral administration of the MRPs to mice suppressed the stress marker (e.g., hydroperoxide value of serum). Also, continuous oral administration of the MRPs to the spontaneously hypertensive rats decreased the blood pressure compared to collagen peptides without the heat treatment. Since this collagen peptide-based product prepared by the Maillard reaction showed good sensory properties, it will be a possible functional food ingredient.

Ējiāo (*Asini Corii Collas*) is a Chinese crude drug made of dry skin, bone, tendons, and ligaments of donkeys (*Equus asinus*) by decoction, degreasing, and concentration (Kumeta et al., 2014). The main component of Ējiāo is collagen. It has been mainly used for promoting hematopoiesis and arresting bleeding. Although it is specified as being made of dry skin derived from donkeys in the Chinese Pharmacopoeia, adulteration with skins or bones from other animals such as horses, cattle, and pigs has been found. Since it is brown in color, the Maillard reaction seems to have occurred during its processing. The advanced glycation end products (AGE), such as Nε-(carboxymethyl) lysine was detected in Ējiāo. Oral administration of Ējiāo to spontaneously hypertensive rats decreased blood pressure. At least a part of this activity would proceed from MRPs of Ējiāo.

12.4.4 Bioactivities of Volatile MRPs from Peptides

As described earlier, MRPs include a wide variety of odor components, which can contribute to the perceived quality of foods. However, very few studies have been conducted on the bioactivities of the odor components generated by the Maillard reaction (Zhou et al., 2016). Initially, Ohata et al. investigated the effects of the odor produced by the Maillard reaction on blood pressure (Ohata et al., 2014). Chicken meat protein digests (peptides) prepared by papain treatment were mixed with xylose in buffer. They were heated at 90°C for 240 minutes at pH 5 or pH 10. Wistar rats were exposed to the vapor of these solutions for 5 min, and systolic blood pressure (SBP) was measured. The pH 10 sample significantly decreased SBP after 10–15 minutes exposure. Volatile compounds generated in the pH 10 sample would have the effect of decreasing SBP of rats. Further experiments indicated that the pH 10 sample odor caused parasympathetic nervous system dominance and induced a relaxed and sedated state (Ohata et al., 2014). 2,3-dimethyl-pyrazine, acetic acid, 2-furancarboxyaldehyde, 2-hydroxy-3-methyl-2-cyclopenten-1-one (cyclotene), 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone (DMHF), and 5-methylpyrazine-2-methanol were

Downloaded From Vet-eBooks.com

identified as odor components contributing to the odor of the pH 10 sample. Since 2-furanoncarboxyaldehyde, cyclotene, DMHF, and 5-methylpyrazine-2-methanol were absent in the sample before heat treatment, these compounds were generated by the Maillard reaction. Some of these compounds seem to be implicated in the effects on the autonomic system in rats.

12.5 Conclusions

Bioactive peptides generated from meat proteins are promising food ingredients for developing functional foods (Arihara, 2014; Arihara and Ohata, 2011b; Olmedilla-Alonso et al., 2013; Young et al., 2013). Also, since traditional meat products, such as fermented sausages and dry-cured ham, have been revealed to contain various bioactive peptides, they have a great possibility to be reborn as functional foods. Apart from bioactivities, meat protein–derived peptides also contribute to the sensory properties of meat and meat products. Since peptides have a wide range of taste from sweet to umami (Temussi, 2012), generation of peptides from meat proteins would lead to development of novel functional meat products with favorable sensory characteristics.

In addition to conventional research techniques (Martínez-Maqueda et al., 2013), the modern nutrigenomics approach have been rapidly applied to the field of nutrition and health (Figure 12.5) (Arihara, 2013; Cevallos-Cevallos and Reyes-De-Corcuera, 2012; Ortea et al., 2016; Pavlidis et al., 2015). Nutrigenomics seeks to find out the interaction between dietary factors and host genes and how genes and their products metabo-

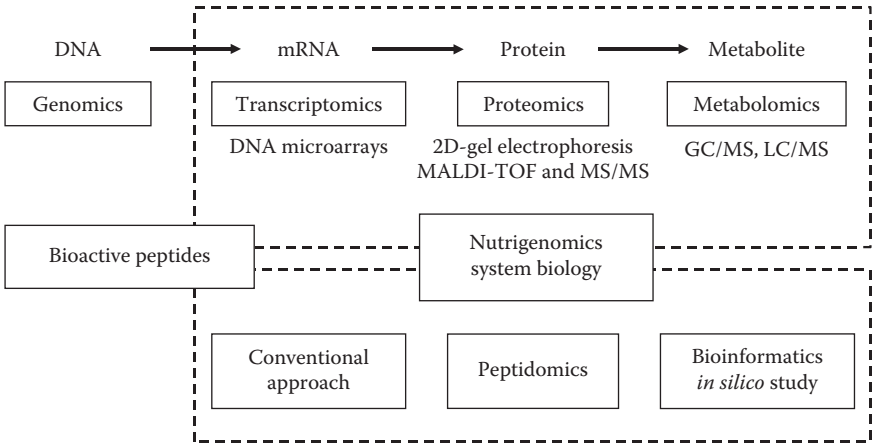


FIGURE 12.5
Strategy for approaching bioactivity of meat protein-derived peptides.

Downloaded From Vet-eBooks.com

lize these constituents into health-promoting nutrients or disease-causing antinutrients, and bioactive compounds. Omics technologies including transcriptomics, proteomics, and metabolomics offer exciting opportunities to address complex issues related to human health. Although application of this strategy for studying meat protein–derived bioactive peptides is still limited, it has great potential to help us understand and utilize bioactive peptides (Carrasco-Castilla et al., 2012). The identification of peptides using mass spectrometry and searching for their sequences in the database is one representative approach. Mora and Toldrá (2013) described the application of proteomic tools to dry-cured ham in order to better understand the intense proteolysis phenomena that occur during its processing. They pointed out that modern mass spectrometry techniques with the analyzers in tandem allows a faster and more reliable identification of the peptide sequences because the obtained MS/MS spectra are matched with the theoretical sequences contained in the databases.

Along with analytical methods, computer-aided techniques are utilized to evaluate food components including bioactive peptides (Dziuba et al., 2012). Bioinformatics combines computers, software tools, statistics, and databases and has been used for *in silico* analyses of biological queries in the omics, including genomics, proteomics, transcriptomics, metabolomics, and peptidomics. Furthermore, system biology involves the integration of genomics, proteomics, and bioinformatics information to create a whole-system view of a biological entity. Bioinformatics and proteomics are useful tools toward the identification of peptides with potent biological activities (Carrasco-Castilla et al., 2012; Khaldi, 2012; Udenigwe, 2014). The hydrolysis of the selected protein can be simulated to generate *in silico* peptides using an open-access database, such as BIOPEP, PEPBANK, or ERP-Moscow. The BIOPEP database provides a useful resource when looking for peptides with different bioactivities. Lafarga et al. (2015) identified meat-derived prolyl endopeptidase inhibitory peptides using *in silico* methods. They evaluated five proteins commonly found in meat by-products (e.g., serum albumin, collagen, and myosin) as a substrate for use in the generation of peptides. These proteins were cleaved *in silico* using the BIOPEP and ExPASy PeptideCutter and generated peptides were compared to known peptides in the BIOPEP. This study demonstrated the usefulness of *in silico* analysis for predicting the release of bioactive peptides from meat proteins.

By combining various techniques and information, it will be possible to understand all aspects and implications of bioactive peptides generated from meat proteins. Also, with the advent of new techniques, the mechanisms underlying numerous biological properties of meat protein–derived peptides will be revealed. Such efforts will contribute to the demonstration of the scientific benefits of meat and meat products and the development of novel functional meat products with bioactive peptides.

Downloaded From Vet-eBooks.com

References

Agyei, D. and Danquah, M. K. (2011). Industrial-scale manufacturing of pharmaceutical-grade bioactive peptides. *Biotechnology Advances,* **29**, 272–277.

Ahhmed, A. M. and Muguruma, M. (2010). A review of meat protein hydrolysates and hypertension. *Meat Science*, **86**, 110–118.

Aleixandre, A. and Miguel, M. (2012). Food proteins and peptide as bioactive agents. *In:* Hettiarachchy, N. S., Sato, K., Marshall, M. R. and Kannan A. (eds.) *Bioactive Food Proteins and Peptides: Applications in Human Health*. Boca Raton, FL: CRC Press, pp. 131–180.

Arihara, K. (2006a). Strategies for designing novel functional meat products. *Meat Science*, **74**, 219–229.

Arihara, K. (2006b). Functional properties of bioactive peptides derived from meat proteins. *In:* Nollet, L. M. L. and Toldrá, F. (eds.) *Advanced Technologies for Meat Processing*. Boca Raton, FL: CRC Press, pp. 245–274.

Arihara, K. (2013). Relevance of peptides bioactivity in foods. *In:* Toldrá, F. and Nollet, L. M. L. (eds.) *Proteomics in Foods*. New York, NY: Springer, pp. 447–463.

Arihara, K. (2014). Functional foods. *In:* Dikeman, M. and Devine, C. (eds.) *Encyclopedia of Meat Sciences* (2nd Ed., vol. 2). Oxford: Elsevier/Academic Press, pp. 32–36.

Arihara, K., Arai, T., Ohata, M., Ishikawa, S. and Itoh, M. (2013). Food/pet food material having health functionality and linking-improving effect with collagen as raw material. Japan Patent No. 2013-5326489.

Arihara, K., Ishikawa, S. and Itoh, M. (2011). *Bifidobacterium* growth promoting peptides derived from meat proteins. Japan Patent No. 2011-4726129.

Arihara, K., Nakashima, Y., Ishikawa, S. and Itoh, M. (2004). Antihypertensive activities generated from porcine skeletal muscle proteins by lactic acid bacteria. In *Abstracts of 50th International Congress of Meat Science and Technology*, Helsinki, Finland, p. 236.

Arihara, K., Nakashima, Y., Mukai, T., Ishikawa, S. and Itoh, M. (2001). Peptide inhibitors for angiotensin I-converting enzyme from enzymatic hydrolysates of porcine skeletal muscle proteins. *Meat Science*, **57**, 319–324.

Arihara, K. and Ohata, M. (2011a). Use of probiotics and prebiotics in meat products. *In:* Kerry, J. P. and Kerry. J. F. (eds.) *Processed Meats: Improving Safety, Nutrition and Quality*. Cambridge: Woodhead Publishing, pp. 403–417.

Arihara, K. and Ohata, M. (2011b). Functional meat products. *In:* Saarela, M. (ed.) *Functional Foods: Concept to Products* (2nd Ed.). Cambridge: Woodhead Publishing, pp. 512–533.

Arihara, K. and Ohata, M. (2015). Antihypertensive peptides generated in aged pork. Japan Patent No. 2015-154703.

Arihara, K. and Ohata, M. (2017). Production of bioactives from meat and animal by-products. *In:* Bekhit, A. E.-D. A. (ed.) *Advances in Meat Processing Technology.* Boca Raton, FL: CRC Press.

Arihara, K., Tomita, K., Ishikawa, S., Itoh, M., Akimoto, M. and Sameshima, T. (2011). Anti-fatigue peptides derived from meat proteins. Japan Patent No. 2011-4828890.

Arihara, K., Zhou, L. and Ohata, M. (2017). Bioactive properties of Maillard reaction products generated from food protein-derived peptides. *Advances in Food and Nutrition Research,* **81**, 161–185.

Downloaded From Vet-eBooks.com

Bagchi, D. and Nair, S. (2017). *Developing New Functional Food and Nutraceutical Products*. London: Academic Press.

Bah, C. S. F., Bekhit, E.-D. A., Carne, A. and McConnell, M. A. (2013). Slaughterhouse blood: An emerging source of bioactive compounds. *Comprehensive Reviews in Food Science and Food Safety*, **12**, 314–331.

Banerjee, P. and Shanthi, C. (2012). Isolation of novel bioactive regions from bovine Achilles tendon collagen having angiotensin I-converting enzyme-inhibitory properties. *Process Biochemistry*, **47**, 2335–2346.

Bauchart, C., Remond, D., Chambon, C. et al. (2006). Small peptides (<5 kDa) found in ready-to-eat beef meat. *Meat Science*, **74**, 658–666.

Bernardini, R. D., Harnedy, P., Bolton, D., Kerry, J. and O'Neil, E. (2011). Antioxidant and antimicrobial peptidic hydrolysates from muscle protein sources and by-products. *Food Chemistry*, **124**, 1296–1307.

Blank, I. (2005). Current status of acrylamide research in food: Measurement, safety assessment, and formation. *Annals of the New York Academy of Sciences*, **1043**, 30–40.

Blishchenko, E., Sazonova, O., Surovoy, A. et al. (2002). Antiproliferative action of valorphin in cell cultures. *Journal of Peptide Science*, **8**, 438–452.

Bouhallab, S., Molle, D. and Leonil, J. (1992). Tryptic hydrolysis of caseinomacropeptide in membrane reactor: Preparation of bioactive peptides. *Biotechnology Letters*, **14**, 805–810.

Bowman, L. (2015). *Bioactive Food Peptides: Production and Health Benefits.* Forest Hills, NJ: Foster Academics.

Brantl, V., Teschemacher, H., Henschen, A. and Lottspeich, F. (1979). Novel opioid peptides derived from casein (β-Casomorphins) I. Isolation from bovine casein peptone. *Hoppe-Seyler's Zeitschrift fur physiologische Chemie*, **360**, 1211–1216.

Brody, E. P. (2000). Biological activities of bovine glycomacropeptide. *British Journal of Nutrition*, **84**, S39–S46.

Bruna, J. M., Fernandez, M., Hierro, E. M., Ordonez, J. A. and de la Hoz, L. (2000). Combined use of pronase E and a fungal extract (*Penicillium aurantiogriseum*) to potentiate the sensory characteristics of dry fermented sausages. *Meat Science*, **54**, 135–145.

Bütikofer, U., Meyer, J., Sieber, R., Walther, B. and Wechsler, D. (2008). Occurrence of the angiotensin-converting enzyme-inhibiting tripeptides Val-Pro-Pro and Ile-Pro-Pro in different cheese varieties of Swiss origin. *Journal of Dairy Science*, **91**, 29–38.

Carrasco-Castilla, J., Hernández-Álvarez, A. J., Jiménez-Martínez, C., Gutierrez-López, G. F. and Dávila-Ortiz, G. (2012). Use of proteomics and peptidomics methods in food bioactive peptide science and engineering. *Food Engineering Reviews*, **4**, 224–243.

Castellano, P., Aristoy, M. C., Sentandreu, M. Á., Vignolo, G. and Toldrá, F. (2013). Peptides with angiotensin I converting enzyme (ACE) inhibitory activity generated from porcine skeletal muscle proteins by the action of meat-borne *Lactobacillus*. *Journal of Proteomics*, **89**, 183–190.

Castellano, P., Mora, L., Escudero, E., Vignolo, G., Aznar, R. and Toldrá, F. (2016). Antilisterial peptides from Spanish dry-cured hams: Purification and identification. *Food Microbiology*, **59**, 133–141.

Cevallos-Cevallos, J. M. and Reyes-De-Corcuera, J. I. (2012). Metabolomics in food science. *Advances in Food and Nutrition Research*, **67**, 1–24.

Downloaded From Vet-eBooks.com

Chakraborty, P. D. and Bhattacharyya, D. (2005). Isolation of fibronectin type III like peptide from human placental extract used as wound healer. *Journal of Chromatography B*, **818**, 67–73.

Chang, C.-Y., Wu, K.-C. and Chiang, S.-H. (2007). Antioxidant properties and protein compositions of porcine haemoglobin hydrolysates. *Food Chemistry*, **100**, 1537–1543.

Chen, H.-M., Muramoto, K. and Yamauchi, F. (1996). Antioxidative activity of designed peptides based on the antioxidative peptide isolated from digests of a soybean protein. *Journal of Agricultural and Food Chemistry*, **44**, 2619–2623.

Cheng, L.-K., Song, H.-L. and Wang, P.-X. C. (2011). Degradation of peptides derived from enzymatic hydrolysis of beef during Maillard reaction. *Food Science*, **32**, 46–50.

Cheung, H. S., Wang, F. L., Ondetti, M. A., Sabe, E. F. and Cushman, D. W. (1980). Binding of peptide substrate and inhibition of angiotensin-converting enzyme: Importance of the COOH-terminal dipeptides sequence. *Journal of Biological Chemistry*, **255**, 401–407.

Chiba, H. and Yoshikawa, M. (1986). Biologically functional peptides from food proteins: New opioid peptides from milk proteins. *In:* Feeney, R. E. and Whitaker, J. R. (eds.) *Protein Tailoring for Food and Medical Users.* New York, NY: Marcel Dekker, pp. 123–153.

Dainty, R. and Blom, H. (1995). Flavor chemistry of fermented sausages. *In:* Campbell Platt, G. and Cook, P. E. (eds.) *Fermented Meats.* Glasgow: Blackie Academic & Professional, pp. 176–193.

Daoud, R., Dubois, V., Bors-Dodita, L. et al. (2005). New antibacterial peptide derived from bovine hemoglobin. *Peptides*, **26**, 713–719.

Dellafiora, L., Paolella, S., Dall'Asta, C., Dossena, A., Cozzini, P. and Galaverna, G. (2015). Hybrid *in silico/in vitro* approach for the identification of angiotensin I converting enzyme inhibitory peptides from Parma dry-cured ham. *Journal of Agricultural and Food Chemistry*, **63**, 6366–6375.

Diaz, O., Fernandez, M., Garcia De Fernando, G. D., de la Hoz, L. and Ordonez, J. A. (1997). Proteolysis in dry fermented sausages: The effect of selected exogenous proteases. *Meat Science*, **46**, 115–128.

Di Bernardini, R., Mullen, A. M., Bolton, D., Kerry, J., O'Neill, E. and Hayes, M. (2012). Assessment of the angiotensin-I-converting enzyme (ACE-I) inhibitory and antioxidant activities of hydrolysates of bovine brisket sarcoplasmic proteins produced by papain and characterisation of associated bioactive peptidic fractions. *Meat Science*, **90**, 226–235.

Dransfield, E. and Etherington, D. (1981). Enzymes in the tenderization of meat. *In:* Birch, G. G., Blakebrough, N. and Parker, K. J. (eds.) *Enzymes and Food Processing.* London: Applied Science Publishers, pp. 177–194.

Dziuba, B., Minkiewicz, P. and Darewicz, M. (2012). Database of biologically active proteins and peptides. *In:* Hettiarachchy, N. S. (ed.) *Bioactive Food Proteins and Peptides*. Boca Raton, FL: CRC Press, pp. 331–373.

Escudero, E., Aristoy, M.-C., Nishimura, H., Arihara, K. and Toldrá, F. (2012). Antihypertensive effect and antioxidant activity of peptide fractions extracted from Spanish dry-cured ham. *Meat Science*, **91**, 306–311.

Escudero, E., Mora, L., Fraser, P. D., Aristoy, M. C., Arihara, K. and Toldrá, F. (2013a). Purification and identification of antihypertensive peptides in Spanish dry-cured ham. *Journal of Proteomics*, **78**, 499–507.

Downloaded From Vet-eBooks.com

Escudero, E., Mora, L., Fraser, P. D., Aristoy, M. C., Arihara, K. and Toldrá, F. (2013b). Identification of novel antioxidant peptides generated in Spanish dry-cured ham. *Food Chemistry*, **138**, 1282–1288.

Escudero, E., Mora, L. and Toldrá, F. (2014). Stability of ACE inhibitory ham peptides against heat treatment and *in vitro* digestion. *Food Chemistry*, **161**, 305–311.

Escudero, E., Sentandreu, M., Arihara, K. and Toldrá, F. (2010). Angiotensin I-converting enzyme inhibitory peptides generated from *in vitro* gastrointestinal digestion of pork meat. *Journal of Agricultural and Food Chemistry*, **58**, 2895–2901.

Etherington, D. J. (1984). The contribution of proteolytic enzymes to postmortem changes in muscle. *Journal of Animal Science*, **59**, 1644–1650.

Ferranti, P., Nitride, C., Nicolai, M. A. et al. (2014). *In vitro* digestion of Bresaola proteins and release of potential bioactive peptides. *Food Research International*, **63**, 157–169.

Fiat, A.-M., Migliore-Samour, D., Jollès, P., Drout, L., Collier, C. and Caen, J. (1993). Biologically active peptides from milk proteins with emphasis on two examples concerning antithrombotic and immunomodulating activities. *Journal of Dairy Science*, **76**, 301–310.

Fogaca, A. C., da Silva, P. I., Miranda, M. T. M. et al. (1999). Antimicrobial activity of a bovine hemoglobin fragment in the tick *Boophilus microplus*. *Journal of Biological Chemistry*, **274**, 25330–25334.

Froidevaux, R., Krier, F., Nedjar-Arroume, N. et al. (2001). Antimicrobial activity of a pepsin-derived bovine hemoglobin fragment. *FEBS Letters*, **491**, 159–163.

Fu, Y., Young, J. F. and Therkildsen, M. (2017). Bioactive peptides in beef: Endogenous generation through postmortem aging. *Meat Science*, **123**, 134–142.

Fujita, H. and Yoshikawa, M. (1999). LKPNM: A prodrug-type ACE-inhibitory peptide derived from fish protein. *Immunopharmacology*, **44**, 123–127.

Fujita, H., Yokoyama, K. and Yoshikawa, M. (2000). Classification and antihypertensive activity of angiotensin I-converting enzyme inhibitory peptides derived from food proteins. *Journal of Food Science*, **65**, 564–569.

Fukudome, S. and Yoshikawa, M. (1992). Opioid peptides derived from wheat gluten, their isolation and characterization. *FEBS Letters*, **296**, 107–111.

Fukudome, S. and Yoshikawa, M. (1993). A novel opioid peptide derived from wheat gluten. *FEBS Letters*, **316**, 17–19.

Gagnaire, V., Molle, D., Herrouin, M. and Leonil, J. (2001). Peptides identified during Emmental cheese ripening: Origin and proteolytic systems involved. *Journal of Agricultural and Food Chemistry*, **49**, 4402–4413.

Gallego, M., Mora, L., Fraser, P. D., Aristoy, M. C. and Toldrá, F. (2014). Degradation of LIM domain-binding protein 3 during Spanish dry-cured ham processing. *Food Chemistry*, **149**, 121–128.

Gallego, M., Grootaert, C., Mora, L., Aristoy, M. C., Van Camp, J. and Toldrá, F. (2016). Transepithelial transport of dry-cured ham peptides with ACE inhibitory activity through a Caco-2 cell monolayer. *Journal of Functional Foods*, **21**, 388–395.

Gallego, M., Mora, L., Aristoy, M. C. and Toldrá, F. (2015). Titin-derived peptides as processing time markers in dry-cured ham. *Food Chemistry*, **167**, 326–339.

Gibson, G. R. (2004). From probiotics to prebiotics. *Journal of Food Science*, **69**, M141–M143.

Gobbetti, M., Minervini, F. and Rizzello, C. G. (2007). Bioactive peptides in dairy products. *In:* Hui, Y. H. (ed.) *Handbook of Food Products Manufacturing: Health, Meat, Milk, Poultry, Seafood, and Vegetables.* Hoboken, NJ: John Wiley & Sons, pp. 489–517.

Downloaded From Vet-eBooks.com

Gomez-Guillen, C. M., Gimenez, B., Lopez-Caballero, M. E. and Montero, M. P. (2011). Functional and bioactive properties of collagen and gelatin from alternative sources: A review. *Food Hydrocolloids*, **25**, 1813–1827.

Gomez-Ruiz, J. A., Ramos, M. and Recio, I. (2002). Angiotensin-converting enzyme-inhibitory peptides in Manchego cheeses manufactured with different starter cultures. *International Dairy Journal*, **12**, 697–706.

Gonzalez de Llano, D. and Polo Sanchez, C. (2003). Peptides. *In:* Caballero, B., Trugo, L. C. and Finglas, P. M. (eds.) *Encyclopedia of Food Science and Nutrition* (2nd Ed.). London: Academic Press, pp. 4468–4473.

Gu, Y., Majumder, K. and Wu, J. (2011). QSAR-aided in silico approach in evaluation of food proteins as precursors of ACE inhibitory peptides. *Food Research International*, **44**, 2465–2474.

Hau, J., Cazes, D. and Fay, L. B. (1997). Comprehensive study of the "beefy meaty peptide". *Journal of Agricultural and Food Chemistry*, **45**, 1351–1355.

Hayase, F. (1996). Scavenging of active oxygen by melanoidins. *In:* Ikan, R. (ed.) *The Maillard Reaction, Consequences for the Chemical and Life Sciences.* Hoboken, NJ: John Wiley & Sons, pp. 89–104.

Hayes, M. and García-Vaquero, M. (2016). Bioactive compounds from fermented food products. *In:* Ojha, K. S. and Tiwari, B. K. (eds.) *Novel Food Fermentation Technologies*. Cham, Switzerland: Springer International Publishing, pp. 293–310.

He, H. L., Liu, D. and Ma, C. B. (2013). Review on the angiotensin-I-converting enzyme (ACE) inhibitor peptides from marine proteins. *Applied Biochemistry and Biotechnology*, **169**, 738–749.

Henriksen, A. H. and Stahnke, L. H. (1997). Sensory and chromatographic evaluations of water soluble fractions from dried sausages. *Journal of Agricultural and Food Chemistry*, **45**, 2679–2684.

Hernández-Ledesma, B., Amigo, L., Ramos, M. and Recio, I. (2004). Angiotensin converting enzyme inhibitory activity in commercial fermented products. *Journal of Agricultural and Food Chemistry*, **52**, 1504–1510.

Hettiarachchy, N. S. (2012). *Bioactive Food Proteins and Peptides*. Boca Raton, FL: CRC Press.

Hierro, E., de la Hoz, L. and Ordonez, J. A. (1999). Contribution of the microbial and meat endogenous enzymes to the free amino acid and amine contents of dry fermented sausages. *Journal of Agricultural and Food Chemistry*, **47**, 1156–1161.

Hou, Y., Liu, W., Cheng, Y., Zhou, J., Wu, L. and Yang, G. (2014). Production, optimization and characterization of immunomodulatory peptides obtained from fermented goal placenta. *Food Science and Technology (Campinas)*, **34**, 723–729.

Hu J., Xu, M., Hang, B. et al. (2011). Isolation and characterization of an antimicrobial peptide from bovine hemoglobin α-subunit. *World Journal of Microbiology and Biotechnology*, **27**, 767–771.

Ibrahim, H. R., Sugimoto, Y. and Aoki, T. (2000). Ovotransferrin antimicrobial peptide (OTAP-92) kills bacteria through a membrane damage mechanism. *Biochimica et Biophysica Acta*, **1523**, 196–205.

Idota, T., Kawakami, H. and Nakajima, I. (1994). Growth-promoting effects of N-acetylneuraminic acid containing substances on bifidobacteria. *Bioscience, Biotechnology, and Biochemistry*, **58**, 1720–1722.

Jang, A. and Lee, M. (2005). Purification and identification of angiotensin converting enzyme inhibitory peptides from beef hydrolysates. *Meat Science*, **69**, 653–661.

Downloaded From Vet-eBooks.com

Jayasena, D. D., Ahn, D. U., Nam, K. C. and Jo, C. (2013). Flavour chemistry of chicken meat: A review. *Asian-Australasian Journal of Animal Sciences*, **26**, 732–742.

Jollès, P. and Henschen, A. (1982). Comparison between the clotting of blood and milk. *Trends in Biochemical Sciences*, **7**, 325–328.

Jollès, P., Levy-Toledano, S., Fiat, A. M. et al. (1986). Analogy between fibrinogen and casein. Effect of an undecapeptide isolated from κ-casein on platelet function. *European Journal of Biochemistry,* **158,** 379–384.

Katayama, K., Fuchu, H., Sakata, A. et al. (2003a). Angiotensin I-converting enzyme inhibitory activities of porcine skeletal muscle proteins following enzyme digestion. *Asian-Australasian Journal of Animal Sciences*, **16**, 417–424.

Katayama, K., Tomatsu, M., Fuchu, H. et al. (2003b). Purification and characterization of an angiotensin I-converting enzyme inhibitory peptide derived from porcine troponin C. *Animal Science Journal*, **74**, 53–58.

Katayama, K., Tomatsu, M., Kawahara, S. et al. (2004). Inhibitory profile of nonapeptide derived from porcine troponin C against angiotensin I-converting enzyme. *Journal of Agricultural and Food Chemistry*, **52**, 771–775.

Kato, T., Matsuda, T., Tahara, T., Sugimoto, M., Sato, Y. and Nakamura, R. (1994). Effects of meat conditioning and lactic fermentation on pork muscle protein degradation. *Bioscience, Biotechnology, and Biochemistry*, **58**, 408–410.

Kayser, H. and Meisel, H. (1996). Stimulation of human peripheral blood lymphocytes by bioactive peptides derived from bovine milk proteins. *FEBS Letters*, **383**, 18–20.

Kęska, P. and Stadnik, J. (2016). Porcine myofibrillar proteins as potential precursors of bioactive peptides—An *in silico* study. *Food Function*, **7**, 2878–2885.

Khaldi, N. (2012). Bioinformatics approaches for identifying new therapeutic bioactive peptides in food. *Functional Foods in Health and Disease*, **2**, 325–338.

Kim, S.-K., Kim, Y.-T., Byun, H.-G., Park, P.-J. and Ito, H. (2001). Purification and characterization of antioxidative peptides from bovine skin. *Biochemistry and Molecular Biology*, **34**, 219–224.

Kim, S.-K., Wijesekara, I., Park, E. Y., Matsumura, Y., Nakamura, Y. and Sato, K. (2012). Proteins and peptide as antioxidants. *In:* Hettiarachchy, N. S., Sato, K., Marshall, M. R. and Kannan, A. (eds.) *Bioactive Food Proteins and Peptides, Applications in Human Health*. Boca Raton, FL: CRC Press, pp. 97–115.

Kitamura, S., Muroya, S., Tanabe, S., Okumura, T., Chikuni, K. and Nishimura, T. (2005). Mechanism of production of troponin T fragments during postmortem aging of porcine muscle. *Journal of Agricultural and Food Chemistry*, **53**, 4178–4181.

Koohmaraie, M. (1994). Muscle proteinases and meat aging. *Meat Science*, **36**, 93–104.

Korhonen, H. and Pihlanto, A. (2003). Food-derived bioactive peptides: Opportunities for designing future foods. *Current Pharmaceutical Design*, **9**, 1297–1308.

Kumeta, Y., Maruyama, T., Asama, H., Yamamoto, Y., Hakamatsuka, T. and Goda, Y. (2014). Species identification of *Asini Corii Collas* (donkey glue) by PCR amplification of cytochrome *b* gene. *Journal of Natural Medicines*, **68**, 181–185.

Lafarga, T. and Hayes, M. (2014). Bioactive peptides from meat muscle and by-products: Generation, functionality and application as functional ingredients. *Meat Science*, **98**, 227–239.

Lafarga, T., O'Connor, P. and Hayes, M. (2015). *In silico* methods to identify meat-derived prolyl endopeptidase inhibitors. *Food Chemistry*, **175**, 337–343.

Lahov, E. and Regelson, W. (1996). Antibacterial and immunostimulating casein-derived substances from milk: Casecidin, istacidin peptides. *Food and Chemical Toxicology*, **34**, 131–145.

Downloaded From Vet-eBooks.com

Lertittikula, W., Benjakula, S. and Tanaka, M. (2007). Characteristics and antioxidative activity of Maillard reaction products from a porcine plasma protein–glucose model system as influenced by pH. *Food Chemistry*, **100**, 669–677.

Li, B., Chen, F., Xang, X., Ji, B. and Wu, Y. (2007). Isolation and identification of antioxidative peptides from porcine collagen hydrolysate by consecutive chromatography. *Food Chemistry*, **102**, 1135–1143.

Li, G. H., Le, G. W., Shi, Y. H. and Shrestha, S. (2004). Angiotensin I-converting enzyme inhibitory peptides derived from food proteins and their physiological and pharmacological effects. *Nutrition Research*, **24**, 469–486.

Li, Y., Lu, F., Luo, C., Chen, Z., Mao, J. and Shoemaker, C. (2009). Functional properties of the Maillard reaction products of rice protein with sugar. *Food Chemistry*, **117**, 69–74.

Liepke, C., Adermann, K., Raida, M., Magert, H.-J., Forssman, W.-G. and Zucht, H.-D. (2002). Human milk provides peptides highly stimulating the growth of bifidobacteria. *European Journal of Biochemistry*, **269**, 712–718.

Liu, R., Xing, L., Fu, Q., Zhou, G.-H. and Zhang, W.-G. (2016). A review of antioxidant peptides derived from meat muscle and by-products. *Antioxidants*, **5**, 32–46.

López, C. M., Bru, E., Vignolo, G. and Fedda, S. G. (2015). Identification of small peptides arising from hydrolysis of meat proteins in dry fermented sausages. *Meat Science*, **104**, 20–29.

Losso, J. N. (2016). *The Maillard Reaction Reconsidered*. Boca Raton, FL: CRC Press.

Maehashi, K., Matsuzaki, M., Yamamoto, Y. and Udaka, S. (1999). Isolation of peptides from an enzymatic hydrolysate of food proteins and characterization of their taste properties. *Bioscience, Biotechnology, and Biochemistry*, **63**, 555–559.

Martínez-Maqueda, D., Hernández-Ledesma, B., Amigo, L., Miralles, B. and Gómez-Ruiz, J. Á. (2013). Extraction/fractionation techniques for proteins and peptides and protein digestion. *In:* Toldrá, F. and Nollet, L. M. L. (eds.) *Proteomics in Foods*. New York, NY: Springer, pp. 21–50.

Maruyama, S., Mitachi, H., Awaya, J., Kurono, M., Tomizuka, N. and Suzuki, H. (1987). Angiotensin I converting enzyme inhibitory activity of the C-terminal hexapeptide of αs1-casein. *Agricultural and Biological Chemistry*, **51**, 2557–2561.

Matsubayashi, Y. and Sakagami, Y. (1999). Characterization of specific binding sites for a mitogenic sulfated peptide, phytosulfokine-α, in the plasma-membrane fraction derived from *Oryza sativa* L. *European Journal of Biochemistry*, **262**, 666–671.

Meisel, H. (1986). Chemical characterization and opioid activity of an exorphin isolated from *in vivo* digests of casein. *FEBS Letters*, **196**, 223–227.

Meisel, H., Walsh, D. J., Murry, B. and FitzGerald, R. J. (2005). ACE inhibitory peptides. *In:* Mine, Y. and Shahidi, F. (eds.) *Nutraceutical Proteins and Peptides in Health and Disease*. Boca Raton, FL: CRC Press, pp. 269–315.

Mellander, O. (1950). The physiological importance of the casein phosphopeptide calcium salts II: Peroral calcium dosage in infants. *Acta Societatis Medicorum Upsaliensis*, **55**, 247–255.

Mikami, M., Nagao, M., Sekikawa, M. and Miura, H. (1995). Changes in peptide and free amino acid contents of different bovine muscle homogenate during storage. *Animal Science and Technology (Japan)*, **66**, 630–638.

Migliore-Samour, D., Floc'h, F. and Jollès, P. (1989). Biologically active casein peptides implicated in immunomodulation. *Journal of Dairy Research*, **56**, 357–362.

Downloaded From Vet-eBooks.com

Mirdhayati, I., Hermanianto, J., Wijaya, C. H., Sajuthi, D. and Arihara, K. (2015). Angiotensin converting enzyme (ACE) inhibitory and antihypertensive activities of protein hydrolysate from meat of *Kacang* goat (*Capra aegagrus hircus*). *Journal of the Science of Food and Agriculture*, **96**, 3536–3542.

Miyoshi, S., Ishikawa, H., Kaneko, T., Fukui, F., Tanaka, H. and Maruyama, S. (1991). Structures and activity of angiotensin-converting enzyme inhibitors α-zein hydrolysate. *Agricultural and Biological Chemistry*, **55**, 1313–1318.

Mora, L., Escudero, E., Arihara, K. and Toldrá, F. (2015). Antihypertensive effect of peptides naturally generated during Iberian dry-cured ham processing. *Food Research International*, **78**, 71–78.

Mora, L., Escudero, E., Fraser, P. D., Aristoy, M. C. and Toldrá, F. (2014). Proteomic identification of antioxidant peptides from 400 to 2500 Da generated in Spanish dry-cured ham contained in a size-exclusion chromatography. *Food Research International*, **56**, 68–76.

Mora, L., Escudero, E. and Toldrá, F. (2016). Characterization of the peptide profile in Spanish Teruel, Italian Parma and Belgian dry-cured hams and its potential bioactivity. *Food Research International*, **89**, 638–646.

Mora, L., Sentandreu, M. A., Fraser, P. D., Toldrá, F. and Bramley, P. M. (2009). Oligopeptides arising the degradation of creatine kinase in Spanish dry-cured ham. *Journal of Agricultural and Food Chemistry*, **57**, 8982–8988.

Mora, L. and Toldrá, F. (2013). Dry-cured ham. *In:* Toldrá, F. and Nollet, L. M. L. (eds.) *Proteomics in Foods*. New York, NY: Springer, pp. 147–160.

Mora, L. and Toldrá, F. (2014). Bioactive peptides generated from meat industry by-products. *Food Research International*, **65**, 344–349.

Morales, F., Somoza, V. and Fogliano, V. (2012). Physiological relevance of dietary melanoidins. *Amino Acids*, **42**, 1097–1109.

Morimatsu, F., Ito, M., Budijanto, S., Watanabe, I., Furukawa, Y. and Kimura, S. (1996). Plasma cholesterol-suppressing effect of papain-hydrolyzed pork meat in rats fed hypercholesterolemic diet. *Journal of Nutritional Science and Vitaminology*, **42**, 145–153.

Mottram, D. S., Wedzicha, B. L. and Dodson, A. T. (2002). Food chemistry: Acrylamide is formed in the Maillard reaction. *Nature*, **419**, 448–449.

Nagaoka, S. (2012). Peptide-lipid interactions and functionalities. *In:* Hettiarachchy, N. S., Sato, K., Marshall, M. R. and Kannan, A. (eds.) *Food Proteins and Peptides: Chemistry, Functionality, Interactions and Commercialization*. Boca Raton, FL: CRC Press, pp. 263–276.

Nagaoka, S., Futamura, Y., Miwa, K. et al. (2001). Identification of novel hypocholesterolemic peptides derived from bovine milk β-lactoglobulin. *Biochemical and Biophysical Research Communications*, **281**, 11–17.

Nagaoka, S., Kanamaru, Y. and Kuzuya, Y. (1992). Comparative studies on the serum cholesterol lowering action of whey protein and soyprotein in rats. *Bioscience, Biotechnology, and Biochemistry*, **56**, 1484–1485.

Nakashima, Y., Arihara, K., Sasaki, A., Ishikawa, S. and Itoh, M. (2002). Antihypertensive activities of peptides derived from porcine skeletal muscle myosin in spontaneously hypertensive rats. *Journal of Food Science*, **67**, 434–437.

Nedjar-Arroume, N., Dubois-Delval, V., Miloudi, K. et al. (2006). Isolation and characterization of four antibacterial peptides from bovine hemoglobin. *Peptides*, **27**, 2082–2089.

Downloaded From Vet-eBooks.com

Nishimura, T. and Kato, H. (1988). Mechanisms involved in the improvement of meat taste during postmortem aging. *Food Science and Technology International (Tokyo)*, **4**, 241–249.

Nishimura, T., Rhue, M. R., Okitani, A. and Kato, H. (1988). Components contributing to the improvement of meat taste during storage. *Agricultural and Biological Chemistry*, **52**, 2323–2330.

Noguchi, M., Arai, S., Yamashita, M., Kato, H. and Fujimaki, M. (1975). Isolation and identification of acidic oligopeptides occurring in a flavor potentiating fraction from a fish protein hydrolysate. *Journal of Agricultural and Food Chemistry*, **23**, 49–53.

Norris, R. and FitzGerald, J. R. (2015). Antihypertensive peptides from food proteins. *In:* Bowman, L. (ed.) *Bioactive Food Peptides: Production and Health Benefits*. Forest Hills, NJ: Foster Academics, pp. 3–30.

Nurminen, M.-L., Sipola, M., Kaarto, H. et al. (2000). α-Lactophin lowers blood pressure via radiotelemetry in normotensive and spontaneously hypertensive rats. *Life Sciences*, **66**, 1535–1543.

Nyberg, F., Sanderson, K. and Glamsta, E.-L. (1997). The hemorphins: A new class of opioid peptides derived from the blood protein hemoglobin. *Biopolymers*, **43**, 147–156.

Ohata, M., Uchida, S., Zhou, L. and Arihara, K. (2016). Antioxidant activity of fermented meat sauce and isolation of an associated antioxidant peptide. *Food Chemistry*, **194**, 1034–1039.

Ohata, M., Zhou, L., Owashi, C. and Arihara, K. (2014). The effect of odor generated from protein digests and reducing sugars by the Maillard reaction on blood pressure. *IMARS News Letter*, **9**, 21–25.

Okumura, T., Yamada, R. and Nishimura, T. (2004). Sourness-suppressing peptides in cooked pork loins. *Bioscience, Biotechnology, and Biochemistry*, **68**, 1657–1662.

Olmedilla-Alonso, B., Jiménez-Colmenero, F. and Sánchez-Muniz, F. J. (2013). Development and assessment of healthy properties of meat and meat products designed as functional foods. *Meat Science*, **95**, 919–930.

Ondetti, M. A. and Cushman, D. W. (1982). Enzymes of the renin-angiotensin system and their inhibitors. *Annual Review of Biochemistry*, **51**, 283–308.

Ordonez, J. A., Hierro, E. M., Bruna, J. M. and de la Hoz, L. (1998). Changes in the components of dry fermented sausages during ripening. *Critical Reviews in Food Science and Nutrition*, **39**, 329–367.

Ortea, I., O'Connor, G. and Maquet, A. (2016). Review on proteomics for food authentication. *Journal of Proteomics*, **147**, 212–225.

Oshima, G., Shimabukuro, H. and Nagasawa, K. (1979). Peptide inhibitors of angiotensin I-converting enzyme in digests of gelatin by bacterial collagenase. *Biochimica et Biophysica Acta*, **566**, 128–137.

Otani, H. and Hata, I. (1995). Inhibition of proliferative responses of mouse spleen lymphocytes and rabbit Peyer's patch cells by bovine milk caseins and their digests. *Journal of Dairy Research*, **62**, 339–348.

Owusu-Apenten, R. (2010). *Bioactive Peptides Applications for Improving Nutrition and Health*. Baca Raton, FL: CRC Press.

Parés, D., Saguer, E. and Carretero, C. (2011). Blood by-products as ingredients in processed meat. *In:* Kerry, J. P. and Kerry, J. F. (eds.) *Processed Meats: Improving Safety, Nutrition and Quality*. Oxford: Woodhead Publishing, pp. 219–242.

Downloaded From Vet-eBooks.com

Pathera, A. K., Jairath, G., Singh, P. K. and Yadav, S. (2017). Health promoting functional properties of meat and meat products. *In:* Mudgil, D. and Barak, S. (eds.) *Functional Foods: Sources and Health Benefits*. Jodhpur, India: Scientific Publishers, pp. 333–362.

Pavlidis, C., Patrinos, G. P. and Katsila, T. (2015). Nutrigenomics: A controversy. *Applied & Translational Genomics*, **4**, 50–53.

Pegg, R. B. and Shahidi, F. (2014). Flavor development. *In: Encyclopedia of Meat Sciences* (2nd Ed., vol. 1), Dikeman, M. and Devine, C (eds.). Oxford: Elsevier/Academic Press, pp. 377–384.

Pihlanto-Leppälä, A. (2001). Bioactive peptides derived from bovine whey proteins: Opioid and ACE inhibitory peptides. *Trends in Food Science & Technology*, **11**, 347–356.

Pihlanto, A. and Korhonen, H. (2003). Bioactive peptides and proteins. *Advances in Food and Nutrition Research*, **47**, 175–276.

Poljak, A., McLean, C. A., Sachdev, P., Brodaty, H. and Smythe, G. A. (2004). Quantification of hemorphins in Alzheimer's disease brains. *Journal of Neuroscience Research*, **75**, 704–714.

Przybylski, R., Firdaous, L., Châtaigné, G., Dhulster, P. and Nedjar, N. (2016). Production of an antimicrobial peptide derived from slaughterhouse by-product and its potential application on meat as preservative. *Food Chemistry*, **211**, 306–313.

Ryan, J. T., Ross, R. P., Bolton, D., Fitzgerald, G. F. and Stanton, C. (2011). Bioactive peptides from muscle sources: Meat and fish (review). *Nutrients*, **3**, 765–791.

Ryder, K., Bekhit, A. E.-D., McConnell, M. and Carne, A. (2016). Towards generation of bioactive peptides from meat industry waste proteins: Generation of peptides using commercial microbial proteases. *Food Chemistry*, **208**, 42–50.

Saiga, A., Okumura, T., Makihara, T. et al. (2003a). Angiotensin I-converting enzyme inhibitory peptides in a hydrolyzed chicken breast muscle extract. *Journal of Agricultural and Food Chemistry*, **51**, 1741–1745.

Saiga, A., Tanabe, S. and Nishimura, T. (2003b). Antioxidant activity of peptides obtained from porcine myofibrillar proteins by protease treatment. *Journal of Agricultural and Food Chemistry*, **51**, 3661–3667.

Saito, T., Nakamura, T., Kitazawa, H., Kawai, Y. and Itoh, T. (2000). Isolation and structural analysis of antihypertensive peptides that exist naturally in Gouda cheese. *Journal of Dairy Science*, **83**, 1434–1440.

Sarraga, C., Gil, M., Arnau, J., Monfort, J. M. and Cusso, R. (1989). Effect of curing salt and phosphate on the activity of porcine muscle proteases. *Meat Science*, **25**, 241–249.

Sayd, T., Chambon, C. and Santé-Lhoutellier, V. (2016). Quantification of peptides released during *in vitro* digestion of cooked meat. *Food Chemistry*, **197**, 1311–1323.

Sentandreu, M. A., Stoeva, S., Aristoy, M. C., Laib, K., Voelter, W. and Toldrá, F. (2003). Identification of small peptides generated in Spanish dry-cured ham. *Journal of Food Science*, **68**, 64–69.

Sentandreu, M. A. and Toldrá, F. (2007). Evaluation of ACE inhibitory activity of dipeptides generated by the action of porcine muscle dipeptidyl peptidases. *Food Chemistry*, **102**, 511–515.

Shahidi, F., Samaranayaka, A. G. P. and Pegg, R. B. (2014). Flavor development. *In:* Dikeman, M. and Devine, C. (eds.) *Encyclopedia of Meat Sciences* (2nd Ed., vol. 1). London: Elsevier/Academic Press, pp. 391–403.

Downloaded From Vet-eBooks.com

Shimizu, M., Sawashita, N., Morimatsu, F. et al. (2009). Antithrombotic papain-hydrolyzed peptides isolated from pork meat. *Thrombosis Research*, **123**, 753–757.

Shin, K., Yamauchi, K., Teraguchi, S. et al. (1998). Antibacterial activity of bovine lactoferrin and its peptides against enterohaemorrhagic *Escherichia coli* O157:H7. *Letters in Applied Microbiology*, **6**, 407–411.

Simonetti, A., Gambacorta, E. and Perna, A. (2016). Antioxidative and antihypertensive activities of pig meat before and after cooking and *in vitro* gastrointestinal digestion: Comparison between Italian autochthonous pig Suino Nero Lucano and a modern crossbred pig. *Food Chemistry*, **212**, 590–595.

Somoza, V. (2005). Five years of research on health risks and benefits of Maillard reaction products: An update. *Molecular Nutrition & Food Research*, **49**, 663–672.

Stadler, R. H., Blank, I., Varga, N. et al. (2002). Food chemistry: Acrylamide from Maillard reaction products. *Nature*, **419**, 449–450.

Stadnik, J. and Kęska, P. (2015). Meat and fermented meat products as a source of bioactive peptides. *Acta Scientiarum Polonorum Technologia Alimentaria*, **14**, 181–190.

Sugano, M., Goto, S., Yamada, Y. et al. (1990). Cholesterol-lowering activity of various undigested fraction of soybean protein in rats. *Journal of Nutrition*, **120**, 977–985.

Temussi, P. A. (2012). The good taste of peptides. *Journal of Peptide Science*, **18**, 73–82.

Terashima, M., Baba, T., Ikemoto, N., Katayama, M., Morimoto, T. and Matsumura, S. (2010). Novel angiotensin-converting enzyme (ACE) inhibitory peptides derived from boneless chicken leg meat. *Journal of Agricultural and Food Chemistry*, **58**, 7432–7436.

Toldrá, F. (2014). Dry. *In:* Dikeman, M. and Devine, C. (eds.) *Encyclopedia of Meat Sciences* (2nd Ed., vol. 1). Oxford: Elsevier/Academic Press, pp. 425–429.

Toldrá, F., Aristoy, M.-C., Mora, L. and Reig, M. (2012). Innovations in value-addition of edible meat by-products. *Meat Science*, **92**, 290–296.

Toldrá, F. and Flores, M. (1988). The role of muscle proteases and lipases in flavor development during the processing of dry-cured ham. *Critical Reviews in Food Science and Nutrition*, **38**, 331–352.

Tomita, M., Takase, M., Bellamy, W. R. and Shimamura, S. (1994). A review, the active peptide of lactoferrin. *Acta Paediatrica Japonica*, **36**, 585–591.

Udenigwe, C. C. (2014). Bioinformatics approaches, prospects and challenges of food bioactive peptide research. *Trends in Food Science & Technology*, **36**, 137–143.

Udenigwe, C. C. and Howard, A. (2013). Meat proteome as source of functional biopeptides. *Food Research International*, **54**, 1021–1032.

Van Lancker, F., Adams, A. and De Kimpe, N. (2011). Chemical modifications of peptides and their impact on food properties. *Chemical Reviews*, **111**, 7876–7903.

Ventanas, J., Cordoba, J. J., Antequera, T., Garcia, C., López-bote, C. and Asensio, M. A. (1992). Hydrolysis and Maillard reactions during ripening of Iberian ham. *Journal of Food Science*, **57**, 813–815.

Vercruysse, L., Van Camp, J. and Smagghe, G. (2005). ACE inhibitory peptides derived from enzymatic hydrolysates of animal muscles protein: A review. *Journal of Agricultural and Food Chemistry*, **53**, 8106–8115.

Watanabe, K., Tsuji, Y., Shimoyamada, M., Ogama, N. and Ebina, T. (1998). Antitumor effects of pronase-treated fragments, glycopeptides, from ovomucin in hen egg white in a double grafted tumor system. *Journal of Agricultural and Food Chemistry*, **46**, 3033–3038.

Downloaded From Vet-eBooks.com

Weerawatanakorn, M., Wu, J.-C., Pan, M.-H. and Ho, C.-T. (2015). Reactivity and stability of selected flavor compounds. *Journal of Food and Drug Analysis*, **23**, 176–190.

Wu, J., Jahandideh, F., Yu, W. and Majumder, K. (2015). Bioactive peptides from meat proteins as functional food components. *In:* Girillo, G., Gianfranco Spizzirri, U. and Iemma, F. (eds.) *Functional Polymers in Food Science.* Beverly, MA: Scrivener Publishing, pp. 181–208.

Xing, L.-J., Hu, Y. Y., Hu, H.-Y., Ge, Q.-F., Zhou, G.-H. and Zhang, W.-G. (2016). Purification and identification of antioxidative peptides from dry-cured Xuanwei ham. *Food Chemistry*, **194**, 951–958.

Yamazaki, Y. and Maekawa, K. (1980). Synthesis of a peptide with delicious taste. *Agricultural and Biological Chemistry*, **44**, 93–97.

Yoshikawa, M. (1996). Selection of proteases for production of biologically active peptides from food proteins. *Japanese Journal of Dairy and Food Science*, **45**, A51–A60.

Yoshikawa, M., Kishi, K., Takahashi, M. et al. (1993). Immunostimulating peptide derived from soybean protein. *Annals of the New York Academy of Sciences*, **685**, 375–376.

Young, D. and Mine, Y. (2009). Functional bioactive proteins and peptides in nutrigenomics. *In:* Mine, Y., Miyashita, K. and Shahidi, F. (eds.) *Nutrigenomics and Proteomics in Health and Disease*. Ames, IA: Wiley-Blackwell, pp. 129–144.

Young, J. F., Therkildsen, M., Ekstrand, B. et al. (2013). Novel aspects of health promoting compounds in meat. *Meat Science*, **95**, 904–911.

Yu, Y., Hu, J. N., Miyaguchi, Y., Bai, X. F., Du, Y. G. and Lin, B. C. (2006). Isolation and characterization of angiotensin I-converting enzyme inhibitory peptides derived from porcine hemoglobin. *Peptides*, **27**, 2950–2956.

Zhao, Q., Garreau, I., Sannier, F. and Piot, J. M. (1997). Opioid peptides derived from hemoglobin: Hemorphins. *Biopolymers*, **43**, 75–98.

Zhou, L., Ohata, M. and Arihara, K. (2016). Effects of odor generated from the glycine/glucose Maillard reaction on human mood and brainwaves. *Food Function*, **7**, 2574–2581.

Downloaded From Vet-eBooks.com

13

New Approaches for the Development of Functional Meat Products

Francisco Jiménez-Colmenero, Milagro Reig, and Fidel Toldrá

CONTENTS

13.1 Introduction: A Global Perspective on Functional Foods 404
13.2 Options to Design Functional Meat and Meat Products 405
13.3 Animal Production Strategies for Improving the Nutritional Profiles of Raw Meat 407
13.3.1 Genetics for the Reduction of Animal Fats 407
13.3.2 Dietary Feed Formulation for the Modification of Animal Fats 408
13.3.2.1 Manipulation of Fatty Acids in Pigs 409
13.3.2.2 Manipulation of Fatty Acids in Ruminants 412
13.3.3 Dietary Enrichment in Compounds of Specific Nutritional Interest 413
13.3.3.1 Vitamins and Antioxidants 413
13.3.3.2 Minerals 415
13.4 Processing Strategies for Developing Functional Meat Products 415
13.4.1 Reduction of Specific Unhealthy Compounds 417
13.4.1.1 Fat and Cholesterol 417
13.4.1.2 Sodium and Phosphate 419
13.4.1.3 Nitrite 419
13.4.1.4 Allergens 419
13.4.2 Promoting the Presence of Specific Healthy Compounds 420
13.4.2.1 Fat and Fatty Acid Profile 420
13.4.2.2 Proteins, Peptides, and Amino Acids 422
13.4.2.3 Prebiotics and Probiotics 423
13.4.2.4 Antioxidants and Vitamins 425
13.4.2.5 Minerals 427
13.4.2.6 Plant Sterols and Stanols 427
13.4.2.7 Other Compounds 428
13.5 Safety and Scientific Criteria for Nutrition and Health Claims: Legislative Implications 428
13.6 Final Considerations 429
Acknowledgments 430
References 430

Downloaded From Vet-eBooks.com

13.1 Introduction: A Global Perspective on Functional Foods

The last few decades have seen rapid expansion of knowledge about the influence of diet on health and well-being. The concept of food in the developed world is changing drastically from the classical idea whereby the purpose of food was to supply the nutrients required for proper metabolism by consumers and to satisfy their expectations; now, the emphasis is increasingly on the beneficial and psychological effects of the diet. In this sense, an adequate diet can not only help to achieve optimal health and development, but it can also play an important role in reducing the risk of disease (Diplock et al., 1999).

The influence of certain foods on human health has long been known, but the scientific basis for the role of certain physiologically active food compounds in the modulation of specific bodily functions was only established recently. Such knowledge has contributed to a concept of optimal nutrition in developed countries and the development of functional foods within that context. This new concept focuses on optimizing the quality of the daily diet. This is achieved by controlling nutrient and nonnutrient contents, and also other food properties that favor health maintenance (Ashwell, 2002).

There is no clear universally accepted definition of functional foods. However, several organizations have attempted to define this emerging food category. Japan was the first country to introduce specific regulatory approval, in 1991. Foods for specified health use (FOSHU) were defined as "foods which, based on knowledge concerning the relationship between foods or food components and health, are expected to have certain health benefits and have been licensed to bear labeling claiming that a person using them may expect to obtain that health through the consumption of these foods" (Arihara, 2004). The American Dietetic Association considers functional foods, including whole foods and fortified, enriched, or enhanced foods, as those having a potential benefit for health when consumed as part of a varied diet on a regular basis, at effective levels (Hasler et al., 2004). A European Commission Concerted Action coordinated by the International Life Science Institute (ILSI Europe) produced a consensus document concerning Scientific Concepts of Functional Foods in Europe and proposed the following definition: "A food can be regarded as a functional food if it is satisfactorily demonstrated to affect beneficially one or more target functions in the body, beyond adequate nutritional effects, in a way that is relevant to an improved health status and well-being and/or reduction of risk of disease. Functional foods must remain as foods and must demonstrate their effects in amounts that can normally be expected to be consumed in the diet: they are not pills or capsules, but part of a normal food pattern" (Diplock et al., 1999). A functional food may be a natural food or one that can be made functional by means of different technological approaches. This refers to new foods specifically designed for health enhancement, but it also includes traditional, family foods where recent research has highlighted health benefits,

Downloaded From Vet-eBooks.com

previously unknown, or dispelled old dogma about potential adverse health effects (Hasler, 2000).

Functional food effects are based on the way in which specific nutrients and food components positively affect target functions in the organism. The substantiation of these effects should be based on the knowledge of the mechanisms by which functional foods can modulate the target function and their relevance to the state of well-being and health and/or reduction of a disease. Although the substantiation should be based on a systematic review of all available relevant evidences, in general, experimental human trial is the most useful experimental study (Diplock et al., 1999; Ashwell, 2002).

13.2 Options to Design Functional Meat and Meat Products

A food can be made functional by using appropriate technologies to optimize beneficial properties. Depending on the origin and targeted modification, within the field of meat these include the following approaches (Roberfroid, 2000; Ashwell, 2002; Holm, 2003): (1) to raise the concentration of the beneficial natural compound (either nutrient or nonnutrient) in the food up to the desired effective level, where such enhancement can be naturally induced (e.g., by increasing specific compounds like n-3 fatty acids or conjugated linoleic acid [CLA] in raw meat by animal production practices) or produced by reformulation (e.g., calcium-fortified meat products); (2) to add a compound that is not normally present in the food but has proven benefits (e.g., a prebiotic in meat products); (3) to replace a compound, usually a macronutrient (e.g., a fat) whose intake is usually excessive and hence harmful to health, by a compound with demonstrated health benefits; (4) to remove specific compounds so as to reduce adverse health effects, where these compounds may be naturally present or produced by processing (e.g., the reduction of saturated fatty acids [SFAs], trans-fatty acids (TFAs), a toxic compound, or a food allergen); (5) to improve the bioavailability (e.g., improved iron absorption) or stability of a compound that is known to produce a functional effect or to reduce the potential disease risk of the food; and (6) any combination of the preceding possibilities.

These different approaches to the production of meat-based functional foods entail the use of a wide range of common bioactive components, both phytochemicals and zoochemicals (see Table 13.1). The more promising target functions for these foods concern: (1) growth, development, and differentiation; (2) substrate metabolism; (3) defense against reactive oxidative species; (4) cardiovascular system; (5) intestinal physiology; and (6) behavioral and psychological functions (Diplock et al., 1999).

Meat and meat products are generally recognized as being contributors to nutrition because they constitute an important source of high biological

Downloaded From Vet-eBooks.com

TABLE 13.1

Examples of Major Classes of Bioactive Food Components

Bioactive Component	Examples
Lipids and fatty acids	n-3 polyunsaturated fatty acids (eicosapentaenoic and docosahexaenoic acids), monounsaturated fatty acids, conjugated linoleic acid
Proteins and peptides	Soy protein, bioactive peptides (inhibitors of the angiotensin I-converting enzyme, antioxidants, carnitine, carnosine, anserine, etc.)
Prebiotic	Dietary fibers (oats, soy, citrus, etc.), oligosaccharides
Probiotic	Lactic acid bacteria (*Lactobacillus casei*, *Lactobacillus acidophilus*, etc.), *Bifidobacteria*
Vitamins	Tocopherols, folic acid, ascorbic acid
Minerals	Calcium, magnesium, selenium, zinc, iron
Phytochemicals	Phytosterols (sterol and stanol esters), carotenoids (β-carotene, lycopene, zeaxanthin, lutein, etc.), flavonoids (flavones, flavonones, catechins, etc.), phytoestrogens (isoflavones), etc.

value proteins, group B vitamins, minerals, trace elements, and other bioactive compounds including strategies to get them enriched in such compounds in order to get enhanced bioactivity (Toldrá and Reig, 2011). On the other hand, they also contribute to the intake of fat, SFAs, cholesterol, salt, and other substances that in inappropriate amounts may result in negative physiological effects. For many years now, the image of meat and meat products has tended to deteriorate because they are viewed as a serious risk factor (through various of their different constituents) involved in some of the most prevalent diseases of western society, such as cardiovascular disease (CVD), hypertension, obesity/diabetes, and certain types of cancer.

The meat industry must adapt to the new concepts in nutrition. There is now a potential market for functional foods, based on the principle of added value linked to health benefits (Diplock et al., 1999), which is one of the main trends in the development of food (meat) products (Sloan, 2000). The scientific research process must be clearly integrated with insights into consumer needs and demands (Weststrate et al., 2002). Functional meats constitute an excellent opportunity to improve their "image" and help facilitate the implementation of strategies (in different areas such as family, health, education, etc.) for updating the recommendations relating to nutrient and dietary goals (WHO, 2003). It is an essential task for the meat sector to promote the production of foods for a healthy and balanced diet. At the same time, leaving aside their current relative importance, this type of food could also furnish a means to achieve more diversification in the sector and to take up a solid position in emerging markets with considerable promise for the future. As a result, more and more work is being done on food processing and technology for the production of functional foods (Jimenez-Colmenero, 2007a).

Downloaded From Vet-eBooks.com

From farm to table, different strategies can be effectively used to increase or reduce bioactive compounds in meat and meat products and thus produce functional meat products. These strategies basically address such levels as animal production practices, meat processing, and cooking systems (Jimenez-Colmenero, 2007a). This chapter provides an overview of these strategies, focusing on certain aspects of interest for the development of functional meat products. It also discusses the potential benefits of meat components for human health, modifications in bioactive compounds, and their health implications.

13.3 Animal Production Strategies for Improving the Nutritional Profiles of Raw Meat

13.3.1 Genetics for the Reduction of Animal Fats

The genetic type plays an important role in nutritional quality. Many breeding strategies in recent years have focused mainly on increasing lean meat content and reducing intramuscular fat content and back fat thickness. Sometimes, the meat is poorer in quality even though grading traits are better (Hovenier et al., 1992). Some studies with cattle breeds have been directed toward more tender meat (Johnson et al., 1990; Wheeler et al., 1990). There are several strategies for increasing the ratio of lean to fat in farm animals. Some are based on the repartitioning effects of somatotropin, specific β-adrenergic agonists, some growth promoting agents, and gene manipulation techniques (Solomon et al., 2002). Other strategies for increasing the lean to fat ratio are based on different breeding schemes, but most entail a backcross or a three- or four-way cross. A three-way cross is quite usual for pigs in the European Union (EU), using a Landrace × Large White (LR × LW) crossbreed as sow. The terminal sire is chosen from a wide range of possibilities like Duroc, Pietrain, Belgian Landrace, Landrace, etc. The choice is very important as it affects not only production and quality but also the nutritional profile, given that the intramuscular fat content may reach anywhere between 1.2 and 2.9/100 g of meat (Leclerq, 1990). Thus, when Duroc is used, the result is an excess of fat and hence an excess of calories. Belgian Landrace and Pietrain, on the other hand, are heavily muscled and consequently have a lower fat content (Toldrá et al., 1996a; Toldrá, 2002). We would note that leaner carcasses are generally associated with a more glycolytic muscle fiber type and hence more rapid postmortem metabolism, paler meat color, and lower water holding capacity (De Smet et al., 2002). Moreover, some changes in lipids have been reported depending on the sire genetic type (Armero et al., 2002). Enzyme fingerprinting, in the form of reports on the activity of endo- and exoproteases, lipases, and esterases, constitutes a useful tool for understanding

Downloaded From Vet-eBooks.com

the biochemical activity of a given meat and for predicting its sensory and nutritional quality and behavior (expected proteolysis and lipolysis) during processing (Armero et al., 1999a).

The age of the animal correlates directly with increased intramuscular fat content (higher calorie intake) and connective tissue (tougher meat and poorer biological value), even though the meat may have stronger flavor and color due to greater generation of volatile compounds and myoglobin, respectively (Armero et al., 1999b). This is a consequence of the action of muscle proteolytic and lipolytic enzymes, which are affected by age. Thus, muscles from heavy pigs have been reported to exhibit a higher peptidase to proteinase ratio and higher lipase activity than muscles from younger pigs (Toldrá et al., 1996b; Rosell and Toldrá, 1998).

Sex mainly affects the fat content. Thus, meat from barrows has more fat, more marbling, and a thicker subcutaneous fat layer than meat from gilts (Armero et al., 1999a). For example, a fat thickness of 15.4 mm has been reported for barrows and 9.0 mm for boars at 90 kg live weight (Wood et al., 1985). The elimination of castration would help reduce fat content in boars to close to that of gilts (Bass et al., 1990).

13.3.2 Dietary Feed Formulation for the Modification of Animal Fats

There has been a good deal of research since the 1980s on altering the fatty acid composition of meat to attain the levels recommended by nutritionists. Initially, the target was to increase the ratio between polyunsaturated and SFAs (PUFA:SFA ratio) to above 0.4. A few years later, nutritionists also recommended manipulating PUFA composition in the direction of a lower n-6:n-3 ratio so as to reduce the adverse effect of arachidonic acid and its eicosanoid products (Simopoulos et al., 2000). This n-6:n-3 ratio constitutes a risk factor in coronary heart diseases and cancers (Enser et al., 2000), so that a n-6:n-3 ratio below 4 is highly recommended (Wood et al., 2003). Meats evidently far exceed these target values, but there are ways to improve them based on the type of feed, as described below. It should be mentioned that manipulation of fats to attain a higher PUFA content may cause indirect problems during processing, such as softer fats (due to its lower melting point). Other major problems such as oxidation and generation of off-flavors (rancid aromas) and color deterioration (trend toward yellowness in the fat) are also likely to arise unless antioxidants are added (Toldrá and Flores, 2004).

The amount of feed also influences the fat content in meat. Thus, an excess of feed may increase the amount of intramuscular fat, but if animals are deprived of feed, lipolysis may be induced and the amount of fat reduced, especially in glycolytic muscles (Fernández et al., 1995). The amount of cholesterol accretion in tissues of growing pigs is not generally influenced by their serum cholesterol content or by the fat and cholesterol content in the diet (Harris et al., 1993).

Downloaded From Vet-eBooks.com

13.3.2.1 Manipulation of Fatty Acids in Pigs

Pigs are monogastric animals that incorporate part of their dietary fatty acid intake practically unchanged into the adipose tissue and cellular membranes, where desaturation and chain elongation processes may take place (Toldrá et al., 1996a; Jakobsen, 1999). The extent of incorporation may vary depending on the specific fatty acid, the time of feeding, and the type of feed. Maximal effect with essential fatty acids has been reported within 40 days, while only half of the effect was achieved in 14 days (Warnants et al., 1999; Wood et al., 2003).

A large variety of dietary oils and their respective effects on the proportions of fatty acid composition have been studied. When feeds are rich in saturated fats like tallow, the levels of palmitic, palmitoleic, stearic, and oleic acids in pork meat are substantially higher and the PUFA:SFA ratio is lower (Morgan et al., 1992). However, the primordial objective of most of the assayed oils was to raise the PUFA content and hence the PUFA:SFA ratio. Thus, dietary oils rich in linoleic acid (C 18:2), a n-6 fatty acid typically present in soy, maize, sunflower, and barley, significantly increase the concentration of this fatty acid in meat (see Table 13.2). When these oils are used in feeds, the oleic acid content is partly replaced by linoleic acid (Hernández et al., 1998). There is a serious potential problem derived from rapid oxidation during heating, caused by the generation of volatile compounds, like some aldehydes, which produce rancid aromas (Larick et al., 1992). Even more serious is an increase in the n-6:n-3 ratio, commonly observed when feeds contain oils of this type, to well above the nutritional recommendations.

Some strategies have focused on n-3 fatty acids in the dietary supply, as these compounds have been shown to protect against some cancers, reduce CVDs, improve rheumatoid arthritis, and reduce inflammatory bowel diseases (Hoz et al., 2003). Dietary oils rich in linolenic acid (C 18:3), a n-3 fatty acid typically present in canola and linseed oils, have been tested recently. These feeds increase the linolenic acid content, and slightly increase the eicosapentaenoic (EPA, C 22:5) and docosahexaenoic (DHA, C 22:6) acid contents, in pork meat, also decreasing the linoleic acid content. In this way, they raise the PUFA:SFA ratio to 0.4, close to the minimum recommended value for the human diet, and reduce the n-6:n-3 ratio to 5, close to the recommended maximum of 4 (Enser et al., 2000). On the other hand, no significant effects on oxidative stability or sensory characteristics have been reported (Sheard et al., 2000). Other authors, feeding either linseed oil alone or a mixture of linseed and olive oils (see Table 13.3), have reported even higher PUFA:SFA ratios, around 0.6–0.7, and lower n-6:n-3 ratios, close to 2.0 (Hoz et al., 2003), although stability against oxidation was lower. Large differences in linoleic and linolenic acid contents between the leanest and fattest animals are common (Enser et al., 1998). A recent work studied the performance and meat quality of grower-finisher

Downloaded From Vet-eBooks.com

TABLE 13.2

Examples of the Effect of Type of Feed on Fatty Acid Composition (Expressed as % of Total Fatty Acids) of Pork Meat Lipids

	Feed Enriched in:					
Fatty Acid	**Barley + Soya Bean Meal[a]**	**Safflower Oil[b]**	**Tallow Diet[c]**	**Barley + Wheat + Maize[d]**	**Canola Oil[e]**	**6% Fish Oil[f]**
C 14:0	–	–	1.37	1.55	1.6	1.93
C 16:0	23.86	27.82	24.15	25.10	20.6	26.89
C 18:0	10.16	12.53	11.73	12.62	9.8	16.30
C 16:1	3.0	3.56	3.63	2.79	3.6	2.56
C 18:1	39.06	37.81	46.22	36.47	45.9	37.31
C 20:1	–	0.01	0.29	0.47	–	–
C 18:2	17.15	14.60	8.95	16.49	12.3	7.78
C 20:2	–	0.01	0.44	0.49	0.4	0.59
C 18:3	0.91	0.01	0.26	1.14	3.0	2.08
C 20:3	0.21	0.01	0.25	0.30	0.1	0.08
C 20:4	4.26	2.14	2.13	0.25	0.74	0.46
C 22:5	0.64	0.01	–	–	–	0.91
C 22:6	0.75	0.01	–	–	–	1.13
Total SFA	34.02	40.35	37.83	39.42	33.6	45.12
Total MUFA	42.06	42.38	50.26	39.74	49.5	39.87
Total PUFA	23.92	16.79	11.91	20.84	16.6	13.03
PUFA:SFA ratio	0.70	0.42	0.32	0.53	0.49	0.29
n-6:n-3 ratio	9.4	559	45.3	16.6	4.5	2.2

[a] Morgan et al. (1992).
[b] Larick et al. (1992).
[c] Leszczynski et al. (1992).
[d] Toldrá et al. (2004).
[e] Miller et al. (1990).
[f] Irie and Sakimoto (1992).
SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid.

pigs when received a feed containing 0.4% of the basal diet as a herb combination (pomegranate, *Ginkgo biloba*, licorice) either in a natural or fermented form (Ahmed et al., 2016). The feed intake was reduced in both cases as well as the back fat thickness of pigs. The lean production and the content of n-3 fatty acids in the *longissimus* muscle were increased with a reduced ratio of n-6/n-3 while the cholesterol was lower in the natural herb combination group (Ahmed et al., 2016). Other strategies are based on the use of feeds enriched with n-3 fatty acids like EPA and DHA, by the addition of fish oils or algae (Jakobsen, 1999). Feeding 4%–6% fish oil to pigs for two to four weeks before slaughter produced a substantial increase in EPA and DHA (see Table 13.3) and reduced the n-6:n-3 ratio to 2.2 (Irie and Sakimoto, 1992). However, feeding with 6% unrefined fish

Downloaded From Vet-eBooks.com

TABLE 13.3

Examples of the Effect of Type of Feed on Fatty Acid Composition (Expressed as % of Total Fatty Acids) of Beef Meat Lipids

	Feed Enriched in Beef					
Fatty Acid	**Maize-Based Concentrate[a]**	**Pasture[b]**	**Pasture[c]**	**Soybean Meal + Corn + 33% Peanut Cake[d]**	**Soybean Oil[a]**	**Canola Meal[e]**
C 14:0	2.9	2.9	1.6	2.9	3.3	2.8
C 15:0	1.3	–	–	0.3	1.5	0.5
C 16:0	26.7	23.9	21.6	25.4	26.0	24.8
C 17:0	1.6	–	–	0.8	1.6	1.0
C 18:0	9.7	19.4	17.7	18.2	11.0	11.4
C 14:1	–	0.4	0.2	0.1	–	–
C 16:1	4.9	2.4	2.5	1.8	3.9	4.8
C 18:1	40.0	35.4	31.5	38.9	33.4	44.8
C 18:2	6.0	1.8	3.3	3.7	7.4	5.7
C 18:3	0.5	0.6	1.3	0.4	0.6	0.5
C 20:3	2.4	–	–	0.9	2.1	–
CLA	0.23	1.3	0.53	3.8	0.3	–
C 20:4	–	–	–	–	–	1.5
Total SFA	42.2	48.1	49.1	50.3	42.4	40.5
Total MUFA	44.9	38.2	41.0	43.9	37.3	50.5
Total PUFA	9.1	4.3	10.0	5.9	10.4	7.7
PUFA:SFA ratio	0.22	0.09	0.20	0.12	0.24	0.19
n-6:n-3 ratio	16.8	3.0	1.4	1.7	15.8	11.4

[a] Dhiman et al. (2005).
[b] Realini et al. (2004a).
[c] Realini et al. (2004b).
[d] Correia et al. (2016).
[e] Rule et al. (1994).

oil up to 60 kg live weight caused off-flavor (Lauridsen et al., 1999). These fatty acids with large numbers of double bonds are evidently prone to oxidation, so that feeds of this type require addition of antioxidants like vitamin E to prevent any oxidation and rancidity development. The amount depends, for instance, enrichment with 250 g per pig for 22–42 days was reported to have no adverse effects on sensory attributes (Marriott et al., 2002). Dietary CLA has been reported to enrich the CLA isomer content to around 0.35/100 g total fatty acids, and to reduce the intramuscular cholesterol (Lauridsen et al., 2005).

Poultry meat has an n-6:n-3 ratio similar to pork, and for similar reasons: basically, the use of feeds rich in linoleic acid (grain, maize, plant seeds, oils). Higher concentrations of n-3 fatty acids may be attained by feeding

Downloaded From Vet-eBooks.com

canola oil, flax seed, fish oil, or algae. For instance, a feed with 20% white fish meal may enrich the n-3 fatty acid content from 3% (on a basal diet) up to 10.4% and reduce the n-6:n-3 ratio from 8.4 to 1.7 (Jakobsen, 1999).

13.3.2.2 Manipulation of Fatty Acids in Ruminants

Fatty acid profiles are more saturated in ruminants than in pigs and the fat is firmer (Wood et al., 2003). The manipulation of fatty acids through the type of feed is more difficult because the rumen is biohydrogenated, producing mainly stearic acid as the final product. It is therefore difficult to increase the PUFA:SFA ratio since more than 90% of the PUFAs are hydrogenated unless they are protected against hydrogenation in the forestomach. Dietary linoleic and linolenic acids may be partially hydrogenated, forming oleic acid and trans-, odd chain, branched-chain, and conjugated fatty acids (Jakobsen, 1999). Meats from ruminants are rich in CLA, mainly cis-9, trans-11 octadecadienoic acid, which has been reported to exert important health-promoting biological activity (Belury, 2002). CLA has exhibited anticarcinogenic, antioxidative, and antidiabetic effects in animal models. In any case, feeding strategies were recommended to be adapted to the priority target of each farm. This means that goals may be quite different: achieve good carcass performance, or reduce fat in the carcass or even get rumen health (Bello et al., 2016).

Some researchers have attempted to manipulate fatty acids in ruminants and, especially in recent years, to enrich beef with CLA. Some examples of final composition in fatty acids in intramuscular lipids of beef and lamb are shown in Tables 13.3 and 13.4, respectively. Dietary full canola altered the fatty acid composition in beef meat, especially by decreasing palmitic acid and increasing stearic and oleic acids (Rule et al., 1994). Comparison of a maize-based concentrated feed with soybean oil revealed that even though the linolenic content twice as high in soybean oil as in the other feed, the final content in the beef was almost identical (Dhiman et al., 2005). Feeding with high-starch (25%) diets improved the nutritional value of beef because the SFAs were decreased and the unsaturated fatty acids increased (Rossi et al., 2016). The inclusion of 25% soybean in the diets increased the amount of PUFA in the meat (Rossi et al., 2016). Feeding with soybean oil increased the trans-10, cis-12 CLAs in the adipose tissue but did not increase the cis-9, trans-11 CLA isomer that was the target (Dhiman et al., 2005). An increase in this last CLA isomer was achieved by feeding with sunflower oil, although this also raised the n-6:n-3 ratio (Noci et al., 2005).

Lambs on pasture usually present more n-3 fatty acids than when they are fed concentrates (Jakobsen, 1999). When lambs are fed with linseed, the amount of n-3 fatty acids increases; similar findings with EPA and DHA have been reported in lambs fed a mixture of fish oil and marine algae (Elmore et al., 2005). However, the results are better when lambs are fed with protected lipid supplements. Then, the amount of n-6 and n-3 fatty acids

Downloaded From Vet-eBooks.com

TABLE 13.4

Examples of the Effect of Type of Feed on Fatty Acid Composition (Expressed as % of Total Fatty Acids) of Lamb Meat Lipids

	Feed Enriched in Lamb		
Fatty Acid	**Barley, Maize, Wheat and Soybean Meal[a]**	**Concentrate + Forage[b]**	**Concentrate + Corn + Soybean[c]**
C 14:0	2.2	3.7	1.5
C 15:0	–	–	–
C 16:0	21.1	23.2	2.3
C 17:0	1.3	–	0.9
C 18:0	11.6	16.3	17.4
C 14:1	–	–	–
C 16:1	2.1	2.3	1.5
C 18:1	38.7	40.4	40.9
C 18:2	10.2	7.5	3.3
C 18:3	0.56	1.0	0.5
C 20:3	–	–	–
CLA	–	–	0.4
C 20:4	–	2.1	2.4
Total SFA	42.7	43.2	46.1
Total MUFA	46.0	42.7	46.8
Total PUFA	4.8	10.6	7.1
PUFA:SFA ratio	0.11	3.31	0.15
n-6:n-3 ratio	18.1	9.32	12.2

[a] Castro et al. (2005).
[b] Salvatori et al. (2005).
[c] Pereira et al. (2016).

increases substantially (see Table 13.2) due to the effectiveness of the protection system (Elmore et al., 2005). On the other hand, feeding with palm oil supplements enriches the meat lipids with SFAs, especially palmitic acid (Castro et al., 2005). It has been reported that the fatty acid composition is somehow affected by genotype (Salvatori et al., 2004).

13.3.3 Dietary Enrichment in Compounds of Specific Nutritional Interest

13.3.3.1 Vitamins and Antioxidants

Meat contains considerable amounts of water-soluble vitamins like B vitamins. However, its content in vitamins A, D, C, and E is rather poor (Reig and Toldrá, 1998a). The deposition of vitamin E in pig and poultry muscles

Downloaded From Vet-eBooks.com

is dependent on its concentration and time of supplementation (Mercier et al., 1998). Usual levels are around 100–200 mg/kg in the feed for several weeks prior to slaughter. For instance, vitamin E content of 12.9 mg/kg dry matter may be reached after feeding 200 mg of α-tocopheryl acetate per kg diet in pigs between 60 and 93 kg live weight (Isabel et al., 2003). The distribution of vitamin E in the organism is variable; it is higher in the muscles of the thoracic limb, neck, and thorax and lower in the muscles of the pelvic limb and back (O'Sullivan et al., 1997). Dietary supplementation with this lipid-soluble antioxidant improves the oxidative stability of the meat (Buckley et al., 1995), which is important because phospholipids are located in subcellular membranes such as mitochondria, microsomes, etc. Their PUFA content is relatively high and this makes them susceptible to peroxidation due to the proximity of prooxidants like myoglobin, cytochromes, nonheme iron, and trace elements (Buckley et al., 1995). Although meat contains several natural antioxidant enzymes like the superoxide dismutase and glutathione peroxidase, their activity is weakened during postmortem storage. Several antioxidants have therefore been assayed as an effective alternative for protection against oxidation. Vitamin E (α-tocopheryl acetate), which was studied in depth in the 1990s, is a very effective antioxidant because it is accumulated in tissues and subcellular structures, including membranes. It is commonly added to the feed today. There are other endogenous antioxidants like the histidine-containing dipeptides carnosine and anserine, but contents vary according to anatomical location and species. These dipeptides are therefore more abundant in light muscles than in dark muscles (Aristoy and Toldrá, 1998). Carnosine content is higher in beef and pork and anserine content is higher in poultry (Reig and Toldrá, 1998b; Toldrá and Reig, 2004).

The use of essential oils as feed additives for livestock production has been reported to increase feed efficiency and animal productivity due to their antimicrobial, antiinflammatory, and antioxidant effects (Bakkali et al., 2008, Benchaar et al., 2006, 2008; Jayasena and Jo, 2013). The antimicrobial activity of essential oils added to livestock feed can contribute to a decreased ruminal biohydrogenation that consequently results in a higher deposition of PUFA in meat (Martineau et al., 2008). Antioxidant activity is widely observed in spices and herbs, for example, oregano, rosemary, thyme, cinnamon, pepper, nutmeg, licorice, aniseed, cassia bark, fennel, prickly ash, round cardamom, basil, garlic, coriander, and ginger. Such antioxidant activity is attributed to various phenolic compounds, structurally related but different in quantity and type, depending on the respective source (Morán et al., 2012, 2013; Jiang and Xiong, 2016). The addition of 3.5 g/animal/day of an essential oil blend (oregano, garlic, lemon, rosemary, thyme, eucalyptus, and sweet orange) was reported to reduce lipid oxidation in bulls even though higher doses had a prooxidant effect (Rivaroli et al., 2016). Such an essential oil blend did not influence the chemical composition nor the fatty acid profile.

Downloaded From Vet-eBooks.com

13.3.3.2 Minerals

Meat constitutes a rich source of iron, and also of trace elements like selenium, magnesium, and zinc. Red meats, i.e., beef and lamb, contain higher levels of iron than pork and poultry. The iron present is part of the heme molecule, an essential component of myoglobin in the muscle, which is highly bioavailable for humans. Iron content is higher in oxidative than in glycolytic muscles. Iron, phosphorus, copper, and zinc contents are little affected by dietary levels; however, the levels of selenium in meat are highly dependent on dietary intakes (Lynch and Kerry, 2000).

Different magnesium salts, such as magnesium aspartate, magnesium aspartate hydrochloride, and magnesium fumarate, have been used as dietary supplements to improve pork meat quality, but cheaper sources like magnesium sulfate and magnesium chloride can also be effective. Magnesium acts as a cofactor for several metabolic and enzymatic pathways; for instance, it reduces skeletal muscle activity by antagonizing calcium (D'Souza et al., 1999). Selenium is needed in certain metabolic processes like the defense against free radicals and their detoxification (Lener et al., 2012) and contributes to better health status (Fairweather-Tait et al., 2011). Selenium can also regulate adipogenesis and lipolysis in the organism (Wang et al., 2016). Supplementation with sodium selenite or selenium-rich yeast makes for a high selenium content in meat. In fact, dietary selenium supplementation has been reported to delay postmortem oxidation reactions, especially due to its antioxidant are more effective when supplied in the organic form (Mahan et al., 2014) as the selenium reaches tissues more efficiently (Jang et al., 2010). Organic selenium, in the form of selenium-enriched yeast, is taken up via methionine transporter mechanisms and located either into selenoenzymes or in place of methionine in muscle proteins (Suzuki and Ogra, 2002). The water holding capacity was improved with organic selenium because the lowest water losses in pork meat were observed when feeding with selenium-rich yeast in comparison to other inorganic sources of selenium like sodium selenite or selenium and vitamin E (Calvo et al., 2016a,b). Further, the inclusion of 0.4 ppm of organic selenium in pig diets improved the lipid stability against oxidation (Calvo et al., 2017).

It was also reported that supplementation with selenium-enriched yeast in sheep is more efficient compared to inorganic selenium supplementation (Stewart et al., 2012).

13.4 Processing Strategies for Developing Functional Meat Products

Meat technology plays a number of major roles in the development of functional meat products. As well as animal production strategies, processing strategies can be used to alter the composition of meat products. For the

Downloaded From Vet-eBooks.com

production of functional meat products (as with others functional foods), there are a number of different technological approaches that can be adopted to remove, reduce, increase, add, and/or replace different components with physiological activity (functional or bioactive components), any of which may help achieve an improved state of health and well-being and/or reduce the risk of disease (Roberfroid, 2000; Ashwell, 2002; Jimenez-Colmenero, 2007a). The presence, absence, or reduced content of substances known to have physiological effects are cited in the EU proposal for the purposes of health claims (EU Commission, 2003).

The beneficial effects of functional foods derive from bioactive components (phytochemicals and zoochemicals) which may have specific positive and negative implications for health. In some cases, these substances may occur naturally in the product (in meat raw materials, or ingredients or additives used in their preparation), while in others they may be formed or their presence may be modified in the course of processing, storage, or cooking.

It is essential to know the role and source of healthy and unhealthy compounds in order to be able to identify, design, and more effectively implement procedures for the development of functional meat products. There are basically two kinds of procedure with regard to the composition of functional meat products (Jimenez-Colmenero et al., 2001) aimed at:

- Reducing the concentration or limiting the formation of unhealthy compounds
- Promoting the presence of healthy compounds

Several basic approaches can be adopted to successfully induce such effects (Jimenez-Colmenero, 2007a). These focus on aspects relating to: selection and preparation (postslaughter) of meat ingredients in order to secure raw materials with suitable composition (mainly in terms of lipidic components: fat proportion, fatty acid profile, and cholesterol content); reformulation of products to induce certain changes in composition; and adaptation of preparation, storage, and cooking technologies so as to limit the concentration and/or formation of unhealthy components.

The development of functional meat products poses some technological challenges, chiefly as regards optimizing the formulation, processing, and subsequent storage so as to promote the presence and activity of healthy components and reduce or eliminate unhealthy substances (optimization of functional food components) without this affecting the quality of meat products. The technological feasibility of achieving the desired composition with optimum palatability depends very much on the product type, i.e., whether it is composed of identifiable pieces of meat, coarsely or finely ground, emulsions, cooked, cured, etc. To help optimize the desired beneficial properties there are a number of technologies (conventional and emerging) that promise friendly processing conditions.

Downloaded From Vet-eBooks.com

13.4.1 Reduction of Specific Unhealthy Compounds

Meat and meat derivatives, like any other food, may contain a number of components (of highly diverse origins) which can have unhealthy implications if more than a given amount is ingested. Some of these compounds may be naturally present in the meat or its derivatives as part of the various raw materials used in their preparation. Examples include fat, SFAs, cholesterol, salt, and others.

There are a number of steps in the processing and storage of meat products, and also in their preparation and consumption, which can modify the concentration and bioavailability of some of their components. These may equally cause the density of some nutrients to increase (e.g., due to cooking-induced drying, etc.) or that of others to decrease (due to cooking). At the same time, like other complex foods, during processing, storage, and cooking, meat and meat products undergo major chemical changes which cause the formation of a number of nonintrinsic components. Although some are health-promoting components such as antioxidant and antimicrobial agents or are probiotic during fermentation (Knorr, 1998), others may have potentially harmful biological properties. This is true of substances such as nitrosamines, polycyclic aromatic hydrocarbons, heterocyclic amines (HA), biogenic amines, lipid oxidation products, or advanced glycoxidation end products (Hotchkiss and Parker, 1990; Cassens, 1999; Goldberg et al., 2004; Ruiz-Capillas and Jiménez Colmenero, 2004; Vitaglione and Fogliano, 2004). Some of them present mutagenic, carcinogenic, and cytotoxic properties related to the etiology of various forms of human cancer, CVD, arteriosclerosis, renal complications, Alzheimer's disease, etc. The presence of these nonintrinsic substances in meat products has prompted several mechanistic hypotheses in which meat intake is associated with some health problem. It has been suggested that these compounds may have more to do with the risk of disease than the meat itself (Ulrich and Potter, 2004), although the relationship has yet to be demonstrated (Carbajal, 2004; Ulrich and Potter, 2004).

Meat products could potentially be made healthier by applying technology strategies that do not lead to the formation (at least in excess of established limits) of such unhealthy nonintrinsic substances. From a practical point of view, any approach to promoting potential functional meat products must involve reducing the concentration and/or formation of those compounds to within acceptable limits. Following are a number of examples.

13.4.1.1 Fat and Cholesterol

Recent years have seen the development of numerous meat products with lower fat content, which consumers perceive as healthier. As a result of recommendations regarding quantitative and qualitative aspects of fat included in population nutrient intake goals for preventing diet-related chronic diseases (WHO, 2003), many consumers are currently limiting the amount of fat and

Downloaded From Vet-eBooks.com

calories in their diet. These recommendations must therefore be a key consideration when a new composition is designed for any product; this is particularly true of meat products given their importance as a source of fat in the diet.

The proportion of calories in the diet contributed by the intake of fat is around 36%–40%, almost a quarter of which derives from the consumption of meat and meat products (Sheard et al., 1998; Chizzolini et al., 1999; Jiménez-Colmenero et al., 2012).

While meat cannot be considered a high-energy food given the current trends in meat production toward leaner animals (retail cuts frequently contain less than 5%), there are various meat products to which the same does not apply, containing fat levels over 30%. Where it is most desirable to reduce fat is in frequently consumed products with higher fat levels, whose poor image in terms of health benefits would really be improved thereby. Fat is a major determinant of the sensory characteristics of a product, and hence acceptable low-fat meat products cannot be effectively achieved simply by reducing the fat in the formulation. There are generally two basic criteria governing fat reduction technology: the use of leaner meat raw materials, and the reduction of fat and energy density (dilution) through the addition of water and other ingredients. However, as fat content falls and water content rises, increasing attention must be paid to water holding ability. In this context, different protein-, carbohydrate-, or fat-based are used, classified as "fat replacers," "fat substitutes," "fat analogs," "fat mimetics," "fat extenders," or "fat barrier compounds," generally depending on their composition, properties, and the functions that they perform in the meat matrix (Jiménez-Colmenero et al., 2012). The feasibility to reduce fat in meat products depends on a number of factors such as the desired fat level, the nature of the product to be reformulated, and the kind of processing required (emulsifying, heating, curing, and others).

Cholesterol content can vary considerably in meat products. Under the heading of processing strategies, there are two main approaches to cholesterol reduction, one entailing alteration of the composition of meat products and another entailing direct removal of cholesterol by supercritical carbon dioxide extraction (Clarke, 1997). A number of different meat products (ground beef, frankfurters, pork patties, restructured pork, structured beef rolls, etc.) have been reformulated; their original composition has been altered by reducing animal fat and/or partially replacing it with vegetable oils (olive, maize, sunflower, soybean, etc.) and adding various plant-based proteins (soy, maize, oat, wild rice, wheat gluten, etc.) or other fat replacers (free cholesterol). This dilution method has made significant cholesterol reduction (20%–50% less than conventional products) possible in meat products such as low-fat ground beef, pork sausages, frankfurters, and others (Sandrou and Arvanitoyannis, 2000; Jiménez Colmenero, 2007a). Some meat products are currently being marketed with claims relating to fat and cholesterol contents. Further information on fat reduction in processed meats is reported in Chapter 15.

Downloaded From Vet-eBooks.com

13.4.1.2 Sodium and Phosphate

Sodium intake generally exceeds nutritional recommendations in industrialized countries (Antonios and MacGregor, 1997; WHO, 2003). Excessive intake of sodium has been associated with high blood pressure, one of the major risk factors for CVD (Antonios and MacGregor, 1997). Since approximately 20%–30% of common salt intake comes from meat products (Wirth, 1991), there is increasing interest among consumers and processors in reducing the use of salt (minimizing sodium) in meat processing.

Sodium reduction requires partial substitution of the sodium chloride added to meat derivatives by other compounds that have similar effects on sensory, technological, and microbiological properties. Different strategies based on selection of meat raw material, modification of processing techniques, use of salt substitutes and flavor enhancers and masking agents, and optimization of the physical form of salt have been used in order to reduce the salt addition in meat products (Desmond, 2006). A number of compounds have been used to overcome property problems in low-salt products, including problems associated with water- and fat-binding properties and texture. These include chlorides, phosphates, lactates, alginate, transglutaminase, and others (Collins, 1997; Monahan and Troy, 1997; Jimenez-Colmenero et al., 2005). More extensive information on sodium reduction in meats and processed meats can be found in Chapter 14. Since excessive dietary inorganic phosphate may increase the risk of lung cancer (Jin et al., 2009) and bone disease (Shahidi and Synowiecki, 1997), different meat products have been reformulated without added phosphates.

13.4.1.3 Nitrite

It is suggested that dietary nitrites (from processed meats among other foods) considered as human carcinogens because they may be converted in the body to N-nitroso compounds that are known carcinogens (Demeyer et al., 2008). In order to limit this unhealthy effect, various ingredients have been used to reduce or even eliminate the added nitrites to ensure that these additives perform their functions (color, antioxidant, antimicrobial, etc.) in the end product while maintaining quality. In this regard, the utilization of nonmeat ingredients that, by nature, have high nitrate content has been proposed to avoid the direct addition of nitrite to meat products (Weiss et al., 2010). On the other hand, different strategies have been suggested to inhibit damage due to nitros(yl)ation reactions, including the use of dietary components such as pre- and probiotics, resistant starch, vitamin C and E, etc. (Demeyer et al., 2008). More extensive information on nitrite reduction in meats can be found in Chapter 16.

13.4.1.4 Allergens

Some of the ingredients used in the preparation of meat products, like vegetable (i.e., soy), egg, or milk proteins, contain substances that cause allergic

Downloaded From Vet-eBooks.com

reactions in some consumers. Such nonmeat ingredients must be excluded to produce "allergen-free" meat products such as pork sausages, hamburger steak, or meatballs, recently approved (FOSHU) in Japan (Arihara, 2004). Different meat products (turkey breast, sausages, ham, etc.) are marketed as gluten free and lactose free.

13.4.2 Promoting the Presence of Specific Healthy Compounds

Some food components possess potential health-beneficial properties, and efforts are therefore being made to promote increased dietary intake. A good way to increase the dietary intake of a functional ingredient is to incorporate it in common foods, such as meat products, and meat processing offers an excellent opportunity to do this. Although some biologically active compounds occur naturally in meat, most are produced by plants. In meat processing, it is possible to promote the presence of functional ingredients by substituting, adding, or enhancing food components. Whereas replacing basically targets lipidic material, adding or increasing embraces a large group of bioactive substances, and this opens up major prospects. Such substances have been used in the form of specific preparations and as constituents of some nonmeat ingredients (extracts, meals, concentrates, homogenates, etc.) used for various different purposes (technological, sensory, nutritional, microbiological, and economical) in the meat industry. The fact is that many of these ingredients (both common and otherwise) are of vegetable origin (oats, soy, wheat, sunflower, rosemary, apple, mushroom, walnut, etc.) and are composed of a variety of biologically active phytochemicals.

13.4.2.1 Fat and Fatty Acid Profile

The basic requirement for any major advance in the direction of diet optimization and human health is that food fat (and meat products are some of the most important sources of dietary fat) be brought down closer to recommended population nutrient dietary goals (WHO, 2003). By means of a formulation approach, changes in lipid content and fatty acid profile can be tailored to help prevent many human diseases. These changes involve reducing SFA levels, raising mono- and PUFA levels (n-3, CLA), improving n-6:n-3 PUFA balances, and limiting cholesterol contents. In meat products, such modifications can be achieved by means of formulation and technological processing, including raw material selection.

Healthier meat products can be made using meat raw materials with improved composition (higher monounsaturated fatty acid [MUFA] or n-3 PUFA contents and lower n-6:n-3 PUFA ratio) achieved by animal production practices (see Section 13.3). Frankfurters, low-fat sausages, dry fermented sausages, and cooked ham or pork liver pate have been manufactured using materials (back fat and meat) obtained from pigs fed diets enriched with safflower, sunflower and canola, linseed or fish oils (St. John et al., 1986; Shack-

Downloaded From Vet-eBooks.com

elford et al., 1990; Hoz et al., 2004; Jeun-Horng et al., 2002; Santos et al., 2004; D'Arrigo et al., 2004).

In order to achieve healthier meat products, part of the animal fat normally present in the product can be replaced with another component more suited to human needs (Jiménez Colmenero, 2005). Lipids from nonmeat sources, for example, vegetable and fish oils, have been used for that purpose. Vegetable oils are of particular interest for meat product development because they are free of cholesterol and high in unsaturated fatty acids. Various different vegetable oils (maize, cottonseed, palm, peanut, soybean, high-oleic acid sunflower, olive, linseed) have been used to replace animal fats (pork back fat or beef fat) in normal and low-fat meat products such as frankfurters (Marquez et al., 1989; Paneras et al., 1998; Lurueña-Martinez et al., 2004), cooked sausages (Yilmaz et al., 2002), ground beef patties (Liu et al., 1991), fermented sausages (Bloukas et al., 1997; Muguerza et al., 2002; Ansorena and Astiasaran, 2004), and others. Other nonmeat ingredients such as walnut have been used to achieve healthier fatty acid profiles in frankfurters and restructured beef steaks (Jimenez-Colmenero et al., 2003; Serrano et al., 2005). A mixture of crushed linseed and linseed oil has been used to produce a range of basic Finnish meat products: sausage, meatballs, liver paste, etc. (Ahola, 2001). Fish oils (n-3 polyunsaturated oil) have been used in low-fat frankfurters (Park et al., 1989). Generally, the procedures used to incorporate natural or processed plant and marine lipids into meat products range from direct addition in the form of liquid oils and interesterified oils, to incorporation in encapsulated or emulsified forms (simple and multiple emulsions) or as part of plant ingredients (Jimenez-Colmenero, 2007b). Novel approaches of stabilizing and structuring liquid oils to create a plastic fat which retains solid-like properties while possessing a healthier fatty acid profile has been suggested recently as an animal fat alternative in order to improve the fat content of meat products. These strategies include those based on the application of interesterification and organogelation processes, the creation of structured emulsions, and the formation of oil bulking agents (Jiménez-Colmenero et al., 2015). A recent example is the use of structured canola oil consisting of an organogel produced with ethylcellulose and sorbitan monostearate to replace beef fat in frankfurters. The replacement of beef fat with such organogel containing 8% ethylcellulose and 1.5% sorbitan monoestearate was reported to give a hardness value similar to that of beef fat, by both sensory and texture profile analysis (Barbut et al., 2016).

There is evidence of a positive association between levels of TFA intake and the risk of CVD. Population nutrient intake goals recommend less than 1% of the total energy as TFA (WHO, 2003). Dietary surveys indicate that intakes of TFA have decreased in a number of EU countries, mainly thanks to reformulation of food products (EFSA, 2004). Although natural TFAs found in ruminant meat fat appear to have a less unhealthy effect than other processed food fats (Higgs, 2000), in any case TFAs of meat products have been reduced by reformulation (adding oat bran or rye bran) (Yilmaz, 2004).

Downloaded From Vet-eBooks.com

Fatty acid profiles of meat products have been improved by direct addition of CLA to pork patties (Joo et al., 2000), beef patties (Chae et al., 2004; Hur et al., 2004), or liver pâté (Martin et al., 2008). Although feeding rather than processing conditions determine the CLA content in foods (Williams, 2000), endogen or exogen CLA content in meat is not negatively altered by cooking or storage; to the contrary, it is increased by heating (Mulvihill, 2001; Arihara, 2004).

The varying effect of fats (depending in part on fatty acid composition) on satiety signaling could be used to develop fat-containing foods (meat products) that modulate satiety. Specific manipulations of fats (and also of proteins and carbohydrates) have the potential to act as functional foods for appetite control (Dye and Blundell, 2002). Modified-fatty-acid-profile and trans-fat-free meat products are currently being marketed.

13.4.2.2 Proteins, Peptides, and Amino Acids

Meat products contain high levels of both meat protein and nonmeat protein from the numerous ingredients used in their manufacture. Essential nutrients can be considered functional components if in addition to the requisite nutritional effect they provide benefit to the human body (Arihara, 2004). Meat protein is an important factor in permitting antibody synthesis, thus allowing for acquired immunity to disease (Romans et al., 1994). A number of amino acids from meat have beneficial effects on the nervous and immune systems. Peptides like carnosine and anserine, which are found in muscle, are the most abundant antioxidants in meats. They have been reported to play roles in wound healing, recovery from fatigue, and prevention of stress-related diseases (Arihara, 2004). Recent research has been disclosing the role of meat proteins as precursors of bioactive peptides. These are fragments that are inactive within the precursor protein, but once released during food processing or during digestion, they can carry out various different health physiological functions in the organism (Toldrá and Reig, 2011). More detailed information on bioactive peptides is given in Chapter 13. L-Carnitine, which is naturally abundant in skeletal muscle, especially in beef, helps the body to reduce cholesterol levels and absorb calcium (Arihara, 2004). The L-carnitine content in processed meat is normally rather low, but levels similar to those found in muscle have been achieved by adding L-carnitine to such products. The first L-carnitine-enriched meat products (wiener sausage and salami) were introduced in 2002 in Germany (Anonymous, 2002).

Protein-based ingredients are among the principal nonmeat ingredients used in the manufacture of meat products. Nonmeat proteins have been used in meat products for technological purposes and to reduce costs. They have also been used to provide nutritional benefits by lowering the caloric and cholesterol contents (when used as fat replacers) and by increasing the protein level and balancing the amino acid profile (Jiménez Colmenero, 2004).

Downloaded From Vet-eBooks.com

Some also contain health-enhancing components which make for healthier processed meats. The range of applicable nonmeat proteins from animal and plant sources is very large.

Plant proteins from soy, sunflower, wheat, and maize derivatives, and flours from cottonseed and oats have been utilized in the meat industry as fat replacers among other purposes (Keeton, 1994). Soy proteins are a good example of such proteins with health-enhancing activity; they are thought to exert a preventive and therapeutic effect in CVDs, cancer, and osteoporosis, and in the relief of menopausal symptoms (Hasler, 1998). In the United States, the Food and Drug Administration (FDA) has approved health claims on soy protein (containing isoflavons) in connection with a reduced risk of coronary heart disease (Holm, 2003). Low-fat sausage products containing soy proteins (as functional ingredients) have recently been approved in Japan, with the claim that an acceptable blood cholesterol level can be maintained by consumption of this product (Arihara, 2004). Some proteins from vegetal sources (sunflower, walnut, etc.) contain high proportions of arginine and have low lysine/arginine ratios, both characteristics that have beneficial effects for heart failure, blood pressure, and stroke (Feldman, 2002). Lysine/arginine ratios of restructured beef steak proteins have been reported to decrease with the addition of 20% walnut (Serrano et al., 2005).

Nonmeat proteins from animal sources have also been used in meat products. Whey protein products are used in a variety of processed meats (ground meats, emulsion products, coarse ground products, whole muscle products) to improve flavor, texture, emulsification, water binding, and cook yield. Whey protein contains bioactive components that help the release of two appetite-suppressing hormones and may have positive effects on cardiovascular health (Ohr, 2004).

13.4.2.3 Prebiotics and Probiotics

One of the most promising areas for the development of functional foods lies in the use of prebiotics and probiotics. A prebiotic is a "nondigestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon and thus improves the health of the host" (Arihara, 2004). Prebiotics utilized in a number of meat products include dietary fibers and oligosaccharides.

Dietary fibers have frequently been claimed to influence a number of beneficial health characteristics, for instance by increasing fecal volume, limiting caloric intake, to favor regulation of blood glucose levels, and to prevent CVDs , colon cancer, and constipation by regulating intestinal transit (Holm, 2003). Consumption of either insoluble or soluble fiber results in distinct physiological effects. Aside from its potential physiological effects, addition of fiber to meat products has been widely practiced because of its role as a

Downloaded From Vet-eBooks.com

technological coadjuvant: its presence can improve water binding properties, texture, or emulsion stability of meat products and can help overcome the effects produced by changes of composition (e.g., due to fat reduction) on meat product characteristics. Dietary fiber from oats, sugar beet, soy, rice, apples, peas, citrus (lemons, oranges), etc. have been incorporated in the formulations of several meat products including ground meat and sausages (Keeton, 1994; Kim et al., 2000; Jimenez Colmenero et al., 2001; Fernández-López et al., 2004). Antioxidant dietary fiber opens up interesting possibilities for meat products.

Oligosaccharides such as inulin, which is composed of a blend of fructose polymers extracted from chicory, has been used in the preparation of various meat products (Pszczola, 1998; Sloan, 2000; Archer et al., 2004). Intake of oligosaccharides improves beneficial intestinal microflora, reduces blood glucose levels, and reduces the production of carcinogens in the intestine (Anandh et al., 2003). Inulin has been used as a fat replacer in sausage, where it presented interesting satiating properties (Archer et al., 2004). The use of dietary fiber in the development of fiber-enriched meat products has been recently reviewed (Jiménez-Colmenero and Delgado-Pando, 2013; Verma and Banerjee, 2010).

Probiotics are live microbial food ingredients that, when ingested in sufficient quantities, exert health benefits on the consumer (Ashwell, 2002). Health benefits relating to gastrointestinal disorders, food allergies, inflammatory bowel diseases, or immune functions have been attributed to probiotics. These health benefits depend on many factors associated to the microorganism (genus, species, strain, etc.) and the product (processing, temperature, pH, a_w, etc.). The level of probiotics required in food products to warrant health claims is not clear, although the minimum daily ingestion of probiotic bacteria needed to show a health effect is estimated at $10^{6–10}$ viable microbes (Anandh et al., 2003; Työppönen et al., 2003). A variety of probiotic meat products have been prepared with most of the focus on fermented meat products as these provide a more appropriate environment (no heat treatment) for the viability of microorganisms. A number of meat products (dry sausage, meat spreads) have been prepared with lactic acid bacteria (*Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus paracassei, Lactobacillus rhamnosus)* or bifidobacteria (*Bifidobacterium* spp.) (Arihara, 2004; Työppönen et al., 2003). Studies using probiotic microorganisms in other types of meat products such as cooked meat sausages and fresh ground or raw meat are still scarce mainly because of the processing required for these products such as heating, the use of additives (sodium chloride, sodium nitrite, etc.), and consume conditions (Cavalheiro et al., 2015). Probiotic delivery strategies, based on encapsulation and/or entrapping in gelled dispersions, designed to enhance probiotic viability during manufacture, storage, cooking, and gastrointestinal stages in meat matrices, have been recently reviewed (Cavalheiro et al., 2015). Meat products with added prebiotics and probiotics have been marketed in different countries.

Downloaded From Vet-eBooks.com

13.4.2.4 Antioxidants and Vitamins

The best way to achieve a balance between oxidative damage and antioxidant defense in the human body would be to enhance antioxidant capacity by optimizing the dietary intake of antioxidants through increased consumption of antioxidant-rich foods, among them animal-derived foods (Suray, 2003). The biological hypothesis suggests that dietary antioxidants have the capacity to prevent oxidative damage in the body, so that increased levels will also reduce the risk of several diseases, in particular CVD, some cancers, Alzheimer's disease, and impairment of vision (Holm, 2003).

The presence of antioxidants also limits the susceptibility of foods to oxidation by reducing the formation (and hence ingestion) of by-products with negative implications for health. Attempting to produce functional meat products entails changes in food composition; depending on the nature of the products, these may in turn entail alterations in the ratio of oxidizable substrate/prooxidants/antioxidants, and consequently also changes in the rate and extent of the oxidative processes, with undesirable effects as regards oxidative damage. Meat and meat products enriched with PUFAs are generally characterized by increased susceptibility to lipid peroxidation. In fact, although PUFAs elicit a greater cholesterol-lowering response than MUFAs, there has been caution in recommending high-PUFA diets because of potentially adverse health effects of their lipoperoxidation products (Williams, 2000). Since antioxidants are known to be effective in decreasing lipid peroxidation in such products, it seems logical that an increase in the degree of unsaturation should be accompanied by enhancement of antioxidant capacity.

The most prominent dietary antioxidants are tocopherols, vitamin C, carotenoids, flavonoids, and phenolic compounds. Animal feeding strategies (see Section 13.3.3) have been used to promote their presence in intact muscle. Reformed and restructured cured turkey products (Walsh et al., 1998), cooked ham (Santos et al., 2004), and dry fermented sausage (Hoz et al., 2004) have all been made with improved meat raw materials of this kind.

The antioxidant profile of meat products has also been improved by adding antioxidants during the manufacturing process, either as a specific preparation or as a component of nonmeat ingredients. One example of this is the incorporation of a wide variety of synthetic or natural exogenous antioxidants (nitrite, phenolic compounds, tocopherols, chelants, plant extracts, etc.), which in some cases are added in the form of complex mixtures of bioactive compounds with multiple functions. Technological processing strategies can also be used to minimize oxidation in muscle foods (Decker and Xu, 1998).

Vitamin E has been added to several meat products (sausages, ham) (Jiménez Colmenero, 2005; Jensen et al., 1998). Various plant-derived ingredients containing tocopherols (and tocotrienols) have been used in the preparation of meat products, including wheat germ in frankfurters (Gnanasambandam and Zayas, 1992), walnut in restructured steak (Serrano et al., 2005), and rice

Downloaded From Vet-eBooks.com

bran oil in roast beef (Kim et al., 2000). Honey, which possesses important antioxidant properties attributed to the presence of α-tocopherol (along with other substances such as ascorbic acid, catalase, flavonoids, etc.), has been used as an agent against lipid oxidation in muscle food (Pszczola, 1998).

Ascorbic acid supplementation presents health-beneficial effects in connection with improvement of the immune function and the prevention of heart diseases and certain types of cancers (Johnston, 2003). Ascorbic acid has been incorporated as an isolate or as a component of nonmeat ingredients (citrus by-products, honey, etc.) in meat products such as beef patties (Sánchez-Escalante et al., 2001), dry-cured sausages (Fernández-López et al., 2004), roasted chicken (Pszczola, 1998), and cooked pork (O'Connell et al., 2002). Additionally, meat products (cooked sausages, hamburgers) have been enriched with folic acid (Cáceres et al., 2008). Folic acid presents different health benefits, for example a reduction in the risk of the incidence of neural tube defects.

Carotenoids (β-carotene, lycopene, lutein, zeaxanthin, etc.) are naturally present in different vegetables (Pennington, 2002), some of which are used as nonmeat ingredients in processed meats. Potential health-promoting effects of dietary carotenoids include antioxidant activity and a contribution to the prevention of common chronic diseases, including reduction of the risk of cancer, CVD, age-related macular degeneration, and cataracts (Suray, 2003). Several carotenoids have been tested as exogenous antioxidant additives in meat products. Lycopene has been used in the production of beef patties (Desmond and Troy, 2004). Beef patties, restructured beef steak, frankfurters, and meat/liver loaves have been prepared with plant-derived ingredients such as tomato pulp or juice (lycopene rich) (Yilmaz et al., 2002; Sánchez-Escalante et al., 2003), carrot and sweet potato (rich in provitamin A) (Saleh and Ahmed, 1998; Devatkal et al., 2004), or spinach (rich in lutein and zeaxanthin) (Pizzocaro et al., 1998). Lutein has been directly incorporated in meat products (Granado-Lorencio et al., 2010). The ready availability of carotenoids (e.g., lycopene and lutein) as food ingredients means that industrial use is likely to grow fast (Sloan, 2000).

There has been growing interest in the meat industry in the use of some plant-derived materials (from herbs, spices or fruit), as sources of natural phenolic antioxidants. For example, antioxidant activity has been reported for extract of cherry in lean ground beef; green tea in poultry meat; grape, licorine root, and horsetail in pork meat; or coffee, rosemary, and grape skin in precooked pork patties or beef patties (Sánchez-Escalante et al., 2001; Turubatovic and Milatovic-Stevanovic, 2001; Nissen et al., 2004). The antioxidant activity of hydroxytyrosol has been demonstrated in frankfurters enriched with n-3 PUFA (Cofrades et al., 2011). Other compounds like taurine or carnosine have been added to meat products during processing to limit lipid oxidation (Morrissey et al., 1998; Sánchez-Escalante et al., 2001).

Some of the compounds cited above have also been used in meat products for purposes other than enhancing lipid stability. For instance, antioxidants

Downloaded From Vet-eBooks.com

(synthetic and natural, pure compounds or food extracts, and whole foods) are thought to be a promising means of reducing HA exposure because of their ability to inhibit HA formation or to block/suppress HA biotransformation/metabolism (Vitaglione and Fogliano, 2004). Addition of vitamin E in certain cured products has been shown to reduce the production of nitrosamines (Jensen et al., 1998).

13.4.2.5 Minerals

Technological strategies have been employed to increase the concentration of various minerals in meat products. In this regard, different meat products have been enriched with minerals such as calcium (Arihara, 2004; Cáceres et al., 2006), selenium (García-Iñiguez et al., 2010), or iron (Navas-Carretero et al., 2009). At the same time, some nonmeat ingredients (walnut, seaweed, etc.) can promote the presence in processed meat of a number of minerals (Cu, Mg, Mn, K, etc.) (Serrano et al., 2005; Olmedilla-Alonso et al., 2013). Iodine has been incorporated into dry fermented sausage (García-Iñiguez et al., 2010).

13.4.2.6 Plant Sterols and Stanols

Plant sterols are natural constituents of plants (Pennington, 2002). However, they are generally present in the diet in relatively low concentrations (Ashwell, 2002). Structurally, plant sterols and stanols (the saturated derivates) resemble cholesterol. A number of studies have demonstrated the ability of plant sterols/stanols to reduce total and low-density lipoprotein (LDL) cholesterol in humans, inhibit the absorption of dietary cholesterol, and reabsorb endogenous cholesterol from the digestive tract (Holm, 2003). In the United States, the FDA has approved the use of a health claim for plant sterol and plant stanol esters based on evidence that they may help to reduce the risk of CVD when the content of saturated fats and dietary cholesterol is low. Meat products such as frankfurters and broiler meatballs have been developed with stanols (Benecol) for marketing in Finland (Leino, 2001).

Some plant ingredients (e.g., vegetable oils) used in meat processing contain phytosterols such as β-sitosterol, camposterol, and stingmasterol, which pass into meat products. They may be incorporated either as isolated phytosterols or through the use of vegetable oils containing them in the course of meat processing. A mixture of plant sterols and/or their esterified forms and mineral salts has been used to prepare plant sterol-enriched frankfurters, sausage, and cold cuts (SCF, 2003).

Vegetable oils (and other plant products) used as animal fat replacers or in frying processes (either industrially or in domestic food preparation) promote the presence of sterols in meat derivatives.

Downloaded From Vet-eBooks.com

13.4.2.7 Other Compounds

The roles of some phytochemicals have so far been noted individually (chiefly as antioxidants); however, many plant products (spices, condiments, and herbs) used in meat products are sources of phytochemicals (flavonols, lignans, allicin, etc.) with potential health-promoting properties (Gibis et al., 1999; Anandh et al., 2003; Fista et al., 2004; Sadler, 2004), including anticarcinogenic, antihypertensive, antihypercholesterolaemic, antibacterial, and antioxidant properties.

13.5 Safety and Scientific Criteria for Nutrition and Health Claims: Legislative Implications

Food safety is an important and essential aspect for food consumers, especially in the meat sector, which is highly sensitive to such considerations. Safety is therefore an essential factor that must be taken into account in the development of new functional meat products. Functional foods (and functional meat products) are consumed as part of a normal food pattern. However, there are several considerations that must be addressed when assessing food risk: (1) altered consumption behavior; (2) the negative effect of this on particular highly sensitive groups of the population; (3) interactions between components and medicaments; (4) effects of low micronutrient intakes; (5) effects of excessive micronutrient intakes; and (6) long-term consequences of their consumption (Diplock et al., 1999).

Most studies on functional meats have focused chiefly on the improvement of their composition by means of one or more functional components. As mentioned in the introduction, the beneficial effect of a functional food must be demonstrated by scientific methods. Such a demonstration will furnish scientific support for health claims, which normally address (1) improvement of physiological functions or (2) reduced risk of certain diseases. The ultimate purpose of the foods obtained using any of the strategies mentioned above is to obtain a given benefit, to be functional, for the target user, the consumer. In the case of certain claims of health-promoting properties, based on generally accepted scientific proof, it is possible to admit a cause–effect relationship between a food category, a food, or one of its constituents, and the claimed effect (Olmedilla-Alonso et al., 2013). This has enabled the EU to establish a list of authorized claims of health-promoting properties of foods other than those relative to the reduction of disease risk and the development and health of children (European Commission, 2012). When this cause–effect relationship is yet to be convincingly established, or substantiation of it is sought in a specific food matrix, it is necessary to evaluate the functional effect, which can be carried out using *in vitro* and *in vivo* models that provide information on mechanisms of action, the effect

Downloaded From Vet-eBooks.com

on function–response *in vivo*, the dose–response relationship, and acute and chronic effects (Ashwell et al., 2002; Howlett, 2008).

Although no simple matter in practice, regulation of nutrition and health claims with respect to foods would help to achieve a high level of protection of human health and to promote the protection of consumer interests by ensuring that foods bearing nutrition and health claims are labeled and advertised in an appropriate and clear manner that enables consumers to make rational choices (EU Commission, 2003). Obviously, such regulations will have a strong influence on the development and marketing of functional foods. The increasing public interest in dietary health benefits have led to the development of different legislative texts (with important global variations) on nutrition and health claims worldwide. Recently, Boer and Bast (2015) reviewed the current international pieces of legislation on nutrition and health claims in an attempt to show the diverse approaches and to envision ways to optimize procedures from a scientific perspective. As indicated by these authors the international harmonization of claims should globally lead to improved pieces of legislation, stimulate industrial efforts in functional foods, and enhance consumers' opportunity to use health-enhancing products.

In the context of EU legislation, meat and meat products, as other foods, have different possibilities related health claims. Commission Regulation (EU) No 432/2012 established a list of permitted health claims made on foods, other than those referring to the reduction of disease risk and to children's development and health. In this document, meat is directly included in relation to the claim: meat contributes to the improvement of iron absorption when eaten with other foods containing iron. In order to bear the claim, information shall be given to the consumer that the beneficial effect is obtained by consuming 50 g of meat together with food(s) containing nonheme iron. However, taking in account the permitted claims, there are many other possibilities to provide meat products with claims (including those related to the reduction of disease risk and to children's development and health), as the conditions of use of the claim are referred to the nutrient, substance, food, or food category, in general according with the conditions of use for the nutrition claims. In this regard, numerous products has been designed and developed in order to improve the presence/absence of compounds with health implications as described in this chapter. On the other hand, novel perspectives can be considered in meat production and processing strategies, as well in the use of new candidate bioactive components. All this opens up a wide range of possibilities of establishing definite designs to provide specific health claim.

13.6 Final Considerations

Meat and meat products are essential foods in our diet. The main role of diet consists in providing enough nutrients to meet all metabolic requirements and maintain well-being. In addition, diet can help to modulate some specific

Downloaded From Vet-eBooks.com

physiological functions and to reduce the risk of certain diseases. Functional foods have progressed very fast in the last few years and are contributing to the development of new foods, including meats. In this context, functional meats and meat products will most probably gain increased market share in the short term, thus helping to project a better image of meat.

The meat industry possesses the technology with which to produce a good number of potential functional meat and meat products, some of which have actually been on the market for several years. Moreover, emerging technologies may also offer interesting possibilities for new functional meat products in the near future. However, for the meat industry, communication with its consumers could present a major challenge given that meat functional foods are unconventional and consumers by no means always perceive meats as healthy foods.

Acknowledgments

To projects AGL2014-53207-C2-1-R and AGL2014-57367-R of the Plan Nacional de Investigación Cientifica, Desarrollo e Innovación Tecnológica (I+D+I), Ministerio de Economía y Competitividad, FEDER funds, and Intramural CSIC: 201470E056.

References

Ahmed, S. T., Mun, H. S., Islam, M. M., Ko, S.-Y. and Yang, C.-J. (2016). Effects of dietary natural and fermented herb combination on growth performance, carcass traits and meat quality in grower-finisher pigs. *Meat Science*, **122**, 7–15.

Ahola, M. (2001). *Innovation in Foods: Health, Quality and Well Being Links*. Scandinavia: Finnish Food Industry and Research. February/March, pp. 56–57.

Anandh, M. A., Lakshmanan, V. and Anjaneluyu, A. S. R. (2003). Designer meat foods. *Indian Food Industry*, **22**(4), 40–45.

Anonymous. (2002). L-carnitine: Bringing health to functional foods. *Innovative Food Science and Emerging Technologies*, **17**, 14–15.

Ansorena, D. and Astiasarán, I. (2004). The use of linseed oil improves nutritional quality of the lipid fraction of dry-fermented sausages. *Food Chemistry*, **87**, 69–74.

Antonios, T. F. T. and MacGregor, G. A. (1997). Scientific basis for reducing the salt (sodium) content in food products. *In:* Pearson, A. M. and Dutson, T. R. (eds.) *Production and Processing of Healthy Meat, Poultry and Fish Products.* London: Blackie Academic & Professional, pp. 84–100.

Archer, B. J., Johnson, S. K., Devereux, H. M. and Baxter, A. (2004). Effect of fat replacement by inulin or lupin-kernel fibre on sausage patty acceptability, post-meal perceptions of satiety and food intake in men. *British Journal of Nutrition*, **91**, 591–599.

Arihara, K. (2004). Functional foods. *In:* Jensen, W., Devine, C. and Dikemann, M. (eds.) *Encyclopedia of Meat Sciences*, vol. 1. London: Elsevier Science, pp. 492–499.

Downloaded From Vet-eBooks.com

Aristoy, M. C. and Toldrá, F. (1998). Concentration of free amino acids and dipeptides in porcine skeletal muscles with different oxidative patterns. *Meat Science*, **50**, 327–332.

Armero, E., Barbosa, J. A., Toldrá, F., Baselga, M., and Pla, M. (1999a). Effect of the terminal sire and sex on pork muscle cathepsin (B, B+L and H), cysteine proteinase inhibitors and lipolytic enzyme activities. *Meat Science*, **51**, 185–189.

Armero, E., Flores, M., Toldrá, F. et al. (1999b). Effects of pig sire types and sex on carcass traits, meat quality and sensory quality of dry-cured ham. *Journal of the Science of Food and Agriculture*, **79**, 1147–1154.

Armero, E., Navarro, J. L., Nadal, M. I., Baselga, M. and Toldrá, F. (2002). Lipid composition of pork muscle as affected by sire genetic type. *Journal of Food Biochemistry*, **26**, 91–102.

Ashwell, M. 2002. *Concepts of Functional Foods*. *ILSI Europe Concise Monograph Series*. Brussels: International Life Science Institute.

Bakkali, F., Averbeck, S., Averbeck, D. and Idaomar, M. (2008). Biological effects of essential oils—A review. *Food and Chemical Toxicology*, **46**, 446–475.

Barbut, S., Wood, J. and Marangoni, A. (2016). Potential use of organogels to replace animal fat in comminuted meat products. *Meat Science*, **122**, 155–162.

Bass, J. J., Butler-Hogg, B. W. and Kirton, A. H. (1990). Practical methods of controlling fatness in farm animals. *In:* Wood, J. D. and Fischer, A. V. (eds.) *Reducing Fat in Meat Animals*. London: Elsevier Applied Science, pp. 398–436.

Bello, J. M., Mantecón, A. R., Rodríguez, M., Cuestas, R., Beltrán, J. M. and González, J. M. (2016). Fattening lamb nutrition. Approaches and strategies in feedlot. *Small Ruminant Research*, **142**, 78–82.

Belury, M. A. (2002). Dietary conjugated linoleic acid in health: Physiological effects and mechanisms of action. *Annual Review of Nutrition*, **22**, 505–531.

Benchaar, C., Calsamiglia, S., Chaves, A. V. et al. (2008). A review of plant-derived essential oils in ruminant nutrition and production. *Animal Feed Science and Technology*, **145**, 209–228.

Benchaar, C., Duynisveld, J. L. and Charmley, E. (2006). Effects of monensin and increasing dose levels of a mixture of essential oil compound on intake, digestion and growth performance of beef cattle. *Canadian Journal of Animal Science*, **86**, 91–96.

Bloukas, J. G., Paneras, E. D. and Fournitzis, G. C. (1997). Effect of replacing pork backfat with olive oil on processing and quality characteristics of fermented sausages. *Meat Science*, **45**, 133–144.

Boer, A. and Bast, A. (2015). International legislation on nutrition and health claims. *Food Policy*, **55**, 61–70.

Buckley, D. J., Morrisey, P. A. and Gray, J. I. (1995). Influence of dietary vitamin E on the oxidative stability and quality of pig meat. *Journal of Animal Science*, **73**, 3122–3130.

Cáceres, E., García, M. L. and Selgas, M. D. (2006). Design of a new cooked meat sausage enriched with calcium. *Meat Science*, **73**, 368–377.

Cáceres, E., García, M. L. and Selgas, M. D. (2008). Conventional and reduced-fat cooked meat sausages enriched with folic acid. *Fleischwirtschaft*, **23**, 58–60.

Carbajal, A. (2004). Consumo de carne y tendencias. Calidad de vida y epidemiología de enfermedades asociadas. *In:* Jiménez Colmenero, F., Sánchez-Muniz, F. J. and Olmedilla, B. (eds.) *La Carne y Productos Cárnicos Como Alimentos Funcionales.* Madrid, Spain: editec@red, pp. 13–38.

Downloaded From Vet-eBooks.com

Calvo, L., Segura, J., Toldrá, F. et al. (2017). Dietary organic selenium increases post-mortem free fatty acid concentration in pigs. *Food Science and Technology International* (accepted for publication).

Calvo, L., Toldrá, F., Aristoy, M. C., López-Bote, C. J. and Rey, A. I. (2016a). Effect of dietary organic selenium on muscle proteolytic activity and water-holding capacity in pork. *Meat Science*, **121**, 1–11.

Calvo, L., Toldrá, F., Rodríguez, A. I., López-Bote, C. and Rey, A. I. (2016b). Effect of dietary selenium source (organic vs. mineral) and muscle pH on meat quality characteristics of pigs. *Food Science & Nutrition*, **5**(1), 94–102. doi:10.1002/fsn3.368.

Cassens, R. G. 1999. Contribution of meat to human health. *Proceedings of the 45th International Congress of Meat Science and Technology*. Yokohama, Japan, vol. 2, 642–648.

Castro, T., Manso, T., Mantecón, A. R., Guirao, J. and Jimeno, V. (2005). Fatty acid composition and carcass characteristics of growing lamb fed diets containing palm oil supplements. *Meat Science*, **69**, 757–764.

Cavalheiro, C. P., Ruiz-Capillas, C., Herrero, A. M., Jimenez-Colmenero, F., Ragagnin de Menezes, C. and Martins Fries, L. L. (2015). Application of probiotic delivery systems in meat products. *Trends in Food Science & Technology*, **46**, 120–131.

Chae, S. H., Keeton, J. T. and Smith, S. B. (2004). Conjugated linoleic acid reduces lipid oxidation in aerobically stored, cooked ground beef patties. *Journal of Food Science*, **69**, 306–309.

Chizzolini, R., Zanardi, E., Dorigoni, V. and Ghidini, S. (1999). Calorific value and cholesterol content of normal and low-fat meat and meat products. *Trends in Food Science & Technology*, **10**, 119–128.

Clarke, A. D. (1997). Reducing cholesterol levels in meat, poultry and fish products. *In:* Pearson, A. M., Dutson, T. R. (eds.) *Production and Processing of Healthy Meat, Poultry and Fish Products*. London: Blackie Academic & Professional, pp. 101–117.

Cofrades, S., Salcedo Sandoval, L., Delgado-Pando, G., López-López, I., Ruiz-Capillas, C. and Jiménez-Colmenero, F. (2011). Antioxidant activity of hydroxytyrosol in frankfurters enriched with n-3 polyunsaturated fatty acids. *Food Chemistry*, **129**, 429–436.

Collins, J. E. (1997). Reducing salt (sodium) levels in processed meat, poultry and fish products. *In:* Pearson, A. M. and Dutson, T. R. (eds.) *Production and Processing of Healthy Meat, Poultry and Fish Products*. London: Blackie Academic & Professional, pp. 282–297.

Correia, B. R., Carvalho, G. G. P., Oliveira, R. L. et al. (2016). Production and quality of beef from young bulls fed diets supplemented with peanut cake. *Meat Science*, **118**, 157–163.

D'Arrigo, M., Hoz, L., Cambero, I., Lopez-Bote, C. J., Pin, C. and Ordoñez, J. A. (2004). Production of n-3 enriched pork liver paté. *Lebensmittel-Wissenschaft und-Technologie*, **37**, 585–591.

Decker, E. A. and Xu, Z. (1998). Minimizing rancidity in muscle foods. *Food Technology*, **52**, 54–59.

De Smet, S., Claeys, E. and Demeyer, D. (2002). Muscle enzymes in relation to meat quality and muscularity. *In:* Toldrá, F. (ed.) *Research Advances in the Quality of Meat and Meat Products.* Trivandrum, India: Research Signpost, pp. 123–142.

Demeyer, D., Honikel, K. and De Smet, S. (2008). The World Cancer Research Fund report 2007: A challenge for the meat processing industry. *Meat Science*, **80**, 953–959.

Downloaded From Vet-eBooks.com

Desmond, E. (2006). Reducing salt: A challenge for the meat industry. *Meat Science*, **74**, 188–196.

Desmond, E. and Troy, D. (2004). Nutrient claims on packaging. *In:* Jensen, W. K, Devine, C. and Dikeman, M. (eds.) *Encyclopaedia of Meat Science*, vol. 2. Oxford: Elsevier Academic Press, pp. 903–910.

Devatkal, S., Mendiratta, S. K. and Kondaiah, N. (2004). Quality characteristics of loaves from buffalo meat, liver and vegetables. *Meat Science*, **67**, 377–383.

Dhiman, T. R., Zaman, S., Olson, K. C., Bingham, H. R., Ure, A. L. and Pariza, M. W. (2005). Influence of feeding soybean oil on conjugated linoleic acid content in beef. *Journal of Agricultural and Food Chemistry*, **53**, 684–689.

Diplock, A. T., Aggett, P. J., Ashwell, M., Bornet, F., Fern, E. B. and Roberfroid, M. B. (1999). Scientific concept of functional foods in Europe. Consensus document. *British Journal of Nutrition*, **81**, S1–S27.

D'Souza, D. N., Warner, R. D., Dunshea, F. R. and Leury, B. J. (1999). Comparison of different dietary magnesium supplements on pork quality. *Meat Science*, **51**, 221–225.

Dye, L. and Blundell, J. (2002). Functional foods: Psychological and behavioural functions. *British Journal of Nutrition*, **88**(Suppl 2), S187–S211.

EFSA. (2004). Opinion of the Scientific Panel on Dietetic Products, Nutrition and Allergies on a request from the Commission related to the presence of *trans* fatty acids in foods and the effect on human health of the consumption of *trans* fatty acids. (Request N EFSA-Q-2003-022). *EFSA Journal*, **81**, 1–49

Elmore, J. S., Cooper, S. L., Enser, M. et al. (2005). Dietary manipulation of fatty acid composition in lamb meat and its effect on the volatile aroma compounds of grilled lamb. *Meat Science*, **69**, 233–242.

Enser, M., Hallet, K. G., Hewett, B., Fursey, G. A., Wood, J. D. and Harrington, G. (1998). Fatty acid content and composition of UK beef and lamb muscle in relation to production system and implications for human nutrition. *Meat Science*, **49**, 329–341.

Enser, M., Richardson, R. I., Wood, J. D., Gill, B. P. and Sheard, P. R. (2000). Feeding linseed to increase the n-3 PUFA of pork: Fatty acid composition of muscle, adipose tissue, liver and sausages. *Meat Science*, **55**, 201–212.

European Commission (EU). (2012). Regulation (EU) No 432/2012 of 16 May 2012 establishing a list of permitted health claims made on foods, other than those referring to the reduction of disease risk and to children's development and health. *Official Journal of the European Union*, **L136**, 1–40.

European Commission (EU). (2003). Proposal for a Regulation of the European Parliament and of the Council on nutrition and health claims made on food. *COM 424, Commission of the European Communities 2003/0165*, Brussels.

Fairweather-Tait, S. J., Bao, Y., Broadley, M. R. et al. (2011). Selenium in human health and disease. *Antioxidants & Redox Signaling*, **14**, 1337–1383.

Feldman, L. B. (2002). The scientific evidence for a beneficial health relationship between walnuts and coronary disease. *Journal of Nutrition*, **132**, 1062S–1101S.

Fernández, X., Mourot, J., Mounier, A. and Ecolan, P. (1995). Effect of muscle type and food deprivation for 24 hours on the composition of the lipid fraction in muscles of Large White pigs. *Meat Science*, **41**, 335–343.

Fernández-López, J., Fernández-Ginés, J. M., Aleson-Carbonell, L., Sendra, E., Sayas-Barberá, E. and Pérez-Alvarez, J. A. (2004). Application of functional citrus by-products to meat products. *Trends in Food Science & Technology*, **15**, 176–185.

Downloaded From Vet-eBooks.com

Fista, G. A., Bloukas, J. G. and Siomos, A. S. (2004). Effect of leek and onion on processing and quality characteristics of Greek traditional sausages. *Meat Science*, **68**, 163–172.

García-Iñiguez, C., Larequi, E., Rehecho, S. et al. (2010). Selenium, iodine, ω-3 PUFA and natural antioxidant form *Melissa officinalis* L: A combination of components for healthier dry fermented sausage formulation. *Meat Science*, **85**, 274–279.

Gibis, M., Schoch, A. and Fisher, A. (1999). The effect of spices on the reduction of the formation of mutagenic/carcinogenic heterocyclic amines in beef patties. *Proceedings of 45th International Congress of Meat Science and Technology*, 2, (716–717) Yokohama, Japan.

Gnanasambandam, R. and Zayas, J. F. (1992). Functionality of wheat germ protein in comminuted meat products as compared with corn germ and soy proteins. *Journal of Food Science*, **57**, 829–833.

Goldberg, T., Cai, W., Peppa, M. et al. (2004). Advanced glycoxidation end products in commonly consumed foods. *Journal of the American Dietetic Association*, **104**(8), 1287–1291.

Granado-Lorencio, F., López-López, I., Herrero-Barbudo, C. et al. (2010). Lutein-enriched frankfurter-type products: Physicochemical characteristics and lutein in vitro bioaccessibility. *Food Chemistry*, **120**, 741–748.

Harris K. B., Cross, H. R., Pond, W. G. and Mersmann, H. J. (1993). Effect of dietary fat and cholesterol level on tissue cholesterol concentrations of growing pigs selected for high or low serum cholesterol. *Journal of Animal Science*, **71**, 807–810.

Hasler, C. M. (1998). Functional foods: Their role in disease prevention and health promotion. *Food Technology*, **52**, 63–70.

Hasler, C. M. (2000). The changing face of functional foods. *Journal of the American College of Nutrition*, **19**, 499S–506S.

Hasler, C. M., Bloch, A. S., Thomson, C. A., Enrione, E. and Manning, C. (2004). Position of the American Dietetic Association: Functional foods. *Journal of the American Dietetic Association*, **104**, 814–826.

Hernández, P., Navarro, J. L. and Toldrá, F. (1998). Lipid composition and lipolytic enzyme activities in porcine skeletal muscles with different oxidative pattern. *Meat Science*, **49**, 1–10.

Higgs, J. D. (2000). The changing nature of red meat: 20 years of improving nutritional quality. *Trends in Food Science & Technology*, **11**, 85–95.

Holm, F. (2003). New Functional Foods Ingredients Cardiovascular Health. Project no. QLK1-CT-2000-00040. SMEs no.5. Available at www.nutrition.org.uk (accessed October 2004).

Hotchkiss, J. H. and Parker, R. S. (1990). Toxic compounds produced during cooking and meat processing. *In:* Pearson, A. M. and Dutson, T. R. (eds.) *Advances in Meat Research*, vol. 6. London: Elsevier Applied Science, pp. 105–134.

Hovenier, R., Kanis, E., Asseldonk, T. H. and Westerink, N. G. (1992). Genetic parameters of pig meat quality traits in a halothane negative population. *Meat Science*, **32**, 309–321.

Howlett, J. (2008). *Functional Foods: From Science to Health and Claims. ILSI Europe Concise Monographs Series.* Belgium, Brussels: ILSI Europe.

Hoz, L., D'Arrigo, M. D., Camberro, I. and Ordoñez, J. A. (2004). Development of an n-3 fatty acid and α-tocopherol enriched dry fermented sausage. *Meat Science*, **67**, 485–495.

Downloaded From Vet-eBooks.com

Hoz, L., López-Bote, C. J., Cambero, M. I. et al. (2003). Effect of dietary linseed oil and α-tocopherol on pork tenderloin (*Psoas major*) muscle. *Meat Science*, **65**, 1039–1044.

Hur, S. J., Ye, B. W., Lee, J. L., Ha, Y. L., Park, G. B. and Joo, S. T. (2004). Effects of conjugated linoleic acid on color and lipid oxidation of beef patties during cold storage. *Meat Science*, **66**, 771–775.

Irie, M. and Sakimoto, M. (1992). Fat characteristics of pigs fed fish oil containing eicosapentaenoic and docosahexanoic acids. *Journal of Animal Science*, **70**, 470–477.

Isabel, B., López-Bote, C. J., Hoz, L., Timón, M., García, C. and Ruiz, J. (2003). Effects of feeding elevated concentrations of monounsaturated fatty acids and vitamin E to swine on characteristics of dry-cured hams. *Meat Science*, **64**, 475–482.

Jakobsen, K. (1999). Dietary modifications of animal fats: Status and future perspectives. *Fett/Lipid*, **101**, 475–483.

Jang, Y. D., Choi, H. B., Durosoy, S., Schlegel, P., Choie, B. R. and Kim, Y. Y. (2010). Comparison of bioavailability of organic selenium sources in finishing pigs. *Asian Australasian Journal of Animal Sciences*, **23**, 931–936.

Jayasena, D. D. and Jo, C. (2013). Essential oils as potential antimicrobial agents in meat and meat products: A review. *Trends in Food Science & Technology*, **34**, 96–108.

Jensen, C., Lauridsen, C. and Bertelsen, G. (1998). Dietary vitamin E: Quality and storage stability of pork and poultry. *Trends in Food Science & Technology*, **9**, 62–72.

Jeun-Horng, L., Yuan-Hui, L. and Chun-Chin, K. (2002). Effect of dietary fish oil on fatty acid composition, lipid oxidation and sensory property of chicken frankfurters during storage. *Meat Science*, **60**, 161–167.

Jiang, J. and Xiong, Y. L. (2016). Natural antioxidants as food and feed additives to promote health benefits and quality of meat products: A review. *Meat Science*, **120**, 107–117.

Jiménez-Colmenero, F. (2004). Chemistry and physics of comminuted meat products. Non-meat proteins. *In:* Jensen, W. K., Devine, C. and Dikeman, M. (eds.) *Encyclopaedia of Meat Science*, vol. 1. Oxford: Elsevier Academic Press, pp. 271–278.

Jimenez-Colmenero, F. (2007a). Meat based functional foods. *In:* Hui, Y. H., Chandan, R. C., Clark, S. et al. (eds.) *Handbook of Food Products Manufacturing.* Hoboken, NJ: John Wiley & Son, pp. 989–1015.

Jimenez-Colmenero, F. (2007b). Healthier lipid formulation approaches in meat based functional foods. Technological options for replacement of meat fats by non-meat fats. *Trends in Food Science & Technology*, **18**, 567–578.

Jimenez-Colmenero, F., Ayo, M. J. and Carballo, J. (2005). Physicochemical properties of low sodium frankfurter with added walnut: Effect of transglutaminase combined with caseinate, KCl and dietary fibre as salt replacers. *Meat Science*, **69**, 781–788.

Jimenez-Colmenero, F., Carballo, J. and Cofrades S. (2001). Healthier meat and meat products: Their role as functional foods. *Meat Science*, **59**, 5–13.

Jiménez-Colmenero, F. and Delgado-Pando, G. (2013). Fibre-enriched meat products. *In:* Delcour, J. A. and Poutanen, K. (eds.) *Fibre-Rich and Wholegrain Foods.* Oxford: Woodhead Publishing, pp. 329–347.

Jiménez-Colmenero, F., Herrero, A., Cofrades, S. and Ruiz-Capillas, C. (2012). Meat and functional foods. *In:* Hui, Y. H. (ed.) *Handbook of Meat and Meat Processing* (2nd Ed.). Boca Raton, FL: Taylor & Francis Group, CRC Press, pp. 225–248.

Downloaded From Vet-eBooks.com

Jiménez-Colmenero, F., Salcedo-Sandoval, L., Bou, R., Cofrades, S., Herrero, A. M. and Ruiz-Capillas, C. (2015). Novel applications of oil-structuring methods as a strategy to improve the fat content of meat products. *Trends in Food Science & Technology*, **44**, 177–188.

Jimenez-Colmenero, F., Serrano, A., Ayo, J., Solas, M. T., Cofrades, S. and Carballo, J. (2003). Physicochemical and sensory characteristics of restructured beef steak with added walnuts. *Meat Science*, **65**, 1391–1397.

Jin, H., Xu, C. X., Lim, H. T. et al. (2009). High dietary inorganic phosphate increases lung tumorigenesis and alters akt signaling. *American Journal of Respiratory and Critical Care Medicine*, **179**, 59–68.

Johnson, M. H., Calkins, C. R., Huffman, R. D., Johnson, D. D. and Hargrove, D. D. (1990). Differences in cathepsin B+L and calcium-dependent protease activities among breed type and their relationship to beef tenderness. *Journal of Animal Science*, **68**, 2371–2379.

Johnston, C. S. (2003). Vitamin C *In:* Bowman, B. A. and Russell, R. M. (eds.) *Conocimientos Actuales sobre Nutrición*. Washington, DC: Organización Panamericana de la Salud, ILSI Press, pp. 191–200.

Joo, S. T., Lee, J. I., Hah, K. H., Ha, Y. L. and Park, G. B. (2000). Effect of conjugated linoleic acid additives on quality characteristics of pork patties. *Korean Journal of Food Science and Technology*, **32**, 62–68.

Keeton, J. T. (1994). Low-fat meat products. Technological problems with processing. *Meat Science*, **36**, 261–276.

Kim, J.S., Godber, J.S. and Prinaywiwatkul, W. (2000) Restructured beef roasts containing rice bran oil and fiber influences cholesterol oxidation and nutritional profile. *Journal of Muscle Foods*, **11**, 111–127.

Knorr, D. (1998). Technology aspects related to microorganisms in functional foods. *Trends in Food Science & Technology*, **9**, 295–306.

Larick, D. K., Turner, B. E., Schoenherr, W. D., Coffey, M. T. and Pilkington, D. H. (1992). Volatile compound contents and fatty acid composition of pork as influenced by linoleic acid content of the diet. *Journal of Animal Science*, **70**, 1397–1403.

Lauridsen, C., Andersen, G., Andersson, M., Danielsen, V., Engberg, R. and Jakobsen, K. (1999). Effect of dietary fish oil supplied to pigs from weaning to 60 kg liveweight on performance, tissue fatty acid composition and palatability of pork when slaughtered at 100 kg liveweight. *Journal of Animal and Feed Sciences*, **8**, 441–456.

Lauridsen, C., Mu, H. and Henckel, P. (2005). Influence of dietary conjugated linoleic acid (CLA) and age at slaughtering on performance, slaughter- and meat quality, lipoproteins, and tissue deposition of CLA in barrows. *Meat Science*, **69**, 393–399.

Leclerq, B. (1990). Facteurs influençant la teneur en lipids des viands de porc et de volailles. *Viandes Produits Carnées*, **11**, 250–254.

Leino, M. 2001. Health trends in meat production. *IFAJ 45th Annual Congress*, Savonlinna, Finland. Available at http://www.maataloustoimittajat.fi/ifaj-2001/speeches/monday/leino_en.htm (accessed 5 October 2004).

Lener, M., Jaworska, K., Muszy ska, M. et al. (2012). Selenium as marker for cancer risk and prevention. *Polish Journal of Surgery*, **84**, 470–475.

Leszczynski, D. E., Pikul, J., Easter, R. A. et al. (1992). Characterization of lipid in loin and bacon from finishing pigs fed full-fat soybeans or tallow. *Journal of Animal Science*, **70**, 2175–2181.

Downloaded From Vet-eBooks.com

Liu, M. N., Huffman, D. L. and Egbert, W. R. (1991). Replacement of beef fat with partially hydrogenated plant oil in lean ground beef patties. *Journal of Food Science*, **56**, 861–862.

Lurueña-Martinez, M. A., Vivar-Quintana, A. M. and Revilla, I. (2004). Effect of locust bean/xanthan gum addition and replacement of pork fat with olive oil on the quality characteristics of low-fat frankfurters. *Meat Science*, **68**, 383–389.

Lynch, P. B. and Kerry, J. P. (2000). Utilizing diet to incorporate bioactive compounds and improve the nutritional quality of muscle foods. *In:* Decker, E., Faustman, C. and López-Bote, C. J. (eds.) *Antioxidants in Muscle Foods*. New York, NY: Wiley Interscience, pp. 455–480.

Mahan, D. C., Azain, M., Crenshaw, T. D. et al. (2014). Supplementation of organic and inorganic selenium to diets using grains grown in various regions of the United States with differing natural Se concentrations and fed to grower-finisher swine. *Journal of Animal Science*, **92**, 4991–4997.

Marquez, E. J., Ahmed, E. M., West, R. L. and Johnson, D. D. (1989). Emulsion stability and sensory quality of beef frankfurters produced at different fat and peanut oil levels. *Journal of Food Science*, **54**, 867–870, 873.

Marriott, N. G., Garrett, J. E., Sims, M. D., Wang, H. and Abril, J. R. (2002). Characteristics of pork with docosahexanoic acid supplemented in the diet. *Journal of Muscle Foods*, **13**, 253–263.

Martin, D., Ruiz, J., Kivikari, R. and Puolanne E. (2008). Partial replacement of pork fat by conjugated linoleic acid and/or olive oil in liver pâtés: Effect on physicochemical characteristics and oxidative stability. *Meat Science*, **80**, 496–504.

Martineau, R., Petit, H. V., Benchaar, C. et al. (2008). Effects of lasalocid or monensin on in situ biohydrogenation of flaxseed and sunflower seed unsaturated fatty acids. *Canadian Journal of Animal Science*, **88**, 335–339.

Mercier, Y., Gatellier, P., Viau, M., Remignon, H. and Renerre, M. (1998). Effect of dietary fat and vitamin E on colour stability and on lipid and protein oxidation in turkey meat during storage. *Meat Science*, **48**, 301–318.

Miller, M. F., Shackelford, S. D., Hayden, K. D. and Reagan, J. O. (1990). Determination of the alteration in fatty acid profiles, sensory characteristics and carcass traits of swine fed elevated levels of monounsaturated fats in the diet. *Journal of Animal Science*, **68**, 1624–1631.

Monahan, F. J. and Troy, D. J. (1997). Over coming sensory problems in low fat and low salt products. *In:* Pearson, A. M. and Dutson, T. R. (eds.) *Production and Processing of Healthy Meat, Poultry and Fish Products*. London: Blackie Academic & Professional, pp. 257–281.

Morán, L., Andrés, S., Bodas, R., Prieto, N. and Giráldez, F. J. (2012). Meat texture and antioxidant status are improved when carnosic acid is included in the diet of fattening lambs. *Meat Science*, **91**, 430–434.

Morán, L., Giráldez, F. J., Panseri, S. et al. (2013). Effect of dietary carnosic acid on the fatty acid profile and flavour stability of meat from fattening lambs. *Food Chemistry*, **138**, 2407–2414.

Morgan, C. A., Noble, R. C., Cocchi, M. and McCartney, R. (1992). Manipulation of the fatty acid composition of pig meat lipids by dietary means. *Journal of the Science of Food and Agriculture*, **58**, 357–368.

Morrissey, P. A., Sheehy, P. J. A., Galvin, K., Kerry, J. P. and Buckley, D. J. (1998). Lipid stability in meat and meat products. *Meat Science*, **49**(Suppl 1), S73–S86.

Downloaded From Vet-eBooks.com

Muguerza, E., Ansorena, D., Gimeno, O., and Astiasarán, I. (2002). Nutritional advantages of dry fermented sausages elaborated with vegetable oils. In *Proceedings of 48th International Congress of Meat Science and Technology*, Rome, Italy, 2, pp. 1012–1013.

Mulvihill, B. (2001). Rumiant meat as a source of conjugated linoleic acid (CLA). *Nutrition Bulletin*, **26**, 295–299.

Navas-Carretero, S., Pérez-Granados, A. M., Sarriá, B. and Vaquero, M. P. (2009). Iron absorption from meat pâté fortified with ferric pyrophosphate in iron-deficient women. *Nutrition*, **25**, 20–24.

Nissen, L. R., Byrne, D. V., Bertelsen, G. and Skibsted, L. H. (2004). The antioxidative activity of plant extracts in cooked pork patties as evaluated by descriptive sensory profiling and chemical analysis. *Meat Science*, **68**, 485–495.

Noci, F., O'Kiely, P., Monahan, F. J., Stanton, C. and Moloney, A. P. (2005). Conjugated linoleic acid concentration in M. Longissimus dorsi from the heifers offered sunflower oil-based concentrates and conserved forages. *Meat Science*, **69**, 509–518.

O'Connell, M., Kerry, J. P., Fannin, S., and Gilroy, D. (2002). Assessment of honey as a functional ingredient in cooked meat pork. In *Proceeding 48th International Congress of Meat Science and Technology*, Rome, Italy, 2, pp. 1016–1017.

Ohr, L. M. (2004). Nutraceutical & functional foods. *Food Technology*, **58**, 71–76.

Olmedilla-Alonso, B., Jiménez-Colmenero, F. and Sánchez-Muniz, F. (2013). Development and assessment of healthy properties of meat and meat products designed as functional foods. *Meat Science*, **95**, 919–930.

O'Sullivan, M. G., Kerry, J. P., Buckley, D. J., Lynch, P. B. and Morrisey, P. A. (1997). The distribution of dietary vitamin E in the muscles of the porcine carcass. *Meat Science*, **45**, 297–305.

Paneras, E. D., Bloukas, J. G. and Filis, D. G. (1998). Production of low-fat frankfurters with vegetable oils following the dietary guidelines for fatty acids. *Journal of Muscle Foods*, **9**, 111–126.

Park, J., Rhee, K. S., Keeton, J. T. and Rhee, K. C. (1989). Properties of low-fat frankfurters containing monounsaturated and omega-3 polyunsaturated oils. *Journal of Food Science*, **54**, 500–504.

Pennington, J. A. T. (2002). Food composition databases for bioactive food components. *Journal of Food Composition and Analysis*, **15**, 419–434.

Pereira, E. S., Mizubuti, I. Y., Oliveira, R. L. et al. (2016). Supplementation with cashew nut and cottonseed meal to modify fatty acid content in lamb meat. *Journal of Food Science*, **81**, c2143–c2148.

Pizzocaro, F., Senesi, E., Veronese, P. and Gasparoli, A. (1998). Mechanically deboned poultry meat hamburgers. II. Protective and antioxidant effect of the carrot and spinach tissues during frozen storage. *Industrie Alimentari*, **37**, 710–720.

Pszczola, D. E. (1998). Addressing functional problems in fortified foods. *Food Technology*, **52**, 38–46.

Realini, C. E., Duckett, S. K., Brito, G. W., Dalla Rizza, M. and De Mattos, D. (2004b). Effect of pasture vs. concentrate feeding with or without antioxidants on carcass characteristics, fatty acid composition, and quality of Uruguayan beef. *Meat Science*, **66**, 567–577.

Downloaded From Vet-eBooks.com

Realini, C. E., Duckett, S. K. and Windham, W. R. (2004a). Effect of vitamin C addition to ground beef from grass fed or grain-fed sources on color and lipid stability, and prediction of fatty acid composition by near-infrared reflectance analysis. *Meat Science*, **68**, 35–43.

Reig, M. and Toldrá, F. (1998a). Pork meat as a source of vitamins. *Recent Research Developments in Nutrition*, **2**, 29–34.

Reig, M. and Toldrá, F. (1998b). Protein nutritional quality of muscle foods. *Recent Research Developments in Agricultural & Food Chemistry*, **2**, 71–78.

Rivaroli, D. C., Guerrero, A., Valero, M. V. et al. (2016). Effect of essential oils on meat and fat qualities of crossbred young bulls finished in feedlots. *Meat Science*, **121**, 278–284.

Roberfroid, M. B. (2000). Concept and strategies of functional food science: The European perspective. *American Journal of Clinical Nutrition*, **71**(Suppl 6), 1660S–1664S.

Romans, J. R., Costello, W. J., Carlson, C. W., Greaser, M. L. and Jones, K. W. 1994. *The Meat We Eat*. Danville, IL: Interstate Publisher, p. 1193.

Rosell, C. M. and Toldrá, F. (1998). Comparison of muscle proteolytic and lipolytic enzyme levels in raw hams from Iberian and White pigs. *Journal of the Science of Food and Agriculture*, **76**, 117–122.

Rossi, L. G., Fiorentini, G., Netoa, A. J. et al. (2016). Impact of ground soybean and starch levels on the quality of meat from feedlot young Nellore bulls. *Meat Science*, **122**, 1–6.

Ruiz-Capillas, C. and Jiménez Colmenero, F. (2004). Biogenic amines in meat and meat products. *Critical Reviews in Food Science and Nutrition*, **44**, 489–499.

Rule, D. C., Busboom, J. R. and Kercher, C. J. (1994). Effect of dietary canola on fatty acid composition of bovine adipose tissue, muscle, kidney, and liver. *Journal of Animal Science*, **72**, 2735–2744.

Sadler, M. J. (2004). Meat alternatives-market developments and health benefits. *Trends in Food Science & Technology*, **15**, 250–260.

Saleh, N. T. and Ahmed, Z. S. (1998). Impact of natural sources rich in provitamin A on cooking characteristics, color, texture and sensory attributes of beef patties. *Meat Science*, **50**, 285–293.

Salvatori, G., Pantaleo, L., Di Cesare, C., Maiorano, G., Filetti, F. and Oriani, G. (2004). Fatty acid composition and cholesterol content of muscles as related to genotype and vitamin E treatment in crossbred lambs. *Meat Science*, **67**, 45–55.

Sánchez-Escalante, A., Djenane, D., Torrescano, G., Beltrán, J. A. and Roncalés P. (2001). The effects of ascorbic acid, taurine, carnosine and rosemary powder on colour and lipid stability of beef patties packaged in modified atmosphere. *Meat Science*, **58**, 421–429.

Sánchez-Escalante, A., Torrescano, G., Djenane, D., Beltran, J. A. and Roncales, P. (2003). Combined effect of modified atmosphere packaging and addition of lycopene rich tomato pulp, oregano and ascorbic acid and their mixtures on the stability of beef patties. *Food Science and Technology International*, **9**, 77–84.

Santos, C., Ordoñez, J. A., Camberro, I., D'Arrigo, M. and Hoz, L. (2004). Physicochemical characteristics of an α-linolenic acid and α-tocopherol-enriched cooked ham. *Food Chemistry*, **88**, 123–128.

Sandrou, D. K. and Arvanitoyannis, I. S. (2000). Low-fat/calorie foods: Current state and perspectives. *Critical Reviews in Food Science and Nutrition Technology*, **40**, 427–447.

Downloaded From Vet-eBooks.com

SCF (2003). Opinion of the Scientific Committee on Food on an application from MultiBene for approval of plant-sterol enriched foods (Scientific Committee on Food, SFC/CS/NF/DOS/24ADD2 Final). Health and Consumer Protection Directorate-General, European Commission, Brussels, Belgium.

Serrano, A., Cofrades, S., Ruiz-Capillas, C., Olmedilla-Alonso, B., Herrero-Barbudo, C. and Jiménez-Colmenero, F. (2005). Nutritional profile of restructured beef steak with added walnuts. *Meat Science*, **70**, 647–654.

Shackelford, S. D., Miller, M. F., Haydon, K. D. and Reagan, J. O. (1990). Effects of feeding elevated levels of monounsaturated fats to growing-finishing swine on acceptability of low-fat sausage. *Journal of Food Science*, **55**, 1497–1500.

Shahidi, F. and Synowiecki, J. (1997). Protein hydrolyzates from seal meat as phosphate alternatives in food processing applications. *Food Chemistry*, **60**, 29–32.

Sheard, P. R., Enser, M., Wood, D. J., Nute, G. R., Gill, B. P. and Richardson, R. I. (2000). Shelf life and quality of pork and pork products with raised n-3 PUFA. *Meat Science*, **55**, 213–221.

Sheard, P. R., Wood, J. D., Nute, G. R. and Ball, R. C. (1998). Effects of grilling to 80°C on the chemical composition of pork loin chops and some observations on the UK National Food Survey estimate of fat consumption. *Meat Science*, **49**, 193–204.

Simopoulos, A. P., Leaf, A. and Salem, N. (2000). Workshop on the essentiality of and recommended dietary intakes for omega-6 and omega-3 fatty acids. *Food Reviews International*, **16**, 113–117.

Sloan, A. E. (2000). The top ten functional food trends. *Food Technology*, **54**, 33–62.

Solomon, M. B., Pursel, V. G. and Mitchell, A. D. (2002). Biotechnology for meat quality enhancement. *In:* Toldrá, F. (ed.) *Research Advances in the Quality of Meat and Meat Products.* Trivandrum, India: Research Signpost, pp. 17–31.

Stewart, W.C., Bobe, G., Pirelli, G.J., Mosher, W.D. and Hall, J.A. (2012) Organic and inorganic selenium: III. Ewe and progeny performance. *Journal of Animal Science*, **90**, 4536-4543.

St. John, L. C., Buyck, M. J., Keeton, J. T., Leu, R. and Smith, S. B. (1986). Sensory and physical attributes of frankfurters with reduced fat and elevated monounsaturated fats. *Journal of Food Science*, **51**, 1144–1146, 1179.

Suray, P. F. (2003). Natural antioxidants: From the animal feed industry to human health. *In:* Suray, P. F. (ed.) *Natural Antioxidants in Avian Nutrition and Reproduction*. Nottingham: Nottingham University Press, pp. 565–604.

Suzuki, K. T. and Ogra, Y. (2002). Metabolic pathway for selenium in the body: Speciation by HPLC-ICP MS with enriched Se. *Food Additives & Contaminants*, **19**, 974–983.

Toldrá, F. (2002). *Dry-Cured Meat Products*. Trumbull, CT: Food & Nutrition Press, pp. 1–238.

Toldrá, F. and Flores, M. (2004). Meat quality factors. *In:* Nollet, L. M. L. (ed.) *Handbook of Food Analysis.* New York, NY: Marcel Dekker, pp. 1961–1977.

Toldrá, F., Flores, M., Aristoy, M. C., Virgili, R. and Parolari, G. (1996b). Pattern of muscle proteolytic and lipolytic enzymes from light and heavy pigs. *Journal of the Science of Food and Agriculture*, **71**, 124–128.

Toldrá, F. and Reig, M. (2004). Analysis of meat in food. *In:* Nollet, L. M. L. (ed.) *Handbook of Food Analysis.* New York, NY: Marcel Dekker, pp. 1941–1959.

Toldrá, F. and Reig, M. (2011). Innovations for healthier processed meats. *Trends in Food Science & Technology*, **22**, 517–522.

Toldrá, F., Reig, M., Hernández, P. and Navarro, J. L. (1996a). Lipids from pork meat as related to a healthy diet. *Recent Research Development in Nutrition*, **1**, 79–86.

Downloaded From Vet-eBooks.com

Toldrá, F., Rubio, M. A., Navarro, J. L. and Cabrerizo, L. (2004). Quality aspects of pork and its nutritional impact. *In:* Shahidi, F., Spanier, A. M., Ho, C.-T. and Braggins, T. (eds.) *Quality of Fresh and Processed Foods. Advances in Experimental Medicine and Biology*, vol. 542. New York, NY: Kluwer Academic/Plenum, 25–32.

Työppönen, S., Petäjä, E. and Mattila-Sandlhom, T. (2003). Bioprotective and probiotics for dry sausages. *International Journal of Food Microbiology*, **83**, 233–244.

Turubatovic, L. and Milatovic-Stevanovic, M. (2001). Bioactive components—New aspects of applying supplements in the meat industry. *Tehnologija Mesa*, **42**(5–6), 377–397.

Ulrich, C. M. and Potter, J. D. (2004). Diet, genes and cancer risk. *In:* Senate Commission on Food Safety (ed.) *Functional Foods: Safety Aspects*. Weinheim: Wiley-Vch. KgaA, pp. 37–49.

Verma, A. K. and Banerjee, R. (2010). Dietary fibre as functional ingredient in meat products: A novel approach for healthy living—A review. *Journal of Food Science and Technology–Mysore*, **47**, 247–257.

Vitaglione, P. and Fogliano, V. (2004). Use of antioxidants to minimize the human health risk associated to mutagenic/carcinogenic heterocyclic amines in food. *Journal of Chromatography B*, **8002**, 189–199.

Walsh, M. M., Kerry, J. F., Buckley, D. J., Arendt, E. K. and Morrissey, P. A. (1998). Effect of dietary supplementation with α-tocopheryl acetate on stability of reformed and restructured low nitrite cured turkey products. *Meat Science*, **50**, 191–201.

Wang, X., Wu, H., Long, Z. et al. (2016). Differential effect of Se on insulin resistance, regulation of adipogenesis and lipolysis. *Molecular and Cellular Biochemistry*, **415**, 89–102.

Warnants, N., Van Oeckel, M. J. and Boucqué, C. V. (1999). Incorporation of dietary polyunsaturated fatty acids into pork fatty tissues. *Journal of Animal Science*, **77**, 2478–2490.

Weiss, J., Gibis, M., Schuh, V. and Salminen, H. (2010). Advances in ingredient and processing systems for meat and meat products. *Meat Science*, **86**, 196–213.

Weststrate, J. A., van Poppel, G. and Verschuren, P. M. (2002). Functional foods, trends and future. *British Journal of Nutrition*, **88**, s233–s235.

Wheeler, T. L., Savell, J. W., Cross, H. R., Lunt, D. K. and Smith, S. B. (1990). Mechanisms associated with the variation in tenderness of meat from Brahman and Hereford cattle. *Journal of Animal Science*, **68**, 4206–4220.

WHO (2003). *Diet, nutrition and the prevention of chronic diseases*. WHO Library Cataloguing-in-Publication Data, Geneva, WHO Technical Report Series 916.

Williams, C. M. (2000). Dietary fatty acids and human health. *Annales de Zootechnie*, **49**, 165–180.

Wirth, F. (1991). Reducing the fat and sodium content of meat products. What possibilities are there? *Fleischwirtschaft*, **7**, 294–297.

Wood, J. D., Jones, R. C. D., Bayntun, J. A. and Dransfield, E. (1985). Backfat quality in boars and barrows at 90 kg live weight. *Animal Production*, **40**, 481–487.

Wood, J. D., Richardson, R. I., Nute, G. R. et al. (2003). Effects of fatty acids on meat quality: A review. *Meat Science*, **66**, 21–32.

Yılmaz, I. (2004). Effects of rye bran addition on fatty acid composition and quality characteristics of low-fat meatballs. *Meat Science*, **67**, 245–249.

Yılmaz, I., Simsek, O. and Isıkli, M. (2002). Fatty acid composition and quality characteristics of low-fat cooked sausages made with beef and chicken meat, tomato juice and sunflower oil. *Meat Science*, **62**, 253–258.

Downloaded From Vet-eBooks.com



Taylor & Francis
Taylor & Francis Group
http://taylorandfrancis.com

Downloaded From Vet-eBooks.com

14

Salt Reduction in Processed Meats

Fidel Toldrá and José M. Barat

CONTENTS

14.1 Introduction 443
14.2 Manufacturing Strategies for Low-Salt Foods 445
14.2.1 Sodium Reduction 445
14.2.2 Sodium Replacement 445
14.2.3 Change of Size and Shape of Salt Crystals 446
14.2.4 Other Strategies: Masking Agents 446
14.3 Effects of Salt on the Quality and Safety of Meat Products 447
14.3.1 Physical Properties 447
14.3.2 Chemical Properties: Enzyme Inhibition 448
14.3.3 Chemical Properties: Sensory Effects 449
14.3.4 Preservation Properties 452
14.4 Current Regulations on Salt Reduction and Labeling 453
14.5 Conclusions 454
References 454

14.1 Introduction

Western societies are concerned with the increased dietary consumption of sodium and, in fact, it is well recognized that sodium intake exceeds the nutritional recommendations in most industrialized countries. The main reasons for such concern is the evidence related to consumers' health. So, an excess of sodium in the diet may result in a significant rise of blood pressure in significant sectors of population having hypertension or prehypertension. This may contribute to an increased risk of coronary heart disease and both forms of stroke (WHO/FAO, 2003; WHO, 2007). High salt intake has been also related as a possible cause contributing to colorectal cancer (Demeyer et al., 2008), stomach cancer, and osteoporosis due to increases in urinary calcium levels excreted even though solid evidence has yet to be demonstrated (Gilbert and Heiser, 2005; Glass and Doyle, 2010).

The major risk factor for cardiovascular disease and one of the three leading risk factors for global disease burden in 2010 is high blood pressure (Lim et al.,

Downloaded From Vet-eBooks.com

2012). Dietary risk factors, diets low in fruits and high in sodium being the most relevant, and physical inactivity collectively accounted for 100% of the disability-adjusted life years in 2010 (Lim et al., 2012). Excessive sodium intake has been confirmed as an important cause for high blood pressure (Aburto et al., 2013; He and MacGregor, 2003). The amount of dietary salt consumed is an important determinant of blood pressure levels and a major risk factor for coronary heart disease and ischemic as well as hemorrhagic stroke (WHO/FSA, 2010). In fact, raised blood pressure is estimated to cause 7.5 million deaths, about 12.8% of all deaths (WHO, 2011). So, sodium consumption of more than 2 g/day is estimated to cause 1.65 million cardiovascular-related deaths each year, representing around 1 of every 10 deaths from cardiovascular causes (Mozaffarian et al., 2014). Raised blood pressure is positively and progressively related to the risk for stroke and coronary heart disease (Whitworth, 2003).

The efficacy of reduced sodium intake in lowering blood pressure is well established. A reduction in salt intake to less than 5 g/day (2 g/day of sodium) was recommended by WHO (2014) in order to reduce blood pressure and the risk of coronary heart disease and stroke. Seventy-five percent of our sodium dietary intake comes from processed foods (Appel and Anderson, 2010). The main source of sodium in the diet is sodium chloride and the use is estimated to be around 9–12 g of salt per day. Dietary salt intake is recommended to decrease to 5 g/day, since this reduction would also decrease the impact of blood pressure and cardiovascular disease. For instance, a study carried out in the United Kingdom demonstrated that blood pressure was reduced to 3.6–5.6/1.9–3.2 mmHg (systolic/diastolic) in hypertensive volunteers when salt intake was reduced by 3 g/day (He and Macgregor, 2003). It was also estimated that with such salt reduction, strokes could be reduced by 13% and ischemic heart disease by 10% (Gilbert and Heiser, 2005). Member states have been supported by the WHO for the development, implementation, and monitoring of salt reduction strategies (WHO, 2010). In 2014, there were 75 countries with national salt reduction strategies, more than double that in 2010 (WHO, 2014).

The consumption of meat products may represent up to 30% of the total dietary sodium intake. Cooked, fermented, and dry-cured meat products contain high concentrations of sodium that may range from 800 to 1900 mg Na^+/100 g product and an example of typical content in a variety of meat products is shown in Table 14.1 (USDA, 2017). The reduction of salt in meat products is not an easy task due to the multiple technological benefits of salt to the final product quality, especially from sensory and safety point of views (Ruusunen and Puolanne, 2005; Desmond, 2006; Toldrá and Reig, 2011). There is a relevant influence of salt content on flavor, water activity, microbial stability, enzyme activity, water holding capacity (WHC), protein solubility, and texture (Toldrá, 2006a; Weiss et al., 2010; Toldrá and Barat, 2012). The content of salt in the meat products can be reduced through different strategies like reducing the amount of added sodium chloride (Andrés et al., 2004), partially replacing the sodium chloride with other salts (i.e., potassium chloride) having similar technological properties (Sofos, 1989; Aliño

Downloaded From Vet-eBooks.com

TABLE 14.1

Typical Reported Content of Sodium Chloride in Selected Meat Products

Meat Product	Sodium Content (mg Na^+/100 g)	Sodium Chloride Content (g NaCl/100 g)
Pork cured ham	977	2.49
Bacon	1420	3.62
Bologna	920	2.34
Frankfurter	816	2.08
Cooked ham	900	2.29
Cooked sausage	809	2.06
Salami	1889	4.80

Source: Adapted from USDA. (2017). Food National Database. Nutritional composition of foods. Available at http://www.ndb.nal.usda.gov/ndb/nutrients.

et al., 2010b; Toldrá and Barat, 2012), replacing salt with other substances that may mask salt characteristics (Inguglia et al., 2017), or reducing the salt crystal size or changing its shape while maintaining the salty taste (Angus et al., 2006; Hanley, 2005).

There are some recent reviews on the different strategies for salt reduction in processed meats (Barat and Toldrá, 2012; Toldrá and Barat, 2009, 2012, 2015, 2016; Inguglia et al., 2017). This chapter deals with and summarizes the available strategies for salt reduction in the processing of meat products.

14.2 Manufacturing Strategies for Low-Salt Foods

14.2.1 Sodium Reduction

The first and basic strategy to reduce salt content in food is the direct reduction of the added salt (Samapundo et al., 2010). In the case of meat products obtained by mixing all the ingredients, the total amount of salt to be added should be reduced (Aaslyng et al., 2014). In the case of salted products, when placing the meat product in contact with the salting media, a reduction of the salting time or salt concentration would be needed (Aliño et al., 2009a).

Nevertheless, the direct reduction of salt content in food is limited by one or more aspects, depending on the type of meat product: WHC, texture, protein extraction, or solution, enzyme activity, sensory, or preservation aspects.

The problems related to the direct reduction of salt in food could be avoided by using total or partial replacement of NaCl by other types of salts.

14.2.2 Sodium Replacement

NaCl can be partially or totally replaced by a nonsodium ingredient to reduce the sodium content of the meat product. The main replacer is KCl, although

Downloaded From Vet-eBooks.com

TABLE 14.2

Studies about NaCl Replacement by Other Salts in Meat Products

Replacement Approach	References
Replacement of NaCl by a mixture of KCl, $MgCl_2$, and $CaCl_2$ in dry-cured pork loin showed that reductions up to 40%–50% in NaCl could be achieved without significantly affecting sensory and/or safety characteristics of the final product.	Aliño et al. (2009b, 2010a) and Armenteros et al. (2009d, 2009c)
The replacement of NaCl by KCl and/or K-lactate in sausages showed that increased K-lactate replacement gave increased pH, sweetness, crumbliness, and pastiness while piquantness, hardness, cohesiveness, ripened flavor, acid taste, and saltiness decreased.	Guàrdia et al. (2008)
Sensory effects were observed at larger replacements than 40% Na^+, especially some bitter and metallic aftertastes, especially when magnesium or calcium chlorides were added.	Armenteros et al. (2011) and Barat et al. (2013)
The replacement of NaCl has also been assayed in Spanish dry-cured ham by replacement with either K-Lactate, or a mixture of KCl alone or mixed with $MgCl_2$ and $CaCl_2$, obtaining satisfactory results with an approximate 40% Na^+ reduction.	Costa-Corredor et al. (2009), Fulladosa et al. (2009), Aliño et al. (2010c), Armenteros et al. (2011), and Ripollés et al. (2011)

$MgCl_2$, $CaCl_2$, K-lactate, glycine, or a mixture of them have also been used. Table 14.2 shows some studies about NaCl replacement in meat products.

14.2.3 Change of Size and Shape of Salt Crystals

Salt is hydrated and dissolved by saliva and then it is distributed throughout the mouth. So, the salt crystal size and shape may affect both the rate of hydration and dissolution phenomena (Rama et al., 2013). The reduction of size of the salt crystal results in an increase of the surface area. In fact, the particle size of salt crystals when applied to fried potato crisps was reported to have a strong effect on the delivery rate and concentration of sodium into the saliva and on the perceived saltiness (Rama et al., 2013). The use of different particulate sizes was patented for an instant soup with low salt content. Different sodium levels and distribution between the particulate phase and the soluble phase was reported (Busch et al., 2008).

14.2.4 Other Strategies: Masking Agents

Unpleasant tastes (bitter or metallic aftertaste) can appear as a consequence of NaCl replacement by other salts. The use of masking agents to reduce those unpleasant tastes is an option. Some masking agents are natural condiments which act in masking the bitter or metallic aftertaste: pepper, onion, garlic, curry, and so on (Toldrá and Barat, 2012). Table 14.3 shows masking agents that can be used as reported in the literature.

Downloaded From Vet-eBooks.com

TABLE 14.3

Studies about NaCl Replacement in Meat Products Based on Masking Mixture

Masking Mixture	References
Magnesium glutamate and naringin, alone or in combination	Imada et al. (2010) and Yamada (2009)
Combination of carboxymethyl cellulose and carrageenan with sodium citrate	Ruusunen et al. (2003)
Combination of one edible nucleotide monophosphate salt and another substance (low organic acid, low organic acid salt, phosphoric acid, phosphate salt, a magnesium salt, sugar, and burnt sugar)	Zolotov et al. (1997)
A cereal flour such as rice flour and a food grade acidulant like citric acid	Chigurupati (2007)
An organic acid with potassium, calcium, and magnesium salts, or potassium bicarbonate containing magnesium, potassium or calcium carbonate, lactate, citrate, tartrate, succinate, glutamate, or orthophosphate	Burckel et al. (2003)
Minor amounts of magnesium sulfate and calcium carbonate, with trace amounts of folic acid and zinc oxide	Ryberg (2008)

14.3 Effects of Salt on the Quality and Safety of Meat Products

14.3.1 Physical Properties

The interaction of salt with the protein matrix of processed meats has big consequences on its physical properties. The influence of salt on the WHC of proteins is probably the most important technological parameter related to the presence of salt in foods. Differences in WHC may be obtained, depending on the type of salt used (Puolanne and Halonen, 2010).

The penetration of NaCl into the meat during the salting process induces protein–ion interactions and entropic repulsions between the myofibrils contained in the muscle fibers (Offer and Knight, 1983). At low salt concentration (around 5%), muscle fibers or myofibrils swell laterally and myosin is extracted. As a consequence of that an increase in the WHC of meat is observed, and an increase of weight of the salted product occurs (Aliño et al., 2010a). This is due to the existence of a swelling pressure that affects the mass transfer processes (Barat et al., 2009). At high salt concentrations, the phenomenon is the contrary: the competition of Na^+ and Cl^- ions with proteins for water molecules lead to the denaturation and precipitation of proteins, with a maximum effect at saturation concentration (26% w/w).

The mentioned behavior contributes to define the most recommendable brine concentration to obtain a certain product. For instance, in the case of marinated meat, low NaCl concentrations are used, so the obtained product is juicy and a high process yield is achieved. Nevertheless, if a salt-dried

Downloaded From Vet-eBooks.com

product is desired, dry salt or saturated brine is used, because of the high water loss of the salted product.

A similar effect on WHC to that observed when using NaCl can be partially obtained by changing the pH of meat products. Since the minimum WHC is achieved at a pH value equal to the isoelectric point of proteins (Warriss, 1982), the change in the pH of the meat product (e.g., by using acid like lactic acid or citric acid [Sawyer et al., 2008; Ke et al., 2009]) allows for an increase in WHC. This increase is bigger when the difference with the isoelectric point increases (Hultin et al., 2010). Apart from changing the pH, other substances can be added to meat products to increase its water binding properties, such as other salts, modified food starches, phosphates, low-dextrose equivalent corn syrup solids, maltodextrins, gums, wheat, concentrates, flours, edible seaweeds (for gel/emulsion meat systems), and whey or soy protein isolates or protein isolates made from low quality muscle foods (Imer, 2007; Ruusunen et al., 2005; Wang et al., 2009; Schilling et al., 2004; Cofrades et al., 2008).

Physical treatments like high-pressure technology can also contribute to increase WHC as tested in frankfurters (Crehan et al., 2000) and in beef sausage batters (Sikes et al., 2009).

The presence of salt in meat products also affects protein extraction and solubility. For instance, an increase in viscosity of meat batter has been observed due to the presence of salt (Desmond, 2006). Low salt contents also affect the texture in dry-cured hams (Morales et al., 2007) resulting in soft hams due to extensive protein breakdown by increased muscle cathepsins activity (Toldrá, 2007).

The replacement of sodium by other salts, like potassium, calcium, or magnesium chloride, can enhance emulsion stability and/or protein extraction and solubility (Nayak et al., 1998a,b; Piggot et al., 2000). but can also contribute to an increased lipid oxidation (Zanardi et al., 2010). Meat product texture can also be improved by using high-pressure treatments (Carballo et al., 2001; Campus et al., 2008; Totosaus and Pérez-Chabela, 2009).

14.3.2 Chemical Properties: Enzyme Inhibition

Sodium chloride has been reported as an effective inhibitor of the majority of muscle proteolytic enzymes, especially cathepsins, dipeptidylpeptidases, and aminopeptidases (Rico et al., 1991; Toldrá and Flores, 1998). Through this inhibitory effect, the proteolysis phenomena is not so extensive during the processing of dry-cured ham (Toldrá, 2002, 2006b,c).

The effect of chloride salts used as NaCl replacers like KCl, $MgCl_2$, and $CaCl_2$ was tested on muscle peptidases (Armenteros et al., 2009b) and a summary of their effects is shown in Table 14.4. As can be observed in Table 14.4, KCl exerts, in general, an effect similar to NaCl, but the inhibitory effect of divalent salts ($CaCl_2$ and $MgCl_2$) is more pronounced in most of the cases. Cathepsins are quite affected by 0.2 M of NaCl, as reported elsewhere (Rico et al., 1991), but also by all other types of assayed salts. In the case of

Downloaded From Vet-eBooks.com

TABLE 14.4

Relative Activity of Muscle Enzymes (Expressed as Approximate % Range) in the Presence of 0.2 M Salt Concentration

Enzyme/Salt	NaCl	KCl	$MgCl_2$	$CaCl_2$
Cathepsin B	40	40	0–10	10–20
Cathepsin B + L	60–70	60–70	40	40
Cathepsin H	40–50	40–50	0–10	30–40
DPP I	<100	<100	<100	40–50
DPP II	80	80	60	nd
DPP III	50	50	10	0
DPP IV	100	100	40–50	20–30
Alanyl aminopeptidase	70	70	20–30	0–10
Arginyl aminopeptidase	<100	<100	100	0
Leucyl aminopeptidase	80–90	90–100	70	70
Methionyl aminopeptidase	100	100	50	Nd

Source: Adapted from *European Food Research and Technology*, **229**, Armenteros, M. et al., Effect of sodium, potassium, calcium and magnesium chloride salts on pork muscle proteases, 93–98. Copyright 2009 with permission from Elsevier.

nd, no available data.

exopeptidases, dipeptidylpeptidase III (DPP III) is the most affected by all types of salts while DPP I, II, and IV remain quite unaffected by 0.2 M of NaCl or KCl. Alanyl aminopeptidase is quite affected by 0.2 M of NaCl or KCl, although leucyl and methionyl aminopeptidases remain nearly unaffected at such concentration. It must be mentioned that arginyl aminopeptidase, also named aminopeptidase B, has a different behavior because it is a chloride activated enzyme (activated at low amounts of NaCl), and thus is also activated at low amounts of NaCl and KCl and at very low amounts of $MgCl_2$. However, $CaCl_2$ shows a strong inhibitory effect (Armenteros et al., 2009b).

The effect of different salt formulations on the proteolysis and lipolysis phenomena during the processing of dry-cured meats was reported. Most proteolytic enzyme activities from dry-cured loins salted with 50% KCl or with 25% KCL, 15% $CaCl_2$, and 5% $MgCl_2$ replacement were higher than those salted traditionally with only NaCl and a larger release of free amino acids was also observed due to the higher aminopeptidase activity (Armenteros et al., 2009a,c,d). In the case of dry-cured ham, nonsignificant statistical differences were reported for proteolysis after different salting treatments (Armenteros et al., 2012b). On the other hand, there was a slight trend toward a major lipolysis in hams salted with $MgCl_2$ and $CaCl_2$ replacement (Ripollés et al., 2011).

14.3.3 Chemical Properties: Sensory Effects

Salt has a salty taste mainly due to its Na^+ cation, so sodium is perceived in the pores on the tongue via receptor cells that allow the sodium ion to enter through specific ion channels. Such channels are quite specific and this is

Downloaded From Vet-eBooks.com

one of the reasons why alternative salts are less perceived (Rama et al., 2013). It must be taken into account that the complexity of the food matrix such as in the case of meat products also exerts an influence on the saltiness perception (Drake et al., 2011). Saltiness was reported to be related to fat and water concentrations based on a cheese matrix. The water content influenced the sodium release and the fat content influenced saltiness during the chewing process, so that in this way, salt concentration could be reduced without saltiness alteration through an increase of water and a decrease of fat content (Phan et al., 2008).

Another relevant effect of salt is that it contributes to a flavor-enhancing effect in meat products. The most noticeable effect of salt addition to meat products is the perception of saltiness (Ruusunen and Puolanne, 2005) and this effect is more perceived in those products having higher fat content (Matulis et al., 1995) and less perceived in products with more protein content. In fact, a reduction of 17%–20% of salt in slow dry-fermented sausages resulted in sausages perceived as less tasty and less aromatic by panelists even though the inoculation of the yeast *Debaryomices hansenii* improved significantly the aroma and taste quality (Corral et al., 2013). In the case of 17%–20% salt reduction and 10%–16% fat reduction, the sausage quality was affected with an increase in a_w and hardness and chewiness values, and a decrease of staphylococci growth (Corral et al., 2014).

The reduction of salt in meat products affects the sensory quality. For instance, salt levels below 1.5% were reported to have a negative effect on consumer acceptability; however, 2.5% salt content was the most accepted by consumers (Tobin et al., 2012a). Beef patties were also assayed for salt and fat reduction, and the beef patty containing 40% fat and 1% salt (a 50% salt reduction in comparison to standard patties) was reported to be the most acceptable for consumers (Tobin et al., 2012b). Similarly, pork breakfast sausages containing 1.4% and 1.0% salt were reported to be more acceptable to consumers than other salt levels (Tobin et al., 2013).

The aroma perception of meat products depends on the concentration and odor threshold of the available volatile compounds as well as how they interact with the food matrix (Guichard, 2002). Fermented and/or long ripened meat products like dry-fermented sausages and dry-cured hams have a wide variety of aroma volatile compounds generated as a consequence of their long processing and the action of muscle and microbial enzymes and further chemical reactions (Toldrá and Aristoy, 2010; Flores and Hernández, 2007). The effect of salt replacers was assayed and the salting-out effects of KCl were compared to those of NaCl by analyzing the headspace concentration of volatile compounds in water solutions, resulting in similar effects. However, no salting-out effects were reported for $MgCl_2$ and $CaCl_2$ (Pérez-Juan et al., 2007). Dry-cured hams produced with formulations containing NaCl alone or in combination 50/50 with KCl were reported to have significantly higher amounts of lipid-derived volatiles such as hexanal than hams produced with formulations including $MgCl_2$ and $CaCl_2$ (Armenteros et al., 2012a).

Downloaded From Vet-eBooks.com

Salt reduction in dry-fermented sausages was reported to increase lipolysis and also to exert a higher oxidation rate and rancid aroma generation. The inoculation of *D. hansenii* yeast in the low-salt dry-fermented sausages resulted in an increased aroma derived from amino acid degradation (3-methylbutanoic acid and benzothiazole) and ester activities increasing the perception of fruity and cured aroma notes from 2-methylpropanoate, 2-methylbutanoate, and 3-methylbutanoate (Corral et al., 2015). Yeasts were isolated from traditional fermented sausages and were genetically typed. Those with the ability to produce aroma compounds were selected and tested in different systems (culture media, model systems, and dry-fermented sausages) and the results revealed that such yeast strains with aroma potential could be used to improve the sensory characteristics of low-salt fermented sausages (Flores et al., 2015).

An example of the effect on sensory properties of dry-cured ham when sodium chloride is replaced by other chloride salts is shown in Table 14.5. As can be observed, the sensory attributes remained unaffected by replacement of 50% of NaCl by KCl. However, dry-cured hams prepared with magnesium and calcium salts, which are characterized by bitter tastes producing metallic, astringent, and irritative sensations, scored low for all sensory attributes (Armenteros et al., 2012b).

TABLE 14.5

Sensory Analysis (Paired Comparison Test): Results of the 9 Month Dry-Cured Hams Samples with Different Salting Formulations at the End of the Ripening Stage

Sensory Traits	I	II	III	*P*-value[a]
Aroma	24	24		n.s
	33		15	*
		34	14	**
Taste	31	17		**
	37		11	***
		36	12	***
Hardness	28	20		n.s
	35		13	**
		34	14	**
Juiciness	27	21		n.s
	32		16	*
		30	18	n.s

Source: Reprinted from *Meat Science*, **90**, Armenteros, M. et al., Biochemical and sensory changes in dry-cured ham salted with partial replacements of NaCl by other chloride salts, 361–367. Copyright 2012b with permission from Elsevier.

Formulation I, Control, 100% NaCl; Formulation II, 50% NaCl, 50% KCl; Formulation III, 55% NaCl, 25% KCl, 15% $CaCl_2$, and 5% $MgCl^2$. Results based on the number of assessors that preferred each batch (total number equal to 48).

$^{*}p < .05$; $^{**}p < .01$; and $^{***}p < .001$.

Downloaded From Vet-eBooks.com

The interaction of volatile compounds with the food matrix is also very relevant for the aroma perception as discussed earlier. In this way, it was reported that the binding ability of sarcoplasmic proteins to some key volatile compounds (branched aldehydes, hexanal, and methional) was significantly reduced by both NaCl and KCl. No effect was observed for other key volatile compounds like octanal and 2-pentanone (Pérez-Juan et al., 2006). The effects were almost negligible for $MgCl_2$ and $CaCl_2$. Only the branched aldehydes were released in the presence of $MgCl_2$ at 1.0 ionic strength (Pérez-Juan et al., 2006). Similar effects were reported for for the interaction of key volatile compounds with some isolated peptides (Martínez-Arellano et al., 2016). In such a work, peptide extracts from dry-cured ham were defatted and deodorized and then used to study the binding effect of each peptide extract to key volatile compounds. Approximately 20% and 30% interaction was observed for 2-methylpropanal, hexanal, and ethyl acetate and the highest interaction was reported for trimethylpyrazine; there was no binding effect on 2-methylbutanal (Martínez-Arellano et al., 2016).

In any case, the reduction in NaCl by 16% resulted in a lower acceptance of aroma, taste, juiciness, and overall quality. The use of KCl as an NaCl replacer resulted in a similar acceptance but the aroma perception was not improved (Corral et al., 2013).

14.3.4 Preservation Properties

The first use of salt in food was probably because of its preservation action (Sofos, 1984). The reduction of microbial spoilage of foods is mainly due to the reduction of the water activity because of the interactions of ions with water present in food (Desmond, 2006). Usually a drying process follows the salting step, implying a further reduction of water activity.

Nevertheless, the a_w values of processed meats are lower than that needed to achieve a complete inhibition. It is important to note that under those circumstances, a combination of preservation factors contribute to extend the shelf life of meat products, such as pH, storage temperature, vacuum or controlled atmosphere packaging, and so on (Leistner, 2000).

The reduction of salt content in processed meats has a direct consequence on its stability, and as a consequence on safety aspects, due to the increase in a_w values (for the same moisture of the meat products). In those cases, if a lower a_w value needs to be achieved, further drying is needed. Nevertheless, a higher decrease of moisture content implies an increase in the NaCl concentration, which would be contrary to the final objective of reducing the salt content.

Although some authors mention a slight effect of NaCl partial replacement on the growth certain microorganisms (Raccach and Hennigen, 1997), most studies indicate that no significant differences are observed, when compared with products having the same water activity (Blesa et al., 2008).

Downloaded From Vet-eBooks.com

14.4 Current Regulations on Salt Reduction and Labeling

Terms like salt reduction or low salt content in foods and drinks have been the object of regulation in many countries in order to clarify the market and avoid confusion to the consumers. The European Union issued Regulation EC 1924/2006 on nutrition and health claims made on foods (EC, 2006). This regulation describes the terms that may be used in the claims of foods, meats in our case, with low sodium/salt content and the requirements needed for each claim. The following claims can be used depending on the sodium/salt content:

Low in Sodium/Salt: Content of sodium, or the equivalent value for salt, less than 0.12 g/100 g.

Very Low in Sodium/Salt: Content of sodium, or the equivalent value for salt, less than 0.04 g/100 g.

Sodium/Salt-Free: Content of sodium, or the equivalent value for salt, less than 0.005 g/100 g.

Reduced Sodium/Salt: At least 25% of sodium, or the equivalent value for salt, less than in the original meat product.

The Nutrition Facts Label (FDA, 2013) in the United States lists the Percent Daily Value (% DV) of sodium in one serving of a food, which is based on 100% of the recommended amount of sodium, which is less than 2400 mg/day.

The FDA Food Labeling Guide (FDA, 2016) describes the terms that may be used in the claims that can be labeled in the package. The following claims can be used depending on the sodium/salt content:

Salt/Sodium Free: For less than 5 mg of sodium per serving.

Very Low Sodium: For 35 mg of sodium or less per serving.

Low Sodium: For 140 mg of sodium or less per serving.

Reduced Sodium: For at least 25% less sodium than in the original product.

Light in Sodium or Lightly Salted: For at least 50% less sodium than the regular product.

No Salt Added or Unsalted: When no salt is added during processing, but this does not guarantee that the product is sodium free. It would be necessary to check the Nutrition Facts Label to be sure about its sodium content.

If the meat product exceeds 480 mg of sodium per serving but other health claims are made (e.g., low in fat), then a disclosure statement is required.

Downloaded From Vet-eBooks.com

14.5 Conclusions

The consumption of meat products may represent up to 30% of the total dietary sodium intake, and there is a clear trend toward its reduction. Several approaches for salt reduction have been reported in this chapter. The main challenges are related to the technological properties of salt like taste, water retention, protein solubilization, and antimicrobial activity that are difficult to replace. However, the unpleasant taste derived from salt replacers is perhaps the most relevant challenge. New ingredients, processes, and technologies are expected to be developed in the near future.

References

Aaslyng, M. D., Vestergaard, C. and Koch, A. G. (2014). The effect of salt reduction on sensory quality and microbial growth in hotdog sausages, bacon, ham and salami. *Meat Science*, **96**, 47–55.

Aburto, N. J., Ziolkovska, A., Hooper, L., Elliott, P., Cappuccio, F. P. and Meerpohl, J. J. (2013). Effect of lower sodium intake on health: Systematic review and meta-analyses. *British Medical Journal*, **346**, f1326.

Aliño, M., Grau, R., Baigts, D. and Barat, J. M. (2009a). Influence of sodium replacement on the salting kinetics of pork loin. *Journal of Food Engineering*, **95**, 551–557.

Aliño, M., Grau, R., Toldrá, F. and Barat, J. M. (2010a). Physicochemical changes in dry-cured hams salted with potassium, calcium and magnesium chloride as a partial replacement for sodium chloride. *Meat Science*, **86**, 331–336.

Aliño, M., Grau, R., Toldrá, F., Blesa, E., Pagán, M. J. and Barat, J. M. (2009b). Influence of sodium replacement on physicochemical properties of dry-cured loin. *Meat Science*, **83**, 423–430.

Aliño, M., Grau, R., Toldrá, F., Blesa, E., Pagán, M. J. and Barat, J. M. (2010b). Physicochemical properties and microbiology of dry-cured loins obtained by partial sodium replacement with potassium, calcium and magnesium. *Meat Science*, **85**, 580–588.

Aliño, M., Grau, R., Toldrá, F., Blesa, E., Pagán, M. J. and Barat, J. M. (2010c). Physicochemical properties and microbiology of dry-cured loins obtained by partial sodium replacement with potassium, calcium and magnesium. *Meat Science*, **85**, 580–588.

Andrés, A. I., Cava, R., Ventanas, J., Thovar, V. and Ruiz, J. (2004). Sensory characteristics of Iberian ham: Influence of salt content and processing conditions. *Meat Science*, **68**, 45–51.

Angus, F., Phelps, T., Clegg, S., Narain, C., Den Ridder, C. and Kilcast, D. (2006). *Salt in Processed Foods: Collaborative Research Project*. Leatherhead: Leatherhead Food International.

Appel, L. J. and Anderson, C. A. M. (2010). Compelling evidence for public health action to reduce salt intake. *New England Journal of Medicine*, **362**, 650–652.

Downloaded From Vet-eBooks.com

Armenteros, M., Aristoy, M. C., Barat, J. M. and Toldrá, F. (2009a). Biochemical and sensory properties of dry-cured loins as affected by partial replacement of sodium by potassium, calcium and magnesium. *Journal of Agricultural and Food Chemistry*, **57**, 9699–9705.

Armenteros, M., Aristoy, M. C. and Toldrá, F. (2009b). Effect of sodium, potassium, calcium and magnesium chloride salts on pork muscle proteases. *European Food Research and Technology*, **229**, 93–98.

Armenteros, M., Aristoy, M. C., Barat, J. M. and Toldrá, F. (2009c). Biochemical and sensory properties of dry-cured loins as affected by partial replacement of sodium by potassium, calcium and magnesium. *Journal of Agricultural and Food Chemistry*, **57**, 9699–9705.

Armenteros, M., Aristoy, M. C., Barat, J. M. and Toldrá, F. (2011). Biochemical and sensory changes in dry-cured ham salted with partial replacement of sodium by a mixture of potassium, calcium and magnesium. *Meat Science*, **90**, 361–367.

Armenteros, M., Aristoy, M. C., Barat, J. M. and Toldrá, F. (2009d). Biochemical changes in dry-cured loins salted with partial replacements of NaCl by KCl. *Food Chemistry*, **117**, 627–633.

Armenteros, M., Toldrá, F., Aristoy, M. C., Ventanas, J. and Estevez, M. (2012a). Effect of the partial replacement of sodium chloride by other salts on the formation of volatile compounds during ripening of dry-cured ham. *Journal of Agricultural and Food Chemistry*, **60**, 7607–7615.

Armenteros, M., Aristoy, M. C., Barat, J. M. and Toldrá, F. (2012b). Biochemical and sensory changes in dry-cured ham salted with partial replacements of NaCl by other chloride salts. *Meat Science*, **90**, 361–367.

Barat, J. M., Aliño, M., Fuentes, A., Grau, R. and Romero, J. B. (2009). Measurement of swelling pressure in pork meat brining. *Journal of Food Engineering*, **93**, 455–459.

Barat, J. M., Pérez-Esteve, E., Aristoy, M. C. and Toldrá, F. (2013). Partial replacement of sodium in meat and fish products by using magnesium salts. *Plant and Soil*, **368**, 179–188.

Blesa, E., Aliño, M., Barat, J. M., Grau, R., Toldrá, F. and Pagán, M. J. (2008). Microbiology and physico-chemical changes of dry-cured ham during the post-salting stage as affected by partial replacement of NaCl by other salts. *Meat Science*, **78**, 135–142.

Burckel, A., Martin, F. and Leclerc, C. (2003). Food supplement composition and use thereof. *Patent WO03053163*.

Busch, J. L., Keulemans, J., Van Den, O. G. J. and Reckweg, F. (2008). Food composition. *Patent WO2008074606*.

Campus, M., Flores, M., Martinez, A. and Toldrá, F. (2008). Effect of high pressure treatment on colour, microbial and chemical characteristics of dry cured loin. *Meat Science*, **80**, 1174–1181.

Carballo, J., Cofrades, S., Fernández-Martín, F. and Jiménez-Colmenero, F. (2001). Pressure-assisted gelation of chemically modified poultry meat batters. *Food Chemistry*, **75**, 203–209.

Chigurupati, S. R. (2007). Low-sodium salt composition. *Patent WO2007032941*.

Cofrades, S., López-López, I., Solas, M. T., Bravo, L. and Jiménez-Colmenero, F. (2008). Influence of different types and proportions of added edible seaweeds on characteristics of low-saltgel/emulsion meat systems. *Meat Science*, **79**, 767–776.

Corral, S., Salvador, A. and Flores, M. (2013). Salt reduction in slow fermented sausages affects the generation of aroma active compounds. *Meat Science*, **93**, 776–785.

Downloaded From Vet-eBooks.com

Corral, S., Salvador, A., Belloch, C. and Flores, M. (2014). Effect of fat and salt reduction on the sensory quality of slow fermented sausages inoculated with *Debaryomyces hansenii* yeast. *Food Control*, **45**, 1–7.

Corral, S., Salvador, A., Belloch, C. and Flores, M. (2015). Improvement the aroma of reduced fat and salt fermented sausages by *Debaryomyces hansenii* inoculation. *Food Control*, **47**, 526–535.

Costa-Corredor, A., Serra, X., Arnau, J. and Gou, P. (2009). Reduction of NaCl content in restructured dry-cured hams: Post-resting temperature and drying level effects on physicochemical and sensory parameters. *Meat Science*, **83**, 390–397.

Crehan, M., Troy, D. J. and Buckley, D. J. (2000). Effects of salt level and high hydrostatic pressure processing on frankfurters formulated with 1.5 and 2.5% salt. *Meat Science*, **55**(1), 123–130.

Demeyer, D., Honikel, K. and De Smet, S. (2008). The World Cancer Research Fund report 2007: A challenge for the meat processing industry. *Meat Science*, **80**, 953–959.

Desmond, E. (2006). Reducing salt: A challenge for the meat industry. *Meat Science*, **74**, 188–196.

Doyle, M. E. and Glass, K. A. (2010). Sodium reduction and its effect on food safety, food quality, and human health. *Comprehensive Reviews in Food Science and Food Safety*, **9**, 44–56.

Drake, S. L., Lopetcharat, K. and Drake, M. A. (2011). Salty taste in dairy foods: Can we reduce the salt? *Journal of Dairy Science*, **94**, 636–645.

EC (2006). Regulation (EC) No 1924/2006 of the European Parliament and of the Council of 20 December 2006 on nutrition and health claims made on foods. *Official Journal of the European Union*, 30th December 2006, **L404**, 9–25.

FDA (2013). Guidance for industries: A food labelling guide. 1–129. Available at http://www.fda.gov/FoodLabelingGuide

FDA (2016). Changes to the nutrition facts label. Available at http://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/LabelingNutrition

Flores, M. and Hernández, D. (2007). Optimization of multiple headspace dolid-phase microextraction for the quantification of volatile compounds in dry fermented sausages. *Journal of Agricultural and Food Chemistry*, **55**, 8688–8695.

Flores, M., Corral, S., Cano-García, L., Salvador, A. and Belloch, C. (2015). Yeast strains as potential aroma enhancers in dry fermented sausages. *International Journal of Food Microbiology*, **212**, 16–24.

Fulladosa, E., Serra, X., Gou, P. and Arnau, J. (2009). Effects of potassium lactate and high pressure on transglutaminase restructured dry-cured hams with reduced salt content. *Meat Science*, **82**, 213–218.

Gilbert, P. A. and Heiser, G. (2005). Salt and health: The CASH and BPR perspective. *British Foundation Nutrition Bulletin*, **30**, 62–69.

Guàrdia, M. D., Guerrero, L., Gelabert, J., Gou, P. and Arnau, J. (2008). Sensory characterisation and consumer acceptability of small calibre fermented sausages with 50% substitution of NaCl by mixtures of KCl and potassium lactate. *Meat Science*, **80**, 1225–1230.

Guichard, E. (2002) Interactions between flavor compounds and food ingredients and their influence on flavor perception. *Food Research International*, 18, 49-70.

Hanley, B. (2005). Salt, sugar and fat reduction in foods—Technological challenges. *Presentation Given at Fat, Sugar and Salt: A Question of Balance Event*. Dublin: Leatherhead Food International.

Downloaded From Vet-eBooks.com

He, F. J. and Macgregor, G. A. (2003). How far should salt intake be reduced? *Hypertension*, **42**, 1093–1099.

Hultin, H. O., Ke, S., Huang, Y., Imer, S. and Vareltzis, P. (2010). Process for improving water holding capacity and tenderness in cooked protein food products. USPTO Applicaton No: #20100009048—Class: 426281 (USPTO).

Imada, T., Ishida, M., Kono, R., Arai, H., So, R. and Kimura, E. (2010). Salty taste enhancer containing magnesium glutamate. *Patent JP 2010011807A*.

Imer, S. (2007). Increasing water holding capacity of muscle foods: Protein isolate effect. Electronic doctoral dissertations for UMass Amherst. Paper AAI3254954. http://scholarworks.umass.edu/dissertations/AAI3254954

Inguglia, E. S., Zhang, Z., Tiwari, B. K., Kerry, J. P. and Burgess, C. M. (2017). Salt reduction strategies in processed meat products—A review. *Trends in Food Science and Technology*, **59**, 70–78.

Ke, S., Huang, Y., Decker, E. A. and Hultin, H. O. (2009). Impact of citric acid on the tenderness, microstructure and oxidative stability of beef muscle. *Meat Science*, **82**(1), 113–118.

Leistner, L. (2000). Basic aspects of food preservation by hurdle technology. *International Journal of Food Microbiology*, **55**(1–3), 181–186.

Lim, S. S., Vos, T., Flaxman, A. D. et al. (2012). A comparative risk assessment of burden of disease and injury attributable to 67 risk factors and risk factor clusters in 21 regions, 1990–2010: A systematic analysis for the global burden of disease study2010. *Lancet*, **380**, 2224–2260.

Martínez-Arellano, I., Flores, M. and Toldrá, F. (2016). Ability of peptide extracts obtained at different dry cured ham ripening stages to bind aroma compounds. *Food Chemistry*, **196**, 9–16.

Matulis, R. J., McKeith, F. K., Sutherland, J. W. and Brewer, M. S. (1995). Sensory characteristics of frankfurters as affected by fat, salt and pH. *Journal of Food Science*, **60**, 42–47.

Morales, R., Serra, X., Guerrero, L. and Gou, P. (2007). Softness in dry-cured porcine *biceps femoris* muscles in relation to meat quality characteristics and processing conditions. *Meat Science*, **77**, 662–669.

Mozaffarian, D., Fahimi, S., Singh, G. M. et al. (2014). Global sodium consumption and death from cardiovascular causes. *New England Journal of Medicine*, **371**, 624–34.

Nayak, R., Kenney, P. B., Slider, S., Head, M. K. and Killefer, J. (1998a). Cook yield, texture and gel ultrastructure of model beef batters as affected by low levels of calcium, magnesium and zinc chloride. *Journal of Food Science*, **63**, 945–950.

Nayak, R., Kenney, P. B., Slider, S., Head, M. K. and Killefer, J. (1998b). Myofibrillar protein solubility affected by low levels of calcium, magnesium and zinc chloride. *Journal of Food Science*, **63**, 951–954.

Offer, G. and Trinick, J. (1983). On the mechanism of water holding in meat: The swelling and shrinking of myofibrils. *Meat Science*, **8**, 245–281.

Pérez-Juan, M., Flores, M. and Toldrá, F. (2006). Model studies on the efficacy of protein homogenates from raw pork muscle and dry-cured ham in binding selected flavor compounds. *Journal of Agricultural and Food Chemistry*, **54**, 4802–4808.

Pérez-Juan, M., Flores, M. and Toldrá, F. (2007). Effect of ionic strength of different salts on the binding of volatile compounds to sarcoplasmic protein homogenates in model systems. *Food Research International*, **40**, 687–693.

Downloaded From Vet-eBooks.com

Phan, V. A., Yvena, C., Lawrence, G., Chabanet, C., Reparet, J. M. and Salles, C. (2008). In vivo sodium release related to salty perception during eating model cheeses of different textures. *International Dairy Journal*, **18**, 956–963.

Piggot, R. S., Kenney, P. B., Slider, S. and Head, M. K. (2000). Formulation protocol and dicationic salts affect protein functionality on model systems beef batters. *Journal of Food Science*, **65**, 1151–1154.

Puolanne, E. and Halonen, M. (2010). Theoretical aspects of water-holding in meat. *Meat Science*, **86**, 151–165.

Raccach, M. and Henningen, E. C. (1997). The effect of chloride salts on *Yersinia enterocolitica* in meat. *Food Microbiology*, **14**, 431–438.

Rama, R., Chiu, N., Da Silva, M. C., Hewson, L., Hort, J. and Fisk, I. D. (2013). Impact of salt crystal size on in-mouth delivery of sodium and saltiness perception from snack foods. *Journal of Texture Studies*, **44**, 338–345.

Rico, E., Flores, J. and Toldrá, F. (1991). Effect of dry-curing process parameters on pork muscle cathepsin B, H and L activity. *Z Lebensm Unters Forsch*, **193**, 541–544.

Ripollés, S., Campagnol, P. C. B., Armenteros, M., Aristoy, M. C. and Toldrá, F. (2011). Influence of partial replacement of NaCl for KCl, $CaCl_2$ and $MgCl_2$ in lipolysis and lipid oxidation on dry-cured ham. *Meat Science*, **89**, 58–64.

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

Ruusunen, M., Vainionpää, J., Lyly, M. et al. (2005). Reducing the sodium content in meat products: The effect of the formulation in low-sodium ground meat patties. *Meat Science*, **69**(1), 53–60.

Ruusunen, M., Vainionpää, J., Puolanne, E., Lyly, M., Lahteenmaki, L. and Niemisto, M. (2003). Effect of sodium citrate, carboxymethyl cellulose and carrageenan levels on quality characteristics of low-salt and low-fat bologna type sausages. *Meat Science*, **64**, 371–381.

Ryberg, P. (2008). A salt mixture with low sodium content for human consumption. *Patent WO08024050*.

Samapundo, S., Ampofo-Asiama, J., Anthierens, T. et al. (2010). Influence of NaCl reduction and replacement on the growth of Lactobacillus sakei in broth, cooked ham and white sauce. *International Journal of Food Microbiology*, **143**(1–2), 9–16.

Sawyer, J. T., Apple, J. K. and Johnson, Z. B. (2008). The impact of lactic acid concentration and sodium chloride on pH, water-holding capacity, and cooked color of injection-enhanced dark-cutting beef. *Meat Science*, **79**, 317–325.

Schilling, M. W., Marriott, N. G., Acton, J. C., Anderson-Cook, C., Alvarado, C.Z. and Wang, H. (2004). Utilization of response surface modeling to evaluate the effects of non-meat adjuncts and combinations of PSE and RFN pork on water holding capacity and cooked color in the production of boneless cured pork. *Meat Science*, **66**, 371–381.

Sikes, A. L., Tobin, A. B. and Tume, R. K. (2009). Use of high pressure to reduce cook loss and improve texture of low-salt beef sausage batters. *Innovative Food Science and Emerging Technologies*, **10**, 405–412.

Sofos, J. N. (1984). Antimicrobial effects of sodium and other ions in foods: A review. *Journal of Food Safety*, **6**, 45–78.

Sofos, J. N. (1989). Phosphates in meat products. *In:* Thorne, S., (ed.) *Developments in Food Preservation—5*. New York, NY: Elsevier Applied Science, pp. 207–252.

Downloaded From Vet-eBooks.com

Tobin, B. D., O'Sullivan, M. G., Hamill, R. M. and Kerry, J. P. (2012a). Effect of varying salt and fat levels on the sensory and physiochemical quality of frankfurters. *Meat Science*, **92**, 659–666.

Tobin, B. D., O'Sullivan, M. G., Hamill, R. M. and Kerry, J. P. (2012b). Effect of varying salt and fat levels on the sensory quality of beef patties. *Meat Science*, **91**, 460–465.

Tobin, B. D., O'Sullivan, M. G., Hamill, R. M. and Kerry, J. P. (2013). The impact of salt and fat level variation on the physiochemical properties and sensory quality of pork breakfast sausages. *Meat Science*, **93**, 145–152.

Toldrá, F. (2002). *Dry-Cured Meat Products*. Ames, IA: Wiley-Blackwell.

Toldrá, F. (2006a). Dry-cured ham. *In:* Hui, Y. H., Castell-Perez, E., Cunha, L. M. et al. (eds.) *Handbook of Food Science, Technology and Engineering*, vol. 4. Boca Raton, FL: CRC Press, pp. 164-1–164-11.

Toldrá, F. (2006b). Meat: Chemistry and biochemistry. *In:* Hui, Y. H, Culbertson, J. D., Duncan, S. et al. (eds.) *Handbook of Food Science, Technology and Engineering*, vol. 1. Boca Raton, FL: CRC Press, pp. 28-1–28-18. ISBN 1-57444-551–0.

Toldrá, F. (2006c). Biochemical proteolysis basis for improved processing of dry-cured meats. *In:* Nollet, L. M. L. and Toldrá, Y. F. (eds.) *Advanced Technologies for Meat Processing*. Boca Raton, FL: CRC Press, pp. 329–351.

Toldrá, F. (2007). Biochemistry of muscle and fat. *In:* Toldrá, F., Hui, Y. H., Astiasarán, I. et al. (eds.) *Handbook of Fermented Meat and Poultry*. Ames, IA: Blackwell Publishing, pp. 51–58.

Toldrá, F. and Aristoy, M. C. (2010). Dry-cured ham. *In:* Toldrá, F. (ed.) *Handbook of Meat Processing*. Ames, IA: Wiley-Blackwell, pp. 351–362.

Toldrá, F. and Barat, J. M. (2009). Recent patents for sodium reduction in foods. *Recent Patens on Food, Nutrition and Agriculture*, **1**, 80–86.

Toldrá, F. and Barat, J. M. (2012). Strategies for salt reduction in foods. *Recent Patents in Food, Nutrition and Agriculture*, **4**, 19–25.

Toldrá, F. and Barat, J. M. (2015). Low-sodium products. *In:* Toldrá, F., Hui, Y. H., Astiasarán, I., Sebranek, J. G. and Talon, R. (eds.) *Handbook of Fermented Meat and Poultry* (2nd Ed.). Chichester, West Sussex: Wiley-Blackwell, pp. 251–257.

Toldrá, F. and Barat, J. M. (2016). Low-salt foods: Types and manufacture. *In:* Caballero, B., Finglas, P. M. and Toldrá, F. (eds.) *Encyclopedia of Food and Health*, vol. 3. London: Academic Press/Elsevier Science Ltd, pp. 756–758.

Toldrá, F. and Flores, M. (1998). The role of muscle proteases and lipases in flavor development during the processing of dry-cured ham. *Critical Reviews in Food Science and Nutrition*, **38**, 331–352.

Toldrá, F. and Reig, M. (2011). Innovations for healthier processed meats. *Trends in Food Science and Technology*, **22**, 517–522.

Totosaus, A. and Pérez-Chabela, M. L. (2009). Textural properties and microstructure of low-fat and sodium-reduced meatbatters formulated with gellan gum and dicationic salts. *LWT—Food Science and Technology*, **42**, 563–569.

USDA. (2017). Food National Database. Nutritional composition of foods. Available at http://www.ndb.nal.usda.gov/ndb/nutrients

Wang, P., Xu, X.-L. and Zhou, G. H. (2009). Effects of meat and phosphate level on water-holding capacity and texture of emulsion-type sausage during storage. *Agricultural Sciences in China*, **8**, 1475–1481.

Warriss, P. D. (1982). The relationship between pH and drip in pig muscle. *Journal of Food Technology*, **17**, 573–578.

Downloaded From Vet-eBooks.com

Weiss, J., Gibis, M., Schuh, V. and Salminen, H. (2010). Advances in ingredient and processing systems for meat and meat products. *Meat Science*, **86**, 196–213.

Whitworth, J. A. (2003). World Health Organization/International Society of Hypertension statement on management of hypertension. *Journal of Hypertension*, **21**, 1983–1992.

WHO/FAO. (2003). *Diet, Nutrition and the Prevention of Chronic Diseases*. WHO Technical Report Series 916. Geneva: World Health Organization/Food and Agriculture Organisation. Available at http://www.who.int/dietphysicalactivity/publications/trs916/en/

WHO. (2007). *Prevention of Cardiovascular Disease: Pocket Guidelines for Assessment and Management of Cardiovascular Risk*. Geneva: World Health Organization. Available at http://www.who.int/cardiovascular_diseases/guidelines/Full%20text.pdf

WHO. (2010). *Creating an Enabling Environment for Population-Based Salt Reduction Strategies*. Geneva: World Health Organization. Available at http://whqlibdoc.who.int/publications/2010/9789241500777_eng.pdf

WHO/FSA. (2010). *Creating an Enabling Environment for Population-Based Salt Reduction Strategies: Report of a Joint Technical Meeting Held by WHO and the Food Standards Agency, United Kingdom*. Geneva: World Health Organization. Available at http://whqlibdoc.who.int/publications/2010/9789241500777_eng.pdf

WHO. (2011). *Global Status Report on Noncommunicable Diseases 2010*. Available at http://www.who.int/nmh/publications/ncd_report_full_en.pdf

WHO (2014). *Global Status Report on Noncommunicable Diseases 2014*. Available at http://www.who.int/nmh/publications/ncd-status-report-2014/en/

Yamada, T. (2009). Drink, and method for reducing bitter taste. *Patent JP 2009279013A*.

Zanardi, E., Ghidini, S., Conter, M. and Ianieri, A. (2010). Mineral composition of Italian salami and effect of NaCl partial replacement on compositional, physicochemical and sensory parameters. *Meat Science*, **86**, 742–747.

Zolotov, S., Braverman, O., Genis, M. and Biale, D. (1997). Low sodium edible salt mixture. *Patent EP809942*.

Downloaded From Vet-eBooks.com

15

Fat Reduction in Processed Meats

Marise A. Rodrigues Pollonio

CONTENTS

15.1 Introduction 462
15.2 Implications of Fat Consumption on Health 462
15.2.1 Saturated Fatty Acids 463
15.2.2 Mono and Polyunsaturated Fatty Acids 464
15.2.3 Meat Products as a Fat Source 465
15.3 The Role of Fat in Processed Meats 466
15.3.1 Texture 466
15.3.2 Flavor and Taste 467
15.3.3 Physicochemical Properties 467
15.3.4 Perception of Satiety 469
15.4 Strategies for Reducing Fat in Meat Products 469
15.4.1 Simple Fat Reduction and Selection of Lean Meat Cuts 470
15.4.2 Use of Fat Replacers 471
15.4.2.1 Gums 471
15.4.2.2 Starch Derivatives 472
15.4.2.3 Protein-Based Fat Replacers 473
15.4.3 Use of Fibers as Functional Ingredients and Fat Replacers 474
15.4.3.1 Insoluble Fibers 474
15.4.3.2 Soluble Fibers 478
15.4.4 Lipid Reformulation Using Vegetable Oils 484
15.4.5 Use of Structured Lipids 488
15.5 Challenges of Fat Reduction in Different Meat Products 492
15.5.1 Emulsified Meat Products 492
15.5.2 Fermented Meat Products 493
15.5.3 Restructured Injected and Marinated Meat Products 494
15.5.4 Restructured and Breaded Meat Products 495
15.6 Final Considerations 496
References 496

Downloaded From Vet-eBooks.com

15.1 Introduction

Fat is a macronutrient in foods responsible for various functions in the human body including energy supply (9 kcal/g); it also serves as a source of fat-soluble vitamins (A, D, E, and K) and essential fatty acids (linoleic and linolenic), and is a precursor of important hormones that participate in different biochemical and metabolic pathways (Pearson and Gillett, 1996; Varnam and Sutherland, 1995). However, the association between high-fat intake and increase in the incidence of chronic diseases such as obesity, hypertension, certain types of cancer, hypercholesterolemia, and cardiovascular and cerebral accidents, among others (Rothstein, 2006; Zhang et al., 2010) requires a strict control of dietary fat intake.

Meat and processed meat products are generally recognized as good sources of proteins with high biological value, B vitamins, minerals and trace elements, and bioactive compounds (Biesalski, 2005; Olmedilla-Alonso et al., 2013). However, these products are poor sources of fiber and contain high-sodium levels, various additives, and fat, particularly cholesterol and saturated fat, which make their regular consumption quite criticized (Whitney and Rolfes, 2002).

In this scenario, the reformulation of processed meat products aims at reducing, suppressing, and/or incorporating ingredients that promote healthier appeals to consumers, without losing the identity and quality standards (Arihara, 2006). In the search for healthier meat products, the reduction of total fat and saturated fat and the reformulation of the lipid profile have been widely studied, as well as fat substitutes with adequate technological and sensory performance and nutritional benefits that are safe and allowed by regulatory agencies.

This chapter addresses the principles for reducing fat in different processed meat products, describing the difficulties and possible strategies considering the sensory acceptance, overall stability, and safe consumption of the reformulated products.

15.2 Implications of Fat Consumption on Health

Although the pathogenesis of diseases associated with excess fat intakes, such as obesity, hypertension, and cardiovascular diseases, has not yet been fully elucidated, several studies have shown that dietary fat may be an important modulator of morbidity and mortality (Vessby, 2003; Rubio-Rodríguez et al., 2010).

However, the simple reduction of total fat is not enough to reduce the risk of these pathologies, since this complex class of macromolecules, including saturated, mono, polyunsaturated fatty acids (PUFAs), and cholesterol, is

Downloaded From Vet-eBooks.com

subdivided into several groups of compounds with specific and diversified physiological properties. Several authors have reported that the balanced intake of these components is more relevant than the simple suppression of total fat (Lamarche and Couture, 2014. This reference is listed in the References Recent studies have strongly criticized fat replacement by high levels of carbohydrates, responsible for the occurrence of other pathologies, thus various scientific authorities have established nutritional recommendations to guide the intake of this class of compounds. According to many studies, only 15%–35% of total calories should come from dietary fat; the intake of saturated fatty acids (SFAs) should not exceed 10%, with 6%–11% coming from PUFAs, 10%–15% from monounsaturated fatty acids (MUFAs), and less than 1% from trans-fatty acids. It is also recommended that cholesterol intake be less than 300 mg per day (Jiménez-Colmenero, 1996; Cengiz and Gokoglu, 2005; WHO, 2003). In this context, the reduction and/or reformulation of processed products with high fat content that are rich in SFAs becomes the target of many studies.

15.2.1 Saturated Fatty Acids

There is no absolute consensus on the role of total fat and saturated fat in increasing the risk of chronic diseases, especially cardiovascular diseases; however, all guidelines from different countries and health agencies are unanimous in recommending a control in the dietary SFAs intake. The U.S. Department of Agriculture (USDA) has suggested a dietary SFA intake of less than 7% of daily calories (Eckel et al., 2014), while European Committees have proposed that the recommended SFAs and trans-fatty acids intake should be as low as possible (EFSA Panel and Dietetic Products, 2010), and as previously described, the WHO indicates that fatty acids intake should be less than 10% of daily calories.

This lipid fraction, which mainly comprises triacylglycerols of animal origin, is known to increase levels of total cholesterol and low-density lipoprotein (LDL) cholesterol (Valsta et al., 2005). Palmitic (C16:0) and myristic (C14:0) fatty acids are among the main SFAs that contribute to the increase in cholesterol levels (Spady and Dietschy, 1985). Studies have suggested that palmitic acid increases the plasma total cholesterol levels to a greater extent when compared to other unsaturated fatty acids and carbohydrates (Mattson and Grundy, 1985; Grundy and Vega, 1988). Some authors have also reported that palmitic acid is also capable of raising the risk of obesity and insulin resistance (Kien et al., 2005).

According to Schaefer (2002), although myristic acid is more hypercholesterolemic than palmitic acid, it is less relevant once it is present in most diets at lower concentrations when compared to palmitic acid (Grundy, 1994). With regard to the hypercholesterolemic role of lauric acid (C12:0), contradictory data are found in the literature (Pronczuk et al., 1995; Denke and Grundy, 1992) and *in vivo* studies have shown that stearic acid (C:18:0) is partially

Downloaded From Vet-eBooks.com

converted to oleic acid and has not been shown to raise plasma cholesterol levels (Bonanome et al., 1992; Bonanome and Grundy, 1988).

Whereas most of the fat from meat products comes from fatty cuts or back fat rich in SFAs, the reduction of saturated fat in processed meat products is an urgent challenge for the meat processing industry.

15.2.2 Mono and Polyunsaturated Fatty Acids

Along with the reduction of saturated fat intake, the reformulation of the lipid profile of processed foods has stood out as one of the main strategies to produce healthier products, once the MUFAs and PUFAs have specific functions in the human diet and the balanced intake results in physiological benefits.

Oleic acid (C18:1 ω-9) is the main fatty acid present in the diet, corresponding to approximately 45% of total fatty acids that make up most of the processed meat products. According to Keys et al. (1965) and Mattson and Grundy (1985), total plasma cholesterol levels decrease around 2.7 mg/dL for each 1% calories from diet when oleic acid (C18:1) is ingested in place of SFA, exclusively due to a reduction in the LDL fraction, with no changes in high-density lipoprotein (HDL).

Although most of the fatty acids can be synthesized by the organism, the precursors of the ω-6 and ω-3 PUFAs are considered essential, since they cannot be endogenously produced in humans and some animals due to deficiency of certain enzymes known as desaturases, which are capable of inserting double bonds at the Δ12 and Δ15 positions (Moreira et al., 2002).

The intake recommendations for these essential fatty acids are due to the main benefits associated with eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), resulting from the conversion of linoleic acid (w6) and α-linolenic acid (w3), including anti-inflammatory action (Vedin et al., 2008; Furuhjelm et al., 2009), cardiovascular protective effect (Kris-Elherton et al., 2002), reduced risk of depression (Appleton et al., 2010), delay in the onset of degenerative diseases caused by aging (Dangour et al., 2012), reduced risk for certain types of cancer, and promotion of adequate fetal development and improvement of baby's cognition (Dunstan et al., 2008).

With regard to the cholesterolemic effect, the replacement of SFAs by PUFAs has been reported in several studies as a very effective way to lower plasma cholesterol levels in humans, although this effect is still unclear (Lamarche and Coture, 2014) and varies according to the ω-6 and ω-3 families. For many years, linoleic acid (C18:2) was recommended for a healthy diet due to its effectiveness in reducing cholesterol levels. However, in humans, a high linoleic acid (C18:2) intake can reduce HDL levels (Mattson and Grundy, 1985) and increase the risk of gallstone cholesterol (Sturdevant et al., 1973). On the other hand, high concentrations of ω-3 fatty acids may reduce plasma triacylglycerides, which may be responsible for reduction of the risk of cardiovascular diseases. Omega-3 PUFAs blood levels have been

Downloaded From Vet-eBooks.com

associated with reduced risk of cardiovascular disease in epidemiological studies due to their protective antiarrhythmic, anti-inflammatory, hypotensive, and hypocholesterolemic effects (Balk et al., 2006; Harris and Von Schacky, 2004). Although the benefits of ω-3 fatty acids on cardiovascular diseases are unclear or referring exclusively to the metabolic transformations of EPA (C20:5) and DHA (C22:6) (Barceló-Coblijn and Murphy, 2009), the ω-6/ω-3 ratio has become increasingly important in human nutrition, with a recommended ratio of 5:1 or less (Simopoulos, 1999; Schaefer, 2002; Chardigny et al., 2001; Kris-Etherton et al., 2000). However, the ω-6/ω-3 ratio has reached alarming proportions, being higher than 20:1 (Simopoulos, 2004) probably due to the low concentration of ω-3 in foods, suggesting strategic reformulations with enrichment with this nutrient.

15.2.3 Meat Products as a Fat Source

The fat content in lean meats is generally less than 5%. However, as a rule, besides the fatty cuts, pork back fat is a key ingredient for the development of texture and flavor in meat product formulations. Meat fats contain less than 50% SFAs and above 50% unsaturated fatty acids (50%–52% bovine, 55%–57% swine, 70% chicken, and 62% rabbit). Although meat contains cholesterol levels less than 75 mg/100 g, except kidney, brain, and heart (Jiménez-Colmenero et al., 2001), these levels are higher in processed meat products.

In meat products, the lipid content depends on both the proportion of fat in meat raw materials and mainly the amount of fatty cuts and back fat added to the formulations during processing. Although traditionally meat products contain between 20% and 35% total fat, fermented meat products such as dry fermented sausages can contain up to 45%–50% (Pearson and Gillett, 1996; Jiménez-Colmenero, 1996).

Emulsified meat products vary widely in their fat content, with mean percentages of 20%–35%. More popular products that contain less noble raw materials such as mechanically separated chicken, organ meats, and skin permitted by law are characterized by higher fat contents. The meat matrix stabilized by myofibrillar proteins as gelling agents and emulsifiers, as well as additives such as phosphates and extenders (starch and protein derivatives), can provide high fat levels in the network. These products are the target of studies to develop new strategies for fat reduction and reformulation of lipid profile.

In fermented meat products, after the fermentation, the drying stage allows important proteolytic and lipolytic reactions, with weight losses that can reach up to 45%–50%, which increases the fat content, making these products the most caloric within this category. However, this characteristic is expected and determines the identity and quality standards for the different categories produced worldwide. On the other hand, for using whole cuts in its manufacture, raw ham requires an adequate meat toilet in the food processing to completely remove the fat covering from the shank, will have its fat content defined by the intermuscular fat (marbling). High-quality

Downloaded From Vet-eBooks.com

standards have established adequate fat contents to control the lipolytic and proteolytic reactions.

Restructured meat products have physical characteristics that allow the use of fat cuts, leading to great variations of fat content, in addition to losses of functional quality. These characteristics highlight the complexity of reducing fat in different meat products from a technological and sensory point of view to minimize the changes that can lead to a product's rejection.

15.3 The Role of Fat in Processed Meats

Fat is one of the main constituents of meat and processed meat products, mainly affecting the sensory (flavor, juiciness, texture), physicochemical and technological characteristics (batter stability and shelf life, viscosity, yield), and microbiological safety, since it contributes to the reduction of water activity in certain processed products (Jiménez-Colmenero, 2000; Pearson and Gillett, 1996). Some of the most important functions are briefly discussed below.

15.3.1 Texture

One of the most important attributes of fat in processed meat products is its recognized role in texture properties, as it strongly affects the homogeneity, chewing, hardness, cohesiveness, crispness, and creaminess of the processed product (Drewnowski, 1997). In reduced-fat products, the texture characteristics are defined according to the technological strategy used, including the type of processing (emulsion formation, fermentation/dehydration, raw material injection/restructuring systems, baking systems), cooking, type of packaging, expected shelf life, raw materials, and fat substitutes, among others.

Several studies have shown a modification of the texture profile when using different types of fat substitutes in meat products (Mendoza et al., 2001; García et al., 2002; Cáceres et al., 2004; Nowak et al., 2007; Fernández-López et al., 2008; Salazar et al., 2009). In addition, when water is used to compensate for the reduced fat, the products tend to become stiffer and firmer, showing yield loss and changes in physicochemical, microbiological, and sensory stability. In fermented meat products, the decrease in fat levels provides a rubber-like texture (Keeton, 1994) and the external appearance of the fermented sausage, for example, is impaired by a wrinkled and hardened surface (Muguerza et al., 2002). Many studies have pointed out that the reduction of lipid content above 20% may result in an unacceptable product to the consumer due to texture defects, mainly accompanied by changes in flavor and appearance, short shelf life, and palatability defects (Yang et al., 2001).

In emulsified meat products, the reduction of saturated fat and/or reformulation of lipid profile by the addition of vegetable oils reduces emulsion

Downloaded From Vet-eBooks.com

stability, leading to loss of juiciness and liquid release during the shelf life of the products, with serious consequences on microbiological safety. In restructured products such as hamburgers, the cooking losses increase significantly, and texture is the most affected parameter (Yang et al., 2001), leading to rejection by consumers.

15.3.2 Flavor and Taste

In addition to texture, flavor and taste can also be altered by the fat reduction in meat products. The fat is responsible for the development of a characteristic flavor, which depends on several factors, including the composition of the animal raw material, processing steps, heat treatment, fermentation and drying stages, storage conditions, and packaging. The components that make up the lipid fraction can interact with amino acids, sugars, and minerals in desirable or undesirable reactions involving or not heat treatments. In fermented and/or ripened meat products, fat has a highly relevant role in the lipolysis reactions that define, along with proteolysis, the characteristics of a particular product, which are also used to differentiate items of the same category around the world.

Fat in the meat products acts as a carrier of the compounds responsible for the aroma, stimulating some senses during the food ingestion. The quantity, composition, and physical state are determining factors for a dynamic release of flavor components during consumption (Hort and Cook, 2007; Cierach et al., 2009).

The fat content is also related to the alteration of the perception of salinity, spicing, and smoking, which can drastically reduce the acceptance of these products when compared to traditional ones (Hughes et al., 1997; Chevance et al., 2000).

Reformulated meat products with replacement of animal fat (back fat) by vegetable oils require additional procedures, such as the use of herbs and spices to improve the final flavor (Tan et al., 2006; Pelser et al., 2007) and reduce the effects of a possible lipid oxidation. These reactions are particularly relevant when fat is a precursor in the generation of volatile compounds (Jiménez-Colmenero, 1996).

15.3.3 Physicochemical Properties

Fat provides various physical characteristics in the meat products, with the development of specific functional properties. Thus, several factors including crystallization capacity, changes in the melting point, and the hydrophobic interactions between meat components and water should be carefully evaluated in reduced-fat meat products.

In cooked meat emulsions, fat is associated with the formation and stability of the emulsion, improvement in the process yield, and increased water retention capacity, which directly interfere with the texture, color,

Downloaded From Vet-eBooks.com

and sensory acceptance of the processed products (Keeton, 1994; Jiménez-Colmenero, 2000). These effects result from the interaction of the saturated lipid fraction with the myofibrillar proteins extracted during the comminution step, under an adequate ionic strength. After cooking and cooling, a stable network containing water, protein, and fat is formed (Pietrasik and Duda, 2000; Yoo et al., 2007; Hughes et al., 1997). This set of reactions affects both the sensory and physicochemical properties of the processed meat products.

The color attributes and the overall appearance are commonly altered in reduced-fat meat products. Many studies have reported the development of darker coloring, probably due to the greater yield loss caused by the fluid exudation accompanying this cycle of events, as previously described in the texture changes, which make the products drier, harder, and more elastic (Cierach et al., 2009). The physicochemical changes may also affect the microbiological stability, since the liquid released from improperly conducted reformulations may accelerate the development of spoilage microorganisms in emulsified and restructured injected meats (Pearson and Gillet, 1996; Keeton, 1994), leading to short shelf life and negative effects on appearance. Some authors have suggested that reducing the fat content in meat products by more than 10%, especially in emulsified products such as sausages, leads to a reduction of palatability, besides increasing hardness and depreciating taste and aroma (Claus, 1991; Rust and Olson, 1988).

In fermented sausages, fat regulates the continuous water release from the inner layers of the product during the manufacturing process, which is very important for effective fermentation and drying. Thus, the fat reduction in fermented products provides greater weight loss since lean meat has a higher moisture content when compared to pork back fat, which is the most common fat source used in traditional formulations (Muguerza et al., 2002; Papadima and Bloukas, 1999).

The fat composition in meat formulations is a determining factor in the physicochemical properties of the processed products. Due to the higher melting point of certain animal fats, especially pork back fat, which is widely used in a variety of meat products, the reduction of total fat contents or their partial substitution by more unsaturated fats can lead to technological problems, probably due to a reduction in viscosity of the reformulated batters.

Heat treatment is another common operation in the manufacturing of emulsified products, and its extent can be affected by the reduced fat in the formulations. When fat is partially replaced by water, processing changes are required, including a longer cooking time for these products to reach the same internal temperature as conventional formulations. However, the increase in the cooking time or temperature to guarantee microbiological safety can change the palatability of the processed product (Jiménez-Colmenero, 2000), thus significantly affecting the product's acceptance (Giese, 1996).

Downloaded From Vet-eBooks.com

15.3.4 Perception of Satiety

From the physiological point of view, fat is responsible for several sensory impressions that include pleasure, overall palatability and, among the most recently studied, the feeling of satiety. Meat products are characterized by a high satiety index, largely due to their high fat content. The development of reformulated products with fat reduction with equivalent satiety index, that is, those products with greater capacity to inhibit appetite for a longer period after consumption, would attract the interest of industry and consumers (Halford and Harrold, 2012), constituting an additional technological challenge.

Satiety is the feeling of fullness that persists after eating, suppressing further consumption of food. Factors affecting satiety can be characterized according to cognitive, postingestion, and postabsorption sensory impressions. Initially, sensory and cognitive factors influence expectations about the food to be consumed, including aspects such as flavor, texture, and aroma or associations with previous experiences. As the food or drink reaches the stomach, postingestion effects begin. First, the distension of the stomach sends signals to the brain, initiating the sensation of satiety. As the digestion process continues in the intestines, hormones that promote satiety are released at this location. In the postabsorption stage, the nutrients are detected by specialized receptors in various parts of the body, including the brain, providing information on nutritional status that can also affect satiety (Blundell, 2006).

In this context, fat reduction should be accompanied by a product reformulation that produces physiological effects on satiety, constituting an additional challenge. Fibers are considered as suitable vehicles to fulfill this objective since they increase volume and weight due to their great water absorption capacity, and trigger gastric and postgastric mechanisms that send signals from the stomach to the brain, leading to the perception of satiety (Slavin, 2005).

Therefore, studies on soluble and plant-derived fibers such as mucilages should gain a foothold among advanced research in reducing fat in meat products. Reducing fat levels and preserving the perception of satiety of meat products is among the key challenges that must be achieved in obtaining healthier products.

15.4 Strategies for Reducing Fat in Meat Products

Despite the technological and sensory challenges, the development of meat products with reduced fat content is an irreversible trend in the industry worldwide. The use of substitute ingredients and the development of new processing technologies may be effective alternatives to maintain the

Downloaded From Vet-eBooks.com

identity of the reduced-fat meat product (Mendoza et al., 2001; García et al., 2002, Weiss et al., 2010).

The strategies for fat reduction vary for each category of products, according to their specific characteristics (raw materials, ingredients, processing aids, packaging, etc.). In addition, the sensory characteristics of the reformulated product should be accepted by the consumer, who can reject products with altered identity and quality standards. For a successful fat reduction in meat products, each category of processed product requires a substitute and/or manufacturing method that results in appropriate technological properties with low impact on overall quality. Nutritional factors, technological properties (lower water retention capacity), cost, acceptance, convenience, legislation, and marketing should also be considered. Some strategies to improve the lipid profile of processed meat products are described below.

15.4.1 Simple Fat Reduction and Selection of Lean Meat Cuts

One of the most common strategies to reduce fat in processed meat products, in a simple way, consists in the selection of leaner meat cuts, adipose tissue removal, and use of raw materials from animals fed diets aiming at the modification of fatty acids composition (Decker and Park, 2010; de Oliveira Faria et al., 2015). Although this reformulation is one of the pioneers in the development of low-fat meat products, some critical points should be investigated.

The selection of lean cuts and the reduction of the energy value cannot guarantee a better nutritional profile of the final products. According to Novakofski et al. (1989), the composition of the lean pork portion was not affected by a good meat toilet before grilling, due to the presence of the marbled fat in the cuts studied. In general, meat contains an approximate PUFA/MUFA ratio of 0.1, indicating an unbalanced fatty acids intake (Webb and O'Neill, 2008). On the other hand, the pork back fat, which is the main component to be reduced, has a PUFA/MUFA ratio of 0.4 more favorable when compared to the use of meat as a fat substitute in the formulation (Bernardi et al., 2016). In addition, the w6/w3 ratio, which is important to ensure many health benefits, is quite inadequate in meat and meat products, thus the consumption of foods that can improve this ratio has been widely recommended (Valencia et al., 2007).

Another limitation to reduce fat in meat products is the use of cuts without marbling, which implies the selection of a few parts of the carcass, mainly pork, which may not be economically feasible due to the high cost.

In addition to nutritional and cost constraints, simple fat reduction and the increase in meat content to compensate for fat in emulsified meat products gives rise to negative changes in appearance, flavor, and texture, such as increased red color intensity and hardness (Weiss et al., 2010). The safety of the product should also be considered when some of the fat is replaced with water and lean meat since the product's stability can be compromised.

Downloaded From Vet-eBooks.com

15.4.2 Use of Fat Replacers

Many ingredients of different chemical classes can be used as fat replacers in processed meat products. Its ranking varies greatly since some compounds have more than one specific property to define it. In essence, for an ingredient to be considered a fat replacer, it should contribute a minimum of calories without drastically altering the sensory and functional characteristics of the product, in addition to being in compliance with the current legislation and having a low cost (Hachmeister and Herald, 1998).

According to Akoh (1998), fat replacers can be classified into two groups. The first are fat substitutes, defined as macromolecules that resemble triacylglycerols in the physicochemical aspect, that can theoretically replace fat in foods, by weight. They are chemically synthesized or derived from conventional fat or oils by enzymatic modification and must be stable on cooking and freezing. Second are fat mimetics, defined as substances that mimic the sensory or physical properties of triacylglycerols, but cannot replace by weight. They are made from proteins or carbohydrates present in common food components, which undergo physicochemical modifications to provide the fat characteristics. In general, these ingredients have no stability to freezing and heating at high temperatures, due to their high water-binding ability.

The wide variety of fat replacers available in the market can be commercially classified into protein extenders (high protein) and nonprotein extenders (high carbohydrate). Protein extenders contribute to increased water retention, gelling, and emulsification of fats, whereas the nonprotein extenders contribute significantly to a higher water retention capacity and are less involved in fat emulsification (Lucca and Tepper, 1994; Brewer, 2012). The following sections discuss fat replacers according to their importance as commonly used ingredients, as well as those that represent nutritional trends with respect to healthier calories and calorie reduction.

15.4.2.1 Gums

Gums are high-molecular-weight carbohydrates classified as hydrocolloids, used as thickeners and/or gelling agents in various meat products, generally in low concentrations. The gums most commonly used in low-fat meat products include carrageenan, agar, alginate, gum arabic, galactomannan (guar gum and locust bean), and xanthan gum, because of their water retention properties (Candogan and Kolsarici, 2003). Although some authors classify these compounds as soluble fibers, this chapter assesses them as a differentiated item, considering their technological relevance and citation in works in the literature.

Carrageenan gum has been widely used as a fat substitute and consists of a group of sulfated polysaccharides extracted from various red algae formed by three major kappa, iota, and lambda fractions. The kappa and iota fractions form thermoreversible gels, while the lambda fraction does not form a

Downloaded From Vet-eBooks.com

gel. Because of its ionic nature, the formation of kappa and iota carrageenan gel is strongly influenced by the presence of electrolytes, showing synergy with other gums. The kappa carrageenan and calcium ions form a rigid, thermoreversible, and highly resistant gel; in contrast, an elastic gel is formed with potassium ions. The iota carrageenan produces a flexible gel, stable to freezing, and forms an elastic gel in the presence of calcium ions (Shand et al., 1990). The functional properties of carrageenan include the water retention capacity and better slice ability, constituting an excellent fat substitute (Bater et al., 1992; Brewer, 2012; Egbert et al., 1991; Trius et al., 1994; Cierach et al., 2009); thus, it has been applied in a broad category of meat products such as hamburgers, sausages, and hams.

Galactomannans (guar gum and locust) can be used as fat substitutes for their ability to control viscosity through water retention, which is relevant in low-fat products that do not form gels (Setser and Racette, 1992). Guar gum produces solutions with high viscosity, with good stabilizing effect and water retention capacity. It confers greater juiciness and creaminess to meat products as it is able to interact synergistically with xanthan gum. Locust gum is soluble in cold water with a maximum viscosity at 95°C, which limits its use in meat products, other than when used in combination with other gums, for example, xanthan gum, when it forms very elastic gels after heating and cooling (Symes, 1980). Lurueña-Martínez et al. (2004) showed that a blend containing xanthan gum and locust gum was effective in reducing fat of Frankfurt sausages, reducing cooking losses, and improving emulsion yield and stability, due to its ability to form hydrogen bonds with water, without affecting the sensory properties of the product.

Several other hydrocolloids derived from algae can be used to stabilize meat batters. Jiménez-Colmenero et al. (2010) used edible algae, and Konjac used algae derivatives as fat substitutes in sausages and found that the emulsion stability was dependent on the replacement ratio. That study demonstrated that a fat substitution up to 15% showed no visible changes in the sensory quality of sausages.

15.4.2.2 Starch Derivatives

Starch is one of the most widely used ingredients in meat processing due to its extensor properties, low cost, and high availability in the production chain. Its properties include increased water retention capacity, reduction of cooking losses, emulsion stabilization, positive effect on the texture of some meat products, and cost reduction, among others (Carballo et al., 1995; Claus and Hunt, 1991; Khalil, 2000; Lyons et al., 1999).

This ingredient is composed of two main fractions, amylose and amylopectin, but its main gelatinization property depends mainly on the content of the amylose component, responsible for forming hydrogen bonds for the construction of the three-dimensional network and the water retention capacity. After gel formation and cooling, the retrogradation of the starch

Downloaded From Vet-eBooks.com

gels is a phenomenon commonly characterized by the reassociation of the gelatinized molecules, causing water release known as syneresis and formation of precipitates that can reduce the quality and safety of vacuum-packed and sliced products. The intensity of the retrogradation depends on the amylose content in each type of starch, increasing in the following order: cassava > rice > potato > maize > wheat (Murphy, 2000).

Starches may undergo chemical or physical modifications such as acid or enzymatic hydrolysis, oxidation, dextrinization, cross-linking, or monosubstitution to alter their functional properties, becoming ingredients widely used in different segments of food processing (Shand et al., 1990).

Native potato and cassava starches have limited dispersibility in cold water and are applied in emulsified products to reduce cooking losses, and increase yield and shelf life, whereas the modified potato starches produce a firm texture gel with potential application in injected, tumbled, and marinated meats, with a strong synergistic effect with soybean proteins. Regarding cassava starch, Hart and Price (1993) also observed an increase in the juiciness of low-fat cooked meat products, probably due to the greater water retention capacity. Rice starch is characterized by its more neutral flavor.

Maltodextrin, resulting from the partial hydrolysis of corn or potato starch, is also considered Generally Recognized As Safe (GRAS) and presents a great application in meat systems requiring increased viscosity, particularly in emulsified products, since it does not gel, favoring filling operations (Akoh, 1998).

15.4.2.3 Protein-Based Fat Replacers

Protein-based fat replacers can be from milk, egg, whey, or plant proteins (Giese, 1996). Some of these proteins are microparticulate (denatured by heating) and form microscopic coagulated round particles that mimic the mouth feel and texture of fat, providing lubricity and better palatability (Roller and Jones, 1996). Low-molecular-weight proteins can develop fat-like physical properties and favorably modify the texture of a lipid-lowering product.

The most commonly used fat substitutes in the meat industry are soy protein isolates, concentrates, and texturized soy protein; defatted milk powder, caseinates, and whey proteins; and wheat flour and gluten. Flours (50% protein) and concentrates (70% protein) are used at levels of 3%–5% (on dry basis), while the isolates (90% protein) are limited to 2% of the total formulation. Each country has regulatory standards to control these percentages and to curb fraud by reducing the content of meat raw materials while maintaining the protein content in a particular meat product.

The use of by-products of the meat production chain such as porcine and bovine skin gels has been explored as potential fat substitutes to add value to the meat and meat production chain since they constitute rich sources of collagen (Alves et al., 2016). Pintado et al. (2016) evaluated different strategies for the incorporation of chia flour (10%) and olive oil in frankfurters and found

Downloaded From Vet-eBooks.com

an increase in fiber and linolenic acid levels, reduction of purge losses, and a 26% reduction of fat in all samples.

15.4.3 Use of Fibers as Functional Ingredients and Fat Replacers

Functional ingredients have been recognized for their beneficial physiological effects on human diet, and are widely used to modify nutritional properties and aggregate healthier consistent appeals in many processed food products (Jiménez-Colmenero et al., 2001). Studies have shown changes in the nutritional profile of meat products as delivery vehicles for bioactive compounds (Zhuang et al., 2016; Alves et al., 2016; Turp, 2016), such as phenolic compounds, flavonoids, PUFAs, prebiotics, and probiotics.

In addition to the high fat content of meat and meat products, one of the main complaints is the absence of fibers in their composition. The World Health Organization has recommended that more than 55% of the energy consumed by humans come from carbohydrates, suggesting fibers as a part of food intake (Talukder, 2015). The American Dietetic Association recommends the intake of 25–30 g of fiber/adult/day, or 10–13 g/1000 kcal, and the insoluble to soluble fiber ratio should be 3:1. In Europe, the recommendation is 20 g fiber/day/person (Borderías et al., 2005). The USDA and US Food and drug administration (USFDA) have established the consumption of 38 g fiber for men and 26 g for women (Mehta et al., 2015).

Thus, the use of plant fibers of different botanical origins to reformulate meat products may be a promising alternative, since they can make the product healthier as a fat substitute and add value due to thier physicochemical properties (Weiss et al., 2010). In this context, dietary fibers can be very useful for their technological properties in meat products, which briefly include (1) increasing water holding capacity, (2) fat binding capacity, (3) increasing viscosity, (4) gelling properties, (5) chelating effects, (6) fermentative capacity, (7) positive effects on texture and slicing, (8) stabilization in frozen products, and (9) prevention of deformation and shrinkage of restructured products during cooking (Fernández-López et al., 2008; Sendra et al., 2008; Campagnol et al., 2013; Choi et al., 2009).

15.4.3.1 Insoluble Fibers

The water solubility of dietary fibers is one of the criteria most used for its classification. Soluble fibers consist of pectins, gums, mucilages, alginates, beta-glucans, and some hemicelluloses, whereas insoluble fibers are represented by lignin, cellulose, and most hemicelluloses (Chawla and Patil, 2010; Brennan, 2005).

Insoluble fibers increase the volume of the fecal bulk, decreasing the time of intestinal transit, facilitating fecal elimination, and also contributing to glucose absorption and retarding the starch hydrolysis. This effect on intestinal regulation makes them relevant to well-being and contributes to

Downloaded From Vet-eBooks.com

the reduction of the risk of various pathologies (Maffei, 2004; Brennan and Cleary, 2005; Mira et al., 2009).

Despite their wide availability, insoluble fibers require very careful studies for their application in meat products, since they may interact negatively with ingredients of the meat matrix, depending on the type of process used. Grittiness, loss of texture, and development of off-flavors are the most common defects, especially in emulsified and fermented meat products (Catalani et al., 2003). On the other hand, the technological advantages are related to the high water and fat holding capacity, as well as cost reduction. Grains and their derivatives, such as whole flours and bran, along with fruits and vegetables, are the main sources of dietary fiber and are among the most studied for application in meat products. Table 15.1 shows some of the major studies on the addition of insoluble fibers in meat products and the critical points of this reformulation.

TABLE 15.1

Insoluble Fibers from Cereals, Legumes, and Fruits as Fat Substitutes in Processed Meats

Insoluble Fiber Source (%)	Meat Product	Effects Resulted from Fiber Addition	Reference
Oat bran (5%–20%)	Meatballs	Lower total fat and trans-fatty acid contents, higher protein, salt and ash contents, lightness, yellowness, and lower moisture and redness. High acceptability.	Yilmaz and Dağlıoğlu (2003)
Oat fiber (13%, 45%)	Beef patties	Higher cooking yield and fat and moisture retention due to the water binding capacity of β-glucan. No changes in appearance, tenderness, and color.	Piñero et al. (2008)
Soy flour (5%), rice flour (5%)	Nuggets	Both soy flour and rice flour provided reduced oil absorption. Soy flour had the highest apparent viscosity and was found to be an effective ingredient in improving batters in crispness and color.	Dogan et al. (2005)
Oat bran (0%, 2%, 4%, 6%)	Beef patties	No changes were observed for protein and fat. Reduction of moisture content in raw patties but increase in cooked patties with improvement of sensory quality.	Serdaroglu (2006)
Carrot fiber (3%, 6%, 9%, 12%)	Sobrassada	Over 3%, the fermentation process in these samples was not successful and the textural parameters were critically affected. Only over 6% was the lipolytic process was influenced.	Eim et al. (2008)
Orange fiber 1% and 2%	Spanish salami	No changes during fermentation and dehydration stages, reducing residual nitrite.	Fernández-López et al. (2008)

(*Continued*)

Downloaded From Vet-eBooks.com

TABLE 15.1 (*Continued*)

Insoluble Fibers from Cereals, Legumes, and Fruits as Fat Substitutes in Processed Meats

Insoluble Fiber Source (%)	Meat Product	Effects Resulted from Fiber Addition	Reference
Cereal grain (wheat and oat) and fruit (peach, apple, and orange) (1.5%–3.0%)	Fermented sausage	Reduction of 35% energy value. Sensory and texture properties of sausages with 3% dietary fiber were not good, due to high hardness and cohesiveness value. The best results for organoleptic characteristics were obtained for 1.5% fruit fiber.	García et al. (2002)
Soy fiber 1% and 2%	Brazilian salamis	No changes in global and technological properties up to 1% addition.	Campagnol et al. (2013)
Wheat fiber 2.5%–5.5%	Hamburger	No changes in sensory properties	Mansour and Kalil (1999)
Rye bran 2.1%–2.95%, oat bran 4.3%–6.0%, and barley fiber 1.3%–1.7%	Sausages and meatballs	Rye bran was more suitable in meatballs due to its particulate nature; oat bran was the best alternative in low-fat sausages due to its gelling ability upon heating. The barley fiber (highest amount of soluble β-glucan) resulted in higher losses and the lowest firmness.	Petersson et al. (2014)
Apple pulp 8%, 10%, 12%	Reduced sodium chicken nuggets	Increasing pH and dietary fiber content, redness, yellowness, and chroma index. Sensory evaluation showed significant reduction in texture, but overall acceptability of the treated samples, with scores in the range of very good.	Verma et al. (2010)
Wheat, rye, oat, and corn bran 5%–20%	Meatballs	Decreasing in weight loss; overall acceptability decreased as bran content increased specially due to higher firmness; redness of meatballs gradually decreased and lightness increased as the level of oat bran increased from 5% to 20%.	Yasarlar et al. (2007)
Lemon albedo 2.5%–10%	Bologna sausage	Good sensory acceptance of the fat-reduction treatments. Sausages containing containing 2.5%, 5.0%, or 7.5% cooked albedo had similar sensory properties when compared to the control.	Fernández-Ginés et al. (2004)
Barley flour (4%, 7%, 10%)	v	Increase in cooking yield and moisture retention. The incorporation of 4% barley flour had higher flavor and texture scores when compared to 7% and 10% levels.	Kumar and Sharma (2004)
Wheat bran (5%, 10%, 15%, 20%)	Meatballs	Lower total trans-fatty acids and higher total unsaturated fatty acids to total saturated fatty acids ratio. Samples with wheat bran added were lighter and yellower than the control.	Yilmaz (2005)

(*Continued*)

Downloaded From Vet-eBooks.com

TABLE 15.1 (*Continued*)

Insoluble Fibers from Cereals, Legumes, and Fruits as Fat Substitutes in Processed Meats

Insoluble Fiber Source (%)	Meat Product	Effects Resulted from Fiber Addition	Reference
Pea flour	Chicken nuggets	Higher emulsion stability up to 10% levels; decrease in moisture, protein, and fat contents and shear force value at 10% pea flour level. No significant changes in the sensory properties.	Singh et al. (2008)
Peach dietary fiber suspensions (17% and 29%)	Frankfurter	No changes in protein and collagen contents; increase of viscosity of the meat batters at two levels. Dietary fiber was effective in retaining water in the final product.	Grigelmo-Miguel et al. (1999)
Tomato, beet root, and inulin (1%, 2%, 3%)	Chopped chicken products	Increase of water-holding capacity and reducing in cook loss for tomato fiber; significant changes in color and texture parameters (higher hardness); tomato fiber reduced the extent of lipid oxidation.	Cava et al. (2012)
Chia (*Salvia hispanica* L.) (10%)	Frankfurter	Increase of total dietary fiber and minerals; reduction in fat and energy content (>265); lower purge; good sensorial acceptance and suitable for health claims.	Pintado et al. (2016)
Pea flour, starch-rich and fiber-rich fractions	Low fat bologna	Adding fibers improved the poor texture of low fat bologna sausage and increased water holding capacity. Consumer acceptance was not influenced, but pea fiber had lowest texture and flavor.	Pietrasik and Janz (2010)
Brown rice fiber (1%, 2%, 3%, 6%)	Pork meat emulsion systems with canola oil	Lower emulsion stability and higher cooking yield and hardness of meat emulsion systems were observed in all treatments. Partially replacing pork back fat with a mix of 10% back fat, 10% canola oil, and 2% brown rice fiber was most similar to the control with 30% pork back fat.	Choi et al. (2015)

Bran is among the most studied fibers. Rice bran is mainly composed of arabinoxylans and cellulose (Andersson et al., 2003), while oat and barley bran, in addition to their insoluble fractions, are rich in β-glucans, soluble fibers related to the reduction of plasma cholesterol when consumed regularly (Beer et al., 1997).

Fibers from beets, soybeans, carrots, pears, apples, oranges, and peas, among others, have been studied as ingredients for the reformulation of meat products, especially in hamburgers and sausages (Table 15.1). In most

Downloaded From Vet-eBooks.com

applications, despite the good performance in the functional properties (Jiménez-Colmenero et al., 2001), some attributes such as flavor and texture can be negatively affected.

Various types of cellulose can be obtained industrially by different processes and used as fat replacers. Microcrystalline cellulose is considered GRAS and noncaloric, contributes to a product's consistency, and acts as emulsion stabilizers, besides controlling baking losses, and conferring viscosity in emulsified products. Various commercial celluloses are available in the market, for example, Z-trim® and Oatrim®, obtained from oat, soy, corn, or wheat by-products, which have been widely used in meat products (Warner and Inglett 1997; Akoh, 1998, Campagnol et al., 2012).

One of the major challenges in the use of insoluble fibers is the sensory acceptance and the regulatory aspects that vary between countries since they can be considered fraud promoters due to their wide water holding capacity. Insoluble fibers can be incorporated into restructured products such as hamburgers and nuggets, as they are compatible with the typical texture of such products.

15.4.3.2 Soluble Fibers

In general, soluble fibers have similar effects to the insoluble fibers in small intestinal transit, and consequently, reduce the rate of digestion and absorption of macronutrients, resulting in higher glucose tolerance and higher starch content available for colonic fermentation. They also indirectly affect the absorption of lipids by the organism, due to their capacity to sequester the bile salts (Roberfroid, 1993). Many studies have suggested that the greatest physiological benefit of soluble fibers results from the action of short-chain fatty acids, which, when in the bloodstream, reach the liver and/or peripheral tissues, causing a decrease in postprandial glycemia, thus reducing the free fatty acid levels (Trowell et al., 1976; Prosky and Hoebregs, 1999; AACC, 2001).

In addition to the solubility criterion, the fibers can be classified based on their physiological properties as digestible oligosaccharides (DOs) and nondigestible oligosaccharides (NDOs), according to the anomeric carbon in the monosaccharide, which makes them available or not for the hydrolytic activity of the digestive enzymes (Roberfroid and Slavin, 2000). Great prominence has been given to NDOs for their important physicochemical properties and health benefits, including the prebiotic effect due to their ability to stimulate the growth of *Bifidobacteria* (Saad, 2006; Mussatto and Mancilha, 2007; Finocchiaro et al., 2009; Monsivais et al., 2011). Some of the most important NDOs for use in meat products are inulin (IN), fructooligosaccharides (FOSs), resistant starch (AR), and polydextrose (PD), which are briefly described below; select applications in processed meats are given in Table 15.2.

Downloaded From Vet-eBooks.com

TABLE 15.2

Using FOS, IN, RS, and PD as Fat Replacers in Meat Products

Prebiotic Fiber	Product	Effects on Meat Products	Reference
FOS (3%, 6%, 9%)	Pepperoni	Reduction of 50% pork back fat without altering texture due to the ability of FOS to form a gel at the interface of meat and fat particles. Good sensorial acceptance and microbial stability.	Dos Santos et al. 2012
FOS (2%–12%)	Sausages	Reduction of 40% fat and 35% energy value; good acceptance scores for 12% FOS; no changes in texture, pH, aw, and weight losses.	Cáceres et al. (2004)
FOS (2%, 4%, 6%)	Dry fermented sausage	50% and 80% fat reduction with good sensorial properties, but with few changes on texture and color as reduction of hardness and increase in lightness.	Salazar et al. (2009)
FOS (2.5% and 5.0%)	Meatballs	No changes in the texture properties (2.5% and 5.0%); higher scores were observed for 2.5% regarding sensory properties when compared to the control without addition of FOS.	Ergönül et al. (2009)
IN	Mortadella	Reduction of up to 47.5% in energy value with use of 12% IN; increase in hardness and adhesiveness, dark color as negative effect.	Nowak et al. (2007)
IN (powder/gel) (2.5%–7.5%)	Mortadella	Powdered IN increased the hardness of reduced fat in sausages even at 2.5% level while the gel form affected texture of product only at 7.5% level. Although the sensory properties remained similar for all products, IN in the form of gel was preferred.	García et al. (2006)
IN (5%, 10%, 15%, 20%)	Veal meatball	At 20% IN, significant changes in physicochemical properties. Lower sensory scores observed with 10%, 15%, and 20% IN were due to hardness, low juiciness, and low flavor intensity. 5% level was found to be optimum.	Yilmaz and Geçgel (2009)
IN	Dry fermented chicken sausage + corn oil	No changes in the physicochemical and microbiological parameters and the product's acceptability, but resulted in an altered texture profile. The products were stable during storage at 4°C for 45 days for physicochemical and microbial parameters.	Menegas et al. (2013)

(*Continued*)

Downloaded From Vet-eBooks.com

TABLE 15.2 (*Continued*)

Using FOS, IN, RS, and PD as Fat Replacers in Meat Products

Prebiotic Fiber	Product	Effects on Meat Products	Reference
IN	Cooked meat batters	Better stability of emulsion with addition of water to replace fat, reduction of cooking losses when using the IN powder and greater creaminess and softness when using the gel form.	Álvarez and Barbut (2013)
IN	Comminuted meat products	Better softness and overall texture, emulsion stability, with no changes in sensory quality, improvement of lipid profile by substitution of pork back fat for vegetable oil.	Keenan et al. (2014)
IN	Low salt Pamplona-style chorizo	The reformulated chorizo with olive alginate emulsion and 6% IN resulted in a product with 20% less fat than the control, 10% richer in MUFAs, and sensory properties similar to the traditional chorizo used as a control, achieving a good acceptability rating.	Beriain et al. (2011)
IN (7.5%–12.5%)	Dry fermented sausage	The IN led to a softer texture and tenderness, springiness, and adhesiveness very similar to that of conventional sausages. A low-calorie product (30% of the original) was obtained with approximately 10% IN without significant changes in sensory properties.	Mendoza et al. (2001)
PD + FOS + IN + RS (3% and 6% for each one)	Mortadella	Prebiotic fibers reduced the emulsion stability of mortadellas when cassava starch was suppressed from the formulations. There was a significant increase in softness. The gelation process of myofibrillar proteins occurred at lower temperatures when compared with control.	Felisberto et al. (2015)
PD	Nuggets	Increased juiciness and moisture with preservation of crispness after cooking.	Stowell (2009)
PD (5% and 10%)	Hamburger	Lower cooking losses and reduced firmness. Cohesiveness was similar to the control formulation (20% fat), even with the addition of PD in combination with potato starch, beet sugar, oat fiber, or pea fiber.	Troutt et al. (1992)

(*Continued*)

Downloaded From Vet-eBooks.com

TABLE 15.2 (*Continued*)
Using FOS, IN, RS, and PD as Fat Replacers in Meat Products

Prebiotic Fiber	Product	Effects on Meat Products	Reference
PD	Cod surimi	Cryoprotectant effect of myofibrillar proteins on cod surimi, due to the high availability of hydroxyl groups, which act on the hydrogen bonds with the protein chains, leading to a greater hydration of the protein chains and reducing aggregation.	Sych et al. (1990)
RS	Sausage	Addition of resistant starch led to an undesirable effect on the sensory properties and cooking yield, with increased hardness. However, an appropriate technological performance was observed when RS was combined with beta-glucan, contributing to the prebiotic appeal of the meat product.	Sarteshnizi et al. (2015)

FOS, fructooligossacharide; IN, inulin; MUFAs, monounsaturated fatty acids; RS, resistant; PD, polydextrose.

15.4.3.2.1 FOS and IN

IN is a soluble fiber, formed by fructose units joined by linear bonds with a terminal glucose unit, and can be found in several plants as an energy reserve, especially in chicory (Meyer et al., 2011). FOSs are oligosaccharides known as unconventional sugars that originate from plants, and their natural biosynthesis occurs in asparagus, beet, garlic, chicory, onion, wheat, barley, and rye (Spiegel et al., 1994; Wang, 2009). FOS can be produced either by enzymatic hydrolysis of IN or by transfructosylation of sucrose, consisting basically of linear units of fructose, with or without the final glucose unit (Passos and Park, 2003).

From the chemical point of view, there is no difference between IN and FOS: both are fructose oligomers whose monomers are joined by β-(2,1) glycosidic bonds. However, while IN chains contain 2 ± 60 units, the length of FOS chains ranges from 2 to 10 units with an average degree of polymerization of four. The molecular weight and size of the chains of these two compounds are responsible for their different functional physicochemical properties. The long IN chain forms microcrystals when mixed with water. On the other hand, the FOS present short chains, which are hygroscopic, soluble, and have good water retention capacity (Niness, 1999).

Both IN and FOS travel through the upper intestinal tract and can be fermented in the colon and selectively used by certain types of beneficial bacteria, such as *Bifidobacterium* and *Lactobacillus*, reducing the number of undesirable bacteria, such as *Clostridium* and *Escherichia*. For these reasons, both fibers are considered prebiotic ingredients (Gibson et al., 1995; Tungland and Meyer, 2002).

Downloaded From Vet-eBooks.com

Many studies have reported the use of IN and FOS as fat replacers in various categories of meat products, as shown in Table 15.2. They are highly stable, supporting pH above 3 and temperatures above 140°C (Bornet, 1994). FOS, in particular, can be used at higher levels because of its greater solubility when compared to IN (Dos Santos et al., 2013). In fermented products, FOSs do not undergo hydrolysis, preventing the development of foreign microorganisms during the fermentation process.

IN can be incorporated at high levels into emulsified meat products without considerable sensory changes, acting as an excellent fat replacer. Because of its ability to form viscous gels, some technological problems can occur when IN is mixed with fat in the cutter, which can be minimized with a correct formulation and the order of addition of the ingredients. Flavor and objective texture measured by texture profile analysis (TPA) are the attributes most affected in emulsified and fermented meat products. Although there are few studies in the literature, both FOS and IN can be components of brines in injected products aimed at the elaboration of healthier restructured meat products with fat reduction.

15.4.3.2.2 Polydextrose

PD is a compound formed by the polycondensation of approximately 89% D-glucose, 10% sorbitol, and 1% citric or phosphoric acid. Due to its glycosidic bonds, the molecule has a complex and compact structure which resists enzymatic digestion in the body; it has a prebiotic effect and ensures a healthy and balanced intestinal microbiota, thus positively affecting the physiological functions (Stowell, 2009). Its mechanism of action is similar to that of the other soluble fibers such as pectins, β-glucans, and IN since they contribute to lower cholesterol levels and blood glucose (Montenegro et al., 2008). PD also has a low energy value (1 kcal/g), low glycemic index, and evidence of reduced risk of colon cancer (Saad, 2006).

In some countries, it has achieved additive status due to its texturizing, stabilizing, thickening, and humectant properties (Burdock and Flamm, 1999), which gives it favorable technological properties as a functional filler. In comminuted and restructured meat products, such as hamburgers, it can be used to replace fat without compromising flavor and aroma; this allows for the creation of low-fat products with reduced energy value (Stowell, 2009) and increased juiciness that maintain the crispness of the product even after cooking.

15.4.3.2.3 Resistant Starch

Resistant starches are defined as compounds capable of resisting digestion by the small intestine, passing into the large intestine, where they act as a dietary fiber (Annison and Topping, 1994; Champ, 2004; Finocchiaro et al., 2009) presenting a different behavior when compared to the conventional highly digestible starches. According to their resistance ratio, they can be classified into five types. Resistant starch type I (RS I) is physically inaccessible, since it is trapped in plant tissues, and is not susceptible to enzymatic

Downloaded From Vet-eBooks.com

hydrolysis, such as starches from whole grains; the resistant starch type II (RS II) is defined as native granular, occurring in raw grain and having type B polymorphism, such as potato, peas, and high amylose starches, also resistant to enzymatic hydrolysis; type III (RS III) is retrograded starch, that is, it has been cooked and cooled. All these types of starches can be found naturally or produced during processing, whereas resistant starch type IV (RS IV) is produced only by chemical modifications, providing linkages nondigestible by the digestive enzymes (Li et al., 2008; Adam-Perrot et al., 2009). On the other hand, resistant starch type V (RS V), developed more recently, is formed by an amylose-lipid complex, for example, through the complexation of maize starch with high amylose and lipid contents (Hasjim et al., 2010).

This resistant portion of the starch passes through the colon and is used by microflora as energy for growth and fermentation, producing short-chain fatty acids (Champ, 2004; Hasjim et al., 2010), accompanied by a reduction of pH, increasing the blood flow of the colon, and reducing the development of abnormal cells, with effects on the reduction or in the treatment of colon diseases, such as colon cancer and inflammatory diseases (Raigond et al., 2015). Studies have shown its effectiveness on the reduction of blood glucose levels and absorption of nutrients, such as calcium and magnesium, due to the higher solubility of these minerals in the cecum and large intestine, which is promoted by the acidification induced by fermentation.

Some resistant starches are commercially available, for example, resistant starches type II (RS II), which include products with higher resistant starch levels and smaller particles. Hi-Maize® 260 is a high-amylose-resistant corn starch produced without chemical or enzymatic treatment, containing at least 60% dietary fiber. When subjected to moderate processes, its granules remain resistant and its organoleptic characteristics intact (Finocchiaro et al., 2009).

The performance of resistant starches as fat substitutes is due to the extensor properties and low energy value. In addition, RS does not change the flavor, texture, and appearance of foods (Yue and Waring, 1998).

Carraro et al. (2012) have reported the technological benefits of using RS by replacing swine fat with RS II, which exhibited the best performance among all resistant starches tested. Felisberto et al. (2015) evaluated the rheological properties of RS II as a substitute for fat and cassava starch and reported a loss of texture in bologna sausages with 50% reduction of pork back fat and the addition of 6% RS II. Although the emulsion stability of the meat batters was also affected, with higher liquid release after the cooking step, the products were considered quite stable. The authors suggested the use of an extender associated with the RS into less robust reformulations.

The use of resistant starches as fat substitutes in meat products should be investigated due to their prebiotic (Sajilata et al., 2006) and technological properties, including their swelling, gel-forming and water-binding capacities, and increased viscosity. In breaded products, resistant starches can act

Downloaded From Vet-eBooks.com

as a coating ingredient as predust and breader due to their crispness property, providing an excellent opportunity to promote healthy approaches

15.4.4 Lipid Reformulation Using Vegetable Oils

The improvement of the fatty acid profile of processed meat products as well as the reduction of total fat and, mainly, saturated fat, has been the subject of much research and is a great challenge for the meat industry (Hur et al., 2008; Jiménez-Colmenero, 2007; Hoz et al., 2004; Ansorena and Astiasaran, 2004).

The addition of vegetable or marine oils to meat products is a relevant strategy to introduce a new source of PUFAs into the diet with a potential health benefit, since the body needs a balance between the consumption of saturated, monounsaturated, and PUFAs (López-López et al., 2009).

Vegetable oils reported in the numerous studies in the literature were selected for their sources of MUFA and PUFA (Pelser et al., 2007; Serrano et al., 2005). Although some of these oils have been used to increase MUFA levels, others have been used essentially to increase PUFAs, or more specifically ω-3, as observed for flaxseed oil (Ansorena and Astiasaran, 2004; Rubio et al., 2008). Table 15.3 shows some relevant studies on the improvement of the fatty acid profile in meat products. Olive oil, rich in oleic acid (C18:1), is one of the most studied vegetable oils, and is used in meat products to

TABLE 15.3

Reformulating Lipid Profile of Meat Products by Adding Vegetable Oils

Authors	Processed Meat Product	Vegetable Oil/l/% Reduced or Replaced Animal Fat	Most Important Results
Muguerza et al. (2002)	Dry fermented sausages	Olive oil/10%, 20%, and 30% pork back fat	Changes in lightness and yellowness but the products had a high sensorial score for odor and taste for 20% olive oil. However, the appearance was just acceptable because the surface was wrinkled and case hardening had developed.
Kayaardı and Gök (2004)	Soudjouk (sucuk)	Pre-emulsified fat (soy protein isolate)/20%, 40%, and 60% of beef fat	Moisture, cholesterol, and pH decreased. No significant difference in appearance, texture, color, and general acceptability upto 40% olive oil. The highest TBA value was found with the 60% olive oil replacement. Oleic and linoleic acid contents were high while other fatty acid contents were low for the 40% and 60% olive oil samples compared with controls.

(*Continued*)

Downloaded From Vet-eBooks.com

TABLE 15.3 (*Continued*)

Reformulating Lipid Profile of Meat Products by Adding Vegetable Oils

Authors	Processed Meat Product	Vegetable Oil/l/% Reduced or Replaced Animal Fat	Most Important Results
Lurueña-Martínez (2004)	Frankfurters	Olive oil added to locust bean/xanthan gum (0.5% and 0.6%)/25% and 40% pork back fat	The reformulation did not affect emulsion stability, jelly and fat separation, processing yield, cook loss, texture, and sensory characteristics.
Muguerza et al. (2003)	Dry fermented sausages	Pre-emulsified soy oil/15%, 20%, and 25% of pork back fat	Significant increase in polyunsaturated fatty acids (25%) and reduction in saturated fatty acids. No increase in lipid oxidation. There were no considerable effects on hardness and springiness, color, and sensory attributes.
Severini et al. (2003)	Salami	Extra-virgin olive oil/33.5%, 50%, and 66.5% of pork back fat	Significant effects on water activity and texture. No changes in the shelf life regarding both oxidation and loss of organoleptic quality. The formulation with 5% olive oil (33%, 3% substitution of pork back fat) was judged best.
Ansorena and Astiasaran (2004)	Dry-fermented sausages	Linseed/25% pork back fat	Increased P/S ratio from 0.4 (control) to 0.6–0.7 (with linseed oil). The $n-6/n-3$ ratio decreased from 14.1 (control) to 1.7–2.1 in modified products as a consequence of the α-linolenic acid increment. No oxidation changes were detected during the ripening process.
Rodríguez-Carpena (2012)	Porcine burger patties	Avocado, sunflower, and olive oils/100% pork back fat	Lowered percentage of SFA (from 36.96% to ~25.30%) and reduction of the atherogenic index (from 0.41 to ~0.24) and significantly lower amounts of lipid and protein oxidation products than control patties. Avocado oil contributed with specific aroma-active terpenes to patties and had a significant impact on particular color and texture parameters.
Tan et al. (2006)	Chicken frankfurters	Palm oil and palm stearin/chicken fat	Good emulsion stability and strong gels after heating; the increment of palm oil raised hardness, chewiness, and shear hardness; the reduction of CF in the formulation resulted in lower scores in chicken flavor, juiciness, oiliness, and overall acceptance of the frankfurters.

(*Continued*)

Downloaded From Vet-eBooks.com

TABLE 15.3 (*Continued*)

Reformulating Lipid Profile of Meat Products by Adding Vegetable Oils

Authors	Processed Meat Product	Vegetable Oil/l/% Reduced or Replaced Animal Fat	Most Important Results
Choi et al. (2009)	Meat emulsion systems	Olive, corn, soybean, canola, or grape seed, and rice bran fiber/66.6% pork back fat	Higher values for moisture, protein, ash content, uncooked and cooked pH values, b^*-value, hardness, cohesiveness, gumminess, chewiness, and viscosity compared with control containing 30% pork back fat. Decrease of cooking loss and better emulsion stability.
Youssef and Barbut (2009)	Comminuted meat products	Canola oil or pre-emulsified canola oil (soy protein, caseinate, or whey protein isolate)/25.0%, 17.5%, and 10.0% beef fat	Canola oil or pre-emulsified treatments showed a positive effect on improving yield and textural parameters, but resulted in a significant reduction in redness and meat batters became darker. The pre-emulsification oil helps to produce more stable meat matrix.
Hur et al. (2008)	Pork patty	Virgin olive oil (isolated soy protein and carrageenan)/50% pork back fat	Reduction in water-holding capacity in treatments control and in those containing isolated soy protein and carrageenan, and increase in hardness in samples with virgin oil. Sensory evaluation scores were higher in control compared to virgin olive oil-substituted pork patty samples.
Paneras and Bloukas (1994)	Frankfurter	Olive, corn, sunflower, or soybean oils/100% pork back fat	Reformulated frankfurters (10% fat + different oils) had 67% lower total fat, 40%–45% lower saturated fatty acids, 50%–53% lower calories, reduced cholesterol, and 20% higher meat protein. They had a darker red color, and were 6%–7.2% lower in processing yield, but they were firmer and less juicy. The type of oil had no effect on these characteristics. Frankfurters with olive oil had 41.8% higher monounsaturated fatty acids and those with seed oils had 5–7 times higher polyunsaturated fatty acids. Soybean oil negatively affected overall acceptability and shelf life due to the increased linolenic acid content.

(*Continued*)

Downloaded From Vet-eBooks.com

TABLE 15.3 (*Continued*)

Reformulating Lipid Profile of Meat Products by Adding Vegetable Oils

Authors	Processed Meat Product	Vegetable Oil/l/% Reduced or Replaced Animal Fat	Most Important Results
Valencia et al. (2008)	Fresh pork sausages	Linseed oil or fish oil added to natural antioxidants/15% pork back fat	Increase in α-linolenic, EPA, and DHA and no changes in lightness, but redness was lower after 7 days of storage. Reformulated cooked pork sausages had no effects in sensory properties, indicating a potential application to enhance nutritional properties.
Backes et al. (2013)	Italian-type salami	Emulsified canola oil/15% and 30% pork back fat	No changes in all treatments for pH, color, and Aw during manufacture process and storage period. The modified salamis had lower weight loss and in substituting 30% pork back fat, an elevation of lipid oxidation was observed. At 15% pork back fat substitution no effect was reported regarding the sensorial acceptance of the product for aroma, flavor, color, texture, and visual appearance.
Mora-Gallego et al. (2016)	Nonacid fermented sausages	Sunflower oil + diacylglycerols/ pork back fat	Fat reduction higher than 70% compared with the standard fermented sausages; higher sensory ratings and improved texture for sunflower oil. Fermented sausages containing less than 12.5% of fat added of sunflower oil had a good overall sensory quality.
Delgado-Pando et al. (2012)	Pork liver pâtés	Olive, linseed, and fish oils added to konjac gels/pork back fat	The increase of unsaturated fatty acid levels resulted in a higher level of lipid oxidation, despite the low values reported at the end of shelf life. The products were considered safe regarding biogenic amine contents of products during shelf life. No changes in microbial stability were observed in chilling storage.

partially replace pork back fat. Some authors recommend between 1 and 10 g of olive oil per 100 g of meatballs (Hsu and Yu, 2002), frankfurters (Lurueña-Martínez et al., 2004), and dry fermented sausages (Muguerza et al., 2001; Severini et al., 2003). It was found that olive oil increases the MUFAs in meat products without significantly altering the ω-6/ω-3 ratio (Muguerza et al., 2002; Ansorena and Astiasaran, 2004).

Downloaded From Vet-eBooks.com

Other oils used to reformulate the lipid profile of meat products include sunflower, cotton, corn, soybean, peanut, linseed, and palm oils, which stand out by raising the MUFA and PUFA levels, reducing cholesterol, and improving PUFA/SFA and ω-6/ω-3 ratios (Paneras and Bloukas, 1994; Ambrosiadis et al., 1996; Hsu and Yu, 2002). Cotton and corn oils are very rich in PUFAs and contain high linoleic acid (C18:2 ω-6) levels (>56% total fatty acids). Their addition to meat products improves the PUFA/SFA ratio, with a negative effect on increasing the ω-6/ω-3 ratio (Paneras and Bloukas, 1994; Paneras et al., 1998). In most studies, these vegetable oils partially replaced pork back fat at levels up to 50%–100%.

Due to the technological and functional limitations of vegetable oils in liquid form, many studies recommend the incorporation of these components in the form of pre-emulsions (Berasategi et al., 2011; Beriain et al., 2011; García-Íñiguez de Ciriano et al., 2010; Rodríguez-Carpena et al., 2012; Jiménez-Colmenero, 2007). In emulsified products, the application of oil in liquid form may result in loss of functionality of the batter, with exudation losses, due to the low melting point, thus requiring a higher content of myofibrillar proteins to encompass all lipid content widely dispersed. In addition, oil in liquid form can negatively affect the emulsion forming and gelling properties. This phenomenon is also observed in fermented products, with an extensive oxidative deterioration when vegetable oils in liquid form are used in the formulations. In products subjected to high temperatures such as hamburgers and nuggets, the lipid fraction will be easily separated, with a loss of texture and sensory attributes.

The pre-emulsions used to overcome these technological problems are oil-in-water type containing an emulsifying agent, generally a nonmeat protein, which results in increased stability during the shelf life of the product subjected to processing and storage under adequate conditions (Djordjevic et al., 2004).

Generally, sodium caseinate and soy protein isolate are the protein extenders most commonly used in this type of reformulation, as listed in Table 15.3.

Regardless of the strategy of the addition of these components rich in MUFAs and PUFAs in the reformulation, many preliminary studies are required, since they can accelerate the lipid oxidation process, resulting in the development of off-flavor and toxic compounds due to the degradation of fatty acids (Jacobsen, 1999).

15.4.5 Use of Structured Lipids

Several strategies to reduce saturated fat and improve the fatty acid profile may result in defects and technological limitations associated with loss of flavor, aroma, juiciness, and texture, heat transfer problems, and other processing operations as a function of the solid fats in these products. These challenges have focused on developing liquid oils restructured by different processes to form functional plastic fats with better technological performance

Downloaded From Vet-eBooks.com

TABLE 15.4

Ingredients Used in Emulsified Lipid Gels and Their Respective Effects

Ingredient	Effects	Concentration (%) (References)
Soy protein isolate	It forms a gel when subjected to heating and cooling, with protein participating in three distinct phases: denaturation, aggregation, and gelation. A preheating stage induces the molecular changes (unfolding of the protein structure and exposure of reactive groups), necessary to form the soluble aggregates. The initial pH of the solution should be sufficiently different from the isoelectric point of the proteins, and the ionic strength should be sufficiently low to avoid an immediate aggregation of the protein molecules during preheating. *T* required to form gel: >70°C. Isoelectric point: 4.5.	5.0%–10.0% (Pires Vilela et al., 2011; Lee et al., 2006; Delgado-Pando et al., 2010)
Inulin	It has different applications in the food industry, including gelling properties, increase in viscosity, improvement of organoleptic properties, and as a nondigestible fiber in meat products. It is water soluble, but dependent on the temperature and degree of polymerization. It is practically insoluble in cold water at $T < 10$°C. It is stable at high temperatures.	2.0%–10.0% (Herrero et al., 2014; Felisberto et al., 2015)
Sodium caseinate	It is widely used due to its functional properties such as emulsification and water and fat holding capacity, as well as the thickening and gelling properties. It gives good stability to emulsions at neutral pH by combination of electrostatic and steric stabilization mechanisms. β-casein and α1-casein, which together account for more than 75% of the total casein, are particularly disordered and substantially hydrophobic, and contribute to its rapid absorption during emulsification, leading to the rapid establishment of a thick and sterically stable layer that protects the newly formed droplets against flocculation and coalescence. Isoelectric point: 4.5. Stable at very high temperatures.	0.5%–5.0% (Lee et al., 2006; Jourdain et al., 2008; Jiménez-Colmenero et al., 2010; Perrechil and Cunha, 2013)
Carrageenan	It is part of a group of water-soluble anionic sulfated polysaccharides that interact with proteins at pH above and below their isoelectric point. It can affect the characteristics of the formed gels, depending on its composition and type (number/position of sulfate groups and number of 3,6-anhydrogalactose rings per disaccharide). In emulsion gels, its stabilizing and/or emulsifying capacity is mainly due to the greater electrostatic repulsion from the interaction between the carrageenan (anionic polysaccharide) and the fat droplets coated by proteins. This interaction depends mainly on polymer concentration, pH, and ionic strength. It is subjected to gelation at temperatures > 65°C.	0.5%–1.5% (Jourdain et al., 2008, Perrechil and Cunha, 2013; Poyato et al., 2014)

(*Continued*)

Downloaded From Vet-eBooks.com

TABLE 15.4 (*Continued*)
Ingredients Used in Emulsified Lipid Gels and Their Respective Effects

Ingredient	Effects	Concentration (%) (References)
Pectin	Anionic polysaccharide composed of polygalacturonic acid and its methyl esters which can generate highly viscous or gel-like materials by heteroaggregation in systems with electrically charged colloidal particles (protein-coated fat droplets).	0.07%–0.67% (Simo et al., 2012)
Soy lecithin	Emulsifying agent with amphoteric character found in different natural sources, such as soybean and egg, composed by a mixture of phospholipids. It contributes as a stabilizing/gelling agent in systems with polar solvents (usually water); it is well accepted by the consumer and considered safe for consumption by the FDA.	0.5% (Lupi et al., 2012)

FDA, Food and Drug Administration.

and healthier characteristics when compared to the pre-emulsions previously discussed. Among the strategies to improve lipid profile emulsion gels have been used as fat substitutes in emulsified meat products. (Jiménez-Colmenero et al., 2015; Delgado-Pando et al., 2010).

An emulsified lipid gel is defined as an emulsion containing a gel-like network structure with mechanical properties similar to that of a solid compound (Dickinson, 2012, 2013). Its development occurs briefly in two stages:

1. *Formation of an oil + water emulsion* containing a protein source as a surfactant by homogenization under high pressure or high speed (potent mixers, ultra-turrax, cutters), whose stability can be improved by the addition of other extender ingredients to the newly formed emulsion.
2. *Formation of a gel from the emulsion obtained in the first step*, obtained by the aggregation of emulsion droplets or gelation of the continuous phase through an appropriate heat treatment. The transformation of the liquid state to the soft solid state of protein-based emulsions occurs during the processing steps, such as heating, acidification, or enzymatic treatment (Dickinson, 2013).

In meat products, the use of emulsified lipid gels is a very promising strategy for fat reduction. Proteins, different hydrocolloids and functional ingredients, with emphasis on sodium caseinate, whey proteins, soy proteins, gelatin, lecithin, carrageenan, and IN, among others (Jiménez-Colmenero et al., 2010; Delgado-Pando et al., 2010) have been evaluated as structuring agents in the development of lipid gels. Because many of these compounds have been used either as extenders in meat product formulations or as fat

Downloaded From Vet-eBooks.com

substitutes in single formulations, the potential use of these new ingredients becomes highly promising.

The proteins can be used as emulsifiers, gelling agents, and thickeners in the formation of lipid gels due to their ability to facilitate the formation and improve the stability of O/W emulsions. Several factors influence protein functionality throughout the lipid gel formation process, which is related to the solution conditions (pH, ionic strength, surfactants, biopolymers), interactions of the ingredients, and process steps (heat treatment, cooling, freezing, drying, homogenization, and mechanical agitation) (McClements, 2004). In inadequate conditions, proteins may suffer electrostatic repulsion, which can destabilize the meat system (Picone et al., 2011). Whey protein isolates, soy protein isolates, and gluten proteins have been extensively studied for this purpose.

In addition to proteins, other ingredients can be added to emulsion gels, especially hydrocolloids (polysaccharides), for their effects on texture and stability properties (Lal et al., 2006). The protein–polysaccharide interactions are mainly electrostatic and thus their intensity and strength are dependent on pH and ionic strength (Dickinson, 1994), which affects the emulsification and gelling properties (Dickinson, 2012). The use of structured lipids in meat products containing substitute salts may affect the technological performance of these compounds as fat substitutes and has been the subject of many studies.

In meat products, examples of polysaccharides to stabilize emulsion gels include alginate, IN, and carrageenan (Paradiso et al., 2015). Several factors can induce gelling of a hydrocolloid, including the addition of calcium ions (pectin, alginate), temperature reduction (agar, carrageenan, gellan), or increase in temperature (methylcellulose, hydroxypropylmethylcellulose). In starches, gelatinization occurs by heating, forming an opaque thermoreversible gel during cooling. The control of syneresis is one of the points that deserves great attention. IN gels are formed by a network of small crystals of approximately 100 nm in diameter that aggregate to form clusters larger than 1–5 μm, which retain a large amount of water in the network. The properties of this network resemble a fat-in-oil crystal network. Due to this similarity, IN has been identified as an alternative ingredient for structuring low-fat and fat-free products (Bot et al., 2004). Table 15.4 presents some of the gelling agents for gel formation and their potential application in meat products considering improvement in the nutritional properties in addition to fat reduction.

The emulsion gels may be a better alternative to mimic the hardness and water retention capacity conferred by pork back fat in meat products when compared to the O/W emulsions (Poyato et al., 2014). Various studies have reported the elaboration of emulsion gels based on olive oil, chia oil, and cold gelling agents (microblial transglutaminase, alginate, or gelatin) to replace saturated fat in emulsified products. As positive points, the authors stated an improvement in the fatty acid profile and reduction of saturated fat content

Downloaded From Vet-eBooks.com

by up to 50%. Very positive results on the oxidative stability, sensory performance, and physicochemical properties have also been reported.

15.5 Challenges of Fat Reduction in Different Meat Products

Pork back fat is the fat source most widely used in different categories of meat products, representing one of the most suitable raw materials of meat products. It has a high melting point, differentiated texture, and distinct flavor characteristics due to its high SFA composition (Ospina-E et al., 2012), presenting excellent stability. On the other hand, "soft fats" or liquid oils have a high content of MUFAs and PUFAs, which can cause technological and sensory problems in meat products, affecting the appearance and slice ability, with a tendency toward lipid oxidation (Maw et al., 2003). Likewise, some sensory problems can take place when fatty cuts are replaced by lean cuts, affecting the texture, softness, and juiciness of meat products. Each product category has its specific challenges, making it necessary to know them for a senspory successful lipid reformulations with stability and safety throughout the shelf life.

15.5.1 Emulsified Meat Products

Meat emulsions are obtained from a mixture of raw meat, fat/fat tissue, water in the form of ice, and various additives and ingredients (such as nitrite, salt, phosphates, and condiments), subjected to a reduction in particle size (cutter comminution or high-pressure homogenization), processed in a single phase, stuffed into natural or artificial casings, and subjected to adequate heat treatment and cooling. Myofibrillar proteins are extracted by increasing the ionic strength in the presence of sodium chloride, which includes fat and water particles, forming a network stabilized by the gel formation during cooking and cooling. A wide variety of products consumed throughout the world belongs to this category, including bologna sausage, sausage, meatloaf, among others (Shimokomaki et al., 2006).

Fat reduction strongly affects emulsion stability, water-binding capacity, and gel formation, with negative effects on the quality of the final products. It is associated with various factors, such as fat particle size, emulsion pH, nonmeat ingredients, meat species (bovine, pork, or poultry), processing time and temperature, cooking method, etc. (Sorapukdee et al., 2013). The higher the fat reduction with partial replacement by water or vegetable oils, the greater the cooking losses, providing a very soft and sandy texture to the product (Sorapukdee et al., 2013). In formulations containing high levels of vegetable oil, higher protein concentrations are needed to recover the lipid content, keeping it in the net of the batter.

Carbohydrate-based extenders or proteins can be used in systems with partial replacement of fat by water, which contributes to stabilize the bound

Downloaded From Vet-eBooks.com

water, avoiding water release during cooking or storage. Such extensors, as previously discussed, may be hydrocolloids, fibers, or nonmeat proteins, but an inadequate selection can result in an increased size of the emulsion particles, darker color of the product, reduction of the meat flavor attributes, and decrease in the shelf life, with loss of microbiological stability (Cengiz and Gokoglu, 2005). The major challenges are the maintenance of the identity of the traditional product, especially firmness and cohesiveness, as well as avoiding water release in the package during storage (Claus et al., 1990).

These technological challenges stand out in low-cost formulations containing high levels of mechanically separated chicken meat, which is a technological resource widely used in many countries and permitted by law. In this case, the lower supply of myofibrillar proteins and the higher fat content of the low-cost raw materials together with the prooxidant compounds that normally occur during the mechanical deboning impair the fat reduction in popular products.

15.5.2 Fermented Meat Products

A set of barriers is used to maintain the safety of fermented meat sausages, including water activity at around 0.90, a decrease in pH, and the addition of nitrite and salt (Leistner, 1992).

Whereas the drying stage leads to high water loss and concentration of other components, the fat reduction in fermented sausages, as is the case of dry fermented sausages, represents a greater challenge when compared to other meat products, concerning the physicochemical stability and sensory properties (Mendoza et al., 2001).

The major technological challenge of fat reduction in fermented sausages is the maintenance of the characteristic texture, due to the addition of pork back fat. The fat content of the fermented sausages can reach up to 50% of the formulation after the drying stage (Wirth, 1988), which markedly affects the speed and water evaporation during the process. This is one of the reasons for the low sensory acceptance of fat-reduced sausages (Muguerza et al., 2002), due to the harder and more elastic texture with undesirable appearance caused by a greater weight loss and fat reduction (Muguerza et al., 2002). In addition, fat is responsible for the generation of volatile flavor and aroma compounds. Lipolysis has an important role in the generation of free fatty acids from the enzymatic hydrolysis, which are substrates for oxidation reactions, leading to the formation of compounds responsible for the aroma of the product (Lücke, 1985; Verplaetse, 1994).

However, these defects can be minimized through adequate processing and correct control of moisture and temperature of the maturation chamber (Jiménez-Colmenero, 1996). Different types of packaging were also studied by Liaros et al. (2009) and Koutsopoulos et al. (2008) to improve the external

Downloaded From Vet-eBooks.com

appearance and to control the lipid oxidation improved by the addition of vegetable oils to dry fermented sausages with reduced fat content.

As regards the reformulation of lipid profile, some strategies suggest the use of vegetable oils in the form of pre-emulsions (Muguerza et al., 2002), which may be an additional challenge, once the pre-emulsions require the use of stabilizing ingredients, such as caseinates, soy protein isolates, carrageenan, etc., which can also interfere with the texture profile and sensory properties (Dickinson, 2013; Zóia, 2011).

15.5.3 Restructured Injected and Marinated Meat Products

Restructured injected meat products include hams, loins, perennis, canopy, turkey breast, and a series of ready-to-eat seasoned cuts. Generally, the fat reduction in such products occurs preferentially by the use of lean cuts, for example, cuts from the rear back portion of the animals. However, the search for healthier claims and lower costs (Brashear et al., 2002) suggests the use of functional ingredients as fat substitutes.

Fibers and a number of soluble hydrocolloids capable of interacting with the myofibrillar proteins, water, and fat portion are the preferred ingredients for reduction and/or partial replacement of fat in restructured products. Such compounds are introduced into the process through the steps of marinating, tumbling, and massaging, or simply in the injection step. The resulting effects are increased water retention capacity and higher protein–protein interaction, with the advantage of shortening the processing time, and increasing the color uniformity, texture, fat distribution, and binding, especially when the injection stage is not conducted (Sheard, 2002).

In general, soluble fibers and hydrocolloids, maltodextrins, and certain additives such as phosphates are added to the brine in brine-mixing tanks, and then incorporated into meat cuts through marinate injectors. The retention of the functional compounds will depend on the compatibility between meat cuts and these ingredients and additives. The ingredients may be selected according to their solubility, dispersibility, and the viscosity at low temperatures. In most cases, brine should be kept at relatively low temperatures to increase muscle retention and process yield (Varnam and Sutherland, 1995) and to ensure microbiological safety. The main problem that can arise in the manufacturing of restructured products is the need to couple a blender to disperse the ingredients, particularly the starch derivatives and soy protein, due to their longer dispersion, which may cause difficulties in cleaning the circuit the brine passes through. During cooking, the product should be kept stable, and the fat reduction should not promote the separation of the restructured cuts after cooling. When subjected to high temperatures, the gelation phenomenon occurs and the exudate liquid forms a film, which retains moisture, improving texture and juiciness (Varnam and Sutherland, 1995). However, a large release caused by functional ingredients that don't have suitable binding properties and reduction of saturated

Downloaded From Vet-eBooks.com

fat with a high melting point can seriously compromise the physicochemical, sensory, and microbiological stability of the products.

15.5.4 Restructured and Breaded Meat Products

Breaded meat products subjected to the frying process are among the most accepted by consumers since the oil introduced into the product confers special crispness, flavor, succulence, and aroma when replacing part of the water. However, excessive fat in the final product with an increase of up to one-third of the energy value leads to many nutritional restrictions.

According to Ordóñez (2005), the process of elaborating the breaded meat products consists basically of the following operations: (1) preparation of the meat matrix with particle size reduction (grinding of raw material) in the case of nuggets, blends of ingredients and spices, molding or marination for small cuts, and tumbling; (2) coating through a specific coating system; and (3) partial prefrying or total frying, freezing, and packaging.

The traditional breading system consists of predust, liquid mixtures (batter), and coating flours. The order of addition and use of these components may vary; there is no correct order and not all of these components are used (Bortoluzzi, 2006; GL, 2002; Uemura and Luz, 2003).

Strategies have been proposed to reduce the absorbed oil in the coating systems and meat batters (Huebner-Keese et al., 2016). According to Brannan et al. (2014), the phenomenon of oil absorption is explained by a series of events that include (1) replacement of water by oil (mass transfer) at temperatures around 180°C, (2) a cooling effect, when the fried product is removed from the hot oil with sudden drop in pore pressure, creating a vacuum effect that sucks the oil that adhered to the surface into the pores, and (3) triacylglycerol degradation in the oil to more polar compounds (diacylglycerols, monoglycerides, free fatty acids, and glycerol) that act as surfactants, reducing the tension between oil and water, collaborating to increase oil absorption, particularly in aged oils.

Based on these events, many compounds have been added to the predust, batter, or breader to reduce oil absorption and to act as barriers, facilitating water release and reducing oil absorption, often as a consequence of reduced vapor escape during frying. Some compounds can favor the formation of thermally induced gels and alteration of the surface hydrophobicity, contributing to the reduction of fat absorbed during the frying process. In meat products, these compounds include sodium caseinate, whey protein concentrate, starch, methyl cellulose, xanthan gum (Altunakar et al., 2006), modified cellulose derivatives, PD, hydroxymethyl cellulose (Jamshidi and Shabanpour, 2014), pregelatinized rice flour, phosphorylated rice starch, pregelatinized acetylated rice starch, pectates, gelatin, and alginate esters (Sahin et al., 2005).

Functional ingredients such as soluble or insoluble fibers have also been used as excellent carriers in the batters of restructured breaded and

Downloaded From Vet-eBooks.com

nonbreaded products, such as hamburgers, in general as a model system to evaluate new fat substitutes.

15.6 Final Considerations

Fat reduction in processed meat products is one of the most important reformulations aimed at promoting healthier consumption appeals, contributing to improve their nutritional value. Although these products are sources of proteins, liposoluble vitamins, essential fatty acids, and many minerals important for maintenance of a healthy diet, their high fat content, especially saturated fat, stimulate a negative perception of product quality by consumers.

However, the manufacture of reduced-fat meat products presents many technological, sensory, and safety challenges, since fat plays functions that cannot be easily replaced. The simple reduction of back fat, the main source of fat used in formulations, greatly affects the texture attributes, flavor, perception of satiety, microbiological safety, and physicochemical stability, compromising the product's quality.

Strategies, such as the use of fat replacers based on ingredients compatible with meat matrixes of processed products, restructured lipids, such as emulsion gels, and partial replacement with vegetable oils, are among the most studied alternatives to overcome the technological and sensory challenges in low-fat meat products.

The specific characteristics of each category of processed meat product, including the raw materials used, processing methods, packaging, and estimated shelf life should be evaluated to obtain safe, stable, and well-accepted food products. Finally, simultaneous reformulations, such as sodium or phosphate reduction, can present additional challenges that can be overcome after careful selection of the manufacturing practices, the use of ingredients with functional properties compatible with the new meat matrix, and the competence to choose physicochemical and sensory methodologies to accurately interpret the effects of lipid reformulation on consumers' perception.

References

AACC. (2001). The definition of dietary fiber. *Cereal Foods World*, **46**(3), 112.

Adam-Perrot, A., Gutton, L., Sanders, L., et al. (2009). Resistant starch and starch-derived oligosaccharides as prebiotics. In: Charalampopoulos, D. and Rastall, R. A. (Eds.). *Prebiotics and Probiotics Science and Technology*. New York: Springer, volume 1, pp. 259–291.

Akoh, C. C. (1998). Structured lipids. *In:* Akoh, C. C. and Min, D. B. (eds.) *Food Lipids Chemistry, Nutrition, and Biotechnology*. New York, NY: Marcel Dekker, pp. 699–727.

Downloaded From Vet-eBooks.com

Altunakar, B., Sahin, S. and Sumnu, G. (2006). Effects of hydrocolloids on apparent viscosity of batters and quality of chicken nuggets. *Chemical Engineering Communications*, **193**(6), 675–682. doi:10.1080/00986440500194069.

Álvarez, D. and Barbut, S. (2013). Effect of inulin, β-glucan and their mixtures on emulsion stability, color and textural parameters of cooked meat batters. *Meat Science*, **94**(3), 320–327. doi:10.1016/j.meatsci.2013.02.011.

Alves, L. A. A., dos, S., Lorenzo, J. M., Gonçalves, C. A. A. et al. (2016). Production of healthier bologna type sausages using pork skin and green banana flour as fat replacers. *Meat Science*, **121**, 73–78. doi:10.1016/j.meatsci.2016.06.001.

Ambrosiadis, J., Vareltzis, K. P. and Georgakis, S. A. (1996). Physical, chemical and sensory characteristics of cooked meat emulsion style products containing vegetable oils. *International Journal of Food Science and Technology*, **31**(2), 189–194.

Andersson, R., Eliasson, C., Selenare, M., Kamal-Eldin, A. and Åman, P. (2003). Effect of endo-xylanase-containing enzyme preparations and laccase on the solubility of rye bran arabinoxylan. *Journal of the Science of Food and Agriculture*, **83**(7), 617–623. doi:10.1002/jsfa.1321.

Annison, G. and Topping, D. L. (1994). Nutritional role of resistant starch: Chemical structure vs physiological function. *Annual Review of Nutrition*, **14**(1), 297–320. doi:10.1146/annurev.nu.14.070194.001501.

Ansorena, D. and Astiasaran, I. (2004). Effect of storage and packaging on fatty acid composition and oxidation in dry fermented sausages made with added olive oil and antioxidants. *Meat Science*, **67**, 237–244. doi:10.1016/j.meatsci.2003.10.011.

Appleton, K. M, Rogers, P. J. and Ness, A. R. (2010). Updated systematic review and meta-analysis of the effects of n-3 long-chain polyunsaturated fatty acids on depressed mood. *American Journal of Clinical Nutrition*, **91**(3), 757–770. doi:10.3945/ajcn.2009.28313.

Arihara, K. (2006). Strategies for designing novel functional meat products. *Meat Science*, **74**(1), 219–229. doi:10.1016/j.meatsci.2006.04.028.

Backes, A. M., Nascimento Terra, N., Inês, L., Milani, G., De, A. P., Rezer, S. et al. (2013). Physico-chemical characteristics and sensory acceptance of Italian-type salami with canola oil addition. *Semina: Ciências Agrárias*, **34**(2), 3709–3720. doi:10.5433/1679-0359.2013v34n6Supl2p3709.

Balk, E. M., Lichtenstein, A. H., Chung, M., Kupelnick, B., Chew, P. and Lau, J. (2006). Effects of omega-3 fatty acids on serum markers of cardiovascular disease risk: A systematic review. *Atherosclerosis*, **189**(1), 19–30. doi:10.1016/ j.atherosclerosis.2006.02.012.

Barceló-Coblijn, G. and Murphy, E. J. (2009). Alpha-linolenic acid and its conversion to longer chain n−3 fatty acids: Benefits for human health and a role in maintaining tissue n−3 fatty acid levels. *Progress in Lipid Research*, **48**(6), 355–374. doi:10.1016/j.plipres.2009.07.002.

Bater, B., Descamps, O. and Maurer, A. J. (1992). Quality characteristics roasted of hydrocolloid-added turkey breasts. *Journal of Food Science*, **57**(5), 1068–1070.

Beer, M. U., Wood, P. J. and Weisz, J. (1997). Molecular weight distribution and (1→3)(1→4)-β-D-glucan content of consecutive extracts of various oat and barley cultivars. Cereal Chemistry. *American Association of Cereal Chemists*, **74**(4), 476–480. doi:10.1094/CCHEM.1997.74.4.476.

Downloaded From Vet-eBooks.com

Berasategi, I., Legarra, S., de Ciriano, M. G. et al. (2011). "High in omega-3 fatty acids" bologna-type sausages stabilized with an aqueous-ethanol extract of *Melissa officinalis*. *Meat Science*, **88**(4), 705–711. doi:10.1016/j.meatsci.2011.02.035.

Beriain, M. J., Gómez, I., Petri, E., Insausti, K. and Sarriés, M. V. (2011). The effects of olive oil emulsified alginate on the physico-chemical, sensory, microbial, and fatty acid profiles of low-salt, inulin-enriched sausages. *Meat Science*, **88**(1) 189–197. doi:10.1016/j.meatsci.2010.12.024.

Bernardi, D. M., Bertol, T. M., Pflanzer, S. B., Sgarbieri, V. C. and Pollonio, M. A. R. (2016). ω-3 in meat products: Benefits and effects on lipid oxidative stability. *Journal of the Science of Food and Agriculture*, 96(8), 2620–2634. doi:10.1002/jsfa.7559.

Biesalski, H. -K. (2005). Meat as a component of a healthy diet – are there any risks or benefits if meat is avoided in the diet? *Meat Science*, **70**(3), 509–524. doi:10.1016/j.meatsci.2004.07.017.

Blundell, J. E. (2006). Perspective on the central control of appetite. *Obesity* (Silver Spring, Md.) **14**(Suppl 4) (July), 160S–163S. doi:10.1038/oby.2006.298.

Bonanome, A., Pagnan, A., Biffanti, S. et al. (1992). Effect of dietary monounsaturated and polyunsaturated fatty acids on the susceptibility of plasma low density lipoproteins to oxidative modification. *Arteriosclerosis and Thrombosis: A Journal of Vascular Biology*, **12**(4), 529–533. http://www.ncbi.nlm.nih.gov/pubmed/1558840.

Bonanome, A. and Grundy, S. M. (1988). Effect of dietary stearic acid on plasma cholesterol and lipoprotein levels. *New England Journal of Medicine*, **318**(19), 1244–1248. doi:10.1056/NEJM198805123181905.

Borderías, A. J., Sánchez-Alonso, I. and Pérez-Mateos, M. (2005). New applications of fibres in foods: Addition to fishery products. *Trends in Food Science and Technology*, **16**(10), 458–465. doi:10.1016/j.tifs.2005.03.011.

Bornet, F. R. (1994). Undigestible sugars in food products. *American Journal of Clinical Nutrition*, **59**(3 Suppl), 763S–769S. http://www.ncbi.nlm.nih.gov/pubmed/8116563.

Bortoluzzi, R. C. (2006). Empanados. *In:* Olivo, R. (ed.) *O mundo do frango: Cadeia produtiva da carne de frango.* Criciúma: Ed. do Autor, pp. 481–494.

Bot, A., Erle, U., Vreeker, R. and Agterof, W. G. M. (2004). Influence of crystallisation conditions on the large deformation rheology of inulin gels. *Food Hydrocolloids*, **18**(4), 547–556. doi:10.1016/j.foodhyd.2003.09.003.

Brannan, R. G., Mah, E. M. S., Yuan, S., Casher, K. L., Myers, A. and Herrick, C. (2014). Influence of ingredients that reduce oil absorption during immersion frying of battered and breaded foods. *European Journal of Lipid Science and Technology*, **116**(3), 240–254. doi:10.1002/ejlt.201200308.

Brashear, G., Brewer, M. S., Meisinger, D. and McKeith, F. K. (2002). Raw material pH, pump level and pump composition on quality characteristics of pork. *Journal of Muscle Foods*, **13**(3), 189–204. doi:10.1111/j.1745-4573.2002.tb00330.x.

Brennan, C. S. (2005). Dietary fibre, glycaemic response, and diabetes. *Molecular Nutrition & Food Research*, **49**(6), 560–570. doi:10.1002/mnfr.200500025.

Brennan, C. S. and Cleary, L. J. (2005). The potential use of cereal (1→3,1→4)-β-D-glucans as functional food ingredients. *Journal of Cereal Science*, **42**(1), 1–13. doi:10.1016/j.jcs.2005.01.002.

Downloaded From Vet-eBooks.com

Brewer, M. S. (2012). Reducing the fat content in ground beef without sacrificing quality: A review. *Meat Science*, **91**(4), 385–395. doi:10.1016/j.meatsci.2012.02.024.

Burdock, G. A. and Flamm, W. G. (1999). A review of the studies of the safety of polydextrose in food. *Food and Chemical Toxicology*, **37**(2), 233–264. doi:10.1016/S0278-6915(98)00113-6.

Cáceres, E., García, M. L., Toro, J. and Selgas, M. D. (2004). The effect of fructooligosaccharides on the sensory characteristics of cooked sausages. *Meat Science*, **68**(1), 87–96. doi:10.1016/j.meatsci.2004.02.008.

Campagnol, P. C. B., dos Santos, B. A., Wagner, R., Terra, N. N. and Pollonio, M. A. R. (2013). The effect of soy fiber addition on the quality of fermented sausages at low-fat content. *Journal of Food Quality*, **36**(1), 41–50. doi:10.1111/jfq.12013.

Campagnol, P. C. B., Dos Santos, B. A., Wagner, R., Terra, N. N. and Pollonio, M. A. R. (2012). Amorphous cellulose gel as a fat substitute in fermented sausages. *Meat Science*, **90**(1), 36–42. doi:10.1016/j.meatsci.2011.05.026.

Candogan, K. and Kolsarici, N. (2003). The effects of carrageenan and pectin on some quality characteristics of low-fat beef frankfurters. *Meat Science*, **64**(2), 199–206. doi:10.1016/S0309-1740(02)00181-X.

Carballo, J., Barreto, G. and Jiménez-Colmenero, F. (1995). Starch and egg white influence on properties of bologna sausage as related to fat content. *Journal of Food Science*, **60**(4), 673–677.

Carraro, C. I. (2012). The use of resistant starch as fat replacers extenders ingredients in emulsified meat products. Unpublished Master's dissertation, Universidade Estadual de Campinas, Brazil.

Catalani, L. A. et al. 2003. Fibras alimentares. *Revista Brasileira de Nutrição Clínica*, **18**(4): 178–182.

Cava, R., Ladero, L., Cantero, V. and Rosario Ramírez, M. (2012). Assessment of different dietary fibers (tomato fiber, beet root fiber, and inulin) for the manufacture of chopped cooked chicken products. *Journal of Food Science*, **77**(4), C346–C352. doi:10.1111/j.1750-3841.2011.02597.x.

Cengiz, E. and Gokoglu, N. (2005). Changes in energy and cholesterol contents of frankfurter-type sausages with fat reduction and fat replacer addition. *Food Chemistry*, **91**(3), 443–447. doi:10.1016/j.foochem.2004.06.025.

Champ, M. M. (2004). Physiological aspects of resistant starch and *in vivo* measurements. *Journal of AOAC International*, **87**, 749–755.

Chardigny, J. M., Arnal, M. A., Juanéda, P., Genty, M., Grégoire, S. and Sebédio, J. L. (2001). Effect of cis-9, trans-11 and 10-trans, 12cis-CLA isomers in two strains of mice. *Annals of Nutrition and Metabolism*, **45**(Suppl 1), 559.

Chawla, R. and Patil, G. R. (2010). Soluble dietary fiber. *Comprehensive Reviews in Food Science and Food Safety*, **9**(2), 178–196. doi:10.1111/j.1541-4337.2009.00099.x.

Chevance, F. F., Farmer, L. J., Desmond, E. M., Novelli, E., Troy, D. J. and Chizzolini, R. (2000). Effect of some fat replacers on the release of volatile aroma compounds from low-fat meat products. *Journal of Agricultural and Food Chemistry*, **48**(8), 3476–3484. http://www.ncbi.nlm.nih.gov/pubmed/10956136.

Choi, Y. S., Choi, J. H., Han, D. J. et al. (2009). Characteristics of low-fat meat emulsion systems with pork fat replaced by vegetable oils and rice bran fiber. *Meat Science*, **82**(2), 266–271. doi:10.1016/j.meatsci.2009.01.019.

Downloaded From Vet-eBooks.com

Choi, Y. S., Kim, Y. B., Kim, H. W. et al. (2015). Emulsion mapping in pork meat emulsion systems with various lipid types and brown rice fiber. *Korean Journal for Food Science of Animal Resources*, **35**(2), 258–264. doi:10.5851/kosfa.2015.35.2.258.

Cierach, M., Modzelewska-Kapituła, M. and Szaciło, K. (2009). The influence of carrageenan on the properties of low-fat frankfurters. *Meat Science*, **82**(3), 295–299. doi:10.1016/j.meatsci.2009.01.025.

Claus, J. R. (1991). Fat reduction in comminuted meat systems. *Reciprocal Meat Conference Proceedings*, **44**, 93–99.

Claus, J. R. and Hunt, M. C. (1991). Low-fat, high added-water bologna formulated with texture-modifying ingredients. *Journal of Food Science*, **56**(3), 643–647. doi:10.1111/j.1365-2621.1991.tb05347.x.

Claus, J. R., Hunt, M. C. and Kastner, C. L. (1990). Effects of substituting added water for fat on the textural, sensory, and processing characteristics of bologna. *Journal of Muscle Foods*, **1**(1), 1–21. doi:10.1111/j.1745-4573.1990.tb00349.x.

Dangour, A. D., Andreeva, V. A., Sydenham, E. and Uauy, R. (2012). Omega 3 fatty acids and cognitive health in older people. *British Journal of Nutrition*, **107**(S2), S152–S158. doi:10.1017/S0007114512001547.

Decker, E. A. and Park, Y. (2010). Healthier meat products as functional foods. *Meat Science*, **86**(1), 49–55. doi:10.1016/j.meatsci.2010.04.021.

Delgado-Pando, G.; Cofrades, S.; Ruiz-Capillas, C.; et al. (2012). Enriched n-3 PUFA/konjac gel low-fat pork liver pate: Lipid oxidation, microbiological properties and biogenic amine formation during chilling storage. Meat Science 92(4): 762--767. doi.org/10.1016/j.meatsci.2012.06.035

Denke, M. A. and Grundy, S. M. (1992). Comparison of effects of lauric acid and palmitic acid on plasma lipids and lipoproteins. *American Journal of Clinical Nutrition*, **56**(5), 895–898. http://www.ncbi.nlm.nih.gov/pubmed/1415008.

Dickinson, E. (1994). Protein-stabilized emulsions. *Journal of Food Engineering*, **22**, 59–74. doi:10.1016/j.cocis.2004.09.003.

Dickinson, E. (2012). Emulsion gels: The structuring of soft solids with protein-stabilized oil droplets. *Food Hydrocolloids*, **28**(1), 224–241. doi:10.1016/j.foodhyd.2011.12.017.

Dickinson, E. (2013). Stabilising emulsion-based colloidal structures with mixed food ingredients. *Journal of the Science of Food and Agriculture*, **93**(4), 710–721. doi:10.1002/jsfa.6013.

Djordjevic, D., Kim, H. -J., McClements, D. J. and Decker, E. A. (2004). Physical stability of whey protein-stabilized oil-in-water emulsions at pH 3: Potential ω-3 fatty acid delivery systems (part A). *Journal of Food Science*, **69**(5), C351–C355. doi:10.1111/j.1365-2621.2004.tb10696.x.

Dogan, S. F., Sahin, S. and Sumnu, G. (2005). Effects of soy and rice flour addition on batter rheology and quality of deep-fat fried chicken nuggets. *Journal of Food Engineering*, **71**(1), 127–132.

Dos Santos, B. A., Pollonio, M. A. R. and Cruz, A. G. (2013). Ultra-flash profile and projective mapping for describing sensory attributes of prebiotic mortadellas. *Food Research International*, **54**, 1705–1711. doi:10.1016/j.foodres.2013.09.022.

De Oliveira Faria, M., Cipriano, T. M., da Cruz, A. G, Santos, B. A., Pollonio, M. A., Campagnol, P. C. (2015). Properties of bologna-type sausages with pork back-fat replaced with pork skin and amorphous cellulose. *Meat Science* 104: 44--51. doi.org/10.1016/j.meatsci.2015.02.002

Dos Santos, B. A., Bastianello Campagnol, P. C., Bertoldo Pacheco, M. T. et al. (2012) Fructooligosaccharides as a fat replacer in fermented cooked sausages. *International Journal of Food Science and Technology*, 47(6): 1183–1192. doi: 10.1111/j.1365-2621.2012.02958.x

Drewnowski, A. (1997). Taste preferences and food intake. *Annual Review of Nutrition*, **17**(1), 237–253. doi:10.1146/annurev.nutr.17.1.237.

Dunstan, J. A., Simmer, K., Dixon, G. and Prescott, S. L. (2008). Cognitive assessment of children at age 21/2 years after maternal fish oil supplementation in pregnancy: A randomised controlled trial. *Archives of Disease in Childhood—Fetal and Neonatal Edition*, **93**(1), F45–F50. doi:10.1136/adc.2006.099085.

Eckel, R. H., Jakicic, J. M., Ard, J. D. et al. (2014). 2013 AHA/ACC Guideline on lifestyle management to reduce cardiovascular risk: A report of the American College of Cardiology/American Heart Association task force on practice guidelines. *Journal of the American College of Cardiology*, **63**(25 PART B), 2960–2984. doi:10.1016/ j.jacc.2013.11.003.

EFSA Panel and Dietetic Products. (2010). Scientific opinion on establishing food-based dietary guidelines. *EFSA Journal*, 8(3), 1460–1502. doi:10.2903/j. efsa.2010.1460.

Egbert, W., Huffman, R. D. L., Chen, C. M. and Dylewski, D. P. (1991). Development of low-fat ground beef. *Food Technology*, **45**, 64.

Eim, V. S., Simal, S., Rosselló, C. and Femenia, A. (2008). Effects of addition of carrot dietary fibre on the ripening process of a dry fermented sausage (sobrassada). *Meat Science*, **80**(2), 173–182. doi:10.1016/j.meatsci.2007.11.017.

Ergönül, B., Ergönül, P. G. and Obuz, E. (2009). Functional traits of prebiotic ingredients of meat products—chemical, textural and sensory traits of meatballs containing inulin and oligofructose as prebiotic additives. *Fleischwirtschaft*, **89**(2), 140–154.

Felisberto, M. H. F., Galvão, M. T. E. L., Picone, C. S.F. , Cunha, R. L. and Pollonio, M. A. R. (2015). Effect of prebiotic ingredients on the rheological properties and microstructure of reduced-sodium and low-fat meat emulsions. *LWT—Food Science and Technology*, **60**(1), 148–155. doi:10.1016/j.lwt.2014.08.004.

Fernández-Ginés, J. M., Fernández-López, J., Sayas-Barberá, E., Sendra, E. and Pérez-Álvarez, J. A. (2004). Lemon albedo as a new source of dietary fiber: Application to bologna sausages. *Meat Science*, **67**(1), 7–13. doi:10.1016/j. meatsci.2003.08.017.

Fernández-López, J., Sendra, E., Sayas-Barberá, E., Navarro, C. and Pérez-Alvarez, J. A. (2008). Physico-chemical and microbiological profiles of "salchichón" (Spanish dry-fermented sausage) enriched with orange fiber. *Meat Science*, **80**(2), 410–417. doi:10.1016/j.meatsci.2008.01.010.

Finocchiaro, E. T., Birkett, A. and Okoniewska, M. (2009). Resistant starch. *In:* Cho, S. S. and Samuel, P. (eds.) *Fiber Ingredients: Food Applications and Health Benefits*. Boca Raton, FL: CRC Press.

Furuhjelm, C., Warstedt, K., Larsson, J. et al. (2009). Fish oil supplementation in pregnancy and lactation may decrease the risk of infant allergy. *Acta Paediatrica*, **98**(9), 1461–1467. doi:10.1111/j.1651-2227.2009.01355.x.

García, M. L., Cáceres, E. and Selgas, M. D. (2006). Effect of inulin on the textural and sensory properties of mortadella, a Spanish cooked meat product. *International Journal of Food Science and Technology*, **41**(10), 1207–1215. doi:10.1111/j.1365-2621.2006.01186.x.

García, M. L., Dominguez, R., Galvez, M. D., Casas, C. and Selgas, M. D. (2002). Utilization of cereal and fruit fibres in low fat dry fermented sausages. *Meat Science*, **60**(3), 227–236. doi:10.1016/S0309-1740(01)00125-5.

García-Íñiguez de Ciriano, M., Larequi, E., Rehecho, S. et al. (2010). Selenium, iodine, w-3 PUFA and natural antioxidant from *Melissa officinalis* L.: A combination of components from healthier dry fermented sausages formulation. *Meat Science*, **85**(2), 274–279. doi:10.1016/j.meatsci.2010.01.012.

Gibson, G. R., Beatty, E. R. Wang, X. and Cummings, J. H. (1995). Selective stimulation of *Bifidobacteria* in the human colon by oligofructose and inulin. *Gastroenterology*, **108**(4), 975–982. http://www.ncbi.nlm.nih.gov/pubmed/7698613.

Giese, J. (1996). Fats, oils, and fat replacers. *Food Technology*, **61**(4), 78–83.

GL-Laboratories Worldwide. (2002). *Guia Completo Para Sistemas De Cobertura*. Guarulhos: GLLW, 41.

Grigelmo-Miguel, N., Abadías-Serós, M. I. and Martín-Belloso, O. (1999). Characterisation of low-fat high-dietary fibre frankfurters. *Meat Science*, **52**(3), 247–256. doi:10.1016/S0309-1740(98)00173-9.

Grundy, S. M. (1994). Influence of stearic acid on cholesterol metabolism relative to other long-chain fatty acids. *American Journal of Clinical Nutrition*, **60**(6 Suppl), 986S–990S. http://www.ncbi.nlm.nih.gov/pubmed/7977157.

Grundy, S. M. and Vega, G. L. (1988). Plasma cholesterol responsiveness to saturated fatty acids. *American Journal of Clinical Nutrition*, **47**(5), 822–824. http://www.ncbi.nlm.nih.gov/pubmed/3364396.

Hachmeister, K. A. and Herald, T. J. (1998). Thermal and rheological properties and textural attributes of reduced-fat turkey batters. *Poultry Science*, **77**(4), 632–638.

Halford, J. C. G. and Harrold, J. A. (2012). Satiety-enhancing products for appetite control: Science and regulation of functional foods for weight management. *Proceedings of the Nutrition Society*, **71**(2), 350–362. doi:10.1017/S0029665112000134.

Harris, W. S. and Von Schacky, C. (2004). The omega-3 index: A new risk factor for death from coronary heart disease? *Preventive Medicine*, **39**(1), 212–220. doi:10.1016/j.ypmed.2004.02.030.

Hart, B. and Price, K. (1993). New potential for low fat beef burgers. *Food Manufacture*, **68**, 42–43.

Hasjim, J., Lee, S. O., Hendrich, S., Setiawan, S., Ai, Y. and Jane, J. L. (2010). Characterization of a novel resistant-starch and its effects on postprandial plasma-glucose and insulin responses. *Cereal Chemistry*, **87**(4), 257–262. doi:10.1094/CCHEM-87-4-0257.

Herrero, A. M., Carmona, P., Jiménez-Colmenero, F. and Ruiz-Capillas, C. (2014). Polysaccharide gels as oil bulking agents: Technological and structural properties. *Food Hydrocolloids*, **36**, 374–381. doi:10.1016/j.foodhyd.2013.08.008.

Hort, J. and Cook, D. (2007). Formulating low-fat food: The challenge of retaining flavour quality. *In:* Taylor, A. and Hort, J. (eds.) *Modifying Flavour in Food*. Cambridge: Woodhead Publishing in Food Science, Technology and Nutrition, 131–143.

Hoz, L., D'Arrigo, M., Cambero, I. and Ordóñez, J. A. (2004). Development of an n-3 fatty acid and a-tocopherol enriched dry fermented sausage. *Meat Science*, **67**(3), 485–495. doi:10.1016/j.meatsci.2003.12.001.

Hsu, S. Y. and Yu, S. H. (2002). Comparisons on 11 plant oil fat substitutes for low-fat kung-wans. *Journal of Food Engineering*, **51**(3), 9–14.

Huebner-Keese, B., Guo, J., Adden, R. et al. (2016). Enable fat reduced deep fried food and meat products by cellulose ethers. In: Williams, P. A. and Phillips, G. (eds.) *Gums and Stabilisers for the Food Industry 18: Hydrocolloid Functionality for Affordable and Sustainable Global Food Solutions*, UK: Royal Society of Chemistry, pp. 313–320. doi:10.1039/9781782623830-00313.

Hughes, E., Cofrades, S. and Troy, D. J. (1997). Effects of fat level, oat fibre and carrageenan on frankfurters formulated with 5, 12 and 30% fat. *Meat Science*, **45**(3), 273–281. doi:10.1016/S0309-1740(96)00109-X.

Hur, S. J., Jin, S. K. and Kim, I. S. (2008). Effect of extra virgin olive oil substitution for fat on quality of pork patty. *Journal of the Science of Food and Agriculture*, **88**, 1231–1237. doi:10.1002/jsfa.

Jacobsen, C. (1999). Sensory impact of lipid oxidation in complex food systems. *European Journal of Lipid Science and Technology*, **101**(12), 484–492. doi:10.1002/(SICI)1521-4133(199912)101:12<484::AID-LIPI484>3.0.CO;2-H.

Jamshidi, A. and Shabanpour, B. (2014). The effect of hydroxypropyl methylcellulose (HPMC) added pre-dust and batter of talang queenfish (*Scomberoides commersonnianus*) nuggets on the quality and reduction of oil uptake. *Minerva Biotec*, **26**(1), 57–64.

Jiménez-Colmenero, F. (1996). Technologies for developing low-fat meat products. *Trends in Food Science & Technology*, **7**(2), 41–48. doi:10.1016/0924-2244(96)81327-6.

Jiménez-Colmenero, F. (2000). Relevant factors in strategies for fat reduction in meat products. *Trends in Food Science & Technology*, **11**(2), 56–66. doi:10.1016/S0924-2244(00)00042-X.

Jiménez-Colmenero, F. (2007). Healthier lipid formulation approaches in meat-based functional foods. Technological options for replacement of meat fats by non-meat fats. *Trends in Food Science & Technology*, **18**, 567–578. doi:10.1016/j.tifs.2007.05.006.

Jiménez-Colmenero, F., Herrero, A., Pintado, T., Solas, M. T. and Ruiz-Capillas, C. (2010). Influence of emulsified olive oil stabilizing system used for pork backfat replacement in frankfurters. *Food Research International*, **43**(8), 2068–2076. doi:10.1016/j.foodres.2010.06.010.

Jiménez-Colmenero, F., Carballo, J. and Cofrades, S. (2001). Healthier meat and meat products: Their role as functional foods. *Meat Science*, **59**(1), 5–13. doi:10.1016/S0309-1740(01)00053-5.

Jiménez-Colmenero, F., Salcedo-Sandoval, L., Bou, R., Cofrades, S., Herrero, A. M. and Ruiz-Capillas, C. (2015). Novel applications of oil-structuring methods as a strategy to improve the fat content of meat products. *Trends in Food Science & Technology*, **44**(2), 177–188, Elsevier. doi:10.1016/j.tifs.2015.04.011.

Jourdain, L., Leser, M. E., Schmitt, C., Michel, M. and Dickinson, E. (2008). Stability of emulsions containing sodium caseinate and dextran sulfate: Relationship to complexation in solution. *Food Hydrocolloids*, **22**, 647–659. doi:10.1016/j.foodhyd.2007.01.007.

Kayaardı, S. and Gök, V. (2004). Effect of replacing beef fat with olive oil on quality characteristics of turkish soudjouk (Sucuk). *Meat Science*, **66**(1), 249–257. doi:10.1016/S0309-1740(03)00098-6.

Keenan, D. F., Resconi, V. C., Kerry, J. P. and Hamill, R. M. (2014). Modelling the influence of inulin as a fat substitute in comminuted meat products on their physico-chemical characteristics and eating quality using a mixture design approach. *Meat Science*, **96**(3), 1384–1394. doi:10.1016/j.meatsci.2013.11.025.

Keeton, J. T. (1994). Low-fat meat products technological problems with processing. *Meat Science*, **36**, 261–276. doi:10.1016/0309-1740(94)90045-0.

Keys, A., Anderson, J. T. and Grande, F. (1965). Serum cholesterol response to changes in the diet: II. The effect of cholesterol in the diet. *Metabolism: Clinical and Experimental*, **14**(7), 759–765. http://www.ncbi.nlm.nih.gov/pubmed/25286460.

Khalil, A. H. (2000). Quality characteristics of low-fat beef patties formulated with modified corn starch and water. *Food Chemistry*, **68**(1), 61–68. doi:10.1016/S0308-8146(99)00156-9.

Kien, C. L., Bunn, J. Y. and Ugrasbul, F. (2005). Increasing dietary palmitic acid decreases fat oxidation and daily energy expenditure. *American Journal of Clinical Nutrition*, **82**(2), 320–326. http://www.ncbi.nlm.nih.gov/pubmed/16087974.

Koutsopoulos, D. A., Koutsimanis, G. E. and Bloukas, J. G. (2008). Effect of carrageenan level and packaging during ripening on processing and quality characteristics of low-fat fermented sausages produced with olive oil. *Meat Science*, **79**(1), 188–197. doi:10.1016/j.meatsci.2007.08.016.

Kris-Etherton, P. M., Taylor, D. S. Yu-Poth, S. et al. (2000). Polyunsaturated fatty acids in the food chain in the United States. *American Journal of Clinical Nutrition*, **71**(1 Suppl): 179S–188S.

Kris-Etherton, P.M., Harris, W.S., Appel, L.J. (2002). Fish consumption, fish oil, Omega-3 fatty acids, and cardiovascular disease. *Circulation*, **106**(21): 2747–2757.

Kumar, M. and Sharma, B. D. (2004). Quality and storage stability of low-fat pork patties containing barley flour as fat substitute. *Journal of Food Science and Technology*, **41**(5), 496–502.

Lal, S. N. D., O'Connor, C. J. and Eyres, L. (2006). Application of emulsifiers/stabilizers in dairy products of high rheology. *Advances in Colloid and Interface Science*, **123–126**(SPEC. ISS.), 433–437. doi:10.1016/j.cis.2006.05.009.

Lamarche, B. and Couture, P. (2014). It is time to revisit current dietary recommendations for saturated fat. *Applied Physiology Nutrition and Metabolism*, **39**, 1409–1411. doi:10.1139/apnm-2014-0141.

Lee, H. A., Choi, S. J. and Moon, T. W. (2006). Characteristics of sodium caseinate- and soy protein isolate-stabilized emulsion-gels formed by microbial transglutaminase. *Journal of Food Science*, **71**(6), C352–C357. doi:10.1111/j.1750-3841.2006.00110.x.

Leistner, L. (1992). Food preservation by combined processes. *Food Research International*, **25**, 151–158.

Li, L., Jiang, H., Campbell, M., Blanco, M. and Jane, J. (2008). Characterization of maize amylose-extender (AE) mutant starches. Part I: Relationship between resistant starch contents and molecular structures. *Carbohydrate Polymers*, **74**(3), 396–404. doi:10.1016/j.carbpol.2008.03.012.

Liaros, N. G., Katsanidis, E. and Bloukas, J. G. (2009). Effect of the ripening time under vacuum and packaging film permeability on processing and quality characteristics of low-fat fermented sausages. *Meat Science*, **83**(4), 589–598. doi:10.1016/j.meatsci.2009.07.006.

López-López, I., Bastida, S., Ruiz-Capillas, C. et al. (2009). Composition and antioxidant capacity of low-salt meat emulsion model systems containing edible seaweeds. *Meat Science*, **83**(3), 492–498. doi:10.1016/j.meatsci.2009.06.031.

Lucca, P. A. and Tepper, B. J. (1994). Fat replacers and the functionality of fat in foods. *Trends in Food Science and Technology*, **5**(1), 12–19. doi:10.1016/0924-2244(94)90043-4.

Lücke, F.-K. (1985). Fermented sausages. *In:* Wood, B. J. B. (ed.) *Microbiology of Fermented Foods*, vol. 2. Essex: Elsevier Applied Science Publishers, pp. 41–83.

Lupi, F. R., Gabriele, D., Facciolo, D., Baldino, N., Seta, L. and de Cindio, B. (2012). Effect of organogelator and fat source on rheological properties of olive oil-based organogels. *Food Research International*, **46**(1), 177–184. doi:10.1016/j.foodres.2011.11.029.

Lurueña-Martínez, M. A., Vivar-Quintana, A. M. and Revilla, I. (2004). Effect of locust bean/xanthan gum addition and replacement of pork fat with olive oil on the quality characteristics of low-fat frankfurters. *Meat Science*, **68**(3), 383–389. doi:10.1016/j.meatsci.2004.04.005.

Lyons, P. H., Kerry, J. F., Morrissey, P. A. and Buckley, D. J. (1999). The influence of added whey protein/carrageenan gels and tapioca starch on the textural properties of low fat pork sausages. *Meat Science*, **51**(1), 43–52. doi:10.1016/S0309-1740(98)00095-3.

Maffei, H. V. L. (2004). Chronic functional constipation. Which supplementary fiber to choose? *Jornal de Pediatria*, **80**(3), 167–168.

Mansour, E. H. and Khalil, A. H. (1999). Characteristics of low-fat beef burgers as influenced by various types of wheat fibres. *Journal of the Science of Food and Agriculture*, **79**(4), 493–498. doi:10.1002/(SICI)1097-0010(19990315)79:4<493::AID-JSFA4>3.0.CO;2-5.

Mattson, F. H. and Grundy, S. M. (1985). Comparison of effects of dietary saturated, monounsaturated, and polyunsaturated fatty acids on plasma lipids and lipoproteins in man. *Journal of Lipid Research*, **26**(2), 194–202. http://www.ncbi.nlm.nih.gov/pubmed/3989378.

Maw, S. J., Fowler, V. R., Hamilton, M. and Petchey, A. M. (2003). Physical characteristics of pig fat and their relation to fatty acid composition. *Meat Science*, **63**(2), 185–190. doi:10.1016/S0309-1740(02)00069-4.

McClements, D. J. (2004). Protein-stabilized emulsions. *Current Opinion in Colloid and Interface Science*, **9**, 305–313.

Mehta, N., Ahlawat, S. S., Sharma, D. P. and Dabur, R. S. (2015). Novel trends in development of dietary fiber rich meat products—A critical review. *Journal of Food Science and Technology*, **52**(2), 633–647. doi:10.1007/s13197-013-1010-2.

Mendoza, E., García, M. L., Casas, C. and Selgas, M. D. (2001). Inulin as fat substitute in low fat, dry fermented sausages. *Meat Science*, **57**(4), 387–393. doi:10.1016/S0309-1740(00)00116-9.

Menegas, L. Z., Pimentel, T. C., Garcia, S. and Prudencio, S. H. (2013). Dry-fermented chicken sausage produced with inulin and corn oil: Physicochemical, microbiological, and textural characteristics and acceptability during storage. *Meat Science*, **93**(3), 501–506. doi:10.1016/j.meatsci.2012.11.003.

Meyer, D., Bayarri, S., Tárrega, A. and Costell, E. (2011). Inulin as texture modifier in dairy products. *Food Hydrocolloids*, **25**(8), 1881–1890. doi:10.1016/j.foodhyd.2011.04.012.

Mira, G. S., Graf, H. and Cândido, L. M. B. (2009). Visão retrospectiva em fibras alimentares com ênfase em beta-glucanas no tratamento do diabetes. *Brazilian Journal of Pharmaceutical Sciences*, **45**(1), 11–20. doi:10.1590/S1984-82502009000100003.

Monsivais, P., Carter, B. E., Christiansen, M., Perrigue, M. M. and Drewnowski, A. (2011). Soluble fiber dextrin enhances the satiating power of beverages. *Appetite*, **56**(1), 9–14. doi:10.1016/j.appet.2010.10.010.

Montenegro, F. M., Gomes-Ruffi, C. R., Vicente, C. A., Collares-Queiroz, F. P. and Steel, C. J. (2008). Fermented cassava starch biscuits enriched with soluble and insoluble fibers. *Ciência E Tecnologia de Alimentos*, **2863**(9), 184–191.

Mora-Gallego, H., Guàrdia, M. D., Serra, X., Gou, P. and Arnau, J. (2016). Sensory characterisation and consumer acceptability of potassium chloride and sunflower oil addition in small-caliber non-acid fermented sausages with a reduced content of sodium chloride and fat. *Meat Science*, **112**, 9–15. doi:10.1016/j.meatsci.2015.10.008.

Moreira, N. X., Curi, R. and Mancini-Filho, J. (2002). Fatty acids : A review. *Journal Brazilian Social Food Nutrition*, **24**, 105–123.

Muguerza, E., Fista, G., Ansorena, D., Astiasaran, I. and Bloukas, J. G. (2002). Effect of fat level and partial replacement of pork backfat with olive oil on processing and quality characteristics of fermented sausages. *Meat Science*, **61**(4), 397–404. doi:10.1016/S0309-1740(01)00210-8.

Muguerza, E., Gimeno, O., Ansorena, D., Bloukas, J. G. and Astiasarán, I. (2001). Effect of replacing pork backfat with pre-emulsified olive oil on lipid fraction and sensory quality of chorizo de Pamplona—A traditional Spanish fermented sausage. *Meat Science*, **59**(3), 251–258. doi:10.1016/S0309-1740(01)00075-4.

Muguerza, E., Ansorena, D., Astiasaran, I. (2003). Improvement of nutritional properties of Chorizo de Pamplona by replacement of pork backfat with soy oil. *Meat Science*, **65**(4), 1361–1367. doi.org/10.1016/S0309-1740(03)00058-5

Murphy, P. (2000). Starch. In G. O. Phillips & P. A. Williams (Eds.), *Handbook of food hydrocolloids* (pp. 41–65), Cambridge: Woodhead Publishing.

Mussatto, S. I. and Mancilha, I. M. (2007). Non-digestible oligosaccharides: A review. *Carbohydrate Polymers*, **68**(3), 587–597. doi:10.1016/j.carbpol.2006.12.011.

Niness, K. R. (1999). Inulin and oligofructose: What are they? *Journal of Nutrition*, **129**(7 Suppl), 1402S–1406S. http://www.ncbi.nlm.nih.gov/pubmed/10395607.

Novakofski, J., Park, S., Bechtel, P. J. and McKeith, F. K. (1989). Composition of cooked pork chops: Effect of removing subcutaneous fat before cooking. *Journal of Food Science*, **54**(1), 15–17. doi:10.1111/j.1365-2621.1989.tb08556.x.

Nowak, B., von Mueffling, T., Grotheer, J., Klein, G. and Watkinson, B.-M. (2007). Energy content, sensory properties, and microbiological shelf life of German bologna-type sausages produced with citrate or phosphate and with inulin as fat replacer. *Journal of Food Science*, **72**(9), S629–S638. doi:10.1111/j.1750-3841.2007.00566.x.

Olmedilla-Alonso, B., Jiménez-Colmenero, F. and Sánchez-Muniz, F. J. (2013). Development and assessment of healthy properties of meat and meat products designed as functional foods. *Meat Science*, **95**(4), 919–930. doi:10.1016/j.meatsci.2013.03.030.

Ordóñez, J. A. (2005). *Tecnologia de Alimentos: Componentes dos Alimentos e Processos* (1st Ed.). Porto Alegre: Editora Artmed.

Ospina-E, J. C., Sierra-C, A., Ochoa, O., Pérez-Álvarez, J. A. and Fernández-López, J. (2012). Substitution of saturated fat in processed meat products: A review. *Critical Reviews in Food Science and Nutrition*, **52**(2), 113–122. doi:10.1080/10408398.2010.493978.

Paneras, E. D. and Bloukas, J. G. (1994). Vegetable oils replace pork backfat for low-fat frankfurters. *Journal of Food Science*, **59**(4), 725–728. doi:10.1111/j.1365-2621.1994.tb08113.x.

Paneras, E. D., Bloukas, J. G. and Filis, D. G. (1998). Production of low-fat frankfurters with vegetable oils following the dietary guidelines for fatty acids. *Journal of Muscle Foods*, **9**(2), 111–126. doi:10.1111/j.1745-4573.1998.tb00648.x.

Papadima, S. N. and Bloukas, J. G. (1999). Effect of fat level and storage conditions on quality characteristics of traditional Greek sausages. *Meat Science*, **51**(2), 103–113. doi:10.1016/S0309-1740(98)00103-X.

Paradiso, V. M., Giarnetti, M., Summo, C., Pasqualone, A., Minervini, F. and Caponio, F. (2015). Production and characterization of emulsion filled gels based on inulin and extra virgin olive oil. *Food Hydrocolloids*, **45**, 30–40. doi:10.1016/j.foodhyd.2014.10.027.

Passos, L. and Park, Y. K. (2003). Fructooligosaccharides: Implications in human health being and use in foods. *Ciência Rural*, **3333**(22), 385–390.

Pearson, A. M. and Gillett, T. A. (1996). *Processed Meats*. US: Springer, pp. 355–371.

Pelser, W. M, Linssen, J. P. H., Legger, A. and Houben, J. H. (2007). Lipid oxidation in n-3 fatty acid enriched Dutch style fermented sausages. *Meat Science*, **75**(1), 1–11. doi:10.1016/j.meatsci.2006.06.007.

Perrechil, F. A. and Cunha, R. L. (2013). Stabilization of multilayered emulsions by sodium caseinate and κ-carrageenan. *Food Hydrocolloids*, **30**(2), 606–613. doi:10.1016/j.foodhyd.2012.08.006.

Petersson, K., Godard, O., Eliasson, A. and Tornberg, E. (2014). The effects of cereal additives in low-fat sausages and meatballs. Part 2: Rye bran, oat bran and barley fibre. *Meat Science*, **96**(1), 503–508. doi:10.1016/j.meatsci.2013.08.019.

Picone, C. S. F., Takeuchi, K. P. and Cunha, R. L. (2011). Heat-induced whey protein gels: Effects of pH and the addition of sodium caseinate. *Food Biophysics*, **6**(1), 77–83. doi:10.1007/s11483-010-9177-9.

Pietrasik, Z. and Duda, Z. (2000). Effect of fat content and soy protein/carrageenan mix on the quality characteristics of comminuted, scalded sausages. *Meat Science*, **98**(2), 181–188. doi.org/10.1016/S0309-1740(00)00038-3

Pietrasik, Z. and Janz, J. A. M. (2010). Utilization of pea flour, starch-rich and fiber-rich fractions in low fat bologna. *Food Research International*, **43**(2), 602–608. doi:10.1016/j.foodres.2009.07.017.

Piñero, M. P., Parra, K., Huerta-Leidenz, N. et al. (2008). Effect of oat's soluble fibre (B-glucan) as a fat replacer on physical, chemical, microbiological and sensory properties of low-fat beef patties. *Meat Science*, **80**(3), 675–680. doi:10.1016/j.meatsci.2008.03.006.

Pintado, T., Herrero, A. M., Jiménez-Colmenero, F. and Ruiz-Capillas, C. (2016). Strategies for incorporation of chia (*Salvia Hispanica* L.) in frankfurters as a health-promoting ingredient. *Meat Science*, **114**, 75–84. doi:10.1016/j.meatsci.2015.12.009.

Pires Vilela, J. A., Cavallieri, A. L. F. and Lopes da Cunha, R. (2011). The influence of gelation rate on the physical properties/structure of salt-induced gels of soy protein isolate–gellan gum. *Food Hydrocolloids*, **25**(7), 1710–1718. doi:10.1016/j.foodhyd.2011.03.012.

Poyato, C., Ansorena, D., Berasategi, I., Navarro-Blasco, Í. and Astiasarán, I. (2014). Optimization of a gelled emulsion intended to supply ω-3 fatty acids into meat products by means of response surface methodology. *Meat Science*, **98**(4), 615–621. doi:10.1016/j.meatsci.2014.06.016.

Pronczuk, A., Khosla, P., Hajri, T. and Hayes, K. C. (1995). Plasma lipids are affected similarly by dietary lauric or palmitic acid in gerbils and monkeys. *Lipids*, **30**(12), 1157–1161. doi:10.1007/BF02536618.

Prosky, L. and Hoebregs, H. (1999). Methods to determine food inulin and oligofructose. *Journal of Nutrition*, **129**(7 Suppl), 1418S–1423S. http://www.ncbi.nlm.nih.gov/pubmed/10395610.

Raigond, P., Ezekiel, R. and Raigond, B. (2015). Resistant starch in food: A review. *Journal of the Science of Food and Agriculture*, **95**(10), 1968–1978. doi:10.1002/jsfa.6966.

Roberfroid, M. (1993). Dietary fiber, inulin, and oligofructose: A review comparing their physiological effects. *Critical Reviews in Food Science and Nutrition*, **33**(2), 103–148. doi:10.1080/10408399309527616.

Roberfroid, M. and Slavin, J. L. (2000). Nondigestible oligosaccharides. *Critical Reviews in Food Science and Nutrition*, **40**(6), 461–480. doi:10.1080/10408690091189239.

Rodríguez-Carpena, J. G., Morcuende, D. and Estévez, M. (2012). Avocado, sunflower and olive oils as replacers of pork back-fat in burger patties: Effect on lipid composition, oxidative stability and quality traits. *Meat Science*, **90**(1), 106–115. doi:10.1016/j.meatsci.2011.06.007.

Roller, S. and Jones, S. A. (1996). *Handbook of Fat Replacers*. Boca Raton: CRC Press.

Rothstein, W. G. (2006). Dietary fat, coronary heart disease, and cancer: A historical review. *Preventive Medicine*, **43**(5), 356–360. doi:10.1016/j.ypmed.2006.07.013.

Rubio, B., Martínez, B., García-Cachán, M. D., Rovira, J. and Jaime, I. (2008). Effect of the packaging method and the storage time on lipid oxidation and colour stability on dry fermented sausage salchichón manufactured with raw material with a high level of mono and polyunsaturated fatty acids. *Meat Science*, **80**, 1182–1187. doi:10.1016/j.meatsci.2008.05.012.

Rust, R. and Olson, D. (1988). Making good "lite" sausage. *Meat and Poultry*, **34**, 1012.

Saad, S. M. I. (2006). Probiotics and prebiotics: The state of the art. *Revista Brasileira de Ciências Farmacêuticas*, **42**(1), 1–16. doi:10.1590/S1516-93322006000100002.

Sahin, S., Sumnu, G. and Altunakar, B. (2005). Effects of batters containing different gum types on the quality of deep-fat fried chicken nuggets. *Journal of the Science of Food and Agriculture*, **85**(14), 2375–2379. doi:10.1002/jsfa.2258.

Sajilata, M. G., Singhal, R. S. and Kulkarni, P. R. (2006). Resistant starch—A review. *Comprehensive Reviews in Food Science and Food Safety*, **5**(1), 1–17. doi:10.1111/j.1541-4337.2006.tb00076.x.

Salazar, P., García, M. L. and Selgas, M. D. (2009). Short-chain fructooligosaccharides as potential functional ingredient in dry fermented sausages with different fat levels. *International Journal of Food Science and Technology*, **44**(6), 1100–1107. doi:10.1111/j.1365-2621.2009.01923.x.

Sarteshnizi, A. R., Hosseini, H., Bondarianzadeh, D., Jiménez-Colmenero, F. and Khaksar, R. (2015). Optimization of prebiotic sausage formulation: Effect of using β-glucan and resistant starch by D-optimal mixture design approach. *LWT—Food Science and Technology*, **62**(1), 704–710. doi:10.1016/j.lwt.2014.05.014.

Schaefer, E. J. (2002). Lipoproteins, nutrition, and heart disease. *American Journal of Clinical Nutrition*, **75**(2), 191–212. http://www.ncbi.nlm.nih.gov/pubmed/11815309.

Sendra, E., Fayos, P., Lario, Y., Fernández-López, J., Sayas-Barberá, E. and Pérez-Alvarez, J. A. (2008). Incorporation of citrus fibers in fermented milk containing probiotic bacteria. *Food Microbiology*, **25**(1), 13–21. doi:10.1016/j.fm.2007.09.003.

Serdaroglu, M. (2006). The characteristics of beef patties containing different levels of fat and oat flour. *International Journal of Food Science and Technology*, **41**(2), 147–153. doi:10.1111/j.1365-2621.2005.01041.x.

Serrano, A., Cofrades, S., Ruiz-Capillas, C., Olmedilla-Alonso, B., Herrero-Barbudo, C. and Jiménez-Colmenero, F. (2005). Nutritional profile of restructured beef steak with added walnuts. *Meat Science*, **70**(4), 647–654. doi:10.1016/j.meatsci.2005.02.014.

Setser, C. S. and Racette, E. L. (1992). Macromolecule replacers in food products. *Critical Reviews in Food Science and Nutrition*, **32**(3), 275–297.

Severini, C., De Pilli, T. and Baiano, A. (2003). Partial substitution of pork backfat with extra-virgin olive oil in 'salami' products: Effects on chemical, physical and sensorial quality. *Meat Science*, **64**(3), 323–331. doi:10.1016/S0309-1740(02)00204-8.

Shand, J. P., Schmidt, G. R., Mandigo, R. W. and Claus, J. R. (1990). New technology for low-fat meat products. *Reciprocal Meat Conference Proceedings*, **43**, 37–46.

Sheard, P. (2002). Processing and quality control of restructured meat. *Meat Processing*, 332–358. doi:10.1533/9781855736665.3.332.

Shimokomaki, M.; Olivo, P., Terra, N. N. Fermentação cárnea: Princípios e Inovações. *In Atualidades em Ciência e Tecnologia de Carnes*. São Paulo: Editora Varela, 29-36.

Simo, O. K., Mao, Y., Tokle, T., Decker, E. A. and McClements, D. J. (2012). Novel strategies for fabricating reduced fat foods: Heteroaggregation of lipid droplets with polysaccharides. *Food Research International*, **48**(2), 337–345. doi:10.1016/j.foodres.2012.04.018.

Simopoulos, A. P. (1999). Essential fatty acids in health and chronic disease. *American Journal of Clinical Nutrition*, **70**(3 Suppl), 560S–569S. http://www.ncbi.nlm.nih.gov/pubmed/10479232.

Simopoulos, A. P. (2004). Omega-6/omega-3 essential fatty acid ratio and chronic diseases. *Food Reviews International*, **20**(1), 77–90. doi:10.1081/FRI-120028831.

Singh, O. P., Singh, J. N., Barthi, M. K. and Soni, K. (2008). Refrigerated storage stability of chicken nuggets containing pea flour. *Journal of Food Science and Technology*, **45**(5), 460–462.

Slavin, J. L. (2005). Dietary fiber and body weight. *Nutrition*, **21**(3), 411–418. doi:10.1016/j.nut.2004.08.018.

Sorapukdee, S., Kongtasorn, C., Benjakul, S. and Visessanguan, W. (2013). Influences of muscle composition and structure of pork from different breeds on stability and textural properties of cooked meat emulsion. *Food Chemistry*, **138**(2–3), 1892–1901. doi:10.1016/j.foodchem.2012.10.121.

Spady, D. K. and Dietschy, J. M. (1985). Rates of cholesterol synthesis and low-density lipoprotein uptake in the adrenal glands of the rat, hamster and rabbit *in vivo*. *Biochimica et Biophysica Acta (BBA)—Lipids and Lipid Metabolism*, **836**(2), 167–175. doi:10.1016/0005-2760(85)90063-3.

Spiegel, J. E., Rose, R., Karabell, P., Francos, V. H. and Schmitt, D. F. (1994). Safety and benefits of frutooligosaccharides as food ingredients. *Food Technology*, **48**, 85–89.

Stowell, J. D. (2009). Polydextrose. *In:* Cho, S. S. and Samuel, P. (eds.) *Fiber Ingredients: Food Applications and Health Benefits*. Boca Raton, FL: CRC Press.

Sturdevant, R. A. L., Pearce, M. L. and Dayton, S. (1973). Increased prevalence of cholelithiasis in men ingesting a serum-cholesterol-lowering diet. *New England Journal of Medicine*, **288**(1), 24–27, Massachusetts Medical Society. doi:10.1056/NEJM197301042880106.

Sych, J., Lacroix, C., Adambounou, L. T. and Castaigne, F. (1990). Cryoprotective effects of lactitol, palatinit and Polydextrose® on cod surimi proteins during frozen storage. *Journal of Food Science*, **55**(2), 356–360. doi:10.1111/j.1365-2621.1990.tb06762.x.

Symes, K. C. (1980). The relationship between the covalent structure of the *Xanthomonas* polysaccharide (xanthan) and its function as a thickening, suspending and gelling agent. *Food Chemistry*, **6**(1), 63–76.

Talukder, S. (2015). Effect of dietary fiber on properties and acceptance of meat products: A review. *Critical Reviews in Food Science and Nutrition*, **55**(7), 1005–1011. doi :10.1080/10408398.2012.682230.

Tan, S. S., Aminah, A., Zhang, X. G. and Abdul, S. B. (2006). Optimizing palm oil and palm stearin utilization for sensory and textural properties of chicken frankfurters. *Meat Science*, **72**(3), 387–397. doi:10.1016/j.meatsci.2005.07.012.

Trius, A., Sebranek, J. G., Rust, R. E. and Carr, J. M. (1994). Low-fat bologna and beaker sausage: Effects of carrageenans and chloride salts. *Journal of Food Science*, **59**(5), 941–945. doi:10.1111/j.1365-2621.1994.tb08163.x.

Troutt, E. S., Hunt, M. C., Johnson, D. E., Claus, J. R., Kastner, C. L. and Kropf, D. H. (1992). Characteristics of low-fat ground beef containing texture-modifying ingredients. *Journal of Food Science*, **57**(1), 19–24. doi:10.1111/j.1365-2621.1992.tb05415.x.

Trowell, H., Southgate, D. A., Wolever, T. M., Leeds, A. R., Gassull, M. A. and Jenkins, D. J. (1976). Dietary fibre redefined. *Lancet* (London, England), **1**(7966), 967. http://www.ncbi.nlm.nih.gov/pubmed/57372.

Tungland, B. C. and Meyer, D. (2002). Nondigestible oligo- and polysaccharides (dietary fiber): Their physiology and role in human health and food. *Comprehensive Reviews in Food Science and Food Safety*, **1**(3), 90–109. doi:10.1111/j.1541-4337.2002.tb00009.x.

Turp, G. Y. (2016). Effects of four different cooking methods on some quality characteristics of low fat Inegol meatball enriched with flaxseed flour. *Meat Science*, **121**, 40–46. doi:10.1016/j.meatsci.2016.05.016.

Uemura, C. H. and Luz, M. B. (2003). Sistemas de cobertura. *Aditivos & Ingredientes*, **28**, 81–82.

Valencia, I., Ansorena, D. and Astiasaran, I. (2007). Development of dry fermented sausages rich in docosahexaenoic acid with oil from the *Microalgae schizochytrium sp.:* Influence on nutritional properties, sensorial quality and oxidation stability. *Food Chemistry*, **104**(3), 1087–1096. doi:10.1016/j.foodchem.2007.01.021.

Valencia, I., O'Grady, M. N., Ansorena, D., Astiasarán, I. and Kerry, J. P. (2008). Enhancement of the nutritional status and quality of fresh pork sausages following the addition of linseed oil, fish oil and natural antioxidants. *Meat Science*, **80**(4), 1046–1054. doi:10.1016/j.meatsci.2008.04.024.

Valsta, L. M., Tapanainen, H. and Männistö, S. (2005). Meat fats in nutrition. *Meat Science*, **70**(3), 525–530. doi:10.1016/j.meatsci.2004.12.016.

Varnam, A. H. and Sutherland, J. P. (1995). *Meat and Meat Products: Technology, Chemistry, and Microbiology*. New York, NY: Chapman & Hall.

Vedin, I., Cederholm, T., Levi, Y. F. et al. (2008). Effects of docosahexaenoic acid-rich n-3 fatty acid supplementation on cytokine release from blood mononuclear leukocytes: The OmegAD study. *American Journal of Clinical Nutrition*, **87**(6), 1616–1622. http://www.ncbi.nlm.nih.gov/pubmed/18541548.

Verma, A. K., Sharma, B. D. and Banerjee, R. (2010). Effect of sodium chloride replacement and apple pulp inclusion on the physico-chemical, textural and sensory properties of low fat chicken nuggets. *LWT—Food Science and Technology*, **43**(4), 715–719. doi:10.1016/j.lwt.2009.12.006.

Verplaetse, A. (1994). Influence of raw meat properties and processing technology on aroma quality of raw fermented meat products. *Proceedings of the 40th International Congress on Meat Science and Technology*, 45–65, Den Haag.

Vessby, B. (2003). Dietary fat, fatty acid composition in plasma and the metabolic syndrome. *Current Opinion in Lipidology*, **14**(1), 15–19. doi:10.1097/01.mol.0000052859.26236.5f.

Wang, Y. (2009). Prebiotics: Present and future in food science and technology. *Food Research International*, **42**, 8–12.

Warner, K. and Inglett, G. E. (1997). Flavor and texture characteristics of foods containing Z-trim corn and oat fibers as fat and flour replacers. *Cereal Foods World*, **42**(10), 821–825.

Webb, E. C. and O'Neill, H. A. (2008). The animal fat paradox and meat quality. *Meat Science*, **80**(1), 28–36. doi:10.1016/j.meatsci.2008.05.029.

Weiss, J., Gibis, M., Schuh, V. and Salminen, H. (2010). Advances in ingredient and processing systems for meat and meat products. *Meat Science*, **86**(1), 196–213. doi:10.1016/j.meatsci.2010.05.008.

Whitney, E. N. and Rolfes, S. R. (2002). *Understanding Nutrition.* Belmont, CA: Wadsworth.

World Health Organization (WHO). (2003). Diet, nutrition and the prevention of chronic diseases. *World Health Organization Technical Report Series*. Geneva, 916: i–viii-1-149.

Wirth, F. (1988). Technologies for making fat-reduce meat products, what possibilities are there? *Fleischwirtschaf*, **68**, 1153–1156.

Yang, A., Keeton, J. T., Beilken, S. L. and Trout, G. R. (2001). Evaluation of some binders and fat substitutes in low-fat frankfurters. *Journal of Food Science*, **66**(7), 1039–1046. doi:10.1111/j.1365-2621.2001.tb08232.x.

Yasarlar, E. E., Daglioglu, O. and Yilmaz, I. (2007). Effects of cereal bran addition on chemical composition, cooking characteristics and sensory properties of Turkish meatballs. *Asian Journal of Chemistry*, **19**(3), 2353–2361.

Yilmaz, I. (2005). Physicochemical and sensory characteristics of low fat meatballs with added wheat bran. *Journal of Food Engineering*, **69**, 369–373. doi:10.1016/j.jfoodeng.2004.08.028.

Yilmaz, I. and Dağlıoğlu, O. (2003). The effect of replacing fat with oat bran on fatty acid composition and physicochemical properties of meatballs. *Meat Science*, **65**(2), 819–823. doi:10.1016/S0309-1740(02)00286-3.

Yilmaz, I. and Geçgel, U. (2009). Effect of inulin addition on physico-chemical and sensory characteristics of meatballs. *Journal of Food Science and Technology*, **46**(5), 473–476, Association of Food Scientists and Technologists (India).

Yoo, S. S., Kook, S. H., Park, S. Y., Shim, J. H. and Chin, K. B. (2007). Physicochemical characteristics, textural properties and volatile compounds in comminuted sausages as affected by various fat levels and fat replacers. *International Journal of Food Science and Technology*, **42**(9), 1114–1122. doi:10.1111/j.1365-2621.2006.01402.x.

Youssef, M. K. and Barbut, S. (2009). Effects of protein level and fat/oil on emulsion stability, texture, microstructure and color of meat batters. *Meat Science*, **82**(2), 228–233, Elsevier. doi:10.1016/j.meatsci.2009.01.015.

Yue, P. and Waring, S. (1998). Resistant starch in food applications. *Cereal Foods World*, **43**(9), 690–695.

Zhang, W., Xiao, S., Samaraweera, H., Lee, E. J. and Ahn, D. U. (2010). Improving functional value of meat products. *Meat Science*, **86**(1), 15–31. doi:10.1016/j.meatsci.2010.04.018.

Zhuang, X., Han, M., Kang, Z. L. et al. (2016). Effects of the sugarcane dietary fiber and pre-emulsified sesame oil on low-fat meat batter physicochemical property, texture, and microstructure. *Meat Science*, **113**, 107–115, Elsevier B.V. doi:10.1016/j.meatsci.2015.11.007.

Zóia, D. (2011). As gomas exudadas de plantas. *Food Ingredients Brasil*, **17**, 26.



Taylor & Francis
Taylor & Francis Group
http://taylorandfrancis.com

16

Processing of Nitrite-Free Cured Meats

Fereidoon Shahidi and Ronald B. Pegg

CONTENTS

16.1 Origins of Nitrite Curing of Meats 513
16.2 *N*-Nitrosamines 515
16.3 Processing Options When Curing Meats 515
16.3.1 Formulating 515
16.3.2 Curing Methods 516
16.3.2.1 Dry Curing 516
16.3.2.2 Brine Curing 517
16.3.2.3 Multiple-Needle Pumping 518
16.3.2.4 Tumbling or Massaging 518
16.3.2.5 Chopping or Blending 519
16.4 Benefits and Drawbacks of Nitrite Curing 519
16.4.1 Color Characteristics 519
16.4.2 Antioxidant Properties 523
16.4.3 Flavor Characteristics 525
16.4.4 Antimicrobial Properties 526
16.5 Tailor Designing Nitrite-Free Meat Products 528
16.6 Other Developments 529
References 529

16.1 Origins of Nitrite Curing of Meats

The origin of salting meats is lost in antiquity, but it is believed that it was first practiced and flourished in Sumeria during the third and fourth millenniums BC. From a historical perspective, meat curing can be defined as the addition of salt to meats for the sole purpose of preservation, that is, to inhibit or deter microbial spoilage. The preservation of meat resulted from necessity, so that the products could be held for extended periods for later consumption in times of scarcity. It was recognized that fresh cuts of meat could be preserved by treating them with a salt solution or packing them in dry salt (Aberle et al., 2001). Salting prevented bacterial growth on account of salt's direct inhibitory effect or because of the drying action it had on meat (note that most bacteria require

substantial amounts of moisture to survive and proliferate). Thus, rock salt was an important commodity that was routinely employed for muscle food preservation in ancient China, Babylonia, and Sumeria (Jensen, 1953). However, high concentrations of salt promoted the formation of an unattractive brownish-gray color within lean muscle tissue. At some point in the development of this art, more likely by accident than design, it was discovered that certain salts (i.e., those containing saltpeter) could impart or "fix" a unique pink or red color and flavor in meats (Binkerd and Kolari, 1975). A preference developed for the use of this special salt. Granulated or grain salt was formerly called "corn," which comes from the Old Norse, *korn*, meaning grain; thus when beef was sprinkled with these salts, corned beef was the resultant product.

By medieval times, treating meat with salt, saltpeter, and smoke was commonplace, and saltpeter's effect to "fix" the red color was well recognized. Gradually, sweet pickle and sugar cures evolved as sucrose became available as a commodity of trade. Sugar added flavor to the meat and helped to counteract some of the harshness and hardening effects of salt. As the art progressed, the term *meat curing* eventually was understood as the addition of salt, sugar, spices, saltpeter (nitrate), or nitrite to meat for its preservation and flavor enhancement (Townsend and Olson, 1987). Spices and other flavorings were added to achieve distinctive brand flavors.

Scientific principles of meat curing were not applied until the latter half of the nineteenth and the early twentieth century when the growing meatpacking trade began to search for ways to improve quality and to extend the shelf life of products. It was discovered that nitrite, not nitrate as originally thought, plays a multifunctional role in the meat matrix (Haldane, 1901; Polenske, 1891). Nitrite is responsible for developing or "fixing" the characteristic color associated with cured meats; for creating a special flavor so that one can distinguish the flavor of corned beef from roast beef; for imparting antioxidant activity to the cooked product, thereby extending its shelf life; and for suppressing the outgrowth and production of toxin from the anaerobic bacterium, *Clostridium botulinum*. Since nitrite has been added to cure meats, U.S. Centers for Disease Control statistics indicate that botulism is no longer associated with cured meats. The industry has evolved to the point that quite a diverse list of cured meat products offering great taste, convenience, and versatility is available to the consumer. On account of household refrigeration, the original need to cure meats no longer exists; nevertheless, consumers have become accustomed to certain products in their diet and still demand their availability in the market.

Until the late 1960s and early 1970s, the primary technological emphasis of nitrite usage had been to reduce the time required for curing as much as possible to increase production capacity. Modern technology and scientific understanding had made it possible to utilize smaller quantities of nitrite while exercising vastly improved control over the curing of meat and meat products. Suddenly, the technological emphasis shifted to problem solving with particular regard to *N*-nitrosamine production (Sebranek, 1979, 2010).

16.2 *N*-Nitrosamines

Despite all of its desirable effects in processed meat products, nitrite under certain conditions such as pan frying forms *N*-nitrosamines at trace quantities (i.e., parts-per-billion levels). Typical volatile *N*-nitrosamines detected in heat processed cured products include *N*-nitrosodimethylamine (NDMA) and *N*-nitrosopyrrolidine (NPYR). Among cured products, fried bacon has consistently shown the presence of NDMA and NPYR at mean levels of up to 3 and 25 μg/kg, respectively (Glória et al., 1997). These *N*-nitrosamines are carcinogenic, mutagenic, and teratogenic in experimental animals (Preussmann and Stewart, 1984; Tricker and Preussmann, 1991). Although the carcinogenicity of *N*-nitrosamines in humans cannot be tested, epidemiological studies have suggested a possible link to the incidence of various cancers in humans.

N-nitrosamines are formed from the reaction of nitrite with free amino acids and amines in meat products under certain heat processing conditions (e.g., high temperatures associated with frying of bacon), or in the stomach after consumption. As it is difficult to control the level of endogenous factors, such as amino acids and amines, a reduction in the level of nitrite added to products or specifics of reaction and process conditions might be necessary. Thus, the allowable level of nitrite addition in cured meats has been reduced to a maximum of 150–200 mg/kg in most products, with lower maximum addition levels for bacon (about 120 mg/kg). The meat industry has responded adequately and responsibly to concerns expressed about nitrite and the control of *N*-nitrosamine formation in processed meats. Nonetheless, two controversial studies published in the mid- to late 1990s recommended that excessive consumption of hot dogs and cured products be avoided to prevent the occurrence of leukemia in children (Blot et al., 1999; Peters et al., 1994). To keep things in perspective, it should be noted that humans excrete noncarcinogenic *N*-nitrosoproline in their urine, thereby demonstrating that such compounds are also formed within the body (Loeppky, 1994).

16.3 Processing Options When Curing Meats

Before a discussion of processing meats using nitrite-free curing systems can be given, a review of formulation and processing options presently employed for curing meats is required.

16.3.1 Formulating

Formulating any food product is much more than simply recipe development. In fact some even call it an art. It involves detailing the required processing steps and in what particular order they must be carried out to

produce a high-quality finished product. Decisions when formulating meat products can entail the selection of meats and their levels of addition; choice of nonmeat ingredients such as salt, sweeteners, binders, and seasonings; method and length of curing; grind or chop size of meat and fat; oven or smokehouse schedules; type and diameter of casings; and type and method of packaging. Formulating meat products also requires preparing a product that meets recognized characteristics set out by the country's standards of identity for that product. For meat companies, innovation is one of the single most important factors in building and maintaining a successful product or brand. A brand can be well recognized, but if over time that brand does not continue to offer value to consumers it will soon be eclipsed in the market.

When designing meat products it is practical to formulate by ingredient weight, which is based on the amount of the meat block. When formulating the seasonings, the processor must work in the weight of seasoning per 100 lb. or kg of meat. Quantities can then be easily converted to percentages and the seasoning formula determined. For meat blocks not in 100 lb. or kg increments, the percentage usage of spice, oleoresin, or seasoning is calculated per pound or kilogram and then multiplied by the weight desired to determine the quantity required. Many processors weigh out the dry ingredients in a controlled-access room where the temperature and humidity are carefully monitored. Often after weighing, the critical components are packaged and then assembled for batch production. A checklist must be prepared and verified to ensure that all of the materials are accounted for. Meat processors keep very detailed records of all formulas, and most are proprietary. The formulas are designed so that costs can be easily calculated and updated as needed (especially in the case of least cost formulation products). Standard blending or formulation documentation includes a list of ingredients, the weight of the individual ingredients in one seasoning unit, the percentage of each ingredient in a batch, and the laboratory and plant code numbers.

16.3.2 Curing Methods

There are a few means by which meats can be cured. The basic methods include the following.

16.3.2.1 Dry Curing

Dry curing is the oldest technique, traditionally employed in bacon and ham manufacturing. It involves the application of uniform and quantified mixtures of salt, sugar, spices, and saltpeter or sodium nitrite to solid meat cuts. Industrial applications of dry curing are still carried out in Europe, but are generally restricted to the preparation of specialty ham products. The curing agents are rubbed in dry form over the surface of meat cuts, which are then placed in a cool room and allowed to cure. No water is added, so the curing agents are solubilized in the endogenous moisture of the muscle tissue. With

time, slow penetration of the cure into the meat via diffusion (about 2.5 cm/week) and micrococcal reduction of nitrate to nitrite affords the characteristic cured meat color and flavor of the product (Fox, 1974). More than one application of the salt mixture is necessary to affect a cure; the cuts must be "overhauled," or turned over and restacked. This labor-intensive process requires a considerably longer period than curing comminuted meats; this is the main disadvantage of this approach. Another problem is that in thicker pieces of meat, spoilage organisms can begin growing before the preservatives reach all parts of the product. The manufacture of dry-cured hams, nevertheless, provides a class of products with flavor and taste that cannot be re-created by any other nitrite-containing or nitrite-free curing method.

After curing is complete, the excess cure is washed off and the meat is placed under refrigeration (2°C–4°C) for 20–40 days to allow for salt equalization throughout. In the case of hams, the meat is held in natural or air-conditioned drying chambers and ripened for a minimum of 6 months and often 12 months or more, depending on each country's traditional practices. The temperature is usually varied between 14°C and 20°C at relative humidities ranging from 90% to 70%. Complex biochemical reactions that are mainly proteolytic and lipolytic in nature occur and a characteristic flavor is developed (Flores and Toldrá, 1993). Dry curing is used only for specialty items such as country-cured hams and bacon, as well as European-type dry-cured hams such as Spanish Serrano and Iberian hams, Italian Parma, and San Daniele prosciuttos, or French Bayonne hams. These European hams are usually consumed raw, unlike country-style hams in the United States and Westphalia hams in Germany, which are smoked and then thermally processed before consumption (Toldrá and Flores, 1998). Worldwide production of dry-cured products represents an important segment of the processed meat industry because these products possess unique flavor and texture attributes that apparently cannot be developed by any other means (Aberle et al., 2001).

16.3.2.2 Brine Curing

Brine is prepared by combining the salt, cure (i.e., sodium nitrite), and water-soluble seasoning mixtures in water, which serves as a carrier. The strength of a brine solution or "pickle" is determined by the amount of salt present. A salometer is a specially graduated hydrometer that measures the strength of brines at a particular temperature (usually 40°F/4.4°C) and is calibrated to indicate the degree of salinity (this is essentially a measure of the brine's density). A 100° salometer reading is equivalent to a 100% saturated salt solution. Most large processors prepare stock solutions of brine at a 100° salometer reading and then formulate working pickles with additional additives at lower strengths. The presence of sweeteners, phosphates, nitrite, and erythorbate in a brine will affect salometer readings to an extent. Typical pickles have strengths of 60°–70°, with 70° brine being the most common. For brine

immersion or cover pickling, the meat pieces are simply immersed in the brine for a specified period. For example, hams and shoulders are normally cured for 2–2.5 days per pound in 70° brine. Due to high water activity, microbial growth and spoilage can arise during pickling even though the product is refrigerated and salt is present at an appreciable concentration. Although the penetration of ingredients into the muscle tissue is faster than in dry curing, this technique also suffers from slowness and is not widely employed by the industry. Presently, only specialty products such as neck bones, tails, pigs' feet, and salt pork are cured in North America in this way (Aberle et al., 2001).

16.3.2.3 Multiple-Needle Pumping

The practice of pumping or injecting meat with a perforated needle originated in the late nineteenth century and greatly shortened the length of time required to cure meat. The process of multiple-needle injection has become popular and such designed machines have ensured rapid, continuous processing of meat cuts. A brine or pickle is prepared and then injected mechanically under pressure through needles, which are perforated along the stem near the point, into primal cuts of meat. In this multiple-needle injection technique, a conveyor belt carries meat under a bank of offset needles through which brine is pumped until a desired target weight is achieved. The spacing of the needles, their size, and the dwell time between strokes are important variables to ensure good distribution and retention of the pickle. By way of the many channels running throughout the muscle tissue, the cure is rapidly distributed. The brine injected into commercial mild-cured products is typically a 70° pickle. The main advantages of multiple-needle pumping include increased product yield, greatly reduced labor costs, and time required for production. After pumping, some products are cooked immediately, whereas others are further processed by immersing them in a brine cure for a period (e.g., Canadian bacon) or subjecting them to a mechanical operation such as tumbling.

16.3.2.4 Tumbling or Massaging

Tumbling or massaging of pickle-injected meat cuts is generally employed to speed up the curing process, to facilitate extraction of salt-soluble proteins, and to improve the texture, bind, water-holding capacity, and yield of the finished product. Tumblers are large stainless steel units that rotate in a circular fashion for a period of time and may also have vacuum capabilities. Inside, baffles continuously lift up pieces of meat to the upper part of the machine. From here they fall, striking the meat mass below, producing an intense mechanical action suitable for high-yield products. Muscle fibers are disrupted by this mechanical action, which makes cellular membranes more permeable and facilitates the distribution and absorption of brine. Some degree of massaging also occurs as the chunks slide over each other as the tumbler turns. Tumblers typically provide somewhat more of a destructive effect than massagers on

account of the impact force generated from the mechanical action. Thus, not all cured meat products can be tumbled. A fitting example for the benefits of tumbling comes from ham production: hams processed in this fashion are more uniform, as brine uptake is more tightly controlled and pickle pockets are reduced. The tighter control of pickle uptake results from the ability to pump the hams at, or somewhat below, the target pump and then adjusting the product's uptake to the exact percentage pump by adding pickle directly to the tumbler. Tumblers and massagers in meat processing operations have resulted in the higher processing yields and lower production costs for a number of value-added meat products.

16.3.2.5 Chopping or Blending

Dry-curing ingredients are distributed directly into ground meat products during the grinding, chopping, and emulsification steps involved in batter preparation. The employment of nitrite-free curing systems using the preformed cooked cured meat pigment (CCMP), as described later, is best achieved for products using this curing method.

16.4 Benefits and Drawbacks of Nitrite Curing

Two ingredients, salt and nitrite, are essential components in meat curing. Nitrite is the active agent in curing; all reactions taking place have some relationship to nitrite chemistry. For the production of dry-cured or fermented meat products, however, nitrate is still required in this long ripening process for slow nitrite generation by bacterial reduction. Nitrite can therefore be considered unique. This one food additive can afford to meet a characteristic cured color by means of a heat-stable nitrosylprotoheme pigment, a typical cured flavor, an extended period of refrigerated storage of cooked products without the worry of warmed-over flavor development, and bacteriostatic action against *C. botulinum* spores. It seems clear that the possibility of finding a single compound to mimic all functions of nitrite is remote at best. Although the *N*-nitrosamine scare has died down, nitrite-free curing of meat might still be attractive in view of the fact that many of the effects of nitrite can be easily duplicated by the presence of adjuncts, together with refrigeration. The answer lies in the development of composite nonnitrite curing mixtures.

16.4.1 Color Characteristics

Although the bacteriostatic action of nitrite is most important from a health standpoint, the color of the meat product will influence the customer's decision to purchase the product. Numerous studies have supported the

view that certain colors do, in fact, influence food acceptance (Kostyla and Clydesdale, 1978). For meat, it is the quantity of hemoproteins, particularly that of myoglobin and its relationship with the environment surrounding it that determines the meat's color (Ledward, 1992; Livingston and Brown, 1981). The addition of nitrite to meat followed by thermal processing produces a relatively stable pink-colored pigment. If nitrite were eliminated from cured meats, the result would be products with beige or tan color, unless an acceptable colorant is employed.

A number of colorants to replace nitrite such as nicotinic acid, 3- and 4-acylpyridine, *N,N*-diethylnicotinamide, erythrosine, protoporphyrin-IX, cochineal, dried radish chip extracts, betalain pigments from beet root extract, and angkak from *Monascus purpureus* have been tested in meat systems. Unfortunately, color fixation, toxicological, oxidation, or thermal stability issues have prevented their use. In 1975, a U.S. patent was issued to Sweet (1975), who first proposed the use of composite nonnitrite curing mixtures for duplicating the cumulative action of nitrite. His multicomponent system consisted of a red colorant—erythrosine—an antioxidant/chelator, an antimicrobial agent, and all other curing adjuncts except for nitrite. Efforts in the laboratory of Shahidi toward the development of a composite nitrite-free curing system, which bestows the characteristic and desirable attributes of cooked cured meat products without *N*-nitrosamine formation and which may be employed at an industrial level, have been successful. Sweet's approach was employed for preparing nitrite-free products, but the colorant of choice was the natural CCMP. This nitrosylated heme pigment was preformed outside the meat matrix and then applied to meat. Palmin and coworkers first suggested using such a pigment for improving the color of sausages (Palmin et al., 1973, 1975; Palmin and Prizenko, 1974).

The pigment is manufactured from the red blood cells of animals, which are an industrial by-product of abattoirs, and a nitrosating agent in the presence of a reductant. The pigment can be prepared in a direct, one-step process or by an indirect method through a hemin intermediate (Shahidi and Pegg, 1991c,d; Shahidi et al., 1984, 1985, 1994); the preparation of the latter process is depicted in Figure 16.1. The coloring efficacy of CCMP, as part of a composite nitrite-free curing package, has been tested in the meat from a number of species, but mostly for pork. Addition of CCMP to comminuted pork at 3–30 mg/kg levels produced a pink color after thermal processing in all cases, albeit of different intensities that was visually similar to nitrite-treated pork systems. Although various levels of CCMP were employed (Table 16.1), colorimetric data (i.e., Hunter color values) demonstrated that pigment-treated pork samples at a 12–18 mg/kg addition level were not significantly ($p > .05$) different from their nitrite-cured counterpart (Pegg and Shahidi, 2000). Studies have shown that the color intensity of nitrite or CCMP-treated meat products depends on the endogenous myoglobin content of meat (Pegg, 1993; Shahidi and Pegg, 1991a; Stevanović et al., 1997). Whether there is an interaction between myoglobin and the added CCMP is uncertain. Nevertheless,

TABLE 16.1

Concentration Effect of the Preformed CCMP on Hunter Color Values of Cooked Ground Pork[a]

	Hunter Values			
Treatment (mg/kg)	**L**	**a**	**b**	**Hue Angle (arctan b/a)**
No additive	58.2 ± 0.5	4.8 ± 0.1	11.9 ± 0.1	68.0 ± 0.4
$NaNO_2$, 156	57.8 ± 0.2	13.4 ± 0.2	9.2 ± 0.1	34.5 ± 0.5
CCMP, 3	58.4 ± 0.5	12.6 ± 0.2	9.1 ± 0.1	35.8 ± 0.5
CCMP, 6	57.9 ± 0.2	12.8 ± 0.1	9.1 ± 0.1	35.4 ± 0.4
CCMP, 9	57.3 ± 0.3	13.0 ± 0.2	9.1 ± 0.1	35.2 ± 0.5
CCMP, 12	57.1 ± 0.2	13.2 ± 0.1	9.1 ± 0.1	34.6 ± 0.3
CCMP, 18	56.4 ± 0.2	13.5 ± 0.1	9.2 ± 0.1	34.3 ± 0.3
CCMP, 24	56.1 ± 0.4	13.8 ± 0.2	9.1 ± 0.1	33.6 ± 0.4
CCMP, 30	55.8 ± 0.3	14.1 ± 0.2	9.1 ± 0.1	32.8 ± 0.4

Source: Reprinted from Pegg, R. B. Development of nitrite-free meat curing systems. PhD thesis, Memorial University, St. John's, Newfoundland and Labrador, Canada, 1993. With permission.

CCMP, cooked cured meat pigment.

[a] All pork systems were prepared with 20% (w/w) distilled water and 550 mg/kg sodium ascorbate.

meats richer in myoglobin require greater addition levels of CCMP to attain an attractive cured color in the final product (Pegg and Shahidi, 1990).

In a pilot-scale study, nitrite-free cured frankfurter and salami products were prepared. Industry panel members were unable to distinguish the nitrite-cured control (i.e., 200 mg/kg sodium nitrite addition based on formulation) from the nitrite-free test samples (i.e., 21–27 mg/kg CCMP) based on visual observation (Pegg, 1993). Frankfurter and salami products used beef, pork, some organ tissues, and mechanically deboned chicken meat in their formulations. It was only when the two sets of samples were examined under bright daylight that the nitrite-free cured sample was discovered to be slightly redder and darker in appearance (Shahidi et al., 1993).

The preformed CCMP undergoes decomposition in the presence of light and air, as does the pigment present in nitrite-cured meats. Unlike the CCMP in processed products, there is no protein matrix protecting the nitrosylated heme pigment from oxidation. Therefore, its stabilization is crucial. The preformed CCMP was protected by microencapsulation using modified starch, cyclodextrins, and gums followed by spray drying. In this manner, the pigment remains "locked" in a powder format until it is released by the moisture when mixed into ground meat or by its dissolution in water or a pickle. The resultant powdered cooked cured meat pigment (PCCMP) acts as a potent agent for color development in nitrite-free curing mixtures. PCCMP was applied to various meat systems and found to successfully duplicate the color characteristics of nitrite-cured analogues (Shahidi and Pegg, 1991b).

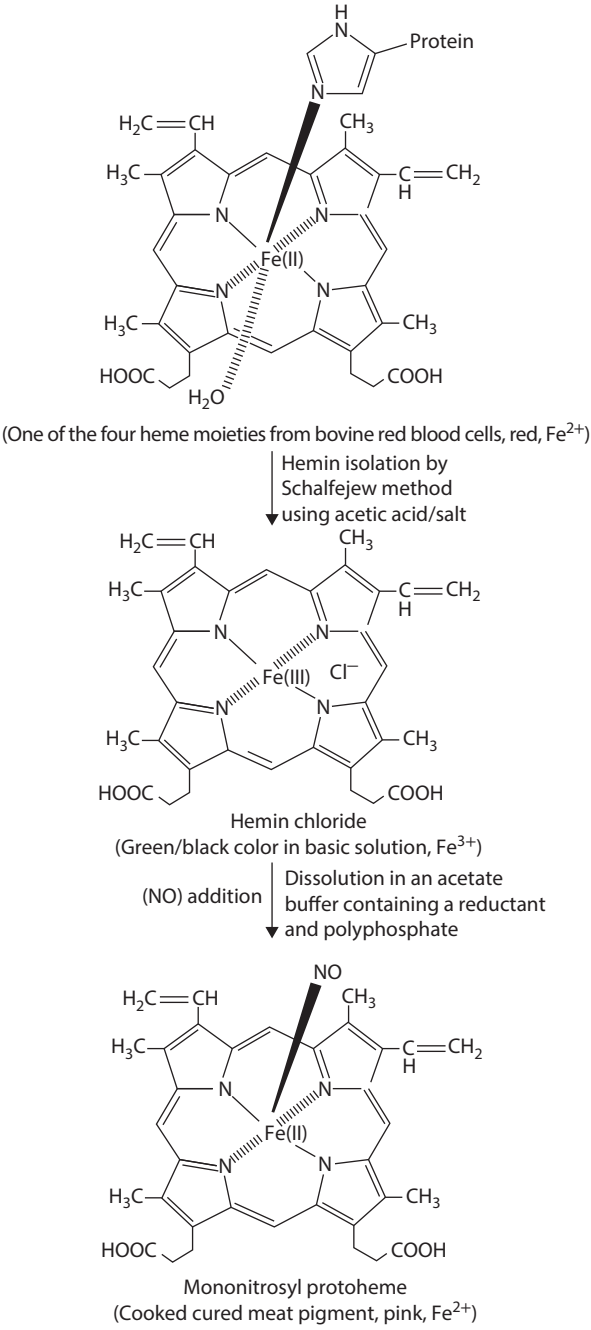


FIGURE 16.1
Preparation and chemical structure of the preformed cooked cured meat pigment from hemin chloride after its isolation from bovine red blood cells.

The optimal addition level of PCCMP to meat depends primarily on its endogenous myoglobin content (Shahidi and Pegg, 1991a). Best performance of the pigment, however, was observed for meat systems containing a low or intermediate concentration of myoglobin.

16.4.2 Antioxidant Properties

Nitrite functions as a strong antioxidant in cured meats and thus prevents lipid oxidation. However, nitrite is not unique in its role as a food-grade antioxidant. Shahidi (1989) proposed that any agent or combination of agents that prevents lipid oxidation, with the exception of nitrite precursors, would in principal duplicate the antioxidant role of nitrite in the curing process.

To reproduce the antioxidative efficacy of nitrite, a number of antioxidants (Shahidi et al., 1987a), sequestrants (Shahidi et al., 1986), and their combinations (Shahidi et al., 1987b, 1988) were examined. Addition of antioxidants to meat and meat products resulted in the preservation of meat quality by retarding autoxidation and rancidity development, as well as discoloration and loss of nutrients. The inhibitory effect of the antioxidants was attributed to their ability to donate a hydrogen atom or an electron to a lipid-free radical as well as possibly to form a complex between the antioxidants themselves and the lipid molecule (Dziezak, 1986). The concentration of carbonyl compounds produced in these systems from autoxidation was markedly reduced when combinations containing polyphosphates, ascorbates, and low levels of an antioxidant were used. The spectrum of notable carbonyl compounds was, however, similar to the nitrite-cured system.

Among synthetic antioxidants, butylated hydroxyanisole (BHA) and *tert*-butylhydroquinone (TBHQ) were most effective, even at 30 mg/kg, in retarding oxidation during a five-week storage period at 4°C. This was confirmed by monitoring the production of 2-thiobarbituric reactive substances (TBARSs) over this period and comparing to those for the nitrite-cured control (Table 16.2). Among the food-grade sequestrants, sodium acid pyrophosphate, tetrasodium pyrophosphate, sodium tripolyphosphate (STPP), and ethylenediaminetetraacetic acid were the most efficacious. Sodium ascorbate and STPP alone retarded lipid oxidation, but together, a strong synergistic action was noted (Shahidi and Pegg, 1992). In fact, the mixture containing sodium ascorbate (550 mg/kg) and STPP (3000 mg/kg) with or without a phenolic antioxidant (30 mg/kg) was as effective as sodium nitrite (150 mg/kg) in the presence of sodium ascorbate (550 mg/kg). Addition of sodium nitrite to meat containing sodium ascorbate and STPP at the abovementioned levels did not have any further effect in controlling lipid oxidation (Shahidi et al., 1987b).

In the food industry, there has been a move to clean label products and hence the use of natural ingredients due to greater sensitivity of consumers to synthetic additives, and especially since BHA and butylated hydroxytoluene (BHT) are suspected to have carcinogenic activity (Valentão et al., 2002). Research has shown that constituents of aromatic plants can

TABLE 16.2

TBARS Values of Cooked Ground Pork Pretreated with Different Additives after a 5-Week Storage Period at 4°C

Exp. No.	Additives (mg/kg)[a]	TBARS Value[b]
1	Control, no additives	15.46
2	Sodium nitrite, 150	0.63
3	Butylated hydroxyanisole, 30	0.44
4	*tert*-Butylhydroquinone, 30	0.35
5	Sodium tripolyphosphate, 3000	1.86
6	Tetrasodium pyrophosphate, 3000	1.66
7	Sodium hexametaphosphate, 3000	7.21
8	(5) + Sodium ascorbate, 550	0.27
9	(6) + Sodium ascorbate, 550	0.23
10	(7) + Sodium ascorbate, 550	0.29
11	(8) + (3)	0.20
12	(8) + (4)	0.18
13	Preformed cooked cured meat pigment, 12	9.89
14	(11) + (13)	0.34
15	(12) + (13)	0.24
16	(14) + Sodium hypophosphite, 3000	0.28
17	(15) + Sodium hypophosphate, 3000	0.21

Source: Reprinted from *Food Chemistry*, 43, Shahidi, F. and Pegg, R. B., Nitrite-free meat curing systems: Update and review, 185–191, Copyright 1992, with permission from Elsevier.

[a] All pork systems were prepared with 20% (w/w) distilled water and listed additive(s).

[b] TBARS (2-thiobarbituric acid reactive substances) values were determined by the classical distillation method and are reported as mg malonaldehyde equivalents/kg meat.

function as natural antioxidants and thereby prevent or retard rancidity of food lipids, improve sensory scores, and offer greater consumer acceptance of food products (Nakatani, 1997). Crude extracts of spices, herbs, and other plant materials rich in polyphenolics are increasingly of interest because they have the capacity to retard oxidative degradation of lipids and thereby improve the quality and nutritional value of food (Amarowicz et al., 2004). Although certain spices and herbs, or their fractions, may possess marked antioxidant activity, their practical application to meat and meat products may be restricted due to a pungent or characteristic flavor imparted by the spice and to their thermal stability in the product.

The antioxidant activity of selected spices and their oleoresins in pork systems has been investigated. The TBARS values for spice-containing samples were lower than those of the control, thus indicating protection to meat by these spices against lipid oxidation. The protection, however, was concentration dependent and a saturated point was reached after a certain amount of spice had been added. For example, the addition of clove at a 500 mg/kg level achieved 96% inhibition of TBARS, but this protection remained unchanged even when higher concentrations were employed (Shahidi et al., 1995). In the

pork systems, clove, sage, rosemary, and oregano appeared quite effective in retarding lipid oxidation as TBARS values remained at less than 1 μg/g sample over the entire three-week storage period at 4°C. Because future research into novel sources of natural antioxidants as a component in functional food formulations is expected to continue, there will be new preparations available to curb lipid oxidation with the potential of being part of a nitrite-free curing system. The use of rosemary extract and green tea in meat product has been a common practice in the more recent formulations (Senanayake, 2013). Furthermore, protein extenders such as meals from mustard, canola, and other grains belonging to the cereal, legume, and oilseeds groups may be used as all of these include considerable amount of antioxidant phenolics (Shahidi, 2016).

16.4.3 Flavor Characteristics

According to the National Academy of Sciences (NAS, 1982), the generation of cured meat flavor is most likely a composite sensation derived from the contribution of many odoriferous compounds. No research has been able to specify a positive contribution by nitrite to flavor in chemical terms, but the NAS suggested that nitrite probably influences the flavor of cured meat by virtue of its antioxidative effects. Because the mechanism involved in the production of the characteristic cured meat flavor is uncertain, there is no known nitrite substitute that can duplicate this flavor.

There were contradicting studies throughout the 1970s and 1980s as to the impact of salt and nitrite on cured meat flavor. What is clear, however, is that the amount of salt used in curing processes plays a vital role in determining the overall flavor of the product. Yun (1984) and Yun et al. (1987) evaluated combinations of ingredients that would effectively prevent lipid oxidation in cooked ground pork systems to be used in the nitrite-free curing of meat products such as frankfurters. The authors reported that sensory evaluation scores of pork systems treated with 3000 mg/kg STPP, 500 mg/kg sodium ascorbate, and 30 mg/kg BHA or TBHQ were not significantly different ($p > .05$) from their nitrite-cured (156 mg/kg) counterparts. Yun (1984) also observed that the concentration of volatiles identified in the distillate of cooked pork samples, notably hexanal, was significantly reduced ($p < .05$) when samples had been pretreated with the preceding antioxidant/chelating agent combinations. The concentration of volatiles in these systems was depressed almost to the level of the nitrite-cured control (Table 16.3).

These two studies suggest, for the most part, that it is possible to prepare nitrite-free cured meat products without seriously compromising their flavor. If one accepts the views of Cross and Ziegler (1965) that cured ham flavor represents the basic or true-to-nature flavor of meat derived from precursors other than triacylglycerols or phospholipids and that the different aromas of the various types of cooked meat depend on the spectrum of carbonyl compounds derived by lipid oxidation, then any agent or combination that suppresses lipid oxidation, would, in principle, duplicate the flavor of

TABLE 16.3

Effect on the Concentration of Dominant Carbonyl Compounds from Cooked Ground Pork When Treated with Either Sodium Nitrite or a Nitrite-Free Preparation

	Relative Concentration		
Carbonyl Compounds	**Uncured Meat[a]**	**Nitrite Cured[b]**	**Nitrite-Free Treated[c]**
Hexanal	100	7.0	6.5
Pentanal	31.3	0.5	0.5
Heptanal	3.8	< 0.5	0.5
Octanal	3.6	< 0.5	0.5
Nonanal	8.8	0.5	0.7
trans-2-Octenal	2.0	–	–
trans-2-Nonenal	1.0	–	–
trans-2-Decenal	1.1	–	<0.1
trans-2-Undecenal	1.4	0.5	0.5
trans,trans-2,4-Decadienal	1.1	—	<0.1

Source: Reprinted from *Food Chemistry*, 43, Shahidi, F. and Pegg, R. B., Nitrite-free meat curing systems: Update and review, 185–191, Copyright 1992, with permission from Elsevier.

[a] Pork system contained 20% (w/w) distilled water.

[b] Pork system contained 20% (w/w) distilled water; sodium nitrite, 150 mg/kg; and sodium ascorbate, 550 mg/kg.

[c] Pork system contained 20% (w/w) distilled water; preformed cooked cured meat pigment, 12 mg/kg; sodium tripolyphosphate, 3000 mg/kg; sodium ascorbate, 550 mg/kg; and *tert*-butylhydroquinone, 30 mg/kg.

nitrite-cured meat. In other words, the antioxidative role of nitrite simply retards the breakdown of unsaturated fatty acids and the formation of secondary lipid oxidation products; this may be the main process involved in modifying the volatile profile of cooked cured meats by suppressing the formation of oxidation products, thereby allowing the unique flavor associated with cured products to be revealed.

16.4.4 Antimicrobial Properties

Nitrite exerts a concentration-dependent antimicrobial effect in cured meat products in the inhibition of the outgrowth of spores of putrefactive and pathogenic bacteria such as *C. botulinum* (NAS, 1982). Irrespective of the fate of nitrite, its removal from or reduction in meat products must be counterbalanced by alternatives that will assure safety from botulinal hazards in abused products (Shahidi and Pegg, 1991d). At the same time, the traditional identity of the cured meat product in question must be retained. According to Sofos and Busta (1980), any substance to be considered as an alternative to nitrite should be suitable for use in all cured meat products and should control other microorganisms of public health significance, delay product spoilage, and not interfere with beneficial microorganisms, such as lactic acid-producing cultures, required in the manufacturing of

fermented meat products. The compound of choice must also be at least as effective as nitrite, safe, heat stable, flavorless, and preferably effective at low concentrations.

Alternatives to nitrite that have been tested include propylparabens, sorbic acid and its potassium salts, esters of fumaric acid, lactic acid and its salts, nisin and other bacteriocins. Sodium hypophosphite has been proposed for use as an antimicrobial agent in foods (Rhodehamel and Pierson, 1990). Microbiological studies have indicated that a total or partial replacement of nitrite with this compound effectively inhibits production of *C. botulinum* toxins (Banner, 1981). At 3000 mg/kg alone or at 1000 mg/kg in combination with 40 mg/kg of nitrite, sodium hypophosphite imparted anticlostridial protection to meat products equivalent to that provided by 120 mg/kg of nitrite. Wood et al. (1986) evaluated the antibotulinal activity of sodium hypophosphite, potassium sorbate, and monomethyl fumarate in nitrite-free curing systems. The treatment containing 3000 mg/kg sodium hypophosphite, together with CCMP, sodium ascorbate, STPP, and TBHQ, most closely resembled that of nitrite at 150 mg/kg in its ability to prevent spore

TABLE 16.4

Effect of Treatment Composition on Gas and Toxin Production by *Clostridium botulinum* in Cooked Ground Pork[a]

Exp No.	Treatment[b]	Incubation at 27°C (Days)								
		1	2	3	4	5	6	7	8	27
1	No additives	34/34 +								
2	$NaNO_2$, 150	0/36 –	11/36 +	5/18 +	8/13 +	3/5 *	2/2 +			
3	(2) + ASC	0/36 –	0/32 –	15/30 –	5/14 +	3/9 +	4/6 +	1/2 +	0/1 –	0/1 –
4	CCMP, 12	17/17 +								
5	(4) + ASC + STPP + TBHQ	12/37 +	25/25 +							
6	(4) + SHP	1/18 –	1/16 +	2/14 +	4/12 +	3/5 +	1/2 +	0/1 –	0/1 –	0/1 –
7	(4) + ASC + SHP	5/17 –	0/11 –	3/11 *	6/8 +	0/2 –	0/2 –	0/2 –	0/2 –	0/2 –
8	(5) + SHP	6/35 –	3/27 *	1/24 –	6/22 +	3/16 *	5/13 +	6/8 +	1/2 +	0/1 –
9	(5) + PS	0/39 –	32/35 +	3/3 +						
10	(5) + MMF	0/37 –	1/33 –	17/31 +	9/18 +	3/7 +	3/4 +	0/1 +	0/1 +	0/1 –

Source: Reprinted from Wood, D. S. et al., *Journal of Food Protection*, 49, 691–695, 1986. Copyright held by the International Association for Food Protection, Des Moines, Iowa, IA. With permission.

[a] Number of packs showing gas production/total number of packs. + = toxin present; – = toxin absent; * = presence of toxin was not tested.

[b] Additives were as follows: sodium ascorbate (ASC), preformed cooked cured meat pigment (CCMP), sodium tripolyphosphate (STPP), *tert*-butylhydroquinone (TBHQ), sodium hypophosphite (SHP), potassium sorbate (PS), and monomethyl fumarate (MMF).

outgrowth and toxin production (Table 16.4). Monomethyl fumarate at 1250 mg/kg was slightly less effective than sodium hypophosphite and these additives had no adverse effect on the oxidative stability or the color of formulated pork products. Moreover, sodium hypophosphite is bland in taste; nitrite-free bacon containing 3000 mg/kg of it had a flavor as desirable as that of its conventionally cured counterpart.

16.5 Tailor Designing Nitrite-Free Meat Products

The use of the nitrite-free meat curing system is highly dependent on the product in question. First, this system cannot be used to dry-cure meat products. Nitrite is a very small anion, whose relative rate of diffusion through muscle tissue utilizing the meat's natural moisture is markedly faster than that of CCMP or PCCMP. The molecular size of CCMP/PCCMP is several orders of magnitude larger than that of nitrite; this affects the pigment's rate of diffusion through the meat. Second, when formulating meat products, nitrite-free curing systems work best in ground or emulsified meat products, as opposed to whole muscle cuts; CCMP or PCCMP is more uniformly distributed throughout the meat matrix. CCMP or PCCMP can also be added to a solution and used in a pickle formulation for treating solid cuts of meat. Unlike nitrite-containing pickles, nitrite-free ones with CCMP or PCCMP are more sensitive to oxidation and therefore need to be prepared in a special manner (i.e., dissolution of the pigment in a polyphosphate and ascorbate solution before mixing it with brine) just before injecting it into pieces of meat. Care must be exercised in the application of nitrite-free curing systems in whole muscle cuts to ensure that the pigment is evenly distributed throughout and that red blotches are avoided. After pumping meats, tumbling or massaging of the product facilitates an even distribution of the CCMP throughout the muscle tissue.

All nitrite-free cured meat products must contain CCMP or PCCMP for proper color fixation and an antioxidant or sequestrant system (i.e., either synthetic or natural antioxidants) for protection of meat lipids against oxidation, but not necessarily an antimicrobial agent. If the meat product being formulated is to be canned, such as a Vienna-type sausage or a meat loaf, the product will be heat sterilized and an antimicrobial agent is not required. If the product is to be stored in a retail display case under a modified atmosphere at refrigerated temperatures, again an antimicrobial agent may not be needed. On the other hand, for a traditional product such as a vacuum-packaged frankfurter, inclusion of an antimicrobial agent, such as sodium hypophosphite, is mandatory in the product for protection against the outgrowth of spores from *C. botulinum*.

To summarize, several nitrite-free combinations consisting of the preformed CCMP or its encapsulated product, PCCMP, a sequestrant, and an antioxidant, as well as an antimicrobial agent for the curing of meat products have been

described. These mixtures successfully reproduced the color, oxidative stability, and flavor as well as the antimicrobial effects of nitrite in meat products. Further details on the process are available in three U.S. patents (Shahidi and Pegg, 1993, 1995a,b). The question now put forward is this: is there any commercial value to these nitrite-free meat curing systems? Limits imposed by regulatory agencies on the level of nitrite permitted in various meat products, coupled with the responsible reductions in nitrite addition implemented by the meat processing industry have reduced the possibility of overcuring and *N*-nitrosamine formation. Today, product innovations and food safety (e.g., hazard analysis critical control points [HACCPs]) drive the meat industry. Benefits from the controlled and responsible use of nitrite overwhelm the possible risks from the outbreak of botulinal food poisoning. Because of nitrite's connection with cancer-causing *N*-nitrosamines, its use, nonetheless, is a potential trouble area. Hence, employment of a nonnitrite cure for a niche market is deemed desirable and also offers a practical solution to address the needs of the industry and consumers.

16.6 Other Developments

Other developments in the field include making use of celery extract as a source of nitrate that is carefully reduced to nitrite, which is then used in the curing of meat. While this process has also been referenced as nitrite-free curing by the user industry, this may be considered as misuse of the term as the process is not nitrite-free and the residual nitrite in the products is not less than those that use nitrite directly. In fact, our own routine testings have shown the reverse trend of increased nitrite residues in such products. Although we have not checked the *N*-nitrosamine content of these products, one may assume that their content could indeed be higher than directly cured products with nitrite. Thus, responsible measures should be considered when developing alternatives to the currently used nitrite curing processing. In this connection, Sebranek et al. (2012) have summarized advances in natural and organic curing processes. Related publications on this topic are also worthy of consideration (Jackson et al., 2011; Sebranek and Bacus, 2007; Sindelar et al., 2007a,b,c). Thus, the solutions put forward in this chapter fill the existing knowledge gap, but their adoption by the industry may need new initiatives.

References

Aberle, E., Forrest, J. C., Gerrard, D. E. and Mills, E. W. (2001). *Principles of Meat Science* (4th Ed.). Dubuque, IA: Kendall/Hunt.

Amarowicz, R., Pegg, R. B., Rahimi-Moghaddam, P., Barl, B. and Weil, J. A. (2004). Free-radical scavenging capacity and antioxidant activity of selected plant species from the Canadian prairies. *Food Chemistry*, **84**, 551–562.

Banner, R. J. (1981). Sodium hypophosphite: New nitrite replacer? *Food Engineering,* **53**(10), 130.

Binkerd, E. F. and Kolari, O. E. (1975). The history and use of nitrate and nitrite in the curing of meat. *Food and Cosmetics Toxicology,* **13**, 655–661.

Blot, W. J., Henderson, B. E. and Boice, J. D., Jr. (1999). Childhood cancer in relation to cured meat intake: Review of the epidemiological evidence. *Nutrition and Cancer,* **34**, 111–118.

Cross, C. K. and Ziegler, P. (1965). A comparison of the volatile fractions from cured and uncured meat. *Journal of Food Science,* **30**, 610–614.

Dziezak, J. D. (1986). Preservatives: Antioxidants. *Food Technology,* **40**(9), 94–97, 100–102.

Flores, J. and Toldrá, F. (1993). Curing. *In:* Macrae, R., Robinson, R. K. and Sadlor, M. J. (eds.) *Encyclopedia of Food Science, Food Technology and Nutrition*. London, UK: Academic Press, pp. 1277–1282.

Fox, J. B., Jr. (1974). Role of cure accelerators. *In: Proceedings of the Meat Industry Research Conference*. Arlington, VA, American Meat Institute Foundation, 17–21.

Glória, M. B. A., Barbour, J. F. and Scanlan, R. A. (1997). Volatile nitrosamines in fried bacon. *Journal of Agricultural and Food Chemistry,* **45**, 1816–1818.

Haldane, J. (1901). The red colour of salted meat. *Journal of Hygiene,* **1**, 115–122.

Jackson, A. L., Sullivan, G. A., Charlwit K., Sebranek, J. G. and Dickson, J. S. (2011). Survival and growth of *Clostridium perfringens* in commercial no-nitrate-or-nitrite-added (natural and organic) frankfurters, hams, and bacon. *Journal of Food Protection,* 74, 410–416.

Jensen, L. B. (1953). *Man's Foods: Nutrition and Environments in Food Gathering Times and Food Producing Times*. Champaign, IL: Garrard Press.

Kostyla, A. S. and Clydesdale, F. M. (1978). The psychophysical relationships between color and flavor. *CRC Critical Reviews in Food Science and Nutrition,* **10**, 303–321.

Ledward, D. A. (1992). Colour of raw and cooked meat. *In:* Johnston, D. W., Knight, M. K. and Ledward, D. A. (eds.) *The Chemistry of Muscle-Based Foods*. Cambridge, UK: The Royal Society of Chemistry, pp. 128–144.

Livingston, D. J. and Brown, W. D. (1981). The chemistry of myoglobin and its reactions. *Food Technology,* **35**, 244–252.

Loeppky, R. N. (1994). Nitrosamine and *N*-nitroso compound chemistry and biochemistry. *In:* Loeppky, R. N. and Michejda, C. J. (eds.) *Nitrosamines and Related N-Nitroso Compounds: Chemistry and Biochemistry*. Washington, DC: American Chemical Society, pp. 1–18.

Nakatani, N. (1997). Antioxidants from spices and herbs. *In:* Shahidi, F. (ed.) *Natural Antioxidants: Chemistry, Health Effects, and Applications*. Champaign, IL: AOCS Press, pp. 64–75.

National Academy of Sciences (NAS). (1982). *Alternatives to the Current Use of Nitrite in Foods.* Washington, DC: National Academy Press.

Palmin, V., Fedorova, T., Prizenko, V. and Loginova, O. (1975). The manufacture of nitrosohaemochromogen and its application as meat product colorant. *Myasnaya Industriya SSSR,* **2**, 40–41.

Palmin, V. V. and Prizenko, V. K. (1974). Stabilization of colour of cured meat using nitric oxide. Izvestia Vysshikh Uchebnykh Zavedenii. *Pishchevaya Teknologiya,* **3**, 51–53.

Palmin, V. V., Prizenko, V. K., Fedorova, T. A. and Loginova, O. A. (1973). Some aspects of colour formation in meat products. In *Proceedings of the 19th European Meeting of Meat Research Workers, Part IV*. Paris, France, 1457–1472.

Pegg, R. B. (1993). Development of nitrite-free meat curing systems. PhD thesis, Memorial University, St. John's, Newfoundland and Labrador, Canada.

Pegg, R. B. and Shahidi, F. (1990). Effects of myoglobin and nitrite or preformed cooked cured-meat pigment concentrations on the colour of cooked meats. In *Paper presented at the 33rd Annual Conference of the Canadian Institute of Food Science and Technology*, Saskatoon, Saskatchewan, Canada.

Pegg, R. B. and Shahidi, F. (2000). *Nitrite Curing of Meat: The N-Nitrosamine Problem and Nitrite Alternatives.* Trumbull, CT: Food & Nutrition Press.

Peters, J. M., Preston-Martin, S., London, S. J., Bowman, J. D., Buckley, J. D. and Thomas, D. C. (1994). Processed meats and risk of childhood leukaemia (California, USA). *Cancer Causes and Control,* **5**, 195–202.

Polenske, E. (1891). Über den Verlust, welchen das Rindfleisch an Nährwerth durch das Pökeln erleidet, sowie über die Veränderungen Salpeter-haltiger Pökellaken. Mitteilungen aus dem chemischen Laboratorium des Kaiserlichen Gesundheitsamtes. *Arbeitslosigkeit Gesundh Amt,* **7**, 471–474.

Preussmann, R. and Stewart, B. W. (1984). *N*-nitroso carcinogens. *In:* Searle, C. E. (ed.) *Chemical Carcinogens* (2nd Ed., vol. 2). Washington, DC: American Chemical Society, pp. 643–838.

Rhodehamel, E. J. and Pierson, M. D. (1990). Sodium hypophosphite inhibition of the growth of selected Gram negative foodborne pathogenic and spoilage bacteria. *Journal of Food Protection,* **53**, 56–63.

Sebranek, J. G. (1979). Advances in the technology of nitrite use and consideration of alternatives. *Food Technology,* **33**(7), 58–62, 93.

Sebranek, J. G. (2010). Basic curing ingredients. *In:* Tarté, R. (ed.) *Ingredients in Meat Products*. New York, NY: Springer Science, pp. 274–289.

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

Sebranek, J. G., Jackson-Davis, A. L., Myers, K. L. and Lavieri, N. A. (2012). Beyond celery and starter culture: Advances in natural/organic curing processes in the United States. *Meat Science,* **92**(3), 267–273.

Senanayake, N. (2013). Green tea extract: Chemistry, antioxidant properties and food applications—A review. *Journal of Functional Foods,* **5**, 1529–1541.

Shahidi, F. (1989). Flavor of cooked meats. *In:* Teranishi, R., Buttery, R. G. and Shahidi, F. (eds.) *Flavor Chemistry: Trends and Developments*. Washington, DC: American Chemical Society, pp. 188–201.

Shahidi, F. (2016). Oxidative stability and shelf-life of meat and meat products. *In:* Hu, M. and Jacobsen, C. (eds.) *Evaluation of Oxidative Stability and Shelf-life of Oils/Fats and Oil/Fat-Containing Foods*. Champaign, IL: AOCS Press, pp. 373–389.

Shahidi, F. and Pegg, R. B. (1991a). Effect of the preformed cooked cured-meat pigment (CCMP) on color parameters of muscle foods. *Journal of Muscle Foods,* **2**, 297–304.

Shahidi, F. and Pegg, R. B. (1991b). Encapsulation of the pre-formed cooked cured-meat pigment. *Journal of Food Science,* **56**, 1500–1504, 1518.

Shahidi, F. and Pegg, R. B. (1991c). Novel synthesis of cooked cured-meat pigment. *Journal of Food Science,* **56**, 1205–1208.

Shahidi, F. and Pegg, R. B. (1991d). Safety and sensory aspects of nitrite alternatives in meat curing. *Canadian Chemical News,* **43**(2), 12–15.

Shahidi, F. and Pegg, R. B. (1992). Nitrite-free meat curing systems: Update and review. *Food Chemistry,* **43**, 185–191.

Shahidi, F. and Pegg, R. B. (1993). Powdered cooked cured-meat pigment which is a non-nitrite meat preservative. US Patent 5,230,915.

Shahidi, F. and Pegg, R. B. (1995a). Proteinaceous food product containing stabilized cooked cured-meat pigment. US Patent 5,443,852.

Shahidi, F. and Pegg, R. B. (1995b). Stabilized cooked cured-meat pigment. US Patent 5,425,956.

Shahidi, F., Pegg, R. B. and Saleemi, Z. O. (1995). Stabilization of meat lipids with ground spices. *Journal of Food Lipids,* **2**, 145–153.

Shahidi, F., Pegg, R. B. and Sen, N. P. (1993). Novel nitrite-free processed meats. *In:* Ali, R. and Barlow, P. J. (eds.) *Impact of Food Research on New Product Development*. Karachi, Pakistan: University of Karachi, pp. 221–245.

Shahidi, F., Pegg, R. B. and Sen, N. P. (1994). Absence of volatile *N*-nitrosamines in cooked nitrite-free cured muscle foods. *Meat Science,* **37**, 327–336.

Shahidi, F., Rubin, L. J., Diosady, L. L., Chew, V. and Wood, D. F. (1984). Preparation of dinitrosyl ferrohemochrome from hemin and sodium nitrite. *Canadian Institute of Food Science and Technology Journal,* **17**, 33–37.

Shahidi, F., Rubin, L. J., Diosady, L. L., Kassam, N. and Li Sui Fong, J. C. (1986). Effect of sequestering agents on lipid oxidation in cooked meats. *Food Chemistry,* **21**, 145–152.

Shahidi, F., Rubin, L. J., Diosady, L. L. and Wood, D. F. (1985). Preparation of the cooked cured-meat pigment, dinitrosyl ferrohemochrome, from hemin and nitric oxide. *Journal of Food Science,* **52**, 564–567.

Shahidi, F., Rubin, L. J. and Wood, D. F. (1987a). Control of lipid oxidation in cooked ground pork with antioxidants and dinitrosyl ferrohemochrome. *Journal of Food Science,* **52**, 564–567.

Shahidi, F., Rubin, L. J. and Wood, D. F. (1987b). Control of lipid oxidation in cooked meats by combinations of antioxidants and chelators. *Food Chemistry,* **23**, 151–157.

Shahidi, F., Rubin, L. J. and Wood, D. F. (1988). Stabilization of meat lipids with nitrite-free curing mixtures. *Meat Science,* **22**, 73–80.

Sindelar, J. J., Cordray, J. C., Sebranek, J. G. Love, J.A. and Ahn, D.U. (2007a). Effects of varying levels of vegetable juice powder and incubation time on colour, residual nitrate and nitrite, pigment, pH, and trained sensory attributes of ready-to-eat uncured ham. *Journal of Food Science,* **72**(6), 388–395.

Sindelar, J. J., Cordray, J. C., Sebranek, J. G., Love, J. A. and Ahn, D. U. (2007b). Effects of vegetable juice powder concentration and storage time on some chemical and sensory quality attributes of uncured, emulsified cooked sausages. *Journal of Food Science,* **72**(5), 324–332.

Sindelar, J. J., Cordray, J. C., Sebranek, J. G., Love, J. A. and Ahn, D. U. (2007c). Investigating quality attributes and consumer acceptance of uncured, no nitrate/nitrite-added commercial hams, bacons, and frankfurters. *Journal of Food Science,* **72**(8), 551–559.

Sofos, J. N. and Busta, F. F. (1980). Alternatives to the use of nitrite as an antibotulinal agent. *Food Technology,* **34**, 244–251.

Stevanović, M., Zlender, B., Abram, V. and Kumperger, U. (1997). The influence of pork muscle's type and addition of pigment CCMP on the colour of meat emulsion coagulates. In: Bass, J. (ed.), *Proceedings of the 43rd International Congress of Meat Science and Technology*. Auckland, New Zealand, July 27–August 1. Hamilton, NZ: Rice Printers, pp. 462–463.

Sweet, C. W. (1975). Additive composition for reduced particle size meats in the curing thereof. US Patent 3,899,600.

Toldrá, F. and Flores, M. (1998). The role of muscle proteases and lipases in flavor development during the processing of dry-cured ham. *CRC Critical Reviews in Food Science and Nutrition,* **38**, 331–352.

Townsend, W. E. and Olson, D. G. (1987). Cured meats and cured meat products processing. *In:* Price, J. F. and Schweigert, B. S. (eds.) *The Science of Meat and Meat Products* (3rd Ed.). Trumbull, CT: Food & Nutrition Press, pp. 431–456.

Tricker, A. R. and Preussmann, R. (1991). Carcinogenic *N*-nitrosamines in the diet: Occurrence, formation, mechanisms and carcinogenic potential. *Mutation Research,* **259**, 277–289.

Valentão, P., Fernandes, E., Carvalho, F., Andrade, P. B., Seabra, R. M. and Bastos, M. L. (2002). Antioxidative properties of cardoon (*Cynara cardunculus* L.) infusion against superoxide radical, hydroxyl radical, and hypochlorous acid. *Journal of Agricultural and Food Chemistry,* **50**, 4989–4993.

Wood, D. S., Collins-Thompson, D. L., Usborne, W. R. and Picard, B. (1986). An evaluation of antibotulinal activity in nitrite-free curing systems containing dinitrosyl ferrohemochrome. *Journal of Food Protection,* **49**, 691–695.

Yun, J. J. (1984). *Alternative Meat Curing System: Flavour and Oxidative Stability*. MASc. thesis, University of Toronto, Toronto, Canada.

Yun, J., Shahidi, F., Rubin, L. J. and Diosady, L. L. (1987). Oxidative stability and flavour acceptability of nitrite-free meat-curing systems. *Canadian Institute of Food Science and Technology Journal,* **20**, 246–251.



Taylor & Francis
Taylor & Francis Group
http://taylorandfrancis.com

17

Proteomic Tools for Improved Processing of Dry-Cured Meats

Leticia Mora and Fidel Toldrá

CONTENTS

17.1 Introduction 535
17.2 Muscle Proteolytic System 536
17.2.1 Endopeptidases 537
17.2.2 Exopeptidases 537
17.3 Microbial Proteolytical System 538
17.3.1 Endopeptidases 540
17.3.2 Exopeptidases 541
17.4 Traditional Techniques for the Follow-Up of Proteolysis in Dry-Cured Meats 542
17.5 Proteomics in Dry-Cured Ham Approaches 543
17.5.1 From Traditional Proteomics to Peptidomics 545
17.5.2 Applications of Peptidomics in Dry-Cured Ham 545
17.5.2.1 Identification of Bioactive Peptides 548
17.5.2.2 Label-Free Relative Quantitation to Determine Differences between Dry-Cured Hams 549
17.5.3 Data Analysis 550
References 552

17.1 Introduction

Dry-cured meats are characterized by a typical flavor, color, and texture that make them a high-quality product that has been consumed for centuries in many countries. The processing of such types of dry-cured meats is very long, lasting a few months for dry-fermented sausages or several months or even years in the case of dry-cured ham (Toldrá, 2012a). There are several biochemical mechanisms taking place, proteolysis through the action of endogenous muscle peptidases the most relevant. The enzymes involved are endopeptidases, mainly cathepsins and calpains, which are able to degrade sarcoplasmic and myofibrillar proteins and generate polypeptides

that are further hydrolyzed into smaller peptides and free amino acids by exopeptidases (Toldrá, 2002; Toldrá and Flores, 1998).

A good control of proteolysis is of primary importance in order to produce a product that is consistent, regular, and high quality. Muscle and/or microbial proteases are mainly responsible for the proteolytical changes and thus, a good knowledge of their properties and mode of action is essential for a controlled proteolysis (Toldrá, 2012b). The proteolysis phenomena involving muscle and microbial proteases and their resulting products, and the latest proteomic tools available for the study of proteolysis in dry-cured meats, are presented in this chapter.

17.2 Muscle Proteolytic System

Skeletal muscle contains a good number of enzymes involved in multiple metabolic pathways. Some of the most important enzymes are related to protein changes. Endopeptidases (calpains and cathepsins) are responsible for protein breakdown, tri- and dipeptidylpeptidases (DPP) are involved in the generation of small tri- and dipeptides, and finally, aminopeptidases and carboxypeptidases release free amino acids (Toldrá, 2012b). A general scheme of the proteolytical chain is shown in Figure 17.1. Most of these enzymes remain very active in postmortem muscle, playing important roles in proteolysis, and thus,

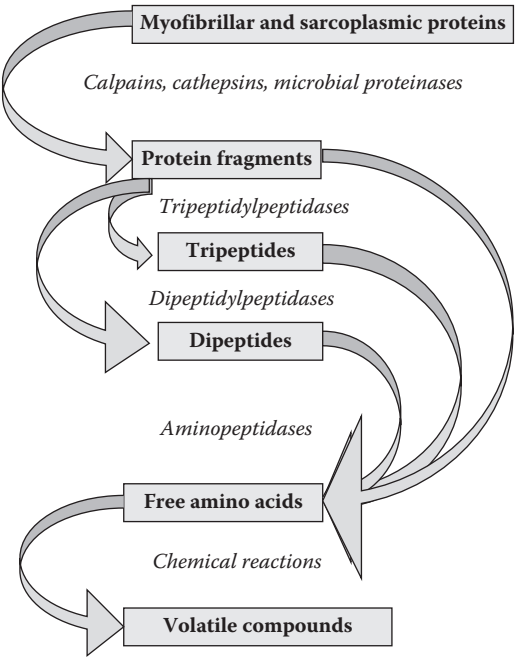


FIGURE 17.1
General scheme of the proteolytical chain in dry-cured meats.

they have an important relevance for the development of meat quality. Some of these peptidases, with optimal acid pH, are located in organules like lysosomes while others are bound to membranes or free in the cytosol (Haard, 1990).

17.2.1 Endopeptidases

There are three important groups of endopeptidases, or proteinases: cathepsins, calpains, and proteasome. Cathepsins B, D, H, and L are located in lysosomes. They are very small in size, with molecular masses within the range 20–40 kDa, that allow them to penetrate into the myofibrillar structure and hydrolyze important proteins like myosin and troponins that experience important changes during dry curing (Toldrá, 1998). Optimal pH for cathepsins B and L is around 6.0, for cathepsin H near 6.8, and for cathepsin D in the range 3.0–5.0 (Toldrá and Flores, 1998). Cathepsins are very active and have high stability in long processed meat products (Toldrá and Etherington, 1988). The second group of proteinases are two calpains, also known as calcium-activated neutral proteinases, calcium-dependent proteases, or calcium-activated factor. Both calpains are located in the cytosol but mainly in the z-line area and differ in their Ca^{2+} requirement for activation, 50–70 μM of Ca^{2+} for calpain I or μ-calpain and 1–5 mM for calpain II or m-calpain. They have an optimal neutral pH, around 7.5, but their activity decreases rapidly at acid pH values such as those found in dry-fermented sausages. The stability is very poor for calpain I and rather poor for calpain II, which may exhibit some activity after a few weeks (Koohmaraie et al., 1987). Calpains have been considered the major contributors to meat tenderization (Lawrie and Ledward, 2006) even though in recent years it has been also attributed to the action of other muscle proteolytic enzymes (Ouali et al., 2006; Kemp et al., 2010). The third group is the proteasome complex, a large protease with multiple catalytic sites, is the third group of proteases. This enzyme is able to exhibit different activities like chymotrypsin-like activity, trypsin-like activity, and peptidyl-glutamyl hydrolyzing activity (Coux et al., 1995). The 20S proteasome may have a role in tenderness because of its ability to degrade myofibrils, specially M and z lines, but this activity is only exhibited at high pH values, far from those found in dry-cured meats (Ouali and Sentandreu, 2002). The mode of action for these enzymes is shown in Figure 17.2.

17.2.2 Exopeptidases

Two important groups of muscle exopeptidases are tripeptidylpeptidases (TPP) and dipeptidylpeptidases (DPP). TPP I and II, with optimal activity at acid and neutral pH, respectively, are able to hydrolyze different tripeptides from the amino termini of peptides and proteins. DPP I and II have optimal acid pH while DPP III and IV have optimal pH around 7.8–8.0 (Sentandreu and Toldrá, 1998, 2000, 2001a,b). Dipeptidases are enzymes able to hydrolyze dipeptides and their names can vary depending on the preference for certain amino acids. Aminopeptidases, which have large molecular masses, are able to release a free amino acid from the amino terminus of peptides and proteins.

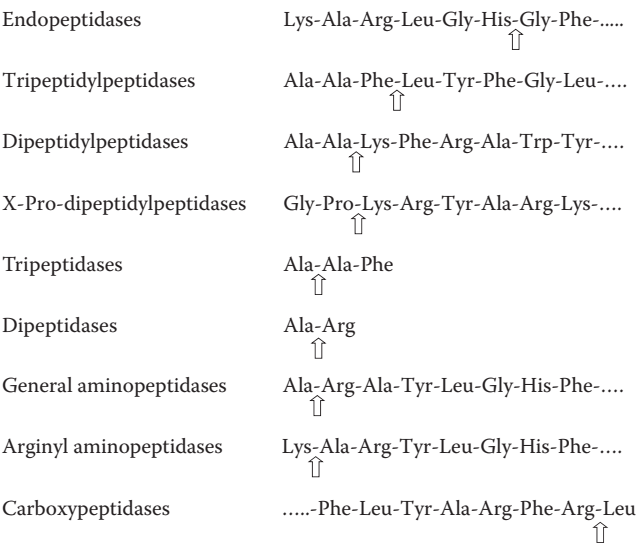


FIGURE 17.2
Example of peptide bond hydrolysis by different types of peptidases.

Arginyl, alanyl, pyroglutamyl, leucyl, and methionyl aminopeptidases are the most important aminopeptidases in skeletal muscle. All of them are active at neutral or basic pH. Alanyl and methionyl aminopeptidases have a wide spectrum of activity while arginyl aminopeptidase, or aminopeptidase B, is only able to hydrolyze arginine and lysine. A minor role is expected for leucyl and pyroglutamyl aminopeptidases due to their optimal basic pH (Toldrá et al., 2000).

There are two carboxypeptidases, both located in the lysosomes, with optimal activity at acid pH. They generate free amino acids from the carboxyl termini of peptides and proteins. Carboxypeptidase B has a wide spectrum of activity against any terminal amino acid but carboxypeptidase A is more specific for hydrophobic amino acids (Barrett et al., 2004). The mode of action of these proteases against different substrates is shown in Figure 17.2 and the substrate preferences are given in Table 17.1.

17.3 Microbial Proteolytical System

The proteolytic system is essential for a number of cellular processes involved in major physiological processes like use of proteins to supply free amino acids as nutrients and gene regulation (Pritchard and Coolbear, 1993). The mechanisms for proteolytic breakdown of proteins by microbial enzymes are similar to those previously described for muscle enzymes (see Figures 17.1 and 17.2). These enzymes are also endopeptidases or proteinases and peptidases.

TABLE 17.1

Main Muscle Exopeptidases: Location and Substrate Preferences

Enzyme	EC Number	Location	Substrates	Optimal pH Range	References
DPP I	EC 3.4.14.1	Lysosome	Ala-Arg-peptide, Gly-Arg-peptide	5.0–6.0	Sentandreu & Toldrá (2000)
DPP II	EC 3.4.14.2.	Lysosome	Gly-Pro-peptide	5.0–6.0	Sentandreu & Toldrá (2001a)
DPP III	EC 3.4.14.4.	Cytosol	Arg-Arg-peptide, Ala-Arg-peptide	7.5–8.0	Sentandreu & Toldrá, (1998)
DPP IV	EC 3.4.14.5.	Membrane	Gly-Pro-peptide	7.5–8.0	Sentandreu & Toldrá (2001b)
TPP I	EC 3.4.14.9.	Lysosome	Gly-Pro-Phe-peptide Ala-Ala-Phe-peptide	3.0–5.0	Toldrá (2002)
TPP II	EC 3.4.14.10.	Cytosol	Gly-Pro-Phe-peptide Ala-Ala-Phe-peptide	6.5–7.5	Toldrá (2002)
Alanyl aminopeptidase	EC 3.4.11.14.	Cytosol	Ala-peptide, amino acids-peptide	6.0–7.0	Flores et al. (1996)
Arginyl aminopeptidase	EC 3.4.11.6.	Cytosol	Arg-peptide, Lys-peptide	6.0–7.0	Flores et al. (1993)
Methionyl aminopeptidase	EC 3.4.11.18.	Cytosol	Met-peptide, Ala-peptide, Lys-peptide, Leu-peptide	7.0–7.5	Flores et al. (2000)
Leucyl aminopeptidase	EC 3.4.11.1.	Cytosol	Leu-peptide	9.0	Toldrá et al. (2012b)
Pyroglutamyl aminopeptidase	EC 3.4.19.3.	Cytosol	Pyroglu-peptide	8.0–8.5	Toldrá et al. (2012b)
Carboxypeptidase A	EC 3.4.16.1.	Lysosome	Peptide-hydrophobic amino acids	5.0–5.5	Barrett et al. (2004)
Carboxypeptidase B	EC 3.4.18.1.	Lysosome	Peptide-amino acid	5.0–5.5	Barrett et al. (2004)

DPP, dipeptidylpeptidases; TPP, tripeptidylpeptidases.

Endopeptidases or proteinases are predominantly extracellular and peptidases are located inside the cell. Extracellular proteinases may be bound either to the cell wall or to the cell membrane (Visser, 1993). In this way, the mode of action may substantially differ if using whole cells or only cell-free extracts. In the case of whole cells, the amino acid and peptide transport system is necessary to supply the cells with the amino acids required for growth (Tan et al., 1993). The cell wall-associated proteolytic activity is the first enzyme to degrade proteins. The generated peptides are then transported into the cell where they are further degraded by different peptidases like tri- and dipeptidases, DPP, and aminopeptidases, to small peptides and free amino acids (Bockelmann, 1995). Many microorganisms have been used as starters for fermented meats. Some

of the most important are *Lactobacillus sakei, Lactobacillus curvatus, Lactobacillus carnosus, Lactobacillus plantarum, Kocuria varians, Staphylococcus xylosus*, and the yeast *Debaryomices hansenii* (Toldrá, 2004, 2012a). Lactic acid bacteria constitute a group of microorganisms widely used in food fermentation. Even though the proteolytic system from dairy lactic acid bacteria has been well characterized, limited information is available on meat *Lactobacilli*.

17.3.1 Endopeptidases

Lactic acid bacteria have some endopeptidases or proteinases associated to the cell envelope. These proteinases are responsible for initial breakdown of proteins into oligopeptides. Several in vitro assays have been performed to elucidate the hydrolysis of sarcoplasmic and myofibrillar meat proteins when incubated with whole cells and cell-free extracts from different *Lactobacilli* like *L. sakei, L. curvatus, L. plantarum*, and *L. carnosus*. The hydrolysis of myofibrillar proteins is rather poor for all the assayed strains (Fadda et al., 1999a,b; Sanz et al., 1999a,b). These *in vitro* observations agree with other reports that bacterial proteinases are less effective than muscle proteinases in hydrolyzing myofibrillar proteins (Molly et al., 1997). However, these *Lactobacilli* strains are able to hydrolyze sarcoplasmic proteins, especially *L. plantarum* and *L. casei,* which show the strongest degradation (Sanz et al., 1999b). The substrate specificity is broad and the proteinase activity appears to be extracellularly located, which is in agreement to the existence of a single cell wall-associated proteinase in dairy lactic acid bacteria, which is responsible for initial caseins hydrolysis (Kunji et al., 1996).

The peptide profiles of the sarcoplasmic and myofibrillar extracts after incubation with these strains show the generation of a large number of hydrophylic peptides. This generation increased under the combined action of whole cells and cell-free extracts. The generation of hydrophilic peptides is important because they are correlated to desirable cured-meat flavors whereas hydrophobic peptides are correlated to bitterness (Aristoy and

TABLE 17.2

Main Purified Exopeptidases in *Lactobacillus sakei*: Biochemical Similarity and Substrate Preferences

Enzyme	Biochemical Similarity	Activation	Substrates	Optimal T (°C)	Optimal pH	Reference
Major aminopeptidase	Pep L	Ca^{2+}, Sn^{2+}, Mg^{2+}, Ba^{2+}, Mn^{2+}	Leu-peptide, Ala-peptide	37	7.5	Sanz and Toldrá (1997)
Arginine aminopeptidase	Pep N like	Reducing agents, salt	Arg-peptide, Lys-peptide	37	5.0	Sanz and Toldrá (2002)
X-prolyl-dipeptidylpeptidase	Pep X		X-Pro-peptides, Ala-Pro-peptide	55	7.5	Sanz and Toldrá (2001)
Dipeptidase	Pep V		Met-Ala	45	7.8	Montel et al. (1995)
Tripeptidase	Pep T		Ala-Ala-Ala	40	7.0	Sanz et al. (1998)

Toldrá, 1995; Henriksen and Stahnke, 1997). Some hydrophobic peptides are generated although in minor amounts.

An endopeptidase, known as protease B, has been purified from *D. hansenii*, a yeast typically found in meat. This protease is active at neutral–basic pH (see Table 17.2) and has homology with proteinase B (PrB) from *Saccharomyces cerevisiae* (Bolumar et al., 2005). It is able to hydrolyze sarcoplasmic proteins when using in vitro assays confirming previous reports incubating sarcoplasmic proteins with whole *D. hansenii* cells and cell-free extracts (Santos et al., 2001). However, this enzyme is inactivated at acid pH, reducing the expectatives for an important role in meat fermentation where pH may easily reach pH 5.0 or even lower.

17.3.2 Exopeptidases

There are important exopeptidases in *L. sakei*, the most prevalent species in European sausages (see Table 17.3). (1) The major or general aminopeptidase, which has an optimal neutral pH (around 7.5) and is similar to PepL from *Lactobacillus delbrueckii*. This aminopeptidase has a broad range of activity against amino acids, especially alanine and leucine, but is unable to hydrolyze basic residues (Sanz and Toldrá, 1997). (2) The arginine aminopeptidase, which has optimal acid pH, is activated by salt, and has a preference for basic residues like arginine and lysine (Sanz and Toldrá, 2002). No similar enzymes have been purified from dairy lactic acid bacteria. (3) A dipeptidase with an optimal basic pH, a broad specificity against dipeptides except those containing Pro or Gly at the N-terminus, and similarity to PepV characterized in other lactic acid bacteria (Montel et al., 1995). (4) A tripeptidase with optimal neutral pH, able to hydrolyze a wide spectrum of tripeptides except those with Pro in the second position; it is similar to PepT from lactococci (Sanz et al., 1998). (5) An X-prolyl-dipeptidylpeptidase, which has optimal neutral pH, the ability to hydrolyze X-Pro dipeptides from the amino terminus from different peptides, and similarity to PepX from dairy lactic acid bacteria (Sanz and Toldrá, 2001). The addition of cell-free extracts from *L. sakei, L. curvatus*,

TABLE 17.3

Main Purified Peptidases in *Debaryomices Hansenii*: Homology to *Saccharomyces cerevisiae* Peptidases and Substrate Preferences

Enzyme	Molecular Mass (kDa)	Homology to *S. cerevisiae* Peptidases	Activation	Substrates	Optimal T (°C)	Optimal pH	Reference
Protease B	430	PrB	–	Sarcoplasmic proteins	37	8.0	Bolumar et al. (2005)
Prolyl aminopeptidase	370	–	–	Pro-peptide	45	7.5	Bolumar et al. (2003a)
Arginyl aminopeptidase	101	ApY	Ca^{2+}, Mg^{2+}, Co^{2+}	Arg-peptide, Lys-peptide	37	7.0	Bolumar et al. (2003b)

and *L. casei* to myofibrillar and sarcoplasmic proteins gave a net increase in free amino acids. The increases were especially significant for glutamic acid, alanine and leucine for *L. sakei*, glutamic acid and alanine for *L. curvatus*, and arginine and glutamic acid for *L. casei* (Fadda et al., 1999b; Sanz et al., 1999a,b). However, this increase was negative when using *L. plantarum* probably due a lower exopeptidase activity or a higher intracellular metabolic activity for amino acid degradation (Fadda et al., 1999a).

In the case of yeasts, like *D. hansenii*, there are also several exopeptidases (see Table 17.3): (1) prolyl aminopeptidase, which has an optimal neutral pH, restricted to the hydrolysis of Pro at the amino terminus of peptides (Bolumar et al., 2003a) and (2) an arginyl aminopeptidase, with an optimal neutral pH, maximum specificity for basic residues like arginine and lysinee, and homology to ApY from *S. cerevisiae* (Bolumar et al., 2003b). Experiences in vitro with whole cells and cell-free extracts of *D. hansenii* have shown that they are able to hydrolyze sarcoplasmic proteins under similar conditions to those found during fermented sausage processing, generating several hydrophilic and hydrophobic peptides and free amino acids. The highest generation rates were observed when using whole cells (Santos et al., 2001).

17.4 Traditional Techniques for the Follow-Up of Proteolysis in Dry-Cured Meats

For many decades, proteolysis has been followed up through the use of several techniques like sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), two-dimensional gel electrophoresis (2-DGE), free solution conjugate electrophoresis (FSCE), size-exclusion chromatography (SEC), ion exchange chromatography (IEC), reversed-phase high-performance liquid chromatography (RP-HPLC), and more recently, hydrophilic interaction liquid chromatography (HILIC). These techniques have been used to study proteolysis and to separate and identify the proteins and major generated fragments from complex mixtures like those found in dry-cured meats as well as the analysis of free amino acids after their derivatization (Lawrie and Ledward, 2006). This has allowed the interpretation of major findings related to peptides generation and the accumulation of released free amino acids (Toldrá, 2012a). An example of the key role of small peptides and free amino acids released and the enzymes involved during the processing of dry-cured meats and its contribution to flavor are shown in Table 17.4. However, there were problems in the identification of the type of peptides generated and their sequences

TABLE 17.4

Main Enzymatic Routes for the Generation of Small Peptides and Free Amino Acids during the Processing of Dry-Cured Meats and their Contributions to Flavor

Compounds	Enzymes Involved	Contribution to Flavor
Tripeptides	Muscle tripeptidyl peptidases	Taste will depend on the specific amino acid composition
Dipeptides	Muscle and microbial dipeptidylpeptidases	Taste will depend on the specific amino acid composition
Phenylalanine, tyrosine, tryptophane, methionine, leucine, valine, and isoleucine	Muscle methionine and alanyl aminopeptidases Major aminopeptidase from *Lactobacillus sakei*	Bitter taste will increase as the concentration of these amino acids arise Generation of volatile branched-chain aldehydes (from Leu, Val, and Ile) that contribute to aroma
Alanine, serine, proline, glycine, and hydroxyproline	Muscle methionine and alanyl aminopeptidases Major aminopeptidase from *L. sakei*	Sweet taste will depend on the final concentration
Proline	Prolyl aminopeptidase from *Debaryomices hansenii*	Slight bitterness reduction
Glutamic and aspartic acids	Muscle methionine and alanyl aminopeptidases Major aminopeptidase from *L. sakei*	Taste enhancement, slight acid taste
Lysine and arginine	Muscle aminopeptidase B Arginine aminopeptidase from *L. sakei* and arginyl aminopetidase from *D. hansenii*	Aged cured taste

Source: Adapted from Toldrá, F., *Dry-Cured Meat Products*, Food and Nutrition Press, Trumbull, CT, 2002; Toldrá, F., *Food Processing: Principles and Applications*, Blackwell Publishing, Ames, IA, 2004; Toldrá, F., *Food Biochemistry and Food Processing*, Blackwell Publishing, Ames, IA, 2012a.

that has been solved with the availability of powerful proteomics tools, as will be described in next sections.

17.5 Proteomics in Dry-Cured Ham Approaches

Traditionally, proteomics has been used to identify proteins from a mix or an extract, obtaining the characterization of the protein profile of the sample. The most frequently used strategy has been the separation of the proteins

using SDS-PAGE and the subsequent digestion of the isolated protein with specific proteases like trypsin that specifically cleaves the protein on the C-terminal side of the basic amino acids arginine and lysine. The peptides obtained after the digestion of the protein are analyzed by mass spectrometry, achieving a list of peak masses. This experimental mass profile is matched against the theoretical masses obtained from the *in silico* digestion of the protein and included in the databases. This strategy is named protein mass fingerprint (PMF) and the results are given as a report where identified proteins are ranked according to the number of peptide masses matching their sequence within a given mass error tolerance. To identify a protein it is necessary that the masses of a certain number of fragments match with the theoretical peptide masses contained in the protein databases. This result is accepted if the data passes a statistical probability threshold of being a correct match (Figure 17.3).

The PMF approach has been used different times in dry-cured ham studies. Regarding the use of this approach in meat-derived matrices, 2D SDS-PAGE and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-ToF MS) analysis were used to study the myofibrillar and water-soluble fraction of raw ham muscles and dry-cured hams with different ripening times (Di Luccia et al., 2005). The two-dimensional maps showed the progressive disappearance of actin, tropomyosin, and myosin light chains during ripening. Some of the sarcoplasmic proteins in water extracts from pork meat markedly decreased in amount or disappeared totally during ripening. The most interesting bands were digested using trypsin enzyme and subsequently analyzed by MALDI-ToF MS. Two fragments of myosin heavy chain and a new form of actin were identified in the myofibrilar fraction whereas α- and β-tropomyosin were detected in the water-soluble protein fraction.

Sarcoplasmic proteins undergoing proteolysis during the ripening of products were also identified by MALDI-ToF MS using the PMF approach after 2D AUT-PAGE/SDS electrophoresis (acetic acid-urea-triton polyacrylamide gel in the first dimension and sodium dodecyl sulfate polyacrylamide gel in the second dimension). This resulted in the identification of thirteen sarcoplasmic proteins and the technique showed higher resolution in comparison

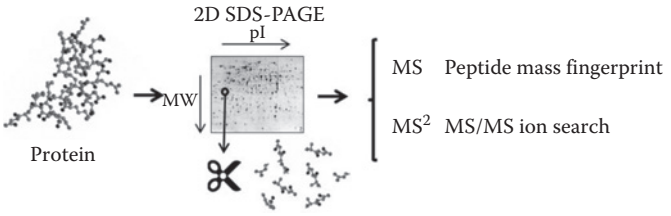


FIGURE 17.3
Scheme of a typical proteomics approach with a previous SDS-PAGE separation of the protein of interest. SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis.

to standard 2D IPG/SGS-PAGE as well as an easier interpretation of the 2D maps (Picariello et al., 2006).

Despite the advantage of using these approaches for a better understanding of the disappearance of proteins, the sequences corresponding to the hydrolysis products were not analyzed in any of these studies. The major difficulty in the study of naturally generated peptides resides is the small size of these fragments that cannot be trypsin digested. This fact gives double difficulty, the small size of the generated peptides that are sometimes at the limit of some standard mass spectrometry techniques, and the impossibility of controlling the hydrolysis, with the consequence being a complex mixture of peptides from different proteins with unspecific cleavage sites. In this sense, it is essential to use advanced proteomic techniques such as mass spectrometry in tandem to elucidate the sequence of these small peptides (see Figure 17.3), as it allows for faster and more reliable identification of the sequences.

17.5.1 From Traditional Proteomics to Peptidomics

Peptidomics is the area of science focused on the comprehensive characterization of peptides present in a biological sample by studying their structure, composition, interactions, and properties. It uses similar methods as proteomics, although the analysis of peptides requires an analytical and experimental adaptation of the strategy to follow in comparison to proteins. Peptidomics has become an essential tool in food science in order to study the biological activity, functional properties, product authenticity and origin, allergenicity, and sensory properties of the peptides (Minkiewicz et al., 2008). Thus, peptidomic approaches constitute the methodologies of choice to identify the specific sequences generated from myofibrillar and sarcoplasmic proteins in dry-cured ham. Thus, peptides from the myofibrillar proteins titin (Gallego et al., 2015), myosin light chain (Mora et al., 2011), troponin T (Mora et al., 2010), LIM domain-binding protein 3 (Gallego et al., 2014), and actin (Sentandreu et al., 2007), as well as sarcoplasmic proteins creatine kinase (Mora et al., 2009), glycolytic enzymes (Mora et al., 2011a), myoglobin (Mora and Toldrá, 2012), and ubiquitin (Mora et al., 2015a) were described using MS/MS analysis with electrospray ionization quadrupole (ESI-Q)/ToF and MALDI-ToF/ToF mass spectrometers. In this sense, Table 17.5 shows the number of peptides that have been sequenced in dry-cured ham including their respective protein of origin.

17.5.2 Applications of Peptidomics in Dry-Cured Ham

Dry-cured ham is a high-quality product with a very long ripening time, reaching in certain cases up to 24 months of processing. The time of curing together with genetics and feed of the animal are main factors determining its final economical value.

TABLE 17.5

Naturally Generated Peptides (in Number) by Respective Protein of Origin during the Processing of Dry-Cured Ham Identified Using Mass Spectrometry in Tandem

Protein Name	Accession Number	Number of Peptides	Analysis	Search Engine	Database	Reference
Small peptides (2, 3, and 4 amino acids)	–	–	Edman degradation	–	–	Sentandreu and Toldrá (2007)
Actin	NP_001161267	4	MALDI-ToF Qtrap	Mascot	UniProt	Sentandreu et al. (2007)
Myosin light chain 1 (MLC 1)	ABK55642	9 137	MALDI-ToFn ESI-LC-Q/ToF	Mascot Mascot	NCBInr NCBInr	Mora et al. (2009, 2011a)
Titin	AAD00528 O97771	5 320	MALDI-ToFn ESI-LC-Q/ToFn ESI-LC-Q/ToF	Mascot Mascot	NCBInr Uniprot	Mora et al. (2009) Gallego et al. (2015)
Creatine kinase (CK)	NP_001123421	58	MALDI-ToFn ESI-LC-Q/ToF	Mascot	NCBInr	Mora et al. (2009)
Slow troponin T (TnT)	Q75ZZ6	2	MALDI-ToF/ToF	Mascot	NCBInr	Mora et al. (2010)
Fast troponin T (TnT)	BAD15381	25	MALDI-ToF/ToF	Mascot	NCBInr	Mora et al. (2010)
Myosin light chain 2 (MLC 2)	NP_001006592	88	nESI-LC-Q/ToF	Mascot	NCBInr	Mora et al. (2011a)
Glycogen phosphorylase (PYGM)	ABF81977	2	nESI-LC-Q/ToF	Mascot Paragon	NCBInr UniProt	Mora et al. (2011b)
Glyceraldehyde 3-phosphate dehydrogenase (GAPDH)	ABI29187	8	nESI-LC-Q/ToF	Mascot Paragon	NCBInr UniProt	Mora et al. (2011b)
Phosphoglycerate kinase 1 (PGK)	NP_001093402	4	nESI-LC-Q/ToF	Mascot Paragon	NCBInr UniProt	Mora et al. (2011b)

(*Continued*)

TABLE 17.5 (*Continued*)

Naturally Generated Peptides (in Number) by Respective Protein of Origin during the Processing of Dry-Cured Ham Identified Using Mass Spectrometry in Tandem

Protein Name	Accession Number	Number of Peptides	Analysis	Search Engine	Database	Reference
Phosphoglycerate mutase 2 (PGAM)	CAD89670	7	nESI-LC-Q/ToF	Mascot Paragon	NCBInr UniProt	Mora et al. (2011b)
Enolase (ENO)	NP_001037992	18	nESI-LC-Q/ToF	Mascot Paragon	NCBInr UniProt	Mora et al. (2011b)
Piruvate kinase 3 isoform 2 (PK)	XP_001929120	2 1	nESI-LC-Q/ToF Edmandegradation	Mascot Paragon –	NCBInr UniProt –	Mora et al. (2011b)
Lactate dehydrogenase (LDH)	P0039	4	nESI-LC-Q/ToF	Mascot Paragon	NCBInr UniProt	Mora et al. (2011b)
LIM domain-binding (LIM)	F1SEN8	107	nESI-LC-Q/ToF	Mascot	UniProt	Gallego et al. (2013)
Myoglobin (MYO)	NP_999401	11	nESI-LC-Q/ToF	Mascot	NCBInr	Mora and Toldrá (2012)
Ubiquitin-60S (UBI)	P63053	68	nESI-LC-Q/ToF	Mascot	UniProt	Mora et al. (2015b)

ESI, electrospray ionization quadrupole (ESI-Q); MALDI/ToF, matrix-assisted laser desorption/ionization time-of-flight; nESI-LC-Q, nano-electrospray ionization/liquid chromatography quadrupole.

Peptidomics have also been used to characterize proteolysis related to food processes such as curing or fermentation (Sforza et al., 2012), to determine peptide biomarkers of time of processing or food quality (Gallego et al., 2016), and to establish differences between genotypes in high-value meat-derived products (Mora et al., 2016). Different empirical and *in silico* approaches have also been used to identify naturally generated bioactive peptides in dry-cured ham. In this sense, peptides showing antihypertensive, antioxidant, and antimicrobial activities were reported (Escudero et al., 2012, 2013a,b). Figure 17.4 shows a scheme of main applications of peptidomics in dry-cured ham samples.

The use of these approaches in dry-cured ham offers many advantages allowing the advance of meat science by both adding extra value to the product with the presence of natural bioactive peptides and controlling the time of curing and thus the final quality of dry-cured hams to prevent fraudulent activities or accidental mislabeling.

17.5.2.1 Identification of Bioactive Peptides

Several bioactive peptides exerting antihypertensive, antioxidant, and antimicrobial activities have been identified in Spanish dry-cured ham. The identification was done after several isolation steps using different chromatographic separations such as SEC or RP-HPLC. From the list of identified peptides in the most active fraction, some of them were selected as potentially bioactive according to their length, amino acid composition, and amino acid location in the sequence (Pihlanto-Leppälä, 2001), and their activity was confirmed *in vitro*.

Regarding the antihypertensive activity, the most active fractions and peptides assayed *in vitro* were also tested *in vivo* for their antihypertensive activity using spontaneous hypertensive rats (SHR). In this respect, fractions obtained from SEC corresponding to molecular masses lower than 1700 Da showed the most intense antihypertensive activity with a decrease in systolic

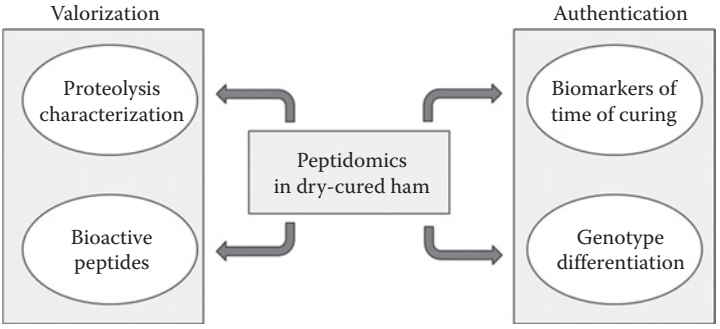


FIGURE 17.4
Main applications of peptidomics in dry-cured ham.

blood pressure (SBP) of 38.38 mmHg in such SHR rats after eight hours of ingestion (Escudero et al., 2012), whereas peptide AAATP with an in vitro IC_{50} value of 100 μM showed a decrease in SBP of 25.62 mmHg after eight-hour administration in SHR (Escudero et al., 2013a). In Parma dry-cured ham, the gastrointestinal digestion of 18 and 24 months of curing samples resulted in the identification of peptides LGL and SFVTT with IC_{50} values of 145 and 395 μM, respectively (Dellafiora et al., 2015). More recently, a peptide extract from Iberian dry-cured ham showed a significant decrease in SBP of 12 mmHg after eight hours of ingestion in spontaneously hypertensive rats (SHR). The analysis resulted in the identification of 2632 sequences of peptides containing the previously described angiotensin-converting enzyme (ACE)-inhibitory fragments PPK (Pro-Pro-Lys), PAP (Pro-Ala-Pro), and AAP (Ala-Ala-Pro) repeated a total of 322, 302, and 119 times, respectively (Mora et al., 2015a).

Regarding antioxidant activity, Jinhua ham and Xuanwei ham from China have been described to show antioxidant activity. The sequence GKFNV was presented as the main peptide playing a key role as a scavenger of free radicals in a Jinhua ham extract (Zhu et al., 2013), whereas the tetrapeptide DLEE was identified in Xuanwei ham as one of the key peptides responsible for the observed antioxidant activity, showing a 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity of 74.45% at a concentration of 0.5 mg/mL (Xing, et al., 2016). In a recent study, different antioxidant peptides from Jinhua ham were isolated and identified such as the sequences DLEE, GKFNV, and LPGGGHGDL, which were tested using DPPH radical scavenging and hydroxyl radical scavenging assays (Zhu et al., 2016). In this sense, Escudero et al. (2013b) reported that the water-soluble extract of Spanish dry-cured ham contained a large amount of potentially antioxidant peptides. This fact was confirmed with the identification of the peptide SNAAC with an IC_{50} value of 75.2 μM in a DPPH radical scavenging assay and 205 μM in ferric-reducing antioxidant power assays (Mora et al., 2014).

Regarding the antimicrobial activity, *Listeria monocytogenes* is an important concern in dry-cured ham products after slicing due to its high resistance. Recently, a novel antilisterial peptide with sequence RHGYM was identified in Spanish dry-cured ham after 10 months of curing showing an MIC value of 6.25 mM (Castellano et al., 2016).

17.5.2.2 Label-Free Relative Quantitation to Determine Differences between Dry-Cured Hams

In peptidomics, the study of peptides often includes their relative quantitation with labeled or label-free methods. In this sense, labeling methodologies provide the most accurate quantitative values, but their use involves costly labeling reagents and multistep experimental protocols. In contrast, label-free methods are a simple, versatile, reliable, and cost-effective alternative. Thus, the use of peak intensity in peptide quantitation is based on the correlation existing between peak areas of peptides with the concentration

of the protein where peptides come from, allowing an accurate and precise evaluation of changes in protein abundance from sample to sample (Zhu et al., 2010).

Peptide quantitation using a label-free approach based on peptidomics results is very useful to detect significant differences between dry-cured ham samples through the generated peptides during hydrolysis as well as to evaluate the main peptides and proteins responsible for these differences. Different approaches based on the relative quantitation of peptides using a label-free methodology have been developed to determine biomarkers of the time of curing (Gallego et al., 2016), to differentiate genetics in high-value meat-derived products (Mora et al., 2016), as well as to establish the degree of posttranslational modifications as oxidation occurred during the processing.

17.5.2.2.1 Biomarkers of the Time of Curing

Time of curing in dry-cured ham is one of the factors determining its final quality as longer processing times are associated with a more intense proteolysis and higher generation of aroma and flavor. Differences obtained in the relative quantification of generated peptides during the processing of dry-cured ham determine that peptides obtained from the degradation of myosin light chain 1 protein were mainly responsible for the observed differences during the last stages of curing. In this sense, two peptides in particular were identified as potential biomarkers of nine months of curing dry-cured ham (Gallego et al., 2016). These results were obtained using principal component analysis (PCA) statistics as indicated in Figure 17.5.

17.5.2.2.2 Differences between Genotypes

The genotype of the pig used in the preparation of dry-cured ham gives extra value to the final product. In this respect, Iberian ham is a product of excellence well valuated for its intramuscular fat and final characteristics of flavor. The use of a peptidomic approach permitted study of the differences between three different genotypes in the final peptide profile of dry-cured ham (Mora et al., 2016). In this case, the most influential peptides were derived from myosin light chain I, creatine kinase, myoglobin, troponin T, and myosin heavy chain 7. This study proved the differences in the action of endogenous enzymes depending on genetics that resulted in a different peptide profile at the end of the processing period.

17.5.3 Data Analysis

In proteomics, the identification of peptides is mostly carried out using database search approaches. This type of data analysis can also be used for the direct analysis of peptides naturally generated from endogenous enzymes (Fricker et al., 2006; Geho et al., 2006; Hardt et al., 2005a,b; Villanueva et al., 2006) although the appropriate choice of the search parameters and sequence database is very important for the successful application of this methodology.

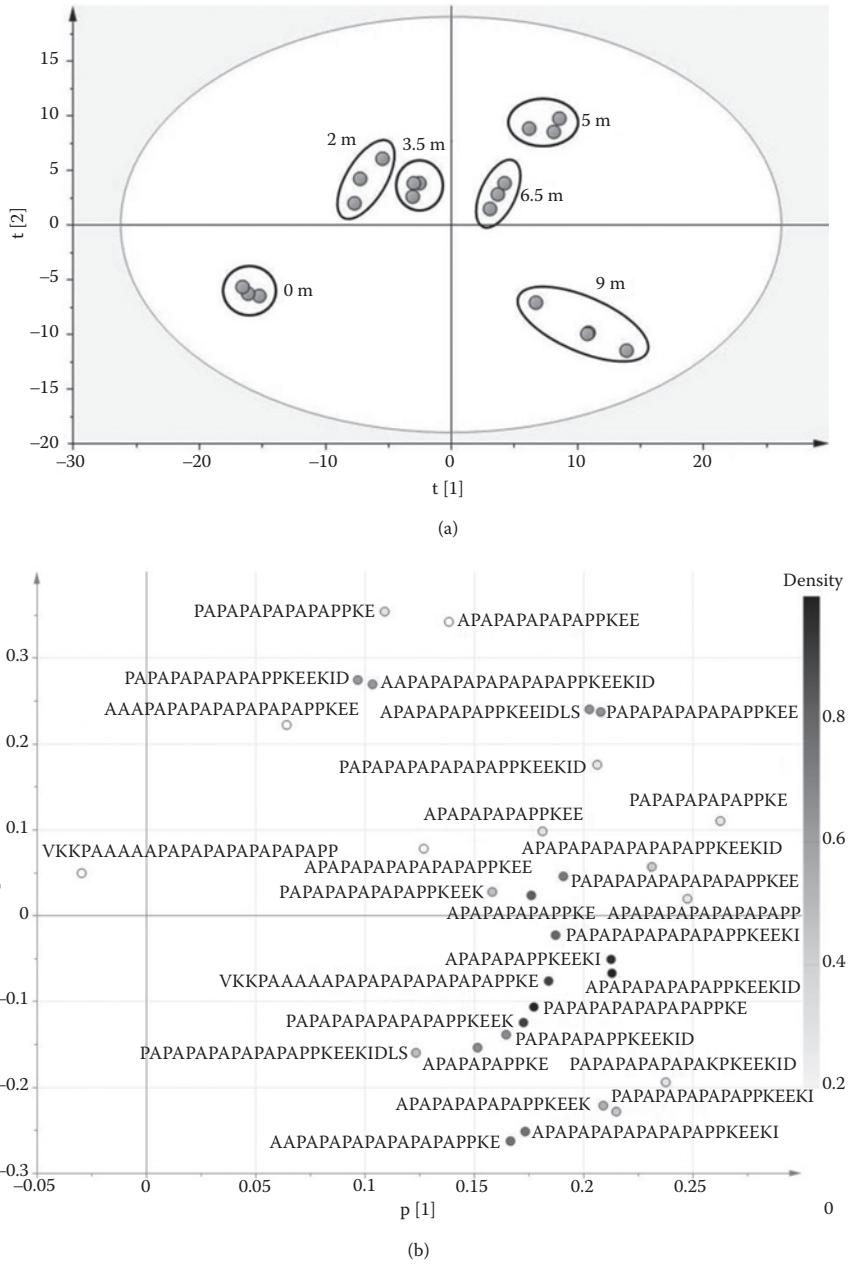


FIGURE 17.5
(a) Principal component analysis (PCA) score plot to assess the variance among the naturally generated peptides at different times of dry-cured ham processing. (b) PCA loading plot showing peptides identified at nine months of curing from myosin light chain 1 protein. Reprinted from *Journal of Proteomics*, **147**, Gallego et al., Peptidomics as a tool for quality control in dry-cured ham processing, 98–107, Copyright 2016, with permission from **Elsevier**.

In fact, database searching of nontryptic peptides is less effective due to the lack of charge localization at the N and C termini of the naturally generated peptides. The uses of nonspecific enzymes as well as the error tolerant option to search the unmatched spectra considering possible modifications are some of the parameters to take into account in the Mascot MS/MS Ions Search when naturally generated peptides are studied.

The most widely used search engines are Mascot (http://www.matrixscience.com/search_form_select.html), SEQUEST (http://fields.scripps.edu/sequest/), Phenyx (http://www.ionsource.com/functional_reviews/Phenyx/phenyx-web.htm), MassWiz (Yadav et al., 2011), Hydra (Lewis et al., 2012), and the free software OMSSA (https://www.ncbi.nlm.nih.gov/Web/Newsltr/V14N2/) and X!Tandem (http://www.thegpm.org/tandem/), but there are more software packages designed to identify the most likely peptide sequence to match a MS/MS spectrum. Mascot from matrixscience (http://www.matrixscience.com/) is the most widely used software but the choice of its search parameters requires some user expertise. There are many available databases, but UniProt and NCBInr are the most commonly used in the identification of meat-derived peptides. Currently, UniProtKB/TrEMBL contains 77,483,538 sequence entries, comprising 25,974,169,949 amino acids, and it is considered the best annotated database although it is important to consider that it is nonredundant so it is ideal for PMF searches, where the loss of one or two peptides is not very important. For MS/MS searches, the NCBInr database is a better choice. This database is large (94 million entries approximately), comprehensive, and nonidentical.

The use of peptidomic approaches in the field of processed meat products such as dry-cured ham has permitted a better understanding of the proteolytic phenomena during its processing through the study of the generated peptides. This also results in an improved value through the identification of bioactive peptides and better control of quality parameters such as time of curing or genotypes. What is more, future aims using peptidomics could apply the obtained information to drive the proteolysis process toward improvements of the final value and quality of the product as well as the development of a new generation of improved processed meat products.

References

Aristoy, M. C. and Toldrá, F. (1995). Isolation of flavor peptides from raw pork meat and dry-cured ham. *In:* Charalambous, G. (ed.) *Food Flavors: Generation, Analysis and Process Influence*. Amsterdam, the Netherlands: Elsevier Science Publishers BV, pp. 1323–1344.

Barrett, A. J., Rawlings, N. D. and Woessner, J.F. (eds.) (2004). *Handbook of Proteolytic Enzymes* (2nd Ed.). Amsterdam, the Netherlands: Elsevier/Academic Press, pp. 831–833, 1923–1929.

Bockelmann, W. (1995). The proteolytic system of starter and non-starter bacteria: Components and their importance for cheese ripening. *International Dairy Journal,* **5**, 977–994.

Bolumar, T., Sanz, Y., Aristoy, M. C. and Toldrá, F. (2003a). Purification and characterization of a prolyl aminopeptidase from *Debaryomyces hansenii*. *Applied and Environmental Microbiology,* **69**, 227–232.

Bolumar, T., Sanz, Y., Aristoy, M. C. and Toldrá, F. (2003b). Purification and properties of an arginyl aminopeptidase from Debaryomyces hansenii. *International Journal of Food Microbiology,* **86**, 141–151.

Bolumar, T., Sanz, Y., Aristoy, M. C. and Toldrá, F. (2005). Protease B from *Debaryomyces hansenii*. Purification and biochemical properties. *International Journal of Food Microbiology,* **98**, 167–177.

Castellano, P., Mora, L., Escudero, E., Vignolo, G., Aznar, R. and Toldrá, F. (2016). Antilisterial peptides from Spanish dry-cured hams: Purification and identification. *Food Microbiology,* **59**, 133–141.

Coux, O., Tanaka, T. and Goldberg, A. (1995). Structure and function of the 20S and 26S proteasomes. *Annual Review of Biochemistry,* **65**, 801–847.

Dellafiora, L., Paolella, S., Dall'Asta, C., Dossena, A., Cozzini, P. and Galaverna, G. (2015). Hybrid in silico/in vitro approach for the identification of angiotensin I converting enzyme inhibitory peptides from parma dry-cured ham. *Journal of Agricultural and Food Chemistry,* **63**(28), 6366–6375.

Di Luccia, A., Picariello, G., Cacace, G. et al. (2005). Proteomic analysis of water soluble and myofibrillar protein changes occurring in dry-cured hams. *Meat Science,* **69**(3), 479–491.

Escudero, E., Aristoy, M. C., Nishimura, H., Arihara, K. and Toldrá, F. (2012). Antihypertensive effect and antioxidant activity of peptide fractions extracted from Spanish dry-cured ham. *Meat Science,* **91**(3), 306–311.

Escudero, E., Mora, L., Fraser, P. D., Aristoy, M. C., Arihara, K. and Toldrá, F. (2013a). Purification and identification of antihypertensive peptides in Spanish dry-cured ham. *Journal of Proteomics,* **78**, 499–507.

Escudero, E., Mora, L., Fraser, P. D., Aristoy, M. C. and Toldrá, F. (2013b). Identification of novel antioxidant peptides generated in Spanish dry-cured ham. *Food Chemistry,* **138**, 1282–1288.

Fadda, S., Sanz, Y., Vignolo, G., Aristoy, M. C., Oliver, G. and Toldrá, F. (1999a). Hydrolysis of pork muscle sarcoplasmic proteins by *Lactobacillus curvatus* and Lactobacillus sake. *Applied and Environmental Microbiology,* **65**, 578–584.

Fadda, S., Sanz, Y., Vignolo, G., Aristoy, M. C., Oliver, G. and Toldrá, F. (1999b). Characterization of muscle sarcoplasmic and myofibrillar protein hydrolysis caused by *Lactobacillus plantarum*. *Applied and Environmental Microbiology,* **65**, 3540–3546.

Flores, M., Aristoy, M. C. and Toldrá, F. (1993). HPLC purification and characterization of porcine muscle aminopeptidase B. *Biochimie,* **75**, 861–867.

Flores, M., Aristoy, M. C. and Toldrá, F. (1996). HPLC purification and characterization of soluble alanyl aminopeptidase from porcine skeletal muscle. *Journal of Agricultural and Food Chemistry,* **44**, 2578–2583.

Flores, M., Marina, M. and Toldrá, F. (2000). Purification and characterization of a soluble methionyl aminopeptidase from porcine skeletal muscle. *Meat Science,* **56**, 247–254.

Fricker, L. D., Lim, J., Pan, H. and Che, F. Y. (2006). Peptidomics: Identification and quantification of endogenous peptides in neuroendocrine tissues. *Mass Spectrometry Reviews,* **25**(2), 327–344.

Gallego, M., Mora, L., Fraser, P. D., Aristoy, M. C. and Toldrá, F. (2014). Degradation of LIM domain-binding protein 3 during Spanish dry-cured ham processing. *Food Chemistry,* **149**, 121–128.

Gallego, M., Mora, L., Aristoy, M. C. and Toldrá, F. (2015). Titin-derived peptides as processing time markers in dry-cured ham. *Food Chemistry,* **167**, 326–339.

Gallego, M., Mora, L. and Toldrá, F. (2016). Peptidomics as a tool for quality control in dry-cured ham processing. *Journal of Proteomics,* **147**, 98–107.

Geho, D. H., Liotta, L. A., Petricoin, E. F., Zhao, W. and Araujo, R. P. (2006). The amplified peptidome: The new treasure chest of candidate biomarkers. *Current Opinion in Chemical Biology,* **10**(1), 50–55.

Haard, N. F. (1990). Enzymes from food myosystems. *Journal of Muscle Foods,* **1**, 293–338.

Hardt, M., Thomas, L. R., Dixon, S. E. et al. (2005a). Toward defining the human parotid gland salivary proteome and peptidome: Identification and characterization using 2D SDS-PAGE, ultrafiltration, HPLC, and mass spectrometry. *Biochemistry,* **44**(8), 2885–2899.

Hardt, M., Witkowska, H. E., Webb, S. et al. (2005b). Assessing the effects of diurnal variation on the composition of human parotid saliva: Quantitative analysis of native peptides using iTRAQ reagents. *Analytical Chemistry,* **77**(15), 4947–4954.

Henriksen, A. P. and Stahnke, L. H. (1997). Sensory and chromatographic evaluations of water soluble fraction from dried sausages. *Journal of Agricultural and Food Chemistry,* **45**, 2679–2684.

Kemp, C. M., Sensky, P. L., Bardsley, R. G., Buttery, P. J. and Parr, T. (2010). Tenderness: An enzymatic view. *Meat Science,* **84**, 248–256.

Koohmaraie, M., Seideman, S. C., Schollmeyer, J. E., Dutson, T. R. and Grouse, J. D. (1987). Effect of postmortem storage on Ca2+ dependent proteases, their inhibitor and myofibril fragmentation. *Meat Science,* **19**, 187–196.

Kunji, E. R. S., Fang, G., Jeronimus-Stratingh, C. M., Bruins, A. P., Poolman, B. and Konings, W. N. (1996). The proteolytic systems of lactic acid bacteria. *Antonie van Leeuwenhoek,* **70**, 187–221.

Lawrie, R. A. and Ledward, D. A. (2006). *Lawrie's Meat Science* (7th Ed). Cambridge, UK: Woodhead Publishing.

Lewis, S., Csordas, A., Killcoyne, S. et al. (2012). Hydra: A scalable proteomic search engine which utilizes the Hadoop distributed computing framework. *BMC Bioinformatics,* **13**, 324.

Minkiewicz, P., Dziuba, J., Darewicz, M., Iwaniak, A., Dziuba, M. and Nałęcz, D. (2008). Food peptidomics. *Food Technology and Biotechnology*, **46**(1), 1–10.

Molly, K., Demeyer, D. I., Johansson, G., Raemaekers, M., Ghistelinck, M. and Geenen, I. (1997). The importance of meat enzymes in ripening and flavor generation in dry fermented sausages. First results of a European project. *Food Chemistry,* **54**, 539–545.

Montel, M. C., Seronine, M. P., Talon, R. and Hebraud, M. (1995). Purification and characterization of a dipeptidase from Lactobacillus sake. *Applied and Environmental Microbiology,* **61**, 837–839.

Mora, L., Calvo, L., Escudero, E. and Toldrá, F. (2016). Differences in pig genotypes influence the generation of peptides in dry-cured ham processing. *Food Research International,* **86**, 74–82.

Mora, L., Escudero, E., Arihara, K. and Toldrá, F. (2015a). Antihypertensive effect of peptides naturally generated during Iberian dry-cured ham processing. *Food Research International*, **78**, 71–78.

Mora, L., Escudero, E., Fraser, P. D., Aristoy, M. C. and Toldrá, F. (2014). Proteomic identification of antioxidant peptides from 400 to 2500 Da generated in Spanish dry-cured ham contained in a size-exclusion chromatography fraction. *Food Research International*, **56**, 68–76.

Mora, L., Gallego, M., Aristoy, M. C., Fraser, P. D. and Toldrá, F. (2015b). Peptides naturally generated from ubiquitin-60S ribosomal protein as potential biomarkers of dry-cured ham processing time. *Food Control*, **48**, 102–107.

Mora, L., Sentandreu, M. A., Fraser, P. D., Toldrá, F. and Bramley, P. M. (2009). Oligopeptides arising the degradation of creatine kinase in Spanish dry-cured ham. *Journal of Agricultural and Food Chemistry*, **57**, 8982–8988.

Mora, L., Sentandreu, M. A. and Toldrá, F. (2010). Identification of small troponin T peptides generated in dry-cured ham. *Food Chemistry*, **123**, 691–697.

Mora, L., Sentandreu, M. A. and Toldrá, F. (2011a). Intense degradation of myosin light chain isoforms in Spanish dry-cured ham. *Journal of Agricultural and Food Chemistry*, **59**, 3884–3892.

Mora, L. and Toldrá, F. (2012). Proteomic identification of small (<2000 Da) myoglobin peptides generated in dry-cured ham. *Food Technology and Biotechnology*, **50**(3), 343–349.

Mora, L., Valero, M. L., Del Pino, M. M. S., Sentandreu, M. A. and Toldrá, F. (2011b). Small peptides released from muscle glycolytic enzymes during dry-cured ham processing. *Journal of Proteomics*, 74, 442–450.

Ouali, A., Herrera-Méndez, C. H., Coulis, G. et al. (2006). Revisiting the conversion of muscle into meat and the underlying mechanisms. *Meat Science*, **74**, 44–58.

Ouali, A. and Sentandreu, M. A. (2002). Overview of muscle peptidases and their potential role in meat texture development. *In:* Toldrá, F. (ed.) *Research Advances in the Quality of Meat and Meat Products*. Trivandrum, India: Research Signpost, pp. 33–63.

Picariello, G., De Martino, A., Mamone, G. et al. (2006). Proteomic study of muscle sarcoplasmic proteins using AUT-PAGE/SDS-PAGE as two-dimensional gel electrophoresis. *Journal of Chromatography B: Analytical Technology Biomedical Life Sciences*, **833**(1), 101–108.

Pihlanto-Leppälä, A. (2001). Bioactive peptides derived from bovine whey proteins: Opioid and ACE-inhibitory peptides. *Trends in Food Science and Technology*, **1**, 347–356.

Pritchard, G. G. and Coolbear, T. (1993). The physiology and biochemistry of the proteolytic system of lactic acid bacteria. *FEMS Microbiology Reviews*, **12**, 179–206.

Santos, N. N., Santos, R. C., Sanz, Y., Bolumar, T., Aristoy, M.-C. and Toldrá, F. (2001). Hydrolysis of muscle sarcoplasmic proteins by *Debaryomyces hansenii*. *International Journal of Food Microbiology*, **68**, 199–206

Sanz, Y., Fadda, S., Vignolo, G., Aristoy, M. C., Oliver, G. and Toldrá, F. (1999a). Hydrolytic action of *Lactobacillus casei* CRL 705 on pork muscle sarcoplasmic and myofibrillar proteins. *Journal of Agricultural and Food Chemistry*, **47**, 3441–3448.

Sanz, Y., Fadda, S., Vignolo, G., Aristoy, M. C., Oliver, G. and Toldrá, F. (1999b). Hydrolysis of muscle myofibrillar proteins by *Lactobacillus curvatus* and *Lactobacillus sake*. *International Journal of Food Microbiology*, **53**, 115–125.

Sanz, Y., Mulholland, F. and Toldrá, F. (1998). Purification and characterization of a tripeptidase from *Lactobacillus sake*. *Journal of Agricultural and Food Chemistry,* **46**, 349–353.

Sanz, Y. and Toldrá, F. (1997). Purification and characterization of an aminopeptidase from *Lactobacillus sake*. *Journal of Agricultural and Food Chemistry,* **45**, 1552–1558.

Sanz, Y. and Toldrá, F. (2001). Purification and characterization of an X-prolyl-dipeptidyl peptidase from Lactobacillus sake. *Applied and Environmental Microbiology,* **67**, 1815–1820.

Sanz, Y. and Toldrá, F. (2002). Purification and characterization of an arginine aminopeptidase from *Lactobacillus sakei*. *Applied and Environmental Microbiology,* **68**, 1980–1987.

Sentandreu, M. A., Armenteros, M., Calvete, J. J., Ouali, A., Aristoy, M. C. and Toldrá, F. (2007). Proteomic identification of actin-derived oligopeptides in dry-cured ham. *Journal of Agricultural and Food Chemistry,* **55**(9), 3613–3619.

Sentandreu, M. A. and Toldrá, F. (1998). Biochemical properties of dipeptidylpeptidase III purified from porcine skeletal muscle. *Journal of Agricultural and Food Chemistry,* **46**, 3977–3984.

Sentandreu, M. A. and Toldrá, F. (2000). Purification and biochemical properties of dipeptidylpeptidase I from porcine skeletal muscle. *Journal of Agricultural and Food Chemistry,* **48**, 5014–5022.

Sentandreu, M. A. and Toldrá, F. (2001a). Importance of dipeptidylpeptidase II in postmortem pork muscle. *Meat Science,* **57**, 93–103.

Sentandreu, M. A. and Toldrá, F. (2001b). Dipeptidylpeptidase IV from porcine skeletal muscle: Purification and biochemical properties. *Food Chemistry,* **75**, 159–168.

Sentandreu, M. A. and Toldrá, F. (2007.) Oligopeptides hydrolysed by muscle dipeptidylpeptidases can generate angiotensin I converting enzyme inhibitory dipeptides. *European Food Research and Technology,* **224**, 785–790.

Sforza, S., Cavatorta, V., Lambertini, F., Galaverna, G., Dossena, A. and Marchelli, R. (2012). Cheese peptidomics: A detailed study on the evolution of the oligopeptide fraction in Parmigiano-Reggiano cheese from curd to 24 months of aging. *Journal of Dairy Science,* **95**(7), 3514–3526.

Tan, P. S. T., Poolman, B. and Konings, W. S. (1993). Proteolytic enzymes of *Lactococcus lactis*. *Journal of Dairy Research,* **60**, 269–286.

Toldrá, F. (1998). Proteolysis and lipolysis in flavour development of dry-cured meat products. *Meat Science,* 49, S101–S110.

Toldrá, F. (2002). *Dry-Cured Meat Products*. Trumbull, CT: Food and Nutrition Press, pp. 7–134.

Toldrá, F. (2004). Meat: Fermented meats. *In:* Scott Smith, J. and Hui, Y. H. (eds.) *Food Processing: Principles and Applications*. Ames, IA: Blackwell Publishing, 399–415.

Toldrá, F. (2012a). Biochemistry of fermented meat. *In:* Simpson, B. K., Nollet, L. M. L., Toldrá, F., Benjakul, S., Paliyath, G. and Hui, Y.H. (eds.) *Food Biochemistry and Food Processing* (2nd Ed.). Ames, IA: Wiley-Blackwell, pp. 331–343.

Toldrá, F. (2012b). Biochemistry of processing meat and poultry. *In:* Simpson, B. K., Nollet, L. M. L., Toldrá, F., Benjakul, S., Paliyath, G. and Hui, Y.H. (eds.) *Food Biochemistry and Food Processing* (2nd Ed.). Ames, IA: Wiley-Blackwell, pp. 303–316.

Toldrá, F., Aristoy, M. C. and Flores, M. (2000). Contribution of muscle aminopeptidases to flavor development in dry-cured ham. *Food Research International,* **33**, 181–185.

Toldrá, F. and Etherington, D. J. (1988). Examination of cathepsins B, D, H and L activities in dry cured hams. *Meat Science,* **23**, 1–7.

Toldrá, F. and Flores, M. (1998). The role of muscle proteases and lipases in flavor development during the processing of dry-cured ham. *CRC Critical Reviews in Food Science and Nutrition,* **38**, 331–352,

Villanueva, J., Martorella, A. J., Lawlor, K. et al. (2006). Serum peptidome patterns that distinguish metastatic thyroid carcinoma from cancer-free controls are unbiased by gender and age. *Molecular and Cellular Proteomics,* **5**(10), 1840–1852.

Visser, S. (1993). Proteolytic enzymes and their relation to cheese ripening. *Journal of Dairy Science*, **76**, 329–350.

Xing, L. J., Hu, Y. Y., Hu, H. Y., Ge, Q. F., Zhou, G. H. and Zhang, W. G. (2016). Purification and identification of antioxidative peptides from dry-cured Xuanwei ham. *Food Chemistry*, **194**, 951–958.

Yadav, A. K., Kumar, D. and Dash, D. (2011). MassWiz: A novel scoring algorithm with target-decoy based analysis pipeline for tandem mass spectrometry. *Journal of Proteome Research,* **10**(5), 2154–2160.

Zhu, W., Smith, J. W. and Huang, C. M. (2010). Mass spectrometry-based label-free quantitative proteomics. *Journal of Biomedicine and Biotechnology,* 840518, 6 pages.

Zhu, C. Z., Zhang, W. G., Zhou, G. H., Xu, X. L., Kang, Z. L. and Yin, Y. (2013). Isolation and identification of antioxidant peptides from jinhua ham. *Journal of Agricultural and Food Chemistry,* **61**, 1265–1271.

Zhu, C. Z., Zhang, W. G., Zhou, G. H. and Xu, X. L. (2016). Identification of antioxidant peptides of Jinhua ham generated in the products and through the simulated gastrointestinal digestion system. *Journal of the Science of Food and Agriculture,* **96**, 99–108.



Taylor & Francis
Taylor & Francis Group
http://taylorandfrancis.com

18

The Use of Bacteriocins against Meat-borne Pathogens

Carmen A. Campos, Marcela P. Castro, María E. Cayre, and Franco P. Rivas

CONTENTS

18.1 Introduction. Microbial Flora Associated with Meat and Meat Products 559
18.2 Application of Bacteriocin and/or Bacteriocinogenic Cultures to Improve the Microbial Safety of Meat Products 563
18.2.1 Raw Meat Products 564
18.2.2 Cooked and Cured-Cooked Meat Products 567
18.2.3 Fermented Sausage Meat Products 571
18.3 Bacteriocins and New Preservation Technologies to Increase the Safety of Meat and Meat Products 575
18.3.1 High Hydrostatic Pressure 575
18.3.2 Active Packaging 577
18.4 Problems to Overcome for the Successful Application of Bacteriocins in Meat and Meat Products 579
18.4.1 Interaction with Meat Components and/or Additives 579
18.4.2 Appearance of Resistant Strains 582
18.5 Overview of Actual Industrial Application and Regulatory Aspects 584
References 585

18.1 Introduction. Microbial Flora Associated with Meat and Meat Products

Meat and meat products are very perishable foods since they have a high content of proteins and other nutrients. Therefore, microbial growth of spoilage and foodborne pathogens can occur at all stages of the production chain including slaughter, preparation, storage, and distribution. Moreover, lipid oxidation and autolytic enzymatic reactions also contribute to the spoilage process leading to economic losses (Dave and Ghaly, 2011). The main pathogenic microorganisms that represent major hazards in meat and meat

products are *Salmonella* spp., *Listeria monocytogenes*, *Escherichia coli O157:H7*, *Clostridium botulinum*, *Clostridium perfringens*, *Campylobacter jejuni*, *Yersinia enterocolítica*, and *Staphylococcus aureus*. Unless products are preserved and handled properly, these microorganisms could grow in the food and/or produce toxins provoking illnesses in consumers.

Particularly, the growth of pathogenic flora is a public health issue. According to fresh reports of the U.S. Centers for Disease Control and Prevention (CDC), the main foodborne illness outbreaks were caused by *norovirus*, *Salmonella*, *E. coli*, and *L. monocytogenes* (CDC, 2015). Herein, the main characteristics of foodborne pathogens related to meat are described.

Salmonella spp. are facultative anaerobic Gram-negative rods that belong to the Enterobacteriaceae family. These bacteria are able to grow in adverse environmental conditions such as refrigerated temperatures and pH values of 4.5 or lower (depending on the strain), and they have the ability to tolerate moderate NaCl levels and heat (D Aoust, 1997). The mentioned characteristics raise concerns about the effectiveness of conservation factors needed to avoid *Salmonella* growth even in fermented foods. *Salmonella* is a widespread bacterium in the food chain. In the case of the meat industry, poultry products are the main reservoirs of *Salmonella* among other meats in many countries. This trend has been growing due to (1) intense animal husbandry practices, (2) the difficulty in controlling salmonellae spread into the production chain, and (3) the selection of resistant strains due to widespread use of antibiotic. To highlight the importance of this bacterium as a pathogen, it can be mentioned that a large outbreak of *Salmonella* infections in 2010 caused nearly 2000 illnesses. Some of the infections were from food origin. The food commodities most often implicated were beef (13%), dairy (12%, nearly all unpasteurized), fish (12%), and poultry (11%) (CDC, 2015).

According to the microbiological criteria for foodstuffs established by the Commission Regulation (EC, No 2073/2005), in minced meat and meat preparations intended to be eaten raw, *Salmonella* spp. must be absent in 10 g. In the case of minced meat and meat preparations intended to be eaten cooked, *Salmonella* spp. must be absent in 25 g.

L. monocytogenes represents the *Listeria* species most commonly associated with illness in animals and humans. As a facultative pathogenic saprotroph, *L. monocytogenes* can live in soil and decaying vegetation, but in an animal or human host, it can cause severe disease in immune-depressed individuals (Orsi et al., 2011). The majority of the infections caused by *L. monocytogenes* are of foodborne origin (Swaminathan and Gerner-Smidt, 2007). Ingestion of contaminated food being the source of infection, governments and food safety agencies worldwide have taken serious measurements to reduce the occurrence of *L. monocytogenes* in the food production chain. The pathogen is psychrotrophic and can support adverse conditions such as refrigeration temperature, low pH, and high salt concentrations (Ahmed et al., 2015). *L. monocytogenes* can be found in a wide range of meat and meat products such as beef, pork, minced meat, smoked, and fermented sausages and ham.

In most circumstances, it involves surface contamination (Rocourt and Cossart, 1997). Major listeriosis outbreaks have been related to contaminated ready-to-eat (RTE) meat and poultry products (Georgaras et al., 2006). In the United States, the average annual incidence was 0.92 cases/100,000 population for 2009–2011 (CDC, 2015). Furthermore, 1600 illnesses and 260 deaths were due to listeriosis (Scallan et al., 2011). In RTE foods intended for infants and for special medical purposes, *L. monocytogenes* must be absent in 25 g (EC, No 2073/2005). While a population of 100 colony forming units (CFUs)/g in RTE foods unable to support *L. monocytogenes* growth at the time of consumption is not considered a risk in some countries, the U.S. regulation demands zero tolerance for this type of food. In recent decades, food processing industries have made considerable progress in reducing the prevalence of *Listeria* in plants and, consequently, a decrease in the incidence of the illness was observed.

E. coli O157:H7 causes hemorrhagic colitis and hemolytic uremic syndrome (HUS), the latter being the most severe foodborne illness. The bacterium is found in cattle, and undercooked meat is the most frequent vehicle of transmission (Doyle et al., 1997). In the case of meat products, *E. coli* O157:H7 must be absent in 65 g of product (EC, No 2073/2005). In 2013, 12 out of 73 outbreaks caused by this bacterium had bovine meat and products thereof as the main food vehicles, followed by vegetables, juices, and cheese (European Food Safety Authority [EFSA], 2015). In order to control this pathogen growth in RTE foods, proper sanitation, cooking, refrigeration at appropriate temperatures, and prevention of cross-contamination are useful tools (Ray and Bhunia, 2014).

S. aureus is a Gram-positive coccus with the ability to grow at adverse environmental conditions, and therefore it is present in many foods where other microorganisms do not grow. This bacterium is present in healthy humans and animals. Food is contaminated by contact with the aforementioned sources. Some strains produce enterotoxins and they are associated with food intoxication. The enterotoxigenic strains can produce serologically different enterotoxins that differ in stability and toxicity. The rate of toxin production depends on growth rate and cell concentration. In general, the toxin can be detected when the population is higher than 10^7 CFU/g. Foods containing the enterotoxin produce severe vomiting and gastroenteritis. Foods implicated in the intoxication include ham, corned beef, salami, bacon, barbecued meat, salad dressings, and cheese. In order to reduce the incidence of *Staphylococcus* intoxication, it is necessary to apply measures to reduce the bacterial population; in this sense, the addition of preservatives can be useful. Furthermore, the acquisition of antibiotic resistance by some strains requires special attention. This trend increases the role of *S. aureus* as a public health concern bacterium (Teramoto et al., 2016).

C. botulinum is a spore-forming, Gram-positive, anaerobic bacterium widely distributed in the environment and in the intestinal tract of animals. There are different types with different physiological characteristics but all

produce neurotoxins. Control of foodborne botulism relies on: (1) thermal destruction of spores, (2) inhibition of bacterial growth, and (3) inhibition of spore germination. The increased consumption of RTE minimally processed foods packaged in modified atmosphere free of oxygen is of special concern, since the preservation factors applied do not assure the inhibition of *C. botulinum* growth (Dods and Austin, 1997). The incorporation of a preservation factor with antibotulinal activity is a must in the mentioned type of foods.

C. perfringens is also a spore-forming Gram-positive anaerobic bacterium which produces different toxins, type A being the more involved in food poisoning. The bacterium is present in the environment, yet meat and poultry are considered the most common food vehicles. Its growth in food is affected by water activity, pH, and curing agents. Therefore, these are the relied-upon factors to control *C. perfringens* growth and to inhibit the germination of spores (McClane, 1997).

Ca. jejuni is an enteric Gram-negative nonsporulating, microaerophilic bacterium. It can enter the food through fecal material from animals or indirectly from sewage or contaminated water. Albeit *Ca. jejuni* has been very frequently isolated from raw meats, it can also be found in milk, vegetables, eggs, mushrooms, and clams. In addition, it is considered the most common causative agent of gastroenteritis worldwide (Ray and Bhunia, 2014). Outbreaks result from the consumption of raw milk and improperly cooked chicken and turkey products. According to Melo et al. (2013), poultry meat is the main disseminator of *Ca. jejuni*. The Panel on Biological Hazards presented by EFSA (2010) revealed a high prevalence of *Campylobacter* spp. in poultry meat, with alarming rates in Europe (of 10 carcasses, 8 were contaminated). There were approximately 200,000 cases of campylobacteriosis in Europe during 2013 (EFSA, 2015).

Y. enterocolitica is one of the three pathogenic species of the genus *Yersinia*. It is a Gram-negative nonspore-forming and facultative anaerobic psychrotroph that causes gastroenteritis. Approximately 100,000 cases of yersinosis are reported annually in the United States and 90% of them are originate in food. *Y. enterocolítica* is found in the environment; however, pork products are the most frequently reported source. Good sanitation during handling and processing and adequate heat treatment are important to prevent infection (Ray and Bhunia, 2014). Furthermore, addition of a preservation factor with activity against this pathogen can be useful to increase the safety of refrigerated meats.

From data previously mentioned, it can be seen that meat and meat products available at the markets nowadays can stand the growth of pathogenic bacteria and that it is necessary to improve the safety of these products. Moreover, consumers demand more fresh tasty foods, free of chemical additives, and with a longer shelf life. The application of new preservation methods is the foundation for the satisfaction of these modern demands. In this context, biopreservation has received increasing attention, since it is a natural way

for controlling the shelf life and safety of meat and meat products. In Section 18.2, the application of biopreservation strategies, particularly the use of bacteriocins to control meatborne pathogens, is discussed.

18.2 Application of Bacteriocin and/or Bacteriocinogenic to Improve the Microbial Safety of Meat Products

Biopreservation refers to the extended shelf life and improvement of the microbial safety of food products using their natural microflora or their metabolites (Stiles, 1996). Lactic acid bacteria (LAB) are the main tool for biopreservation of meat and meat products since they comprise the normal flora of these goods and because of their ability to provide metabolic products with antimicrobial effect against spoilage and pathogenic bacteria as bacteriocins (Gialamas et al., 2010). Bacteriocins are ribosomally synthesized peptides or small proteins with antimicrobial activity, produced by bacteria. The use of bacteriocins in meat products has been extensively investigated since they have been proven to be highly effective against microbial agents causing food poisoning, mainly against the opportunistic psychrotroph foodborne pathogen *L. monocytogenes* (Zhu et al., 2005).

Strategies for meat product biopreservation including bacteriocins comprise the use of different approaches:

1. The inoculation of the meat products with bacteriocinogenic viable strains, as protective cultures, with consequent *in situ* production of bacteriocin. Lücke (2000) defined protective cultures as those added to meat products in order to inhibit pathogens or extend shelf life without changing their sensory profile. These can be applied either by surface inoculation or mixed with the food, and produce antibacterial substances during storage, fermentation, and/or ripening. The appropriate application of protective cultures must be based on the careful selection of well-adapted safe strains that can grow and possess the ability to produce enough bacteriocins to inhibit specific pathogenic or spoilage microorganisms on the conditions prevailing in the meat environment (Vermeiren et al., 2004; Vesković Moračanin et al., 2014).
2. The use of purified or semipurified bacteriocins preparations, obtained by *ex situ* production, incorporated into meat as food additives. In this case, the bacteriocin dosage is precise and predictable but its application is limited by regulations on food additives. To date nisin is the only bacteriocin that has been approved for commercial use in many countries. One drawback of the direct application of bacteriocins to a food matrix is that activity loss arises over time because of enzymatic degradation and interactions with food components

such as proteins and lipids (see details in Section 18.4.1). Furthermore, bacteriocins produced *ex situ* can be applied in the form of immobilized preparations, where a partially purified bacteriocin is bound to a carrier. This strategy helps to protect the bacteriocin and represents a valuable tool to overcome the negative effects of certain food components and potential enzyme inactivation.

3. The development of innovative bioactive packaging with the direct incorporation of bacteriocin or bacteriocin-producing strains into polymeric matrices. The advantage of incorporating them in films is that the bacteriocins are localized at the food surface—where the microbial growth is mostly found—giving more functionality to the biopreservation process (Coma, 2008).

The aforementioned strategies are continuously evolving and being evaluated in order to control spoilage and pathogenic microorganisms in meat and meat products. However, the outgrowth of surviving or bacteriocin-resistant bacteria after prolonged incubation points out that bacteriocins should not be the only preservative principle used in meat. Indeed, they should be part of a system with multiple preservative principles, so-called "hurdles" (Hartmann et al., 2011).

18.2.1 Raw Meat Products

Pathogenic species such as *S. aureus*, *L. monocytogenes*, *C. perfringens*, *Ca. jejuni*, *Y. enterocolitica*, and enteropathogenic bacteria such as *E. coli* and *Salmonella* spp. can contaminate fresh meat during every step of the production and marketing chain, leading to loss of quality and potential public health problems (Nychas et al., 2008; Solomakos et al., 2008). In order to improve the safety of raw meats, the use of bacteriocins and bacteriocinogenic strains is presented herein as an additional hurdle to inhibit and/or inactivate pathogens.

In spite of the studies published by a number of authors on the use of nisin as an antimicrobial in raw meat (Thomas et al., 2000), its use in this matrix is still a matter of controversy. The high dose (750 UI/g) required to be effective, hence, its cost, low solubility at meat pH values, uneven distribution, and lack of stability make nisin the least-desired option for the biopreservation of raw meat. Nevertheless, nisin showed good performance when it was combined with other antimicrobial agents, such as organic acids, chelating agents, and lysozyme, and/or vacuum-packaging or modified atmosphere packaging (MAP). The application of nisin + the aforementioned treatments before manufacturing or packaging of the product enhanced the inactivation of pathogens and spoilage bacteria—*L. monocytogenes*, *Brochothrix thermosphacta*, and *E. coli* O157:H7. (Gálvez et al., 2007; Aymerich et al., 2008; Juncioni de Arauza et al., 2009). Furthermore, the joint use of nisin with new

preservation methods, namely irradiation, high pressure, active packaging, etc., has given effective alternatives to the meat industry. These methods are further described in Section 18.3.

In addition, other bacteriocins have shown antimicrobial activity in raw meat. Nieto-Lozano et al. (2006) reported that use of the pediocin (50, 1000, and 5000 AU mL^{-1}) from *Pediococcus acidilactici* (Chr. Hansen Laboratories) on the surface of raw meat pork was effective for inhibition of *L. monocytogenes* at 4°C and 15°C. A bacteriostatic effect on *C. perfringens* was observed only when the meat treated with 5000 AU mL^{-1} of the bacteriocin was stored at 4°C. Hartmann et al. (2011) tested cell-free culture supernatants (CFS) of different antagonistic bacterial strains containing class IIa bacteriocins, namely leucocin A, leucocin B, mundticin L, pediocin PA-1, sakacin A, and sakacin X for their ability to inhibit *L. monocytogenes* in ground beef at 4°C. Taking into account the minimal effective concentration (MEC), defined as the minimal concentration of CFS that resulted in a reduction of *L. monocytogenes* below the detection limit, pediocin PA-1 and sakacin A resulted in a more effective meat environment.

The effects of pentocin 31-1, a bacteriocin produced by *Lactobacillus pentosus* 31-1, on microbiological counts, physicochemical change, and sensory quality of chill-stored nonvacuum-tray-packaged pork meat was investigated by Zhang et al. (2010). Results from samples treated with 80 AU mL^{-1} of pentocin confirmed that the shelf life of the product could be extended. In addition, good sensory characteristics of the final product were preserved.

The strain *Weissella paramesenteroides* J1 produces a bacteriocin called BacJ1. The analysis of the N-terminal sequence of this active peptide revealed no significant similarity to currently available antimicrobial peptides as informed by Chakchouk-Mtibaa et al. (2014). The effect of BacJ1 on *Salmonella* Typhimurium ATCC14028 growth in turkey escalope was evaluated. The addition of bacteriocin, at a final concentration of 400 AU/g, led to a rapid reduction in the number of *S.* Typhimurium ATCC14028 during storage at 4°C.

The *in situ* bacteriocin production by potential protective cultures has been also evaluated in the preservation of raw meats. The abilities of Sakacin-A producer *Lactobacillus sakei* Lb706 to control the growth of *L. monocytogenes* in vacuum-packaged lamb during 12 weeks storage at –1.5°C was investigated by Jones et al. (2009). In this work, lamb samples were coinoculated with *L. monocytogenes* and *Lb. sakei* Lb706 (0.3 and 3000 CFU cm^{-2}) or *Lb. sakei* Lb706b (bacteriocin-negative variant). Results obtained showed that, albeit both *Lb. sakei* Lb706 and its nonbacteriocinogenic analog predominated in the flora of lamb meat, significant levels of *L. monocytogenes* inhibition were obtained in packs inoculated with the bacteriocin-producing strain. This fact highlighted that the antilisterial effect was predominantly because of bacteriocin activity, rather than other mechanisms. Moreover, the extent to which *L. monocytogenes* was inhibited by *Lb. sakei* Lb706 varied with the size of the inoculum: the higher the size the better the inhibition.

Another bacteriocinogenic strain, *Lactobacillus curvatus* CRL705, evidenced its biopreservative potential in vacuum-packaged fresh meat stored at chilled temperatures (Castellano et al., 2008). This strain produces two bacteriocins: lactocin 705 with antilisterial activity and lactocin AL705 active against closely related LAB and *B. thermosphacta*. Furthermore, the live culture of *Lb. curvatus* CRL705 sprayed onto the surface of the meat disc (10^6 UFC cm^{-2}) showed to be similarly effective in preventing the growth of *B. thermosphacta* and *Listeria innocua* as the use of synthetic lactocin 705 (2.8 μmol L^{-1}) and purified lactocin AL705 (6400 AU mL^{-1}) during storage of vacuum-packaged fresh meat at 2°C (Castellano and Vignolo, 2006). Besides, Castellano et al. (2010) demonstrated that the organoleptic quality of inoculated beef steaks was not negatively affected by the surface spraying of *Lb. curvatus* CRL705 compared to the noninoculated samples.

The combined application of different class IIa bacteriocins produced by LAB has proved to be an effective strategy to enhance the antilisterial activity in raw meats. Dortu et al. (2008) evaluated the effect of *Lb. sakei* CWBI-B1365, a sakacin G producer, *Lb. curvatus* CWBI-B28, which produces sakacin P, and their combination on the survival and growth of *L. monocytogenes* in raw beef and chicken stored at 5°C in sealed bags during 21 days. The meats were surface-inoculated with *L. monocytogenes* and 10^6 CFU g^{-1} of *Lb. sakei* CWBI-B1365, *Lb. curvatus* CWBI-B28, or a combination of both strains at 5×10^5 CFU/g each. While in beef, the joint use of both bacteriocinogenic strains did not represent an advantage in the reduction of *L. monocytogenes* numbers compared to the sole use of the individual strains in poultry meat, only the strains in combination inhibited the growth of the pathogen throughout the storage period. Similarly, the combination of *Lb. curvatus* CWBI-B28wt (sakacin P producer) and *P. acidilactici* H (pediocin Ac/H producer) resulted in more effective control of *L. monocytogenes* in raw pork meat than individual use of *Lb. curvatus* CWBI-B28wt (Kouakou et al., 2008, 2010).

Apart from *Lb. sakei* and *Lb. curvatus*, other bacteriocinogenic species—producing class IIa and IIb bacteriocins—have been tested as protective cultures in raw meats. As an illustration, *Enterococcus faecium* PCD71 and *Leuconostoc pseudomesenteroides* PCK18—which possess multiple enterocin genes—showed antilisterial activity (Maragkoudakis et al., 2009; Carlini et al., 2010). The strain *Ec. faecium* PCD71 has been evaluated as a potential protective culture against *L. monocytogenes* in chicken meat under domestic refrigeration, and it was observed that the growth of the pathogenic microorganism was adversely affected by the presence of the protective cultures without any detrimental effect on biochemical parameters related to spoilage of the chicken meat (Maragkoudakis et al., 2009). On the other hand, the use of *Ln. pseudomesenteroides* PCK 18 as a protective culture combined with MAP was able to control the growth of *L. monocytogenes* in fresh suckling-lamb meat when the differences between them are around 2 log CFU/g (Osés et al., 2015) and chicken meat burgers (Melero et al., 2012).

As a closing remark, growth control of Gram-negative pathogens in raw meats by means of bacteriocinogenic protective culture is more complicated since bacteriocins are not normally active against these microorganisms. However, the combination of protective cultures and chelating agents could favor bacteriocin effectiveness. For instance, reduction in the count of *E. coli* O157:H7 and indigenous coliforms was observed in frozen ground-beef patties under temperature abuse (5°C) in the presence of bacteriocinogenic *Lb. curvatus* CRL705 combined with nisin productor *Lactococcus lactis* CRL1109 and Na_2EDTA (48 mM) (Castellano et al., 2011). Although several studies have reported Gram-negative pathogen inhibition by LAB protective cultures of species as feared as *E. coli* O157:H7, *S.* Typhimurium, and *Ca. jejuni*, these cultures were comprised of more than one bacterial strain, and it is uncertain whether the inhibition could be attributed to the bacteriocins themselves or not. Such is the case of protective culture on lamb (Jones et al., 2010), poultry meat products (Melero et al., 2013), and beef (Chaillou et al., 2014). In these studies, the nature of the inhibitory effect was not attributed to a bacteriocin in particular; consequently, the inhibitory effect has not been completely elucidated. Other inhibitory mechanisms or their combination would probably be responsible for the reported results.

18.2.2 Cooked and Cured-Cooked Meat Products

Sliced cooked and cured meats are one of the most consumed RTE food products around the world. The two major reasons that fuel the demand for such products are convenience and good acceptance by consumers. During preparation and sale, cooked meat products can be contaminated by pathogenic bacteria causing illness in consumers if infective doses are reached at the time of consumption (Garrido el al., 2009; Norrung and Buncic, 2008; Gudbjornsdottir et al., 2004; Gombas et al., 2003; EFSA, 2007). Epidemiological and microbiological studies have identified cross-contamination (during preparation and sale) and subsequent bacterial growth (during storage) as the main causes of RTE food contamination and illnesses. Cooked meat products are likely to be contaminated after processing due to recontamination events during handling at retail points. Particularly, slicing machines and cutting utensils are recognized as important vehicles of contamination of cooked meat products both at factory and sale points (Pérez-Rodríguez et al., 2007, 2010; Vorst et al., 2006). Hence, several outbreaks have been associated to contaminated slicing machines (Public Health Agency of Canada [PHAC], 2008). Improper food handling practices have been most related to distribution and sale of RTE products (Lues and Van Tonder, 2007) and, especially, to small establishments such as delicatessen and butchery premises. Various studies have suggested that foods handled in small- and medium-sized establishments (SMEs) are associated with lower microbiological quality than large-sized establishments (Norrung and Buncic, 2008; Violaris et al., 2008). Deficient education programs, financial constraints, and insufficient

food safety awareness can influence significantly poorer hygiene in SMEs (Violaris et al., 2008).

L. monocytogenes is well adapted to survival on equipment and in production facilities and the occurrence of this microorganism in cooked meat products is known to be connected with cross-contamination after heat treatment (Bredholt et al., 1999). Heat survivors appear to be the main cause of contamination (Samelis and Metaxopoulos, 1999). *Listeria* spp. and *L. monocytogenes* survived in ham and other tumbled meats cooked in boilers at core temperatures below 70°C and in traditional Greek country-style sausages heated to 65°C–68°C. In contrast, *Listeriae* did not survive in samples heated to 72°C–75°C (Samelis and Metaxopoulos, 1999; Mataragas et al., 2003). Hence, the maintenance of the cold chain is an effective means to prevent microbial growth in food products. However, psychrotrophic pathogens such as *L. monocytogenes* can overcome this hurdle due to their capacity to grow at refrigerated storage conditions, reaching infective doses at consumption.

Furthermore, *L. monocytogenes* and other *Listeria* spp. contaminate most areas where raw materials are being processed and colonize certain equipment which are not made from stainless steel or are not disinfected properly (Stiles, 1996).

Cooked cured meat products are sensitive to spoilage. The low salt content (2.0% in water phase), a pH value above 6.0, and a water activity higher than 0.95 are only small hurdles in terms of inhibiting the growth of psychrotrophic pathogens like *L. monocytogenes*. After cooking, the normal flora of the product, consisting of LAB, is too low to protect the products against the growth of pathogens (Pérez-Rodríguez et al., 2010). Since these products are heated to a temperature of 65°C–75°C, most vegetative cells are killed and postheat treatment recontamination determines their shelf life (Borch et al., 1996). Ham and ham-like products are contaminated to a great extent during tumbling since the need to operate tumblers continuously does not enable their proper cleaning and disinfection on a daily basis (Samelis and Metaxopoulos, 1999). Not only product handling after cooking plus slicing prior to packaging recontaminates these products with about 0.5–2-log CFU/g of total bacteria, but also the psychrotrophic pathogen *L. monocytogenes* can be found as a result of postcontamination (Uyttendaele et al., 1999; Samelis et al., 2000).

Several authors reported that the population of *Listeriae* decreased when cooked meat products had been inoculated with bacteriocinogenic LAB, probably due to the production of bacteriocin. Several authors have reported the antilisterial effect of bacteriocinogenic LAB strains as bioprotective cultures in cooked meat products. *Lb. sakei* CTC494, sakacin K producer, diminished the number of *Listeria* from an initial inoculum of 2×10^2 CFU/g to 2 most probable number (MPN)/g in cooked ham after seven days of storage at 7°C (Hugas et al. 1998). Mataragas et al. (2003) showed that two LAB had an antagonistic effect on *L. innocua* growth in sliced cooked cured pork under vacuum conditions. At the beginning of storage (7th day), *L. innocua*

population increased, from 3.5 to 3.9 $\log_{10}$ CFU/g and to 4.4 $\log_{10}$ CFU/g in the presence of *Leuconostoc mesenteroides* L124 and *Lb. curvatus* L442, respectively. After this initial increase, the number of the listeriae population decreased until the end of storage, with final population numbers of 2.5 and 2.9 $\log_{10}$ CFU/g, respectively. The reduction of the listeriae population was the same (1.5 $\log_{10}$ CFU/g) for both LAB strains. In the presence of *Ln. mesenteroides* L153 (bac–), *L. innocua* increased by 1 $\log_{10}$ CFU/g, from 3.3 to 4.0 $\log_{10}$ CFU/g at the end of storage. In the samples inoculated with *L. innocua*, the population reached 7.3, 4 $\log_{10}$ CFU/g more than the initial number. When the bacteriocins were present, listeriae population reduced by 1 $\log_{10}$ CFU/g on the third day of storage and on the fourteenth day the population was below the enumeration limit. In the noninoculated samples, *Listeria* was not detected during storage, reflecting good hygiene during the production of meat product. The results showed that bacteriocins were able to inhibit *L. innocua.*

Mathieu et al. (1994) and Hanlin et al. (1993) studied the use of a combination of two bacteriocins to provide greater antibacterial activity against *L. monocytogenes* and other Gram-positive bacteria and their spores. This study demonstrated that *Ln. mesenteroides* L124 and *Lb. curvatus* L442 have several attributes as potential protective organisms against listeriae. Since the listeriae can grow or survive under vacuum or modified atmosphere, respectively, at low temperatures and the two bacteriocinogenic LAB strains grow rapidly because they are well adapted to growth in the meat products and they can reduce the listeriae numbers, they can be either employed as biopreservatives or as starter cultures with antilisterial properties in meat products. Additionally, their bacteriocins have a bactericidal effect on the listeriae growth and could be used to improve meat safety (Mataragas et al., 2003).

The results obtained by Kouakou et al. (2008) with samples of cooked pork meat showed that when samples were treated with *Lb. curvatus* CWBI-B28wt, the *Listeria* CFU count dropped to an undetectable level by the end of week 1 and remained at that low level until the end of week 2, at which point regrowth occurred. When samples were treated with cell-adsorbed bacteriocin, the count dropped similarly but remained at an undetectable level from the end of week 1 until the end of the experiment (28 days). In parallel, untreated controls, the *Listeria* CFU count increased three-fold in the course of the experiment. Bacteriocin activity was higher in samples treated with cell-adsorbed bacteriocin (4500–2000 AU/g) than in samples treated with the bacteriocin-producing strain (2000–1000 AU/g).

Rivas et al. (2014) evaluated the inhibition of *L. innocua* on the surface of cooked pork using the strain *Lb. curvatus* ACU-1 isolated from artisanal dry sausages manufactured in Argentina, which produces the bacteriocin sakacin Q. The pork meat slices were immersed for five minutes in different suspensions (pH 6.5) containing the bacteriocin-producing strain alone and/or with bacteriocin. Bacteriocin production *in situ* significantly reduced counts of *Listeria* (approximately 5-log cycles at the end of the trial), while

the antilisterial activity decreased after three weeks when the pathogen was only inoculated with the bacteriocin. A larger effect was achieved by the combination of the producer strain together with bacteriocin. However, the greatest effect was accomplished by using freeze-dried bacteriocin.

Jacobsen et al. (2003) reported that the use of living cells of *Leuconostoc carnosum* 4010 was better than the partially purified leucocin 4010 in preventing the growth of *L. monocytogenes* in pork saveloys. In the control batch, *Listeria* grew up to 10^7 CFU/g, whereas in batches with the bacteriocin producer strain, the counts never exceeded 10 CFU/g during the four weeks of storage at 10°C.

A study by Scannell et al. (2000) on keeping the quality of fresh pork sausage found that lacticin and nisin exhibited better antimicrobial action against Gram-positive organisms than sodium metabisulfite. The combination of organic acids with either of these bacteriocins enhanced their antimicrobial activity against *L. innocua* and *Salmonella* Kentucky and was even more effective against *C. perfringens*. This study also suggested that lacticin combined with sodium lactate or sodium citrate can be used as an alternative preservative of fresh pork sausage since it gave lower total aerobic plate counts throughout storage than sodium metabisulfite.

Pediocin is produced by *P. acidilactici* or *Pediococcus pentosaceus* and has been shown to be effective against *L. monocytogenes* and other Gram-positive pathogens on meat surfaces (Coma, 2008). Pediocin has generally recognized as safe (GRAS) status in certain food applications (Juneja et al., 2012). Research on some meat products indicated that pediocins (especially pediocin PA-1) could be more effective than nisin especially when used in combination with nisin, lysoszyme, organic acids, sodium dodecyl sulfate, or ethylenediaminetetraacetic acid (EDTA) (Hugo and Hugo, 2015).

In the experiment of Ananou et al. (2010a), AS-48 effectively inhibited *L. monocytogenes* in cooked ham in a concentration-dependent way at 5°C and 15°C. Nevertheless, even at the higher concentration used (60 μg/g), it was not possible to avoid the regrowth of *Listeria* after 15–30 storage days at 5°C. They attributed the lower effectiveness of AS-48 in cooked ham compared to brain–heart infusion (BHI) broth to a higher retention of the bacteriocin molecules by meat and fat components, to a slower diffusion, and also to the irregular distribution of the bacteriocin molecules and the bacterium in the meat matrix with a higher dry matter content compared to liquid media. Also, results from bacteriocin extraction experiments revealed that bacteriocin levels in the meat decreased markedly after day 7, which could explain regrowth of surviving bacteria. Other previous experiments carried out by these authors in a sausage model demonstrated that it was necessary to apply 450 AU/g (40 μg/g) to a meat mixture to maintain *Listeria* below detection level from six to nine days of incubation at 20°C (Ananou et al., 2005).

Regarding the effect of the bacteriocin AS-48 on *S. aureus* in cooked ham, a slight inhibitory effect was observed. On the contrary, in vegetable sauces, the bacteriocin showed an antistaphylococci effect (Ananou et al., 2010a). Furthermore, the mentioned effect was lower compared to previous results

obtained in liquid media for AS-48 (Ananou et al., 2004; Muñoz et al., 2007) reinforcing the suggestion that interaction of AS-48 with food components can interfere with its efficacy.

Another pathogen of concern in cooked RTE foods is *C. botulinum* and the spores produced by this microorganism, even under refrigerated storage conditions. Okereke and Montville (1991) demonstrated that LAB can produce bacteriocins at refrigeration temperatures and that the bacteriocins produced by *Lc. lactis*, *Lactobacillus plantarum*, and *P. pentosaceus* are inhibitory against proteolytic and nonproteolytic *C. botulinum* spores. Rodgers et al. (2003) have demonstrated the inhibition of nonproteolytic *C. botulinum* with the bacteriocinogenic LAB *Lactobacillus rhamnosus*, *Lc. lactis*, *Ec. faecium*, and *P. pentosaceus* at refrigerated temperatures, as demonstrated by spot-on-the-lawn tests (Kostrzynska and Bachand, 2006).

Nieto-Lozano et al. (2010) found that the treatment of frankfurters with 5000 bacteriocin unit/mL of the pediocin PA-1 had an inhibitory effect against *L. monocytogenes* CECT4031 after storage for 60 days at 4°C. Thus, counts of this target bacteria strain were 8×10^5 CFU/g after 60 days in control samples but only 8×10^3 CFU/g in treated samples. There were significant differences between both treatments for samples stored at 4°C, whereas the inhibitory effect was only 0.6-log cycles (not significant) for samples stored at 15°C after 30 days. Moreover, counts of *C. perfringens* CECT376 were reduced by 2-log cycles in the pediocin-treated frankfurters after storage at 10°C for 60 days. They found significant differences between the samples stored at 10°C and the control, whereas only a small inhibitory effect (only 0.8-log cycles; not significant) was observed in samples stored at 15°C after 30 days compared with the control. These results have shown the inhibitory effect of the pediocin-producing strain and the pediocin PA-1 itself against *L. monocytogenes* CECT4031 and *C. perfringens* CECT376. Inhibition of the target bacterial strains varied with temperature and was greater in refrigerated products.

Reuterin is a broad spectrum antimicrobial substance produced by *Lactobacillus reuteri* during fermentation of glycerol. Reuterin was found capable of significantly decontaminating cooked pork contaminated with *E. coli* O157:H7 and *L. monocytogenes* (El-Ziney et al. 1999). Kuleasan and Cakmakei (2002) also found that the application of reuterin to the surface of sausage significantly reduced the growth of *L. monocytogenes*.

18.2.3 Fermented Sausage Meat Products

The applied manufacturing processes can mainly lead to two distinct groups of fermented meat products: (1) dry sausages (i.e., pepperoni, Genoa salami, dry salami, etc.) and (2) semidry sausages (summer sausage, Lebanon bologna, cervelat, etc.). Whereas dry sausages require lower temperatures and a longer manufacturing process, semidry sausages have a longer shelf-stable life due to their low A_w. Having unfavorable environmental conditions—low pH, low A_w, and salts, among others—does not prevent the growth of pathogenic bacteria

such as *E. coli*, *Salmonella* spp., *L. monocytogenes*, *S. aureus*, and *C. perfringens* (Benito et al., 2007; Ferreira et al., 2007). As Enterobacteriaceae are common contaminants of meat, they can be detected in fermented substrates in different counts depending on the initial microbial load of raw material, type of sausage, and ripening process (Casquete et al., 2012). Bacterial numbers decline persistently under the conditions of ripening, an essential process to inhibit food pathogens such as *Salmonella*, *Shigella*, or *E. coli*. Yet, Moore (2004) reported the survival of *Salmonella* and *E. coli* in this type of product. The use of selected LAB as protective cultures stands as an effective extra hurdle to increase food safety of dry-fermented products without increasing the content of chemical additives. The most regularly identified LAB species in traditionally fermented sausages are *Lb. sakei*, *Lb. curvatus*, and *Lb. plantarum* (Aymerich et al., 2006b; Talon et al., 2008; Urso et al., 2006a). Many strains belonging to these species are bacteriocin producers, hence their application seems not be a drawback for the processing and/or production of these products.

New bioprotective cultures for food fermentation purposes can be selected by means of three steps: (1) isolation and selection of bacteriocinogenic LAB from endogenous microbiota, (2) assessment of antibiotic resistance and production of biogenic amines, and (3) evaluation of technological features, such as acidification profile, proteolytic and lipolytic activities, etc. (Lücke, 2000; Aymerich et al., 2006b).

Several bacteriocin-producing LAB species, including *Lactobacillus*, *Lactococcus*, *Pediococcus*, and *Enterococcus* spp., have been evaluated for improvement of microbial safety of fermented meat products. These species can be applied as starter cultures or as adjunct cultures. In the latter, the bacteriocin producer does not need to contribute to the fermentation; besides, it does not interfere with the function of the starter, which of course needs to be insensitive to the bacteriocin (Gálvez et al., 2007). Below are some examples of the applications implemented during the last 10 years.

Pediocin-producing strains have been used in fermented meat products to improve the quality and safety of these products. Nieto Lozano et al. (2010) evaluated the inhibitory effect of the *P. acidilactici* MCH14, a pediocin-producing strain, in Spanish dry-fermented sausages using *L. monocytogenes* as the target bacteria strain. Inhibition of the pathogen by the bacteriocinogenic *P. acidilactici* MCH14 was effective in the product, the counts being reduced by 2-log cycles compared to the control. In another study, Albano et al. (2009) analyzed the ability of *P. acidilactici* HA-6111-2, a producer of pediocin PA-1, to inhibit a cocktail of *L. innocua* strains during production and shelf life of "Alheira," a traditional naturally fermented meat sausage typical of Trás-os-Montes (Portugal). The results demonstrated that *P. acidilactici* HA-6111-2 has several attributes as a potential bioprotective organism: It is able to inhibit *L. innocua* in "Alheiras" during storage, has a good capacity for colonization of meat, and produces sufficient amounts of bacteriocin. Furthermore, no significant antibiotic resistance was detected and a trained panel considered the treated product as sensorially acceptable.

Successful applications of *Lb. curvatus* strains as biopreservatives in fermented meat products have been published. Benkerroum et al. (2005) used the bacteriocin-producing strains *Lc. lactis* subsp. *lactis* LMG21206 and *Lb. curvatus* LBPE as adjunct cultures for sausage fermentations to control the occurrence and survival of *L. monocytogenes*. In this study, a commercial starter culture (Bel'meatTM SL-25) not inhibitory to *L. monocytogenes* was mixed (1:1) with each of the two lyophilized bacteriocinogenic strains to obtain starters active against the pathogen. The effectiveness of these Bac+ starters was evaluated in dry-fermented sausages experimentally contaminated with a mixture of four different strains of *L. monocytogenes* (10^2–10^3 CFU/g). The results showed that both strains caused a decrease in cell counts. However, the *Lb. curvatus* strain resulted in more efficient pathogen control, reducing *Listeria* counts to below the detectable limit after four hours of fermentation. Recently, Casaburi et al. (2016) informed on the suitability of bacteriocinogenic *Lb. curvatus* 54M16 to be used as starter culture for sausage fermentation. This strain produces multiple bacteriocins as confirmed by molecular mass spectrometry analysis and N-terminal amino acid sequencing, with activity against *L. monocytogenes*, *Bacillus cereus*, and the meat spoilage organism *B. thermosphacta*. Microbiological, physiochemical, and sensorial evaluation of inoculated samples during sausage fermentation showed that the use of *Lb. curvatus* 54M16 might contribute to increase the quality and safety of the traditionally fermented sausages prepared without antimicrobial additives.

Based on the capacity to produce several bacteriocins, meatborne *Lb. sakei* is another strain tested in the biopreservation of fermented meat products. Antilisterial activity was reported for bacteriocinogenic *Lb. sakei* CTC494 in fermented sausages (Ravyts et al., 2008), *Lb. sakei* strains 1155 in Croatian traditionally fermented sausages (Zdolec et al., 2007), and a bacteriocin producer *Lb. sakei* in fermented sausages from four European countries (Drosinos et al., 2006). The *Lb. sakei* strain (sakacin P producer), originally isolated from naturally fermented sausages, was evaluated as a starter culture by Urso et al. (2006b). The strain was able to grow in conditions used in the production line and colonized rapidly the ecosystem. A high number of isolates, capable of producing sakacin P, were isolated after the third day of fermentation and persisted throughout the course of the fermentation. Moreover, total bacterial count and fecal enterococci showed a rapid decrease at the end of the fermentation; in addition, the sensory evaluation panelists preferred the sausages produced with the *Lb. sakei* when compared to a fermented sausage produced with a commercial starter. Similarly, Gao et al. (2014) analyzed the use of bacteriocinogenic *Lb. sakei* C2 in the production of Chinese fermented sausages. This strain can produce a bacteriocin, named after sakacin C2 (Gao et al., 2010), with a broad inhibitory spectrum which has certain probiotic potential (Gao et al., 2012). Results obtained showed that *Lb. sakei* C2 could quickly colonize the meat matrix, effectively control spoilage and pathogenic microorganisms, and decrease the content of lipid oxidation products and nitrite, while improving the sensory characteristics of final products.

The use of enterococci as starter cultures or cocultures has been studied not only for their organoleptic properties, but also for their negative effect on food pathogens because of the production of enterocins (Khan et al., 2010). Rubio et al. (2013) examined the bioprotective effect of three bacteriocinogenic, nonaminogenic, and nonvirulent *Enterococcus* strains (*Ec. faecium* CTC8005, *Enterococcus devriesei* CTC8006, and *Enterococcus casseliflavus* CTC8003) against *L. monocytogenes* and *S. aureus* in the elaboration of Spanish fuets (low-acid fermented sausages) when applied alone, or in combination with an high hydrostatic pressure (HHP) treatment (600 MPa for 5 minutes at 15°C) after ripening. The inoculated strains, monitored by random amplification of polymorphic DNA by polymerase chain reaction (RAPD-PCR), were able to grow, survive, and dominate the endogenous enterococci population. *Ec. devriesei* CTC8006 and *Ec. faecium* CTC8005 predominantly inhibited the growth during the ripening process. *S. aureus* was not affected by the strains. The application of HHP treatment (600 MPa for 5 minutes) at the end of the ripening period (day 21) promoted a decrease of 1-log unit in the counts of *S. aureus* and increased the antilisterial effect. From these results, the authors concluded that the combination of the bacteriocinogenic strain *Ec. faecium* CTC8005 as a bioprotective starter culture with an HHP treatment—applied after ripening—could be recommended in order to produce low-risk, high-quality low-acid fermented sausages.

In addition to the *in situ* production, purified or semipurified bacteriocins have also been explored as preservatives in fermented meat products. In Turkish fermented sausage (sucuk) contaminated with *L. monocytogenes* ATCC 7644 at a concentration of 10^6 CFU/g, the addition of 50 and 100 μg/g commercial nisin resulted in effective control of *L. monocytogenes* since counts were reduced during storage and no surviving cells were detected at day 25 and 20, respectively (Hampikyan and Ugur, 2007). In another study, semipurified bacteriocins, homologous to sakacins P and X from *Lb. curvatus* MBSa2, were incorporated into the batter for production of salami contaminated with *L. monocytogenes* (10^4–10^5 CFU/g). The bacteriocins caused 2-log and 1.5-log reductions in the counts of the pathogen in the product after 10 and 20 days, respectively (de Souza Barbosa et al., 2015).

Furthermore, the application of enterocins resulted in effective control of some pathogens in low-acid fermented sausages. Ananou et al. (2010b) reported that the use of enterocin AS-48 (148 AU/g) applied as an additive in the formulation of fuet caused a drastic 5.5-log CFU/g decrease in *L. monocytogenes* and a significant reduction for *Salmonella enterica* at the end of ripening (10 days) but did not affect *S. aureus*. On the other hand, Jofré et al. (2009) applied the enterocins A and B (2000 AU/g) in raw sausages spiked with 3-log CFU/g of *Salmonella*, *L. monocytogenes*, and *S. aureus*. Both enterocins caused a fast reduction in the counts of *L. monocytogenes*, while *S. aureus* was not inhibited. On the other hand, *Salmonella* was more affected by the endogenous hurdles associated with the ripening process. Regarding the last two studies using enterocins, it can be concluded that neither the enterocin AS-48

nor the enterocins A and B could control the growth *S. aureus* in low-acid fermented sausages.

18.3 Bacteriocins and New Preservation Technologies to Increase the Safety of Meat and Meat Products

Application of bacteriocins in foods is performed as a part of the hurdle technology. The latter takes advantage of the possible synergistic action between stress factors, which allows the use of a lower concentration of antimicrobial agents and/or the application of milder treatments.

Many antimicrobials have been used in combination with bacteriocins in order to inhibit the spoilage flora and decrease the risk of pathogens. There is a trend to select antimicrobials from natural sources and to use GRAS compounds as previously mentioned. Some examples of the use of bacteriocins are presented in Table 18.1. Nisin is mainly applied—in combination with antimicrobials—for the preservation of meat and meat products.

Many new preservation technologies have been proposed in order to increase food safety without damaging sensory and nutritional properties. Among them, active packaging systems and HHP are the most relevant and innovative techniques for raw meat and meat products.

18.3.1 High Hydrostatic Pressure

HHP has probably been the most investigated technology in recent decades. This preservation method is able to destroy microbial vegetative cells and inactivate enzymes without changing sensory and nutritional characteristics of the food. It has been applied at a commercial scale for the production of some products (Ananou et al., 2010b). Since some foodborne pathogens can survive after the application of mild HHP, its use in combination with bacteriocins has been explored. Different studies demonstrated that HHP treatment sensibilize Gram-negative bacterial cells to nisin action, suggesting that HHP and nisin can be a useful combination of stress factors.

Regarding meat products, effectiveness of HHP and bacteriocins has been evaluated mostly in ham and in low-acid fermented sausages. Jofré et al. (2008) studied the effect of a HHP at 600 MPa in combination with nisin, lactate, or both on the growth of *L. moncytogenes*, *Salmonella*, and *S. aureus* in sliced cooked ham at refrigeration temperatures. It was found that the joint use of HHP and nisin and the storage at 6ºC reduced *Salmonella* and *L. monocytogenes* population to less than 10 CFU/g and reduced *S. aureus* population to 2.4 CFU/g for three months. Hereu et al. (2012) evaluated the effect of nisin application (directly applied or through active packaging) combined with HHP (600 MPa for 5 minutes) on the behavior of *L. monocytogenes* CTC1034 onto the surface of RTE sliced dry-cured ham stored for 60 days at 8°C. It was

TABLE 18.1

Compilation of the Use of Bacteriocins in Combination with Antimicrobials for the Preservation of Meat and Meat Products

Bacteriocin Producer Form of Application	Antimicrobial—Other Hurdles	Product—Target Microrganisms	Reference
Carnocin *Carnobacterium piscicola* Meat batter	Chitosan Refrigeration	Pork sausages— Aerobic flora, *Listeria innocua*	Roller et al. (2002)
Nisin Meat batter	Potassium lactate, high hydrostatic pressure Refrigeration	Cooked ham— *Listeria monocytogenes* *Staphylococcus aureus* *Salmonella enterica* serovars	Jofré et al. (2008)
Nisin mixing	Sodium chloride Refrigeration	Raw buffalo meat mince— *Listeria monocytogenes*	Pawar et al. (2000)
Nisin mixing	Thyme essential oil Refrigeration	Minced beef— *Escherichia coli* O157:H7	Solomakos et al. (2008)
Nisin mixing	Sodium chloride, sodium acetate and lactate, hop alpha acids Refrigeration	Meat model system— *Listeria monocytogenes*	Dominic Dussault et al. (2016)
Nisin mixing	Grape seed extract Edible film Refrigeration	Turkey frankfurter— *Listeria monocytogenes*	Theivendran et al. (2006)
Nisin mixing	EDTA and lysozyme	Ham and bologna— Aerobic flora	Gill and Holley (2000)
Nisin mixing	Sodium lactate, sodium diacetate	Ham steaks— *Listeria monocytogenes*	Ye et al. (2008)
Nisin mixing	Oregano essential oil	Minced sheep meat— *Salmonella* Enteritidis	Govaris et al. (2010)

observed that the presence of nisin increased the inactivation induced by HHP when the bacteriocin was applied directly on the surface of ham. Liu et al. (2012) evaluated the effect of HHP (200 and 400 MPa for 10 minutes) and enterocin (256 and 2560 AU/g) on the inhibition of *L. monocytogenes* and *Salmonella enteriditis* inoculated in cooked sliced ham at 4°C. Results obtained showed that the most effective treatment from microbiological and sensorial points of view was achieved with the combination of 400 MPa HHP and 2560 AU/g enterocin.

The slightly fermented sausages exhibit a moderate decrease in pH, and therefore pathogens such as *L. monocytogenes*, *S. aureus*, and *Salmonella* spp. may be able to survive. To increase the safety of these products, the combined use of enterocins and HHP was satisfactorily evaluated. For example,

the application of a HHP treatment at the end of the ripening or its combination with enterocins A and B proved to be necessary to decrease the levels of *Salmonella* and *L. monocytogenes*, respectively, to <1-log CFU/g at the end of storage. It must be remarked that the ability of *S. aureus* to tolerate hurdles such as curing salts, low water activity, enterocins, and HHP (400 MPa) suggests the need of higher intensity HHP treatments to obtain safe low-acid fermented sausages (Jofré et al., 2009). Furthermore, enterocin AS-48 (148 AU/g) applied alone can be slightly effective against *Salmonella* at the end of ripening but very effective for the inhibition of *L. monocytogenes* in low-acid fermented sausages. The combination of this bacteriocin with HHP treatment improved the inhibition of *Salmonella*. However, neither enterocin AS-48 nor HHP treatment inhibits *S. aureus* growth (Ananou et al., 2010b).

The aforementioned trends suggest that the combined use of HHP and bacteriocins provides a useful tool to control *L. monocytogenes* and *Salmonella* during the storage of RTE meat products. But control of *S. aureus* needs the application of other stress factors.

18.3.2 Active Packaging

Active packaging systems are based on the inclusion of active compounds—mainly antimicrobials—into the packaging, with the aim of improving quality and extending food shelf life. There are different ways to incorporate the active compounds: (1) by addition into a pad placed in the package from which the volatile agent is released, (2) by inclusion into the packaging film during its manufacture, (3) by coating the packaging with a matrix carrying the active compound, and (4) by using an active compound possessing film-forming ability (Coma, 2008; Campos et al., 2011). These strategies have been intensively studied in recent years, edible films and coatings being the most developed ones (Campos et al., 2011).

Inclusion of the active compound into the packaging instead of its addition into the food has been proposed as an efficient way to prevent surface microbial growth. Meat from healthy animals is sterile and microorganims are present at the external surface. For this reason, the presence of an antimicrobial where microbial growth happens can be effective using a smaller antimicrobial amount. Furthermore, a controlled released from the external surface to the food can assure the presence of the antimicrobial in the food during storage. This strategy is very useful to prevent the loss of antimicrobial activity due to the reaction or binding of the antimicrobial with food components or other additives.

A compilation of the use of bacteriocins in active packaging systems for the preservation of meat and meat products is shown in Table 18.2. As can be observed, nisin is the most selected bacteriocin to be included within antimicrobial films. The latter were mainly edible and formed by proteins and polysaccharydes. Most of the studies have been conducted to address the inhibition of *L. monocytogenes* in processed meats. It must be stressed that for a

TABLE 18.2

Compilation of the Use of Bacteriocins in Active Packaging Systems for the Preservation of Meat and Meat Products

Bacteriocin—	Polymer Carrier—Other Hurdles Applied	Product—Target Microrganism	Reference
Nisin	Whey protein isolate—organic acids, and natural extracts Refrigeration	Turkey frankfurter—*Listeria monocytogenes*, *E. coli O157:H7*, and *Salmonella* Typhimurium	Gadang et al. (2008)
Nisin	Soy protein film—with grape seed extract or green tea extract Refrigeration	Turkey frankfurters—*Listeria monocytogenes*	Theivendran et al. (2006)
Nisin	Zein protein film Refrigeration	Ready-to-eat chicken—*Listeria monocytogenes*	Janes et al. (2002)
Nisin	Bacterially produced cellulose film Refrigeration	Frankfurters—*Listeria monocytogenes*	Nguyen et al. (2008)
Nisin	Alginate films and beads Refrigeration	Beef—*Staphylococcus aureus*	Millette et al. (2007)
Nisin	Sodium alginate film Refrigeration	Ham slices, frankfurters—*Listeria monocytogenes*	Kapetanakou et al. (2016)
Enterocins A and B, sakacin K, nisin A	Perforated polypropylene, polyamide and nonperforated polypropylene—natural preservatives Refrigeration	Cooked ham—*Salmonella spp*	Jofré et al. (2008)
Nisin	Gelatin film Refrigeration	Turkey bologna—*Listeria monocytogenes*	Min et al. (2010)
Pediocin	Cellulose acetate films Refrigeration	Sliced ham—*Listeria innocua*, *Salmonella spp*	Santiago-Silva et al. (2009)
Lactocin 705 and lactocin AL705	Cryovac® synthetic film, Agroprotein film Refrigeration	Wieners—*Listeria innocua*	Massani et al. (2014)
Nisin Z	Pullulan films—natural preservatives Refrigeration	Fresh and processed meats—*Salmonella* Typhimurium and *Salmonella* Enteritidis, *Listeria monocytogenes*, *E. coli O157:H7*	Pattanayaiying et al. (2015)

successful inhibition of postprocess contamination, the release of the bacteriocin into the food must be controlled in order to have enough inhibitory amount at the surface of the food throughout the storage. The rate of release depends on the characteristics of the film and the food. For example, Massani

et al. (2014) observed that after 45 days of storage, the activity of lactocin 705 and lactocin AL705 added to a multilayer synthetic film and to a wheat gluten/starch film was lost in the case of the multilayer syntethic film but was present in the edible film. Both films were used to wrap wieners, and they exhibited a bacteriostatic effect on *L. innocua* until the fourth week of storage at 5ºC.

Inclusion of a bacteriocin in a film can modify the physical properties of the film. As an example, addition of pediocin to a cellulose acetate matrix caused an increment on film thickness and on the maximum force needed for rupture. These results could be attributed to a possible interaction between pediocin and the cellulosic matrix which became more rigid (Santiago-Silva et al., 2009).

The combined use of bacteriocins with other preservatives and their inclusion in an edible film can be useful to inhibit several pathogens. For example, Pattanayaiying et al. (2015) reported that pullulan films with the inclusion of a mixture of nisin Z and lauric arginate were effective to control the growth of *Salmonella* spp., *L. monocytogenes*, *S. aureus*, and *E. coli* O157:H7 in vacuum-packaged, refrigerated fresh, and RTE muscle foods.

Results discussed herein show that bacteriocins incorporated into films represent a promising tool to control foodborne pathogens in meat products.

18.4 Problems to Overcome for the Successful Application of Bacteriocins in Meat and Meat Products

Bacteriocin application appears to be a laudable aim for preserving food; however, it could also be a double-edged sword. The antimicrobial activity of bacteriocins is uneven and unpredictable, as it depends on a vast array of chemical and physical conditions of foods together with factors involving the inherent microbiota and the target microorganisms. Processing conditions, spontaneous cell loss of the bacteriocin producer bacteria, phage infection of the cell, and the presence of competitive microorganisms in the medium can interfere with the bacteriocin production by LAB (da Silva Sabo et al., 2014). Furthermore, the bacteriocin effectiveness would also be affected by the presence of bacteriocin-resistant microorganisms, enzymes (namely proteases), occurrence of oxidation–reduction reactions, interaction with constituents of the food formula (fats, proteins, surfactants, for instance) and diffusion restraints due to high salt concentration. In addition, the presence of nitrate and nitrite and low water activity can lead to inadequate distribution of the bacteriocin throughout the food product (Alves et al., 2015).

18.4.1 Interaction with Meat Components and/or Additives

From a physicochemical point of view, meat and meat products exhibit a structure that comprises the presence of tissues, and hydrocolloids and lipids which contribute to the viscous, gelled, or emulsified feature

of the product. According to Wilson et al. (2002), food structure is one of the growth constraints that microorganisms find on foods—together with storage conditions, food composition, and presence of additives. Food structure not only modifies water mobility and distribution of solutes such as acidulants, water activity depressors, and preservatives, but also affects the mobility of microorganisms (Brocklehurst et al., 1993; Castro et al., 2003; Wimpenny et al., 1995). On account of close spatial distribution, microorganisms grow as colonies—rather than planktonically—competing for nutrients and oxygen; in addition, their metabolic end products can be accumulated near colonies affecting growth (Wilson et al., 2002). These considerations must be taken into account when using a protective culture destined to produce the bacteriocin *in situ*. Meat tissue consists of long, thin, parallel cells arranged into fiber bundles which are surrounded by elastin fibers. Its surface comprises the simplest physical arrangement affecting the growth of microorganisms. As the aqueous phase is structured among the network of meat fibers and the crevices of the surrounded tissue, microorganisms are immobilized and constrained to grow as colonies. This fact limits nutrient diffusion and produces depletion of oxygen and accumulation of protons beneath the colony; this results in local changes in the concentration of substrates and acidic metabolic end-products, which leads to decreasing growth rates. Hence, the effectiveness of bacteriocinogenic LAB for the biopreservation of meat and meat products critically depends on interactions with meat constituents (mainly fat, proteins, and endogenous enzymes), and the distribution of bacteriocin molecules within the food. Unfortunately, little information has been recorded on this topic, the meat matrix being an unexplored field in this matter. Conversely, many are the studies conducted on cheese matrices that successfully depicted the interaction issue. As an illustration, when nisin is produced *in situ*, bacteria are entrapped in the cheese and grow as colonies (Jeanson et al., 2011). To be effective after being released, nisin has to migrate (Aly et al., 2011) and reach the target colonies. All these steps can fairly be compared to those found in meat products.

Additionally, the use of bacteriocins, i.e., purified and concentrated extracts, as biopreservatives in meat and meat products faces several limitations resulting from random interactions. For instance, application of nisin in meat products may deal with phospholipid emulsifiers and other food components, poor solubility at pH above 6.0, and inactivation by formation of a nisin-glutathione adduct. Nevertheless, this inactivation is lower in cooked meats due to the loss of free sulfhydryl groups during cooking as a result of the reaction of glutathione with proteins (Stergiou et al., 2006).

With the limitations for nisin application in meats as a biopreservative, a search for alternative sources of biopreservative agents is necessary. In this sense, Rivas et al. (2014) assessed antilisterial activity of sakacin Q produced by *Lb. curvatus* ACU-1 on the surface of cooked pork meat. As a preliminary attempt to find out whether this bacteriocin adsorbs to food matrices, fat

and meat particles (cubes bounded by squared faces of approximately 1 cm^2) were added to the CFS of the bacteriocinogenic culture (50 mL). Periodically, samples were withdrawn to measure bacteriocin activity in the supernatant. Antimicrobial activity of sakacin Q was halved in the presence of both food matrices (fat and meat tissues), which could be attributed to bacteriocin adsorption to them. Though the remaining concentration was approximately 400 AU mL^{-1} at the end of the trial (after 24 hours) in both tissues, it was still effective against *L. innocua* ATCC 33090. The authors attributed this phenomenon to adsorption of the bacteriocin to fat and meat particles. Hydrophobic sites of bacteriocins are prone to establishing unspecified unions to the hydrophobic surface of fat particles (Holzapfel et al., 1995). As an illustration, fat in the bulk of CFS from *Lb. sakei* CTC 494 caused a great loss of bacteriocin activity (Leroy and De Vuyst, 2005). Regardless of being bound to the food matrix, part of the bacteriocin sakacin Q could exert its antimicrobial activity. This fact was supported by a simple qualitative assay: small pieces of meat and fat, which were subjected to the aforementioned procedure, were placed onto BHI agar seeded with *L. innocua*. Inhibition halos recorded after incubation of the plates suggested that the adsorption of the bacteriocin to fat and meat tissues did not hinder its antimicrobial activity.

Furthermore, Kouakou et al. (2009) found that high-fat content meat antagonizes the antilisterial effect of bacteriocinogenic *Lb. curvatus* CWBI-B28wt in pork meat systems. The unsatisfactory effect of bacteriocin-producing strains *in situ* is due to hydrophobic interactions of the bacteriocins with fat. However, several factors regarding the type of bacteriocin may interfere with its activity. While some bacteriocins—like nisin—associate reversibly with fat and have a stabilizing effect on the fat–water interface, preserving their antilisterial effect (Bani-Jaber et al., 2000), other bacteriocins—like sakacin P—bind tightly to lipids in the food matrix and lose their antimicrobial activity (Aasen et al., 2003).

On the other hand, endogenous proteases may lead to a pathogen rebound after a certain amount of storage time since proteolytic degradation of bacteriocin can proceed at the end of the culture. Kouakou et al. (2009) observed this listerial rebound; they attributed it also to the presence of nitrites. It has been suggested that this nitrite-triggered repression of bacteriocin production is due to interference of salt molecules with binding of the induction factor to its receptor (Nilsen et al., 1998). Other authors suggested different mechanisms such as direct interference of nitrite with the action of bacteriocin through binding to its pathogen-targeting pole (Dykes and Hastings, 1998; Ennaharet al., 2000) and enhancement of listeria resistance through nitrite-induced stress (Gravesen et al., 2002).

As a whole, *in situ* bacteriocin production for the biopreservation of meat and meat products should be conducted with a technologically competent strain—capable of producing higher levels of bacteriocins—in order to overcome the adverse effects on bacteriocin effectiveness that may occur in some situations.

18.4.2 Appearance of Resistant Strains

The development of bacteriocin resistance (BacR) might hinder bacteriocin application in food preservation. Moreover, an undesirable consequence of an extended use of natural antimicrobials such as nisin in food might be cross-resistance to clinically used antibiotics in foodborne pathogens such as *L. monocytogenes*.

Most LAB bacteriocins impair the overall integrity of the cytoplasmic membrane by pore formation. Moreover, nisin and related lantibiotics use the membrane-bound peptidoglycan precursor lipid II as a docking molecule for pore formation and, thus, cell wall synthesis is inhibited. Target microorganisms may become in some measure resistant to the pore-forming bacteriocins. The resistance may be transient or remain stable. Changes in the membrane composition and fluidity and polysaccharide production are examples of resistance mechanisms toward nisin (Martínez and Rodríguez, 2005). While in nisin-resistant mutants of *L. monocytogenes* an altered gene expression was detected, resistance to class IIa bacteriocins has been related to the absence of one of the components of the mannose transport system (Naghmouchi et al., 2007).

A word of caution must be taken toward this topic since arguments depicted by researchers devoted to these investigations rely on specific antibiotics and pathogen strains. On the one hand, Martínez and Rodríguez (2005) suggested that the development of nisin resistance in *L. monocytogenes* would not hamper antibiotic therapy towards listeriosis based on β-lactam treatment. As found in their study, nisin-resistant strains were more susceptible to β-lactams than the wild types; indeed, a higher sensitivity has been frequently correlated to enhanced nisin resistance in *L. monocytogenes* (Gravesen et al., 2001). On the other hand, Naghmouchi et al. (2006) found an increased resistance of all *L. monocytogenes* bacteriocin-resistant variants to several antibiotics, which target different cellular sites. All variants were more resistant to ampicillin, bacitracin, chloramphenicol, vancomycin, and kanamycin than was the wild-type strain. These authors proposed that increased resistance to these antibiotics may indicate that the compositional changes in the cell wall fraction of variants selected by exposure to bacteriocin can also confer protection to other inhibitory substances such as antibiotics.

It is well known that Gram-negative bacteria have natural resistance to nisin due to the presence of the outer membrane; however, the use of chelating agents, such as EDTA, allows for their sensitization. Nevertheless, *Salmonella* can alter the composition and the structure of their lipopolysaccharide (LPS) layer in response to changes in environmental conditions, diminishing their sensitivity to bacteriocins, such as nisin. Prudêncio et al. (2015) demonstrated and characterized tolerance to nisin combined with EDTA in *S.* Typhimurium cultured under specific conditions of temperature and pH. In this study, it was presumed that the modifications related to the tolerance phenotype of

nisin combined with EDTA possibly involve changes in the phospholipid acyl chains, in the sugar content of LPS, and in the peptidoglycan present in the cell wall. Arguably, these findings demonstrate the importance of better understanding the behavior of the cells treated with nisin and EDTA, especially due to widespread use of this bacteriocin in food preservation.

Resistance to a bacteriocin may extend to other bacteriocins within the same class or even in other classes (Naghmouchi et al., 2007). As an illustration, Kaur et al. (2013) determined that resistance to a bacteriocin extended to other bacteriocins within the same class, e.g., Pediocin 34, conferred cross-resistance to Enterocin FH 99 (class IIa bacteriocins) but not to nisin (class I). The acquirement of resistance to bacteriocins can significantly affect physiological activity, alter cell-envelope lipid composition, and modify the antibiotic susceptibility/resistance profile of *L. monocytogenes*. An interesting work conducted by Macwana and Muriana (2012) took full advantage of this fact, proposing a practical system for grouping LAB bacteriocins based on their mode of action as determined by changes in inhibitory activity to spontaneously acquired BacR. Bacteriocin sensitive wild-type *L. monocytogenes* 39–2, sensitive to five bacteriocins produced by three genera of LAB: pediocin PA-1 and pediocin Bac3 (*Pediococcus*), lacticin FS97 and lacticin FS56 (*Lactococcus*), and curvaticin FS47 (*Lactobacillus*). Spontaneous BacR derivative of *L. monocytogenes* 39–2 obtained by selective recovery against lacticin FS56 provided complete resistance to the bacteriocin made by *Lc. lactis* FS56. The lacticin FS56-resistant strain of *L. monocytogenes* 39–2 was also cross-resistant to curvaticin FS47 and pediocin PA-1, but not to lacticinFS97 or pediocin Bac3. A spontaneous mutation that renders a strain cross-resistant to different bacteriocins indicates that they share a common mechanism of resistance due to similar modes of action of the bacteriocins. Furthermore, these authors also proved that mixtures of bacteriocins of different modes of action provided greater inhibition than mixtures of bacteriocins of the same mode of action. This methodical approach to classify bacteriocins into functional groups based on mechanism of resistance (i.e., mode of action) could be used for identifying the best mixture of bacteriocins for use as biopreservatives.

In conclusion, the occurrence of bacteriocin-resistant variants of meatborne pathogens, mainly *L. monocytogenes* and *Salmonella* spp., became evident in the last decade (Naghmouchi et al., 2006, 2007; Collins et al., 2010; Macwana and Muriana, 2012; Kaur et al., 2013). Knowledge of the features of classes I and IIa bacteriocin-resistant variants and the conditions that prevent their emergence will help to determine optimal conditions for bacteriocin applications in foods and minimize the incidence of resistance. Since bacteriocins are regarded as potential tools for biopreservation, more study is needed to determine the distribution of BacR phenomena among microorganisms that cause food spoilage and among foodborne pathogens, especially *L. monocytogenes*. In addition, the relationship between acquiring resistance to bacteriocins and the pathogenicity of singly or multiply resistant *L. monocytogenes* remains to be elucidated.

18.5 Overview of Actual Industrial Application and Regulatory Aspects

In spite of the GRAS status that many (bacteriocinogenic) protective cultures already have, only two LAB bacteriocins, i.e., nisin and pediocin PA-1, have been authorized for the biopreservation of foods. Nisin is commercially available as a dried concentrated powder called Nisaplin (Danisco, Copenhagen, Denmark) and was admitted into the European food additive list in the early 1980s, where it was assigned the number E234 (EEC, 1983). In 1988, nisin received GRAS status from the Food and Drug Administration (FDA) (Federal Register, 1988), and is the only bacteriocin that has been approved by the World Health Organization for use as a food preservative (Sobrino-López and Martín-Belloso, 2008). Pediocin PA-1 is marketed as a bacteriocin-containing fermentate powder, namely ALTA®2351 (Kerry Bioscience, Carrigaline, Co. Cork, Ireland) (Mills et al., 2011).

The authority under which a given bacteriocin will be regulated for use in food will depend on the foods in which it is used and the purpose for which it is used. International regulatory agencies have different criteria concerning doses and food applications. For instance, an additional suitability assessment by the U.S. Department of Agriculture's (USDA) Food Safety and Inspection Service (FSIS) is required for bacteriocins used in meat products. According to the FAO/WHO Codex Alimentarius (2013), nisin—named as INS 234 by this international organization—application in meats comprise RTE products, such as hot dogs and smoked sausages, 25 ppm being the highest suggested concentration within the products. These regulations take into account that nisin should be part of a multiple hurdle system where sodium lactate or diacetate are added and aid the preservation. When talking about nisin incorporation to a product, FAO/WHO experts consider that there is an extra challenge for the meat industry: the equipment and the technology necessary for the suitable packaging of the product, since the extensive use of nisin implies external application immediately before packaging.

On the other hand, protective cultures from LAB have been included in the manufacturing process of many RTE meat products; the legislation treat them as food additives. The way they have to be incorporated, their concentration, and shelf life of the final products are regulated by many international public health organisms. The determination that specific strains of *Carnobacterium maltaromaticum* are GRAS—on the basis of scientific procedures—can be found as one example from the FDA Federal Register (62 FR 18937–18964, 2004). *Cb. maltaromaticum* strains CB1, CB2, CB3LV17, UAL26, ATCC 35586, and ATCC43225 for use in RTE and fresh comminuted processed meat products, at a maximum inoculation concentration of 1×10^4 CFU/g, are meant to be used as inhibitors of *L. monocytogenes*. Furthermore, commercial protective cultures are already marketed, comprised of a mixture of several

bacteriocinogenic cultures. For instance, the DuPont™ Danisco® protective culture called HOLDBAC® Listeria can be added to semidry-cured meats and cooked and fresh ground meats to inhibit the growth of the aforementioned pathogen.

In addition to the information given, the reader is invited to read Aymerich et al. (2006), since neither regulations nor legislation related to biopreservation of meat and meat products have been substantially changed in the last decade.

References

Aasen, I. M., Markussen, S., Moretro, T., Katla, T., Axelsson L. and Naterstad, L. (2003). Interactions of the bacteriocin sakacin P and nisin with food constituents. *International Journal of Food Microbiology,* **87**, 35–43.

Ahmed, O. M., Pangloli, P., Hwang, C. A. et al. (2015). The occurrence of *Listeria monocytogenes* in retail ready-to-eat meat and poultry products related to the levels of acetate and lactate in the products. *Food Control,* **52**, 43–48.

Albano, H., Pinho, C., Leite, D. et al. (2009). Evaluation of a bacteriocin-producing strain of *Pediococcus acidilactici* as a biopreservative for "Alheira", a fermented meat sausage. *Food Control,* **20**, 764–770.

Alves, V. F., Martinez, R. C. R., Lavrador, M. A. S. and De Martinis, E. C. P. (2006). Antilisterial activity of lactic acid bacteria inoculated on cooked ham. *Meat Science,* **74**, 623–627.

Aly, S., Floury, J., Famelart, M. H. et al. (2011). Nisin quantification by ELISA allows the modeling of its apparent diffusion coefficient in model cheeses. *Journal of Agricultural and Food Chemistry,* **59**, 9484–9490.

Ananou, S., Baños, A., Maqueda, M., Martínez-Bueno, M., Gálvez, A. and Valdivia, E. (2010a). Effect of combined physico-chemical treatments based on enterocin AS-48 on the control of *Listeria monocytogenes and Staphylococcus aureus* in a model cooked ham. *Food Control*, 21, 478–486.

Ananou, S., Garriga, M., Jofré, A. et al. (2010b). Combined effect of enterocin AS-48 and high hydrostatic pressure to control food-borne pathogens inoculated in low acid fermented sausages. *Meat Science,* **84**, 594–600.

Ananou, S., Maqueda, M., Martínez-Bueno, M., Gálvez, A. and Valdivia, E. (2005). Control of *Staphylococcus aureus* in sausages by enterocin AS-48. *Meat Science,* **71**, 549–556.

Ananou, S., Valdivia, E., Martínez-Bueno, M., Gálvez, A. and Maqueda, M. (2004). Effect of combined physico-chemical preservatives on enterocin AS-48 activity against the enterotoxigenic *Staphylococcus aureus* CECT 976 strain. *Journal of Applied Microbiology,* **97**, 48–56.

Aymerich, T., Garriga, M., Jofré, A., Martín, B. and Monfort, J. M. (2006a). The use of bacteriocins against meat-borne pathogens. *In:* Nollet, L. M. L. and Toldra, F. (eds.) *Advanced Technologies for Meat Processing*, Chapter 15. New York, NY: Taylor & Francis Group LLC, pp. 371–399.

Aymerich, T., Martín, B., Garriga, M., Vidal-Carou, M. C., Bover-Cid, S. and Hugas, M. (2006b). Safety properties and molecular strain typing of lactic acid bacteria from slightly fermented sausages. *Journal of Applied Microbiology,* **100**, 40–49.

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

Bani-Jaber, A., McGuire, J., Ayres, J. W. and Daeschel, M. A. (2000). Efficacy of the antimicrobial peptide nisin in emulsifying oil in water. *Journal of Food Science,* **65**, 502–506.

Benito, M. J., Martín, A., Aranda, E., Pérez-Nevado, F., Ruiz-Moyano, S. and Córdoba, M. (2007). Characterization and selection of autochthonous lactic acid bacteria isolated from traditional Iberian dry-fermented salchichón and chorizo sausages. *Journal of Food Science,* **72**, 193–201.

Benkerroum, N., Daoudi, A., Hamraoui, T. et al. (2005). Lyophilized preparations of bacteriocinogenic *Lactobacillus curvatus* and *Lactococcus lactis* subsp. *lactis* as potential protective adjuncts to control *Listeria monocytogenes* in dry-fermented sausages. *Journal of Applied Microbiology,* **98**, 56–63.

Blanco Massani, M., Molina, V., Sanchez, M., Renaud, V., Eisenberg, P. and Vignolo, G. (2014). Active polymers containing Lactobacillus curvatus CRL705 bacteriocins: Effectiveness assessment in Wieners. International Journal of Food Microbiology. **178**, 7–12.

Borch, E., Kant-Muermans, M. and Blixt, Y. (1996). Bacterial spoilage of meat and cured meat products. *International Journal of Food Microbiology,* **33**, 103–120.

Bredholt, S., Nesbakken, T. and Holck, A. (1999). Protective cultures inhibit growth of *Listeria monocytogenes* and *Escherichia coli* O157:H7 in cooked, sliced, vacuum- and gas-packaged meat. *International Journal of Food Microbiology,* **53**, 43–52.

Brocklehurst, T. F., Parker, M. L., Gunning, P. A. and Robins, M. M. (1993). Microbiology of emulsions: Physicochemical aspects. *Lipid Technology*, 83–88.

Campos, C. A., Gerschenson, L. N. and Flores, S. K. (2011). Development of edible films and coatings with antimicrobial activity. *Food and Bioprocess Technology,* **4**(6), 849–875.

Carlini, N., Mogna, L., and Strozzi, G. P. (2010). Reducing foodborne pathogens in the food chain by the use of protective and probiotic cultures. In J. Rovira (Ed.), How to Improve the Food Safety of My Products? Madrid, Spain: International Marketing & Communication, pp. 73–88.

Casaburi, A., Di Martino, V., Ferranti, P., Picariello, L. and Villani, F. (2016). Technological properties and bacteriocins production by *Lactobacillus curvatus* 54M16 and its use as starter culture for fermented sausage manufacture. *Food Control,* **59**, 31–45.

Casquete, R., Benito, M. J., Martín, A., Ruiz-Moyano, S., Aranda, E. and Córdoba, M. G. (2012). Microbiological quality of salchichón and chorizo, traditional Iberian dry-fermented sausages from two different industries, inoculated with autochthonous starter cultures. *Food Control,* **24**, 191–198.

Castellano, P., Belfiore, C., Fadda, S. and Vignolo, G. (2008). A review of bacteriocinogenic lactic acid bacteria used as bioprotective cultures in fresh meat produced in Argentina. *Meat Science,* **79**, 483–499.

Castellano, P., Belfiore, C. and Vignolo, G. (2011). Combination of bioprotective cultures with EDTA to reduce *Escherichia coli* O157:H7 in frozen ground-beef patties. *Food Control,* **22**, 1461–1465.

Castellano, P., González, C., Carduza, F. and Vignolo, G. (2010). Protective action of *Lactobacillus curvatus* CRL 705 on vacuum-packaged raw beef. Effect on sensory and structural characteristics. *Meat Science,* **85**, 394–401.

Castellano, P. and Vignolo, G. (2006). Inhibition of *Listeria innocua* and *Brochothrix thermosphacta* in vacuum-packaged meat by addition of bacteriocinogenic *Lactobacillus curvatus* CRL705 and its bacteriocins. *Letters in Applied Microbiology,* **43**, 194–199.

Castro, M. P., Garro, O., Gerschenson, L. N. and Campos, C. A. (2003). Interaction between potassium sorbate, oil and Tween 20 on the growth and inhibition of *Z. bailii* in model salad dressings. *Journal of Food Safety,* **23**, 47–59.

Centers for Disease Control and Prevention (2015). Centers for disease control and prevention, outbreak surveillance data. Available at http://www.cdc.gov/foodsafety/outbreaks/investigating-outbreaks/index.html (accessed January 2015).

Chaillou, S., Christieans, S., Rivollier, M., Lucquin, I., Champomier-Vergès, M. C. and Zagorec, M. (2014). Quantification and efficiency of *Lactobacillus sakei* strain mixtures used as protective cultures in ground beef. *Meat Science,* **97**, 332–338.

Chakchouk-Mtibaa, A., Elleuch, L., Smaoui, S. et al. (2014). Characterization of the bacteriocin BacJ1 and its effectiveness for the inactivation of *Salmonella typhimurium* during turkey escalope storage. *Food Chemistry,* **152**, 566–572.

Codex Alimentarius. (2013). *FAO/OMS. Secretariat of the Codex Alimentarius Commission*. Roma, Italia. Available at http://www.fao.org/3/a-i3243s.pdf.

Collins, B., Curtis, N., Cotter, P. D., Hill, C. and Ross, R. P. (2010). The ABC transporter AnrAB contributes to the innate resistance of *Listeria monocytogenes*to nisin, bacitracin, and various β-lactam antibiotics. *Antimicrobial Agents and Chemotherapy,* **54**, 4416–4423.

Coma, V. (2008). Bioactive packaging technologies for extended shelf life of meat-based products. *Meat Science,* **78**, 90–103.

D Aoust, J. I. (1997). *Salmonella* species. *In:* Doyle, M. P., Beuchat, L. R. and Montville, T. J. (eds.) *Food Microbiology. Fundamentals and Frontiers*. Washington, DC: ASM Press, pp. 129–158.

da Silva Sabo, S., Vitolo, M., Domínguez González, J. M. and Pinheiro de Souza Oliveira, R. (2014). Overview of *Lactobacillus plantarum* as a promising bacteriocin producer among lactic acid bacteria. *Food Research International,* **64**, 527–536.

Dave, D. and Ghaly, A. E. (2011). Meat spoilage mechanisms and preservation techniques: A critical review. *American Journal of Agricultural and Biological Sciences,* **6**(4), 486–510.

de Souza Barbosa, M., Todorov, S. D., Ivanova, I., Chobert, J-M., Haertle, T. and Gombossy de Melo Franco, B. D. (2015). Improving safety of salami by application of bacteriocins produced by an autochthonous *Lactobacillus curvatus* isolate. *Food Microbiology,* **46**, 254–262.

Dods, K. L. and Austin, J. W. (1997). *Clostridium botulinum*. *In:* Doyle, M. P., Beuchat, L. R. and Montville, T. J. (eds.) *Food Microbiology. Fundamentals and Frontiers*. Washington, DC: ASM Press, pp. 288–304.

Dominic Dussault, D., Vu, K. D. and Lacroix, M. (2016). Development of a model describing the inhibitory effect of selected preservatives on the growth of *Listeria monocytogenes* in a meat model system. *Food Microbiology,* **53**, 115–121.

Dortu, C., Huch, M., Holzapfel, W. H., Franz, C. M. A. P. and Thonart, P. (2008). Antilisterial activity of bacteriocin-producing *Lactobacillus curvatus* CWBI-B28 and *Lactobacillus sakei* CWBI-B1365 on raw beef and poultry meat. *Journal of Applied Microbiology,* **47**, 581–586.

Doyle, M. P., Zhao, T., Meng, J. and Zhao, S. (1997). *Escherichia coli* O157:H7. *In:* Doyle, M. P., Beuchat, L. R. and Montville, T. J. (eds.) *Food Microbiology. Fundamentals and Frontiers*. Washington, DC: ASM Press, pp. 171–191.

Drosinos, E. H., Mataragas, M., Vesković-Moračanin, S., Gasparik-Reichardt, J., Hadžiosmanović, M. and Alagić, D. (2006). Quantifying nonthermal inactivation of *Listeria monocytogenes* in European fermented sausages using bacteriocinogenic lactic acid bacteria or their bacteriocins: A case study for risk assessment. *Journal of Food Protection,* **69**, 648–2663.

Dykes, G. A. and Hastings, J. W. (1998). Fitness costs associated with class IIa bacteriocin resistance in *Listeria monocytogenes* B73. *Letters in Applied Microbiology,* **26**, 1–5.

EC, European Parliament and Council of the European Community. (2005). Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. *Official Journal of the European Union*, **L338**, 1–26. Brussels, Belgium.

European Food Safety Authority (EFSA). (2007). The community summary report of trends and sources of zoonoses, zoonotic agents, antimicrobial resistances and foodborne outbreaks in the European Union in 2006. *EFSA Journal,* **130**, 3–352. Available at http://www.efsa.europa.eu/cs/BlobServer/DocumentSet/Zoonreport2006en,0.pdf?ssbinary=true (accessed October 2009).

European Food Safety Authority (EFSA). (2010). Analysis of the baseline survey on the prevalence of Campylobacter in broiler batches and of Campylobacter and Salmonella on broiler carcasses in the EU, 2008. European Food Safety Authority, Italy. EFSA Journal, 8, 3, 1503.

El-Ziney, M. G., van den Temple, T., Debevere, J. and Jakobsen, M. (1999). Application of reuterin produced by *Lactobacillus reuteri* 12002 for meat decontamination and preservation. *Journal of Food Protection,* **62**, 257–261.

Ennahar, S., Sashihara, T., Sonomoto, K. and Ishizaki, A. (2000). Class IIa bacteriocins: Biosynthesis, structure and activity. *FEMS Microbiology Reviews,* **24**, 85–106.

European Commission. (EEC). (1983). Commission Directive 83/463/EEC 22 of July introducing temporary measures for the designation of certain ingredients in the labeling of foodstuffs for sale to the ultimate consumer. *Official Journal of European Communities,* **L255**, 1–6.

European Food Safety Authority. (2015). The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2013. *European Food Safety Authority Journal,* **2015**(13), 3991. Available at http://ecdc.europa.eu/en/publications/Publications/EU-summary-report-trends-sources-zoonoses-2013.pdf.

Federal Register. (1988). Nisin preparation: Affirmation of GRAS status as a direct human food ingredient. *Federal Register,* **53**, 11247–11251.

Ferreira, V., Barbosa, J., Silva, J. et al. (2007). Chemical and microbiological characterization of "Salpicão de Vinhais" and "Chouriça de Vinhais": Traditional dry sausages produced in the North of Portugal. *Food Microbiology,* **24**, 618–623.

Gadang, V. P., Hettiarachchy, N. S., Johnson, M. G. and Owens, C. (2008). Evaluation of antibacterial activity of whey protein isolate coating incorporated with nisin, grape seed extract, malic acid, and EDTA on a Turkey frankfurter system. Journal of Food Science, **73**, M389–M394.

Gálvez, A., Abriouel, H., López, R. L. et al. (2007). Bacteriocin-based strategies for food biopreservation. *International Journal of Food Microbiology,* **120**, 51–70.

Gao, Y., Jia, S., Gao, Q. and Tan, Z. (2010). A novel bacteriocin with a broad inhibitory spectrum produced by *Lactobacillus sake* C2, isolated from traditional Chinese fermented cabbage. *Food Control,* **21**, 76–81.

Gao, Y., Li, D. and Liu, X. (2014). Bacteriocin-producing *Lactobacillus sakei* C2 as starter culture in fermented sausages. *Food Control,* **35**, 1–6.

Gao, Y., Li, D., Liu, S. and Liu, Y. (2012). Probiotic potential of *L. sake* C2 isolated from traditional Chinese fermented cabbage. *European Food Research and Technology,* **234**, 45–51.

Garrido, V., Vitas, A. I. and García-Jalón, I. (2009). Survey of *Gisteria monocytogenes* in ready-to-eat products: Prevalence by brands and retail establishments for exposure assessment of listeriosis in Northern Spain. *Food Control,* **20**, 986–991.

Georgaras, I., Panagiotis, N., Skandamis, N. et al. (2006). Post-processing application of chemical solutions for control of *Listeria monocytogenes,* cultured under different conditions on commercial smoked sausage formulated with and without lactate-sodium diacetate. *Food Microbiology,* **23**, 762–771.

Gialamas, H., Zinoviadou, K., Biliaderis, C. and Koutsoumanis, K. (2010). Development of a novel bioactive packaging based on the incorporation of *Lactobacillus sakei* into sodium-caseinate films for controlling *Listeria monocytogenes* in foods. *Food Research International,* **43**, 2402–2408.

Gill, A. O. and Holley, R. A. (2000). Inhibition of bacterial growth on ham and bologna by lysozyme, nisin and EDTA. *Food Research International,* **33**, 83–90.

Gombas, D. E., Chen, Y., Clavero, R. S. and Scott, V. N. (2003). Survey of *Listeria monocytogenes* in ready-to-eat foods. *Journal of Food Protection,* **66**, 459–469.

Govaris, A., Solomakos, N., Pexara, A. and Chatzopoulou, P. S. (2010). The antimicrobial effect of oregano essential oil, nisin and their combination against *Salmonella* Enteritidis in minced sheep meat during refrigerated storage. *International Journal of Food Microbiology,* **137**, 175–180.

Gravesen, A., Axelsen, A., da Silva, J. M., Hansen, T. B. and Knøchel, S. (2002). Frequency of bacteriocin resistance development and associated fitness costs in *Listeria monocytogenes*. *Applied and Environmental Microbiology,* **68**, 756–764.

Gravesen, A., Sorensen, K., Aarestrup, F. M. and Knochel, S. (2001). Spontaneous nisin-resistant *Listeria monocytogenes* mutants with increased expression of a putative penicillin-binding protein and their sensitivity to various antibiotics. *Microbial Drug Resistance,* **7**, 127–135.

Gudbjornsdottir, B., Suihko, M. L., Gustavsson, P. et al. (2004). The incidence of *Listeria monocytogenes* in meat, poultry and seafood plants in the Nordic countries. *Food Microbiology,* **21**, 217–225.

Hampikyan, H. and Ugur, M. (2007). The effect of nisin on *L. monocytogenes* in Turkish fermented sausages (sucuks). *Meat Science,* **76**, 327–332.

Hanlin, M. B., Kalchayanand, P. B. and Ray, B. (1993). Bacteriocins of lactic acid bacteria in combination have greater antibacterial activity. *Journal of Food Protection,* **56**, 252–255.

Hartmann, H. A., Wilke, T. and Erdmann, R. (2011). Efficacy of bacteriocin-containing cell-free culture supernatants from lactic acid bacteria to control *Listeria monocytogenes* in food. *International Journal of Food Microbiology,* **146**, 192–199.

Hereu, A., Bover-Cid, A., Garriga, M. and Aymerich, T. (2012). High hydrostatic pressure and biopreservation of dry-cured ham to meet the Food Safety Objectives for *Listeria monocytogenes*. *International Journal of Food Microbiology,* **154**, 107–112.

Holzapfel, W. H., Geisen, R. and Schillinger, U. (1995). Biological preservation of foods with reference to protective cultures, bacteriocins and food-grade enzymes. *International Journal of Food Microbiology,* **24**, 343–362.

Hugas, M., Pages, F., Garriga, M. and Monfort, J. M. (1998). Application of the bacteriocinogenic *Lactobacillus sakei* CTC494 to prevent growth of *Listeria* in fresh and cooked meat products packed with different atmospheres. *Food Microbiology,* **15**(6), 639–650.

Hugo, C. J. and Hugo, A. (2015). Current trends in natural preservatives for fresh sausage products. *Trends in Food Science and Technology,* **45**, 12–23.

Jacobsen, T., Budde, B. B. and Koch, A. G. (2003). Application of *Leuconostoc carnosum* for biopreservation of cooked meat products. *Journal of Applied Microbiology,* **95**(2), 242–249.

Janes, M. E., Kooshesh, S. and Johnson, M. G. (2002). Control of Listeria monocytogenes on the surface of refrigerated, ready-to-eat chicken coated with edible zein film coatings containing nisin and/or calcium propionate. Journal of Food Science, **67**, 2754–2757.

Jeanson, S., Chadoeuf, J., Madec, M. N. et al. (2011). Spatial distribution of bacterial colonies in a model cheese. *Applied and Environmental Microbiology,* **77**(4), 1493–1500.

Jofré, A., Aymerich, T. and Garriga, M. (2009). Improvement of the food safety of low acid fermented sausages by enterocins A and B and high pressure. *Food Control,* **20**, 179–184.

Jofré, A., Garriga, M. and Aymerich, T. (2008). Inhibition of *Salmonella* sp., *Listeria monocytogenes* and *Staphylococcus aureus* in cooked ham by combining antimicrobials, high hydrostatic pressure and refrigeration. *Meat Science,* **78**, 53–59.

Jones, R., Wiklund, E., Zagorec, M. and Tagg, J. (2010). Evaluation of stored lamb bio-preserved using a three-strain cocktail of *Lactobacillus sakei*. *Meat Science,* **86**, 955–959.

Jones, R., Zagorec, M., Brightwell, G. and Tagg, J. (2009). Inhibition by *Lactobacillus sakei* of other species in the flora of vacuum packaged raw meats during prolonged storage. *Food Microbiology,* **26**, 876–881.

Juncioni de Arauza, L., Faustino Jozala, A., GavaMazzola, P. and Vessoni Penna, T. C. (2009). Nisin biotechnological production and application: A review. *Trends in Food Science and Technology,* **20**, 146–154.

Juneja, V. K., Dwivedi, H. P. and Yan, X. (2012). Novel natural food antimicrobials. *Annual Reviews in Food Science and Technology,* **3**, 381–403.

Kaur, G., Singh T. P. and Malik, R. K. (2013). Antibacterial efficacy of Nisin, Pediocin 34 and Enterocin FH99 against *Listeria monocytogenes* and cross resistance of its bacteriocin resistant variants to common food preservatives. *Brazilian Journal of Microbiology,* **44**(1), 63–71.

Kapetanakou, A. E., Karyotis, D. and Skandamis, P. N. (2016). Control of Listeria monocytogenes by applying ethanol-based antimicrobial edible films on ham slices and microwave-reheated frankfurters. Food Microbiology, **54**, 80–90.

Khan, H., Flint, S. and Yu, P.-L. (2010). Enterocins in food preservation. *International Journal of Food Microbiology,* **141**, 1–10.

Kostrzynska, M. and Bachand, A. (2006). Use of microbial antagonism to reduce pathogen levels on produce and meat products: A review. *Canadian Journal of Microbiology,* **52**, 1017–1026.

Kouakou, P., Ghalfi, H., Destain, J., Duboisdauphin, R., Evrard P. and Thonart, P. (2008). Enhancing the antilisterial effect of *Lactobacillus curvatus* CWBI-B28 in pork meat and cocultures by limiting bacteriocin degradation. *Meat Science,* **80**, 640–648.

Kouakou, P., Ghalfi, H., Destain, J., Dubois-Dauphin, R., Evrard, P. and Thonart, P. (2009). Effects of curing sodium nitrite additive and natural meat fat on growth control of *Listeria monocytogenes* by the bacteriocin-producing *Lactobacillus curvatus* strain CWBI-B28. *Food Microbiology,* **26**, 623–628.

Kouakou, P., Ghalfi, H., Dortu, C., Evrard, P. and Thonart, P. (2010). Combined use of bacteriocin-producing strains to control *Listeria monocytogenes* regrowth in raw pork meat. *International Journal of Food Science and Technology,* **45**, 937–943.

Kuleasan, H. and Cakmakei, M. L. (2002). Effect of reuterin produced by *Lactobacillus reuteri* on the surface of sausages to inhibit the growth of *Listeria monocytogenes* and *Salmonella* spp. *Nahrung,* **46**, 408–410.

Leroy, F. and De Vuyst, L. (2005). Simulation of the effect of sausage ingredients and technology on the functionality of the bacteriocin-producing *Lactobacillus sakei* CTC 494 strain. *International Journal of Food Microbiology,* **100**, 141–152.

Liu, G., Wang, Y., Gui, M., Zheng, H., Dai, R. and Li, P. (2012). Combined effect of high hydrostatic pressure and enterocin LM-2 on the refrigerated shelf life of ready-to-eat sliced vacuum-packed cooked ham. *Food Control,* **24**, 64–71.

Lücke, F. K. (2000). Utilization of microbes to process and preserve meat. *Meat Science,* **56**, 105–115.

Lues, J. F. R. and Van Tonder, I. (2007). The occurrence of indicator bacteria on hands and aprons of food handlers in the delicatessen sections of a retail group. *Food Control,* **18**, 326–332.

Macwana, S. and Muriana, P. M. (2012). Spontaneous bacteriocin resistance in *Listeria monocytogenes* as a susceptibility screen for identifying different mechanisms of resistance and modes of action by bacteriocins of lactic acid bacteria. *Journal of Microbiological Methods,* **88**, 7–13.

Maragkoudakis, P. A., Mountzouris, K. C., Psyrras, D. et al. (2009). Functional properties of novel protective lactic acid bacteria and application in raw chicken meat against *Listeria monocytogenes* and *Salmonella enteritidis*. *International Journal of Food Microbiology,* **130**, 219–226.

Martínez, B. and Rodríguez, A. (2005). Antimicrobial susceptibility of nisin resistant *Listeria monocytogenes* of dairy origin. *FEMS Microbiology Letters,* **252**, 67–72.

Mataragas, M., Drosinos, E. H. and Metaxopoulos, J. (2003). Antagonistic activity of lactic acid bacteria against *Listeria monocytogenes* in sliced cooked cured pork shoulder stored under vacuum or modified atmosphere at 4 ± 2°C. *Food Microbiology,* **20**, 259–265.

MCClane, B. A. (1997). *Clostridium perfringens*. *In:* Doyle, M. P., Beuchat, L. R. and Montville, T. J. (eds.) *Food Microbiology. Fundamentals and Frontiers*. Washington, DC: ASM Press, pp. 305–326.

Melero, B., Diez, A. M., Rajkovic, A., Jaime, I. and Rovira, J. (2012). Behaviour of non-stressed and stressed *Listeria monocytogenes* and *Campylobacter jejuni* cells on fresh chicken burger meat packaged under modified atmosphere and inoculated with protective culture. *International Journal of Food Microbiology,* **158**, 107–112.

Melero, B., Vinuesa, R., Diez, A. M., Jaime, I. and Rovira, J. (2013). Application of protective cultures against *Listeria monocytogenes* and *Campylobacter jejuni* in chicken products packaged under modified atmosphere. *Poultry Science,* **92**, 1108–1116.

Melo, R. T., Nalevaiko, P. C., Mendonça, E. P. et al. (2013). *Campylobacter jejuni* strains isolated from chicken meat harbour several virulence factors and represent a potential risk to humans. *Food Control,* **33**, 227–231.

Millette, M., Le Tien, C., Smoragiewicz, W. and Lacroix, M. (2007). Inhibition of Staphylococcus aureus on beef by nisin-containing modified alginate films and beads. Food Control, **18**, 878–884.

Mills, S., Stanton, C., Hill, C. and Ross, R. P. (2011). New developments and applications of bacteriocins and peptides in foods. *Annual Review of Food Science and Technology,* **2**, 299–329.

Min, B. J., Han, I. Y. and Dawson, P. L. (2010). Antimicrobial gelatin films reduce Listeria monocytogenes on turkey bologna. Poultry Science, **89**, 1307–1314.

Moore, J. E. (2004). Gastrointestinal outbreaks associated with fermented meats. *Meat Science,* **67**, 565–568.

Muñoz, A., Ananou, S., Gálvez, A. et al. (2007). Inhibition of *Staphylococcus aureus* in dairy products by enterocin AS-48 produced in situ and ex situ: Bactericidal synergism with heat. *International Dairy Journal,* **17**, 760–769.

Naghmouchi, K., Drider, D., Kheadr, E., Lacroix, C., Prévost, H. and Fliss, I. (2006). Multiple characterizations of *Listeria monocytogenes* sensitive and insensitive variants to divergicin M35, a new pediocin-like bacteriocin. *Journal of Applied Microbiology,* **10**, 29–39.

Naghmouchi, K., Kheadr, E., Lacroix, C. and Fliss, I. (2007). Class I/Class IIa bacteriocin cross-resistance phenomenon in *Listeria monocytogenes*. *Food Microbiology,* **24**, 718–727.

Nguyen, V. T., Gidley, M .J. and Dykes, G. A. (2008). Potential of a nisin-containing bacterial cellulose film to inhibit Listeria monocytogenes on processed meats. Food Microbiology, **25**, 471–478.

Nieto-Lozano, J. C., Reguera-Useros, J. I., Peláez-Martínez, M. C. and Hardisson de la Torre, A. (2006). Effect of a bacteriocin produced by *Pediococcus acidilactici* against *Listeria monocytogenes* and *Clostridium perfringens* on Spanish raw meat. *Meat Science,* **72**, 57–61.

Nieto-Lozano, J. C., Reguera-Useros, J. I., Peláez-Martínez, M. C., Sacristán-Pérez-Minayo, G., Gutiérrez-Fernández, A. J. and Hardisson de la Torre, A. (2010). The effect of the pediocin PA-1 produced by *Pediococcus acidilactici against Listeria monocytogenes* and *Clostridium perfringens* in Spanish dry-fermented sausages and frankfurters. Food Control, 21, 679–685.

Nilsen, T., Nes, I. F. and Holo, H. (1998). An exported inducer peptide regulates bacteriocin production in *Enterococcus faecium* CTC 492. *Journal of Bacteriology,* **180**, 1848–1854.

Norrung, B. and Buncic, S. (2008). Microbial safety of meat in the European Union. *Meat Science,* **78**, 14–24.

Nychas, G. J. E., Skandamis, P. N., Tassou, C. C. and Koutsoumanis, K. P. (2008). Meat spoilage during distribution. *Meat Science,* **78**, 77–89.

Okereke, A. and Montville, T. J. (1991). Bacteriocin-mediated inhibition of *Clostridium botulinum* spores by lactic acid bacteria at refrigeration and abuse temperatures. *Applied and Environmental Microbiology,* **57**, 3423–3428.

Orsi, R. H., den Bakker, H. C. and Wiedmann, M. (2011). *Listeria monocytogenes* lineages: Genomics, evolution, ecology, and phenotypic characteristics. *International Journal of Medical Microbiology,* **301**, 79–96.

Osés, S., Diez A., Gómez, E., et al. (2015). Control of *Escherichia coli* and *Listeria monocytogenes* in suckling-lamb meat evaluated using microbial challenge tests. *Meat Science,* **110**, 262–269.

Pattanayaiying, R. H-Kittikun, A. and Cutter, C. N. (2015). Incorporation of nisin Z and lauric arginate into pullulan films to inhibit foodborne pathogens associated with fresh and ready-to-eat muscle foods. International Journal of Food Microbiology, **207**, 77–82.

Pawar, D. D., Malik, S. V. S., Bhilegaonkar, K. N. and Barbuddhe, S. B. (2000). Effect of nisin and its combination with sodium chloride on the survival of *Listeria monocytogenes* added to raw buffalo meat mince. *Meat Science,* **56**, 215–219.

Pérez-Rodríguez, F., Castro, R., Posada-Izquierdo, G. D. et al. (2010). Evaluation of hygiene practices and microbiological quality of cooked meat products during slicing and handling at retail. *Meat Science,* **86**, 479–485.

Pérez-Rodríguez, F., van Asselt, E. D., García-Gimeno, R. M., Zurera, G. and Zwietering, M. H. (2007). Extracting additional risk managers information from a risk assessment of *Listeria monocytogenes* in deli meats. *Journal of Food Protection,* **70**, 1137–1152.

Prudêncio, C. V., Vanetti, M. C. D. and Prieto, M. (2015). Tolerance of Salmonella enteric serovar Typhimurium to nisin combined with EDTA is accompanied by changes in cellular composition. *Food Research International,* **69**, 281–288.

Public Health Agency of Canada (PHAC). (2008). Listeria Monocytogenes Outbreak. Available at http://www.phac-aspc.gc.ca/alert-alerte/listeria/listeria_2008-eng.php (accessed October 2009).

Ravyts, F., Barbuti, S., Frustoli, M. A. et al. (2008). Competitiveness and antibacterial potential of bacteriocin-producing starter cultures in different types of fermented sausages. *Journal of Food Protection,* **71**, 1817–1827.

Ray, B. and Bhunia, A. (2014). *Fundamental Food Microbiology*. Boca Raton, FL: CRC Press/Taylor & Francis.

Rivas, F. P., Castro, M. P., Vallejo, M., Marguet, E. and Campos, C. A. (2014). Sakacin Q produced by *Lactobacillus curvatus* ACU-1: Functionality characterization and antilisterial activity on cooked meat surface. *Meat Science,* **97**(4), 475–479.

Rocourt, J. and Cossart, P. (1997). *Listeria monocytogenes*. *In:* Doyle, M. P., Beuchat, L. R. and Montville, T. J. (eds.) *Food Microbiology. Fundamentals and Frontiers*. Washington, DC: ASM Press, pp. 337–352.

Rodgers, S., Peiris, P. and Casadei, G. (2003). Inhibition of nonproteolytic *Clostridium botulinum* with lactic acid bacteria and their bacteriocins at refrigeration temperatures. *Journal of Food Protection,* **66**, 674–678.

Roller, S., Sagoo, S., Board, R. et al. (2002). Novel combinations of chitosan, carnocin and sulphite for the preservation of chilled pork sausages. *Meat Science,* **62**, 165–177.

Rubio, R., Bover-Cid, S., Martín, B., Garriga, M. and Aymerich, T. (2013). Assessment of safe enterococci as bioprotective cultures in low-acid fermented sausages combined with high hydrostatic pressure. *Food Microbiology,* **33**, 158–165.

Samelis, J., Kakouri, A. and Rementzis, J. (2000). Selective effect of the product type and the packaging conditions on the species of lactic acid bacteria dominating the spoilage microbial association of cooked meats at 4°C. *Food Microbiology,* **17**, 329–340.

Samelis, J. and Metaxopoulos, J. (1999). Incidence and principal sources of *Listeria* spp. and *Listeria monocytogenes* contamination in processed meats and a meat processing plant. *Food Microbiology,* **16**, 465–477.

Santiago-Silva, P., Soares, N. F .F., Nóbrega, J. E. Júnior, M. A. W. Barbosa, K. B. F., Volp, A. C. P., Zerdas, E. R. M. A. and Würlitzer, N. J. (2009).Antimicrobial efficiency of film incorporated with pediocin (ALTAÒ 2351) on preservation of sliced ham. Food Control, **20**, 85–89.

Scallan, E., Hoekstra, R. M., Angulo, F. J. et al. (2011). Food borne illness acquired in the United States—Major pathogens. *Emerging Infectious Diseases,* **17**, 7–15.

Scannell, A. G. M., Ross, R. P., Hill, C. and Arendt, E. K. (2000). An effective lacticin biopreservative in fresh pork sausage. *Journal of Food Protection,* **63**, 370–375.

Sobrino-López, A. and Martín-Belloso, O. (2008). Use of nisin and other bacteriocins for preservation of dairy products. *International Dairy Journal,* **18**, 329–343.

Solomakos, N., Govaris, A., Koidis, P. and Botsoglou, N. (2008). The antimicrobial effect of thyme essential oil, nisin and their combination against *Listeria monocytogenes* in minced beef during refrigerated storage. *Food Microbiology,* **25**, 120–127.

Stergiou, V. A., Thomas, L. V. and Adams, M. R. (2006). Interactions of nisin with glutathione in a model protein system and meat. *Journal of Food Protection,* **69**, 951–956.

Stiles, M. E. (1996). Biopreservation by lactic acid bacteria. *Anton van Leeuwenhoek,* **70**, 331–345.

Swaminathan, B. and Gerner-Smidt, P. (2007). The epidemiology of human listeriosis. *Microbes Infections,* **9**, 1236–1243.

Talon, R., Leroy, S., Lebert, I. et al. (2008). Safety improvement and preservation of typical sensory qualities of traditional dry fermented sausages using autochthonous starter cultures. *International Journal of Food Microbiology,* **126**, 227–234.

Teramoto, H., Salaheen, S. and Biswas, D. (2016). Contamination of post-harvest poultry products with multidrug resistant *Staphylococcus aureus* in Maryland-Washington DC metro area. *Food Control,* **65**, 132–135.

Theivendran, S., Hettiarachchy, N. S. and Johnson, M. G. (2006). Inhibition of *Listeria monocytogenes* by nisin combined with grape seed extract or green tea extract in soy protein film coated on turkey frankfurters. *Journal of Food Science,* **71**(2), 39–44.

Thomas, L. V., Clarkson, M. R. and Delves-Broughton, J. (2000). Nisin. In: Naidu, A. S. (ed.) *Natural Food Antimicrobial Systems*. Boca Raton, FL: CRC-Press, 463–524.

Urso, R., Comi, G. and Cocolin, L. (2006a). Ecology of lactic acid bacteria in Italian fermented sausages: Isolation, identification and molecular characterization. *Systematic and Applied Microbiology,* **29**, 671–680.

Urso, R., Rantsiou, K., Cantoni, C., Comi, G. and Cocolin, L. (2006b). Technological characterization of a bacteriocin-producing *Lactobacillus sakei* and its use in fermented sausages production. *International Journal of Food Microbiology,* **110**, 232–239.

Uyttendaele, M., De Troy, P. and Debevere, J. (1999). Incidence of *Listeria monocytogenes* in different types of meat products on the Belgian retail market. *International Journal of Food Microbiology,* **53**(1), 75–80.

Vermeiren, L., Devlieghere, F. and Debevere, J. (2004). Evaluation of meat born lactic acid bacteria as protective cultures for the biopreservation of cooked meat products. *International Journal of Food Microbiology,* **96**, 149–164.

Vesković Moračanin, S. M., Đukić, D. A. and Memiši, N. R. (2014). Bacteriocins produced by lactic acid bacteria—A review. *Acta periodica technologica,* **45**, 271–283.

Violaris, Y., Bridges, O. and Bridges, J. (2008). Small businesses—Big risks: Current status and future direction of HACCP in Cyprus. *Food Control,* **19**, 439–448.

Vorst, K. L., Todd, E. C. D. and Ryser, E. T. (2006). Transfer of *Listeria monocytogenes* during mechanical slicing of turkey, bologna, and salami. *Journal of Food Protection,* **69**, 619–626.

Wilson, P. D. G., Brocklehurst, T. F., Arino, S. et al. (2002). Modelling microbial growth in structured foods: Towards a unified approach. *International Journal of Food Microbiology,* **73**, 275–289.

Wimpenny, J. W. T., Leistner, L., Thomas, L. V., Mitchell, A. J., Katsaras, K. and Peetz, P. (1995). Submerged bacterial colonies within food and model systems: Their growth, distribution and interactions. *International Journal of Food Microbiology,* **28**, 299–315.

Ye, M., Meetoo, H. and Chen, H. (2008). Control of *Listeria monocytogenes* on ham steaks by antimicrobials incorporated into chitosan-coated plastic films. *Food Microbiology,* **25**, 260–268.

Zdolec, N., Hadziosmanovic, M., Kozacinski, L. et al. (2007). Protective effect of *Lactobacillus sakei* in fermented sausages. *Archiv furLebensmittelhygiene,* **58**, 152–155.

Zhang, J., Liu, G., Li, P. and Qu, Y. (2010). Pentocin 31-1, a novel meat-borne bacteriocin and its application as biopreservative in chill-stored tray-packaged pork meat. *Food Control,* **21**, 198–202.

Zhu, M., Du, M., Cordray, J. and UkAhn, D. (2005). Control of *Listeria monocytogenes* contamination in ready-to-eat meat products. *Comprehensive Reviews in Food Science and Food Safety,* **4**, 34–42.



Taylor & Francis
Taylor & Francis Group
http://taylorandfrancis.com

19

Functionalities of Meat Bacterial Starters

Régine Talon and Sabine Leroy

CONTENTS

19.1 Introduction 597
19.2 Bacterial Starter Cultures and Competitiveness 598
19.3 Protective Cultures 599
19.4 Starters and Sensory Quality 600
19.4.1 Texture 601
19.4.2 Color 601
19.4.3 Flavor 602
19.4.3.1 Carbohydrate Catabolism 603
19.4.3.2 Protein Hydrolysis 603
19.4.3.3 Amino Acid Catabolism 604
19.4.3.4 Lipid Hydrolysis 605
19.4.3.5 Fatty Acid Oxidation 605
19.4.3.6 Formation of Esters 606
19.5 Probiotic Cultures 606
19.6 Conclusion 608
References 608

19.1 Introduction

The production of fermented foods is one of the oldest food processing technologies known to man. Of course, these processes were first artisanal in nature. Today, even though some of these traditional processes remain in use, large-scale industrial processes have been developed. The advent of retailing and mass marketing required the availability of products with consistent quality and safety. For many fermented foods, thus for fermented meat products, the characterization of microorganisms responsible for the fermentation, toward the end of the nineteenth century, led to the development of starter cultures. This development had a major impact on the process and contributed to ensuring consistency of the product and reliability of fermentation. Research on starter cultures continued to advance at a very impressive rate and considerable knowledge was developed to manipulate and control these bacteria.

The original and primary purpose of fermentation was to ensure shelf life and microbiological safety of the products. Thus, the research on starter cultures was mainly focused on their protective effect. Then as many preservation technologies were developed, fermented meats were manufactured because of their unique flavor, aroma, and texture. Beside preservation and safeguard as objectives of fermentation, the role of starter cultures in the sensory quality of fermented products was questioned. Finally, other aspects such as wholesomeness and acceptability have become increasingly important and valued features to consumers. In this context, probiotic cultures for production of fermented sausages were investigated.

19.2 Bacterial Starter Cultures and Competitiveness

Most fermented sausages formulated with nitrate and/or nitrite are produced with a starter culture, generally consisting of lactic acid bacteria (LAB) and coagulase negative staphylococci (CNS) (Hammes, 2012; Table 19.1). *Lactobacillus sakei*, *Lactobacillus curvatus*, and *Lactobacillus plantarum* (mainly in Europe) and *Pediococcus pentosaceus* and *Pediococcus acidilactici* (mainly

TABLE 19.1

Most Currently Used Bacteria in Meat Fermentation with Published Genomes

Species	References
Lactobacillus curvatus CRL705	AGBU01000000, Hebert et al. (2012)
Lactobacillus plantarum JDM1	CP001617, Zhang et al. (2009)
L. plantarum ST-III	CP002222, Wang et al. (2011)
L. plantarum NC8	AGRI01000000, Axelsson et al. (2012)
L. plantarum ZJ316	CP004082, Li et al. (2013)
Lactobacillus sakei 23 K	CR936503, Chaillou et al. (2005)
L. sakei LS25	ASTI00000000, McLeod et al. (2013)
Pediococcus acidilactici MA18/5M	AGKB00000000, Barreau et al. (2011)
P. acidilactici D3	AQGT00000000, Sturino et al. (2013)
Pediococcus pentosaceus ATCC 25745	CP000422, Makarova et al. (2006)
P. pentosaceus IE-3	CAHU01000001 to CAHU01000091, Midha et al. (2012)
P. pentosaceus SL4	CP006854, Dantoft et al. (2013)
Staphylococcus carnosus subsp. *carnosus* TM300	AM295250, Rosenstein et al. (2009)
S. carnosus subsp. *utilis* LTH 7013	LAIU00000000, Müller et al. (2015)
Staphylococcus equorum Mu2	CAJL01000001 to CAJL01000030, Irlinger et al. (2012)
S. equorum KS1039	CP013114, Jeong et al. (2016)
Staphylococcus xylosus SMQ-121	CP008724, Labrie et al. (2014)
S. xylosus C2a	LN554884, Vermassen et al. (2014, 2015)

in North America) are the starters most currently used for their fermentative role in sausage manufacturing (Talon et al., 2004). *Staphylococcus xylosus* and *Staphylococcus carnosus* are widely used for their involvement in color and aroma development (Talon et al., 2002). *Staphylococcus equorum* is more and more often mentioned in dry-fermented sausages (Mauriello et al., 2004; Leroy et al., 2010). It is also used as a commercial starter culture. In general, the starters are inoculated at 10^6 viable germs/g of product.

Research on starter cultures continued to advance at a very impressive rate through the twentieth and twenty-first centuries. The sequencing of genomes of some of the species used as starters (Table 19.1), and consequently, the development of postgenomics studies (transcriptomic, proteomic) either *in vitro* or *in situ* have provided basic knowledge on their adaptation to the meat environment and their main functionalities.

These genomic studies have revealed that *L. sakei* have a metabolic repertoire that may contribute to their adaptation to meat including nucleotide scavenging, catabolism of arginine, and the use of heme and nonheme iron sources (Chaillou et al., 2005; Duhutrel et al., 2010; Nyquist et al., 2011; Rimaux et al., 2011, 2012). This species was shown to be able to cope with cold temperatures, salt, changing redox, and oxygen levels (Marceau et al., 2004; Chaillou et al., 2005; Nyquist et al., 2011). Comparative genome analysis revealed that *L. curvatus* CRL705 and *L. sakei* 23K strains were highly similar (Hebert et al., 2012). *L. plantarum* is a versatile species that can use a broad range of fermentable carbon sources (Molenaar et al., 2005). The use of nucleotides as an alternative energy source appears to be a common feature in CNS including *S. carnosus*, *S. xylosus,* and *S. equorum* (Janssens et al., 2014) while the use of arginine either by arginine deiminase or arginase pathway varied among the species as well as the strains (Sànchez Mainar et al., 2014). The ability to cope with osmotic stress is well known for staphylococci. Nine systems for osmoprotectant transport or biosynthesis were identified in the genome of *S. carnosus* (Rosenstein et al., 2009) and the overexpression of genes involved in transport and synthesis of osmoprotectants and Na^+/H^+ extrusion were evidenced in *S. xylosus* in a salted meat model (Vermassen et al., 2016). *S. xylosus* was also shown to be able to cope with nitrosative stress generated by nitrate and nitrite in a meat model by modulating the expression of genes involved in iron homeostasis and antioxidant defense (Vermassen et al., 2014).

19.3 Protective Cultures

The use of bacterial starter cultures with protective effects can enhance the safety of meat products and prevent potential foodborne microbial hazards. Strategies using a biopreservation approach are investigated to "naturally" control the growth of pathogenic and spoilage microorganisms. Rapid growth

and production of acids by bacterial meat starter cultures are important for sensory qualities of fermented sausages and for controlling undesirable bacteria. LAB can inhibit the growth of these microorganisms by a variety of antimicrobial agents such as organic acids, bacteriocins, and the competition for nutrients in the product (Granly Koch, 2004). Organic acids including lactic and acetic acids are produced during the fermentation process. These acids under their undissociated form at low pH exert strong antagonistic effects particularly against Gram-negative bacteria (Holzapfel, 2002).

The main LAB used as meat starter cultures can inhibit pathogenic and spoilage bacteria by bacteriocins production. Bacteriocins are heterogeneous groups of peptides and proteins, and the majority produced by LAB belong to two categories: the lanthionine-containing lantibiotics (class I) and the nonlanthionine-containing lantibiotics (class II) (De Vuyst and Leroy, 2007; Vignolo et al., 2015). *L. sakei* strains can produce several sakacins and lactocin S while *L. curvatus* strains produce sakacin P, curvacin, several curvaticins, and lactocins, which are active against LAB, *Clostridium*, *Listeria monocytogenes,* and *Enterococcus* (Vignolo et al., 2015). *L. plantarum* synthesize plantaricin active against LAB and *Li. monocytogenes* while pediococci produce pediocins with a large spectrum of inhibition against other LAB, *Clostridium*, *Li. monocytogenes, Staphylococcus aureus, Enterococcus,* and propionibacteria (Vignolo et al., 2015). However, the role of bacteriocins in meat products appears to be limited because they are often bound to the matrix, and could be degraded by tissue proteases and inhibited by sodium nitrite and sodium chloride (Verluyten et al., 2003; Leroy et al., 2015). Nevertheless, in various sausages, a 1.5–2.5-log reduction of *Li. monocytogenes* was obtained by the presence of bacteriocin-producing strains belonging to *P. acidilactici*, *L. sakei*, *L. plantarum,* and *L. curvatus* species. These bacteria could be used alone or in combination (Vignolo et al., 2015).

Few works mentioned the production of bacteriocins by CNS and only two concerned CNS used as starter cultures. One strain of *S. xylosus* isolated from Italian sausages not only produced an inhibitory substance active against *Li. monocytogenes* on a solid medium but also in Naples-type sausages in which a 2-log reduction of *Li. monocytogenes* was measured (Villani et al., 1997). One strain of *S. equorum* was found to produce a macrocyclic peptide antibiotic, called micrococcin, which is a substance exhibiting a bacteriostatic effect on a variety of Gram-positive bacteria, and in particular *Li. monocytogenes* (Carnio et al., 2000).

19.4 Starters and Sensory Quality

Sausages are made with minced meat and fat, mixed with salt and spices, often inoculated with starter cultures, stuffed into casings, and then ripened

and dried. During the combined, consecutive, and interactive changes that take place during fermentation and drying, the specific color, texture, and flavor of the sausages are developed. Starter cultures are involved in the development of these different attributes.

19.4.1 Texture

During chopping, salt solubilizes muscle proteins (mainly myosin), which on the drop in pH during fermentation coagulate and form a gel surrounding the fat and meat particles that is stabilized during drying (Demeyer and Toldrá, 2004). A pH of 5.3 is required for coagulation at the often-used salt concentration of 3% (Talon et al., 2004). Acidification by LAB during fermentation produces two opposing effects on gel strength: coagulation and the induction of proteolysis of the myosin by cathepsin D, lowering its contribution to gel strength (Demeyer and Toldrá, 2004).

19.4.2 Color

The typical cured meat color is associated with the formation of the nitrosomyoglobin, which results from a series of reactions involving the formation of nitrogen oxide (NO) and its reaction with myoglobin producing nitrosylated pigments, which yield a red color (Hammes, 2012).

The substrate added to produce NO could be nitrate or nitrite. Nitrite acts as a very reactive oxidant and is reduced to NO immediately after preparation of the sausage mix. The reduction of nitrite to NO is favored by the acidification caused by LAB. The use of nitrate involves bacterial reduction to nitrite carried out by the nitrate reductase of CNS (Sànchez Mainar and Leroy, 2015). There is heterogeneity in the capacity of the CNS to reduce nitrate, but high nitrate reductase activity is found for the strains belonging to the three species used as starters (Mauriello et al., 2004; Gøtterup et al., 2007). Furthermore, *S. carnosus* and *S. xylosus* are able to reduce nitrite in ammonia (Hammes, 2012). The nitrite and nitrate reductases of *S. carnosus* are well characterized. They are encoded by the *nir* and *nar* operons, respectively (Rosenstein et al., 2009). These operons are similar in the *S. xylosus* C2a genome (Vermassen et al., 2014).

Safety considerations in meat fermentation relate to the use of nitrate and nitrite based on their potential to form nitrosamines with carcinogenic property (Hammes, 2012). An alternative to these additives could be the exploitation of the nitric oxide synthase (NOS) activity generating NO from arginine catabolism. NOS-like enzymes were identified from several Gram-positive bacteria (Crane et al., 2010). Up to now, the *nos* gene has been identified in many CNS but the NOS activity has been only characterized in *Staphylococcus aureus* (Sapp et al., 2014). The contribution of the NOS activity of CNS to the color development in meat remains to be demonstrated.

Discoloration of cured meat can be observed by the formation of peroxide. This default can be avoided by the catalase activity of CNS that protects the color (Talon et al., 2004).

19.4.3 Flavor

Flavor is one of the most important properties in sausage. It covers the taste, aroma, and odor of the product, and its perception depends on the texture of the product. A large variety of compounds are likely to contribute to the desired aroma and taste of fermented sausages. Some of them are added to sausage mix such as salt, constituents of spices, and smoke. Others result from the catabolism of carbohydrates, proteins, and lipids from tissue and microbial enzyme reactions and chemical reactions (Figure 19.1). It is still difficult to distinguish between tissue enzymes and microbial enzymes, and

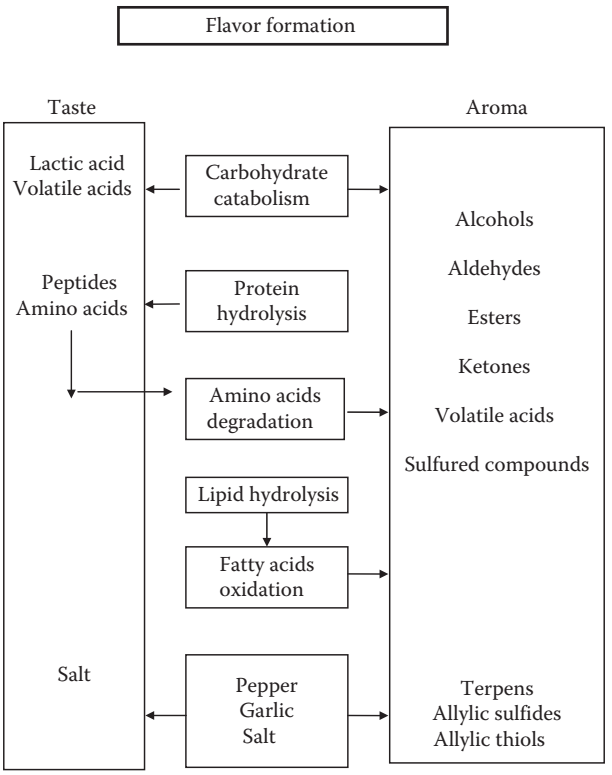


FIGURE 19.1
Formation of important flavor compounds in fermented sausages. (Data from Stahnke, L. H., in Toldrá, F. [ed.], *Research Advances in Quality of Meat and Meat Products*. Trivandrum, Research Signpost, 2002; Talon, R. et al., in Toldrá, F. [ed.], *Research Advances in Quality of Meat and Meat Products*. Trivandrum: Research Signpost, 2002; Flores, M. and Olivares, A., *Handbook of Fermented Meat and Poultry*, 2015, pp. 217–225. Copyright Wiley-VCH Verlag GmbH & Co. KGaA.)

also to determine the origin of certain compounds. (For reviews in this field, see Ravysts et al., 2012; Talon and Leroy, 2014; Cocconcelli and Fontana, 2015; Flores and Olivares, 2015; Leroy et al., 2016.)

19.4.3.1 Carbohydrate Catabolism

The acid taste is an important component of the overall taste of fermented meat products, sought in the northern process, whereas it may be rejected in the southern process. It is positively correlated with lactate and acetate contents (Demeyer and Toldrá, 2004; Lücke, 2000). In 100 g of dry material, lactic acid is present in the range of 0.4–2.8 g in various sausages such as Belgian, Italian, Norwegian, Spanish, and Swedish types (Stahnke, 2002). Many factors influence lactic acid production. The most important factors are temperature, type and amount of carbohydrates, and LAB (Leroy et al., 2015).

L. sakei, L. curvatus, and *L. plantarum*, the most common starters used in meat, produce L- and D-lactate by metabolizing the carbohydrates added to the mixture (glucose, sucrose, etc.) via the homofermentative pathway (Axelsson, 2004). *Pediococcus* also degrades glucose via the homofermentative pathway (Josephsen and Jespersen, 2004). Some lactobacilli such as *L. sakei* ferment pentoses via the heterolactic pathway producing lactate and acetate (Champomier-Vergès et al., 2002).

The genomes of *S. carnosus* and *S. xylosus* are well equipped with functions involved in various sugar degradation pathways such as glucose and ribose occurring in the meat or added to the batter (Rosenstein et al., 2009; Vermassen et al., 2016). *S. xylosus* produced mainly acetate as an end product and simultaneously metabolized glucose and lactate in a meat model supplemented with glucose (Vermassen et al., 2016). Acetate contributes to acidic taste and also plays an important role in sausage aroma by providing a hint of vinegar. In excess, however, it can lead to a pungent, sour taste. This odor is higher in northern than in southern sausages (Talon et al., 2002).

The buttery or dairy product aroma of certain sausages is related to the presence of diacetyl and acetoin, which may result from the metabolism of pyruvate by staphylococci. *S. xylosus* and *S. equorum* produced more diacetyl and acetoin than *S. carnosus* (Sondergaard and Stahnke, 2002). The production of these compounds was dependant on the curing ingredients (Olesen et al., 2004a,b).

19.4.3.2 Protein Hydrolysis

During sausage ripening, both the soluble (sarcoplasmic) and insoluble proteins (myofibrillar) are hydrolyzed into smaller proteins and peptides (Hughes et al., 2002; López et al., 2015). Their breakdown is mainly due to endogenous enzymes but bacteria are involved in this degradation. LAB indirectly contributed to proteolysis by reducing the pH, which increases cathepsin D activity (Fadda et al., 2010). *L. plantarum*, *L. curvatus,* and *L. sakei* have been reported to make a remarkably hydrolytic contribution to the

initial hydrolysis of sarcoplasmic proteins when whole cells, or whole cells combined with cell free extracts (CFEs), are used as an enzyme source (Fadda et al., 2010). The starter cultures (*P. pentosaceus* plus *S. xylosus* or *L. sakei* plus *S. carnosus*) influenced the proteolytic activity and the release of amino acids in sausages (Aro Aro et al., 2010; Hughes et al., 2002).

The degradation of peptides into amino acids in sausage results in microbial and tissue activities, but great differences in the content of amino acids were noticed (Beriain et al., 2000; Aro Aro et al., 2010; Hughes et al., 2002; López et al., 2015). High peptidases and aminopeptidases activities on sarcoplasmic or myofibrillar extracts have been observed in *L. plantarum*, *L. curvatus,* and *L. sakei* strains from meat (Fadda et al., 2010). A comparison between *L. sakei* and *L. curvatus* showed that these two species harbored three similar peptide transport systems and 19 genes encoding peptidases (Freiding et al., 2011). *S. xylosus* grown in a meat model overexpressed genes involved in peptide transport and peptidases (Vermassen et al., 2016).

Peptides and amino acids have an important influence on meat flavor development (Hughes et al., 2002). Hydrophilic peptides are associated with desirable flavor while hydrophobic peptides give rise to off-flavor. Although the intrinsic sensory potential of amino acids is disputed, their role as precursors of aroma compounds is obvious (Leroy et al., 2016).

19.4.3.3 Amino Acid Catabolism

Amino acids can be transformed into amines, ammonia, or various compounds. Some of them have aromatic properties such as methyl aldehydes, -acids, and -alcohols.

Given its contribution to the increase in pH observed during drying, ammonia could also influence the sensory properties of compounds and the metabolic reactions (Flores and Olivares, 2015). Ammonia production is particularly evident in sausages with a long drying phase such as southern-type sausages (Demeyer et al., 2000).

Amino acids, in particular, branched-chain amino acids (leucine, isoleucine, and valine), aromatic amino acids (phenylalanine, tyrosine, and tryptophan), and sulfured amino acids (methionine), are catabolized into aldehydes, alcohols, and acids, which play an important role in acquiring flavor (Stahnke, 2002; Flores and Olivares, 2015). With the exception of dimethyldisufide, which confers a putrid odor, 2- or 3-methyl butanol with fermented fruit odors, 2- or 3-methyl butanal, and 2-methyl propanal with malt and cacao odors and phenyl-alcohol, -aldehyde, -acid with floral and honey notes are important in terms of aroma (Stahnke, 2002; Flores and Olivares, 2015). Acids confer an animal and cheese note on products (Stahnke, 2002; Flores and Olivares, 2015).

The catabolism of amino acids could involve the Strecker reaction that could occur due to the high amino acid content and the low water activity values (Flores and Olivares, 2015). Nonetheless, the production of methyl-

aldehydes, -acids, and -alcohols in sausage is modulated by the starter cultures and, in particular, by staphylococci such as *S. carnosus, S. xylosus,* and *S. equorum* (Sondergaard and Stahnke, 2002; Stravopoulou et al., 2015). The catabolism of branched-chain amino acids by staphylococci is influenced by various parameters such as temperature, salt, nitrate, nitrite, and pH (Olesen and Stahnke, 2003; Olesen et al., 2004a,b; Tjener et al., 2004). The branched-chain amino acid aminotransferase involved in the first step of this catabolism from *S. carnosus* has been characterized (Madsen et al., 2002). The pathway leading to the synthesis of the methyl compounds from the catabolism of leucine has been identified in *S. xylosus* (Beck et al., 2004). *S. xylosus* overexpressed several genes involved in this pathway during its growth in a meat model (Vermassen et al., 2016).

LAB have restricted aromatic potential. Under laboratory conditions, *L. sakei*, *L. plantarum*, *L.curvatus,* and *P. acidilactici* only weakly degrade leucine, mainly into α-ketoisocaproate, a molecule with no odor (Larrouture. et al., 2000). Strains of *L. curvatus* and *L. sakei* lacked known aminotransferases specific for branched-chain amino acids and aromatic amino acids (Freiding et al., 2011).

19.4.3.4 Lipid Hydrolysis

Lipolysis in dry sausages releases free fatty acids with chain length between 16 and 18 carbon atoms and is, to a great extent, of endogenous origin with triglyceride lipases and phospholipases (Talon et al., 2004). Staphylococci could contribute to lipolysis. In *S. xylosus*, two lipases have been identified (Mosbah et al., 2005; Sakinc et al., 2005). Most strains of these species had lipolytic activity on pork fat while few strains of *S. equorum* had this capacity (Mauriello et al., 2004). The strain *S. carnosus* TM300 was devoid of gene coding lipase (Rosenstein et al., 2009) and *S. carnosus* strains appeared unable to hydrolyze pork fat (Casaburi et al., 2005).

Short-chain fatty acids have a taste of sour cheese. However, the longer the chain, the weaker the sensory characteristics are (Flores and Olivares, 2015). In fact, fatty acids are mainly precursors of aroma molecules.

19.4.3.5 Fatty Acid Oxidation

Fatty acid oxidation results in numerous compounds, which belong to six families: alkanes, alkenes, aldehydes, alcohols, ketones, and acids. Although the production of these compounds in sausages is low—in the order of ppm—their low sensory threshold, except for alkanes and alkenes which are odorless, means that they have a real effect (Flores and Olivares, 2015). Oxidation of fatty acids can be either chemical (peroxidation) or enzymatic (β-oxidation).

Peroxidation is affected by many factors such as oxygen content, the presence of pro-oxidative compounds (NaCl, metals) or antioxidative compounds (nitrite, spices), and the amount of unsaturated lipids. Staphylococci via their antioxidant properties contribute to the regulation of oxidation (Talon et al., 2004).

Under laboratory conditions, *S. xylosus* and *S. carnosus* limited the oxidation of linoleic and linolenic unsaturated fatty acids (Talon et al., 2000). The catalase and superoxide dismutase (SOD) activities of *S. carnosus* have been characterized (Barrière et al., 2001a). Mutants deficient in SOD or catalase activity of *S. xylosus* were less efficient than the wild type in limiting the oxidation of unsaturated fatty acids (Barrière et al., 2001b,c). The presence of nitrate and nitrite in a meat model generated a nitrosative stress and *S. xylosus* responded to this stress by the regulation of 12 genes involved in protection and detoxification mechanisms (Vermassen et al., 2014). Among these genes, three encode catalases revealing that the *S. xylosus* strain C2a has three distinct catalases. The *S. carnosus* strain TM300 has two catalases (Rosenstein et al., 2009). Some strains of *S. xylosus* and *S. equorum* have been reported as having two catalases (Blaiotta et al., 2010). In addition, in the *S. xylosus* strain C2a, the genes *ahpC* encoding alkyl hydroperoxide reductase and *trxB* encoding thioredoxin reductase were upregulated (Vermassen et al., 2014). All these genes encode enzymes involved in H_2O_2 detoxification or conferring resistance to other reactive oxygen species (Gaupp et al., 2012).

Methyl ketones have been identified in sausages and could arise from incomplete β-oxidation of fatty acids. In this case, intermediate CoA esters can be freed; they are successively converted into α-ketoacid via thioesterase activity and then into methyl-ketone and secondary alcohol via decarboxylase and dehydrogenase action. Engelvin et al. (2000) have shown that intermediates were freed during the β-oxidation cycle in *S. carnosus*. They also reported thioesterase activity in this strain. Fadda et al. (2003) have highlighted the presence of β-decarboxylase activity in *S. carnosus*. These results suggest that *S. carnosus* may produce ketones via this pathway.

19.4.3.6 Formation of Esters

Esters are present in fermented meat products and their aromatic characteristics contribute to the fruity note of the products (Stahnke, 2002). Most of the esters in sausages are ethyl esters. Their production depends on the presence of ethanol and different acids (from two to eight carbon atoms) as well as on technological factors and microorganisms.

Esters could be of chemical origin as they are found in dry raw ham with a low bacterial count. However, they can also be of bacterial origin. In sausages, esters are associated with the presence of *S. xylosus* or *S. carnosus* strains (Stahnke, 2002).

19.5 Probiotic Cultures

The relationship between certain foods and their health benefits has allowed the development of the concept of functional food as a food or food ingredient

with positive effects on host health. In this context, attention has been directed toward probiotics, which are defined as living microorganisms that beneficially affect the health of the host when ingested in adequate amounts (Arihara, 2015).

Utilization of probiotic bacteria is one of the criteria for the development of healthier meat products. This possibility has been discussed in several papers (Työppönen et al., 2003; Kröckel, 2006; De Vuyst et al., 2008; Arihara, 2015).

The first approach was to consider the probiotic species originating from isolates from the intestinal tract. This ecological niche is the natural habitat for *Bifidobacterium*, *Lactobacillus acidophilus,* and *Lactobacillus rhamnosus* (Arihara, 2015). *Lactobacillus casei* may also be found in the microbiota of the oral cavity, in various fermented foods, and in spoiled foods (Kröckel, 2006). Foremost, these probiotic bacteria must survive in sausages. Indeed, their viability could be affected by various factors including the process, the acidification, the additives, and the competition with starter cultures (De Vuyst et al., 2008). The potentiality of the six probiotic species belonging to the *L. acidophilus* group was investigated for their application in meat fermentation. Among them, *Lactobacillus gasseri* was the most appropriate; it grew in the sausage model and decreased the pH during fermentation, however it was sensitive to sodium chloride and nitrite (Arihara, 2015). *L. rhamnosus* and *Lactobacillus paracasei* subsp. *paracasei* were able to carry out the fermentation as the commercial *L. sakei* starter culture suggesting that intestinal *Lactobacillus* could be used for developing probiotic starter cultures in fermented sausages (Arihara, 2015). Pidcock et al. (2002) showed that nontraditional meat starter (NTMS) cultures survived in salami throughout fermentation and maturation. These NTMS cultures included one strain of *L. acidophilus*, two of *L. paracasei*, one of *Lactobacillus* spp, and one of *Bifidobacterium lactis*. Furthermore, the addition of these NTMS cultures in conjunction with the commercial meat starter increased the safety of Hungarian salami. Similarly, three probiotic strains of *L. rhamnosus* and one of *L. plantarum* were studied for their capacity to act as main fermenting bacteria in sausage manufacturing (Erkkilä et al., 2001a,b). These lactic bacteria decreased the pH from 5.6 to 4.9 or 5.0 and produced a flavor profile similar to that produced by commercial lactic meat starter cultures. Microencapsulation has been shown to protect a probiotic *L. reuteri* strain during sausage manufacturing (Muthukumarasamy and Holley, 2006). From all of these studies, it seems possible to successfully use probiotics as starter cultures for dry-fermented sausages, as there were no significant technological and sensorial differences between the sausages fermented by probiotic or nonprobiotic strains.

A second approach was to consider the LAB used as starters as potential probiotics. Their main requirements are acid and bile tolerance, safety in use, and clinical documentation of health effects (De Vuyst et al., 2008; Arihara, 2015). Considering the criteria of acid and bile tolerance, Erkkilä and Petäjä (2000) screened eight commercial meat starter cultures for their potential probiotic use. One strain of *L. sakei* and one of *P. acidilactici* had the best survival capacities under acidic conditions and high concentration of bile

salts. The selection of potentially probiotic lactobacilli from Greek fermented sausages (Papamanoli et al., 2003) and Italian fermented sausages (Pennacchia et al., 2004) was realized. Twenty-four strains of *L. curvatus* and seven of *L. plantarum* isolated from the Greek products were resistant to 0.3% bile salts. Strains of *L. plantarum* and *L. casei* from Italian sausages were found to be potentially usable in the future as probiotic starter cultures for the manufacture of novel fermented sausages.

The question of usefulness of probiotics in meat products is still in debate. The proof of a beneficial effect should be based on sound studies performed with the probiotics in the meat matrix as it is consumed. Mahoney and Henriksson (2003) investigated if a salami batter inoculated with a meat starter culture, a probiotic (*L. acidophilus*), or a mixture of both reduced the gastrointestinal colonization and virulence of *Li. monocytogenes* in mice. Consumption of salami fermented with the mixture reduced fecal levels of the pathogen by 2.5-log units compared to a reduction of 1.0- and 1.5-log units by the starter and *L. acidophilus*, respectively. The amount of sausage prepared with probiotic that must be ingested to confer the claimed beneficial health effect to consumer is still unknown. Only one study states that a daily consumption of 50 g of sausage, fermented with *L. paracasei*, by human volunteers modulates their immune system (Jahreis et al., 2002). Further studies on the relationship between the consumption of probiotic sausage and human health are needed.

19.6 Conclusion

The exploitation of data on bacterial genomes of technological interest offer new research opportunities by revealing properties that could explain their adaptation to the meat environment, their interaction with a specific substrate, and their competitiveness. This increasing basic knowledge will lead to the development of bacterial meat starter cultures that exhibit protective and probiotic properties as well as achieving the required technological and sensory tasks in the fermented sausages.

References

Arihara, K. (2015). Probiotics. In: Toldrá, F. (ed.) *Handbook of Fermented Meat and Poultry* (2nd Ed.). Chichester, West Sussex, UK: John Wiley & Sons, pp. 155–160.

Aro Aro, J. M., Nyam-Osor, P., Tsuji, K., Shimada, K. I., Fukushima, M. and Sekikawa, M. (2010). The effect of starter cultures on proteolytic changes and amino acid content in fermented sausages. *Food Chemistry,* **119**, 279–285.

Axelsson, L. (2004). Lactic acid bacteria: Classification and physiology. *In:* Salminer, S., Ouwehand, A. and Von Wright, A. (eds.) *Lactic Acid Bacteria: Microbiology and Functional Aspects* (3rd Ed.). New York, NY: Marcel Dekker.

Axelsson, L., Rud, I., Naterstad, K. et al. (2012). Genome sequence of the naturally plasmid-free *Lactobacillus plantarum* strain NC8 (CCUG 61730). *Journal of Bacteriology,* **194**, 2391–2392.

Barreau, G., Tompkins, T. A. and de Carvalho, V. G. (2011). Draft genome sequence of probiotic strain *Pediococcus acidilactici* MA18/5M. *Journal of Bacteriology,* **194**, 901.

Barrière, C., Brückner, R. and Talon, R. (2001b). Characterization of the single superoxide dismutase of *Staphylococcus xylosus*. *Applied and Environmental Microbiology,* **67**, 4096–4104.

Barrière, C., Centeno, D., Lebert, A., Leroy-Sétrin, S., Berdagué, J. L. and Talon, R. (2001c). Roles of superoxide dismutase and catalase of *Staphylococcus xylosus* in the inhibition of linoleic acid oxidation. *FEMS Microbiology Letters,* **201**, 181–185.

Barrière, C., Leroy-Sétrin, S. and Talon, R. (2001a). Characterization of catalase and superoxide dismutase in *Staphylococcus carnosus* 833 strain. *Journal of Applied Microbiology,* **91**, 514–519.

Beck, H. C., Hansen, A. M. and Lauritsen, F. R. (2004). Catabolism of leucine to branched-chain fatty acids in *Staphylococcus xylosus*. *Journal of Applied Microbiology,* **96**, 1185–1193.

Beriain, M. J., Lizaso, G. and Chasco, J. (2000). Free amino acids and proteolysis involved in salchichon processing. *Food Control,* **1**, 41–47.

Blaiotta, G., Fusco, V., Ercolini, D., Pepe, O. and Coppola, S. (2010). Diversity of *Staphylococcus* species strains based on partial *kat* (catalase) gene sequences and design of a PCR-restriction fragment length polymorphism assay for identification and differentiation of coagulase-positive species (*S. aureus, S. delphini, S. hyicus, S. intermedius, S. pseudintermedius,* and *S. schleiferi subsp. coagulans*). *Journal of Clinical Microbiology,* **48**, 192–201.

Carnio, M. C., Holtzel, A., Rudolf, M., Henle, T., Jung, G. and Scherer, S. (2000). The macrocyclic peptide antibiotic micrococcin P(1) is secreted by the food-borne bacterium *Staphylococcus equorum* WS 2733 and inhibits *Listeria monocytogenes* on soft cheese. *Applied and Environmental Microbiology,* **66**, 2378–2384.

Chaillou, S., Champomier-Vergès, M. C., Cornet, M. et al. (2005). Complete genome sequence of the meat-born lactic acid bacterium *Lactobacillus sakei* 23K. *Nature Biotechnology,* **23**, 1527–1533.

Champomier-Vergès, M. C., Chaillou, S., Cornet, M. and Zagorec, M. (2002). *Lactobacillus sakei*: Recent developments and future prospects. *Research in Microbiology,* **153**, 115–123.

Casaburi, A., Blaiotta, G., Mauriello, G., Pepe, O. and Villani, F. (2005). Technological activities of *Staphylococcus* carnosus and *Staphylococcus simulans* strains isolated from fermented sausages. *Meat Science,* **71**, 643–650.

Cocconcelli, P. S. and Fontana, C. (2015). Starter cultures: Bacteria. In: Toldra, F. (ed.) *Handbook of Fermented Meat and Poultry* (2nd Ed.). Chichester, West Sussex, UK: John Wiley & Sons, pp. 117–127.

Crane, B., Sudhamasu, J. and Pantel, B. (2010). Bacterial nitric oxide synthases. *Annual Review of Biochemistry,* **79**, 445–470.

Dantoft, S. H., Bielak, E. M., Seo, J.-G., Chung, M.-J. and Jensen, P. R. (2013). Complete genome sequence of *Pediococcus pentosaceu*s strain SL4. *Genome Announcements,* **1**, e01106–e01113.

Demeyer, D., Raemaekers, M., Rizzo, A. et al. (2000). Control of bioflavour and safety in fermented sausages: First results of a European project. *Food Research International,* **33**, 171–180.

Demeyer, D. and Toldrá, F. (2004). Fermentation. *In:* Devine, C. and Dikeman, M. (eds.) *Encyclopedia of Meat Sciences*. Amsterdam: Elsevier, 467–474.

De Vuyst, L., Falony, G. and Leroy, F. (2008). Probiotics in fermented sausages. *Meat Science,* **80**, 75–78.

De Vuyst, L. and Leroy, F. (2007). Bacteriocins from lactic acid bacteria: Production, purification, and food applications. *Journal of Molecular Microbiology and Biotechnology,* **13**, 194–199.

Duhutrel, P., Bordat, C., Wu, T. D., Zagorec, M., Guerquin-Kern, J. L. and Champomier-Vergès, M. C. (2010). Iron sources used by the non pathogenic lactic acid bacterium *Lactobacillus sakei* as revealed by electron energy loss spectroscopy and secondary-ion mass spectrometry. *Applied and Environmental Microbiology,* **76**, 560–565.

Engelvin, G., Féron, G., Perrin, C., Molle, D. and Talon, R. (2000). Identification of beta-oxidation and thioesterase activities in *Staphylococcus carnosus* 833 strain. *FEMS Microbiology Letters,* **190**, 115–120.

Erkkilä, S. and Petäjä, E. (2000). Screening of commercial meat starter cultures at low pH and in the presence of bile salts for potential probiotic use. *Meat Science,* **55**, 297–300.

Erkkilä, S., Petäjä, E., Eerola, S., Lilleberg, L., Mattila-Sandholm, T. and Suihko, M. L. (2001a). Flavour profiles of dry sausages fermented by selected novel meat starter cultures. *Meat Science,* **58**, 111–116.

Erkkilä, S., Suihko, M. L., Eerola, S., Petaja, E. and Mattila-Sandholm, T. (2001b). Dry sausage fermented by *Lactobacillus rhamnosus* strains. *International Journal of Food Microbiology,* **64**, 205–210.

Fadda, S., Leroy-Sétrin, S. and Talon, R. (2003). Preliminary characterization of β-decarboxylase activities in *Staphylococcus carnosus* 833, a strain used in sausage fermentation. *FEMS Microbiology Letters,* **228**, 143–149.

Fadda, S., López, C. and Vignolo, G. (2010). Role of lactic acid bacteria during meat conditioning and fermentation: Peptides generated as sensorial and hygienic biomarkers. *Meat Science,* **86**, 66–79.

Flores, M. and Olivares, A. (2015). Flavor. In: Toldrá, F. (ed.) *Handbook of Fermented Meat and Poultry* (2nd Ed.). Chichester, West Sussex, UK: John Wiley & Sons, pp. 217–225.

Freiding, S., Gutsche, K. A., Ehrmann, M. A. and Vogel, R. F. (2011). Genetic screening of *Lactobacillus sakei* and *Lactobacillus curvatus* strains for their peptidolytic system and amino acid metabolism, and comparison of their volatilomes in a model system. *Systematic and Applied Microbiology,* **34**, 311–320.

Gaupp, R., Ledala, N. and Somerville, G. A. (2012). Staphylococcal response to oxidative stress. *Frontiers in Cellular and Infection Microbiology,* **2**, 33.

Gøtterup, J., Olsen, K., Knöchel, S., Tjener, K., Stahnke, L. H. and Møller, J. K. S. (2007). Relationship between nitrate/nitrite reductase activities in meat associated staphylococci and nitrosylmyoglobin formation in a cured meat model system. *International Journal of Food Microbiology,* **120**, 303–310.

Granly Koch, A. (2004). Biopreservation. *In:* Devine, C. and Dikeman, M. (eds.) *Encyclopedia of Meat Sciences*. Amsterdam: Elsevier, pp. 68–74.

Holzapfel, W. H. (2002). Appropriate starter culture technologies for small-scale fermentation in developing countries. *International Journal of Food Microbiology,* **75**, 197–212.

Hammes, W. P. (2012). Metabolism of nitrate in fermented meats: The characteristic feature of a specific group of fermented foods. *Food Microbiology,* **29**, 151–156.

Hebert, E. M., Saavedra, L., Taranto, M. P. et al. (2012). Genome sequence of the bacteriocin-producing *Lactobacillus curvatus* strain CRL705. *Journal of Bacteriology,* **194**, 538–539.

Irlinger, F., Loux, V., Bento, P. et al. (2012). Genome sequence of *Staphylococcus equorum* subsp. *equorum* Mu2, isolated from a French smear-ripened cheese. *Journal of Bacteriology,* **194**, 5141–5142.

Jahreis, G., Vogelsang, H., Kiessling, G., Schubert, R., Bunte, C. and Hammes, W. P. (2002). Influence of probiotic sausage (*Lactobacillus paracasei*) on blood lipids and immunological parameters of healthy volunteers. *Food Research International,* **35**, 133–138.

Janssens, J., Van de Mijnsbrugge, A., Sànchez Mainar, M., Bazarini, T., De Vuyst, L. and Leroy, F. (2014). The use of nucleosides and arginine as alternative energy sources by coagulase negative staphylococci in view of meat fermentation. *Food Microbiology,* **39**, 53–60.

Jeong, D. W., Na, H., Ryu, S. and Lee, J. H. (2016). Complete genome sequence of *Staphylococcus equorum* KS1039 isolated from Saeu-jeotgal, Korean high-salt-fermented seafood. *Journal of Biotechnology,* **219**, 88–89.

Hughes, M. C., Kerry, J. P., Arendt, E. K., Kenneally, P. M., McSweeney, P. H. H. and O'Neill, E. E. (2002). Characterization of proteolysis during the ripening of semi-dry fermented sausages. *Meat Science,* **62**, 205–216.

Josephsen, J. and Jespersen, L. (2004). Starters cultures and fermented products. *In:* Hui, Y. H., Meunier-Goddik, L., Hansen, A. S. et al. (eds.) *Handbook of Food and Beverage Fermentation Technology*. New York, NY: Marcel Dekker, pp. 23–49.

Kröckel, L. (2006). Use of probiotic bacteria in meat products. *Fleischwirtschaft,* **86**, 109–113.

Labrie, S. J., El Haddad, L., Tremblay, D. M. et al. (2014). First complete genome sequence of *Staphylococcus xylosus*, a meat starter culture and a host to propagate *Staphylococcus aureus* phages. *Genome Announcements,* **2**, e00671–e00714.

Larrouture, C., Ardaillon, V., Pépin, M. and Montel, M. C. (2000). Ability of meat starter cultures to catabolize leucine and evaluation of the degradation products by using an HPLC method. *Food Microbiology,* **17**, 563–570.

Leroy, S., Giammarinaro, P., Chacornac, J. P., Lebert, I. and Talon, R. (2010). Biodiversity of indigenous staphylococci of naturally fermented dry sausages and manufacturing environments of small-scale processing units. *Food Microbiology,* **27**, 249–301.

Leroy, F., Goudman, T. and De Vuyst, L. (2015). The influence of processing parameters on starter cultures performance. In: Toldrá, F. (ed.) *Handbook of Fermented Meat and Poultry* (2nd Ed.). Chichester, West Sussex, UK: John Wiley & Sons, pp. 169–175.

Leroy, S., Vermassen, A. and Talon, R. (2016). *Staphylococcus*: Occurrence and properties. In: Cabalerro, B., Finglas, P. M. and Toldrá, F. (eds.) *Encyclopedia of Food and Health*, vol. 5. Oxford: Academic Press, pp. 140–145.

Li, X., Gu, Q., Lou, X. et al. (2013). Complete genome sequence of the probiotic *Lactobacillus plantarum* strain ZJ316. *Genome Announcements,* **1**, e00094–e00113.

López, C., Bru, E., Vignolo, G. and Fadda, S. (2015). Identification of small peptides arising from hydrolysis of meat proteins in dry fermented sausages. *Meat Science,* **104**, 20–29.

Lücke, F. K. (2000). Utilization of microbes to process and preserve meat. *Meat Science,* **56**, 105–115.

Madsen, S. M., Beck, H. C., Ravn, P., Vrang, A., Hansen, A. M. and Israelsen, H. (2002). Cloning and inactivation of a branched-chain-amino-acid aminotransferase gene from *Staphylococcus carnosus* and characterization of the enzyme. *Applied and Environmental Microbiology,* **68**, 4007–4014.

Mahoney, M. and Henriksson, A. (2003). The effect of processed meat and meat starter cultures on gastrointestinal colonization and virulence of *Listeria monocytogenes* in mice. *International Journal of Food Microbiology,* **84**, 255–261.

Makarova, K., Slesarev, A., Wolf, Y. et al. (2006). Comparative genomics of the lactic acid bacteria. *Proceedings of the National Academy of Sciences,* **103**, 15611–15616.

Marceau, A., Zagorec, M., Chaillou, S., Méra, T. and Champomier-Vergès, M. C. (2004). Evidence for the involvement of at least six proteins in *Lactobacillus sakei* adaptation to cold temperature and addition of NaCl. *Applied and Environmental Microbiology,* **70**, 7260–7268.

Mauriello, G., Casaburi, A., Blaiotta, G. and Villani, F. (2004). Isolation and technological properties of coagulase negative staphylococci from fermented sausages of Southern Italy. *Meat Science,* **67**, 149–158.

McLeod, A., Brede, D. A., Rud, I. and Axelsson, L. (2013). Genome sequence of *Lactobacillus sakei subsp. sakei* LS25, a commercial starter culture strain for fermented sausage. *Genome Announcements,* **1**, e00475–e00513.

Midha, S., Ranjan, M., Sharma, V. et al. (2012). Genome sequence of *Pediococcus pentosaceus* strain IE-3. *Journal of Bacteriology,* **194**, 4468.

Molenaar, D., Bringel, F., Schuren, F. H., de Vos, W. M., Siezen, R. J. and Kleerebezem, M. (2005). Exploring *Lactobacillus plantarum* genome diversity by using microarrays. *Journal of Bacteriology,* **187**, 6119–6127.

Mosbah, H., Sayari, A., Mejdoub, H., Dhouib, H. and Gargouri, Y. (2005). Biochemical and molecular characterization of *Staphylococcus xylosus* lipase. *Biochemica Biophysica Acta General Subjects,* **1723**, 282–291.

Müller, A., Huptas, C., Wenning, M., Schmidt, H. and Weiss, A. (2015). Draft genome sequence of *Staphylococcus carnosus subsp. utilis* LTH 7013, isolated from South Tyrolean Ham. *Genome Announcements,* **3**, e00456–e00515.

Muthukumarasamy, P. and Holley, R. A. (2006). Microbial and sensory quality of dry fermented sausages containing alginate-microencapsulated *Lactobacillus reuteri*. *International Journal of Food Microbiology,* **111**, 164–169.

Nyquist, O. L., McLeod, A., Brede, D. A., Snipen, L., Aakra, Å. and Nes, I. F. (2011). Comparative genomics of *Lactobacillus sakei* with emphasis on strains from meat. *Molecular Genetics and Genomics,* **285**, 297–311.

Olesen, P. T., Meyer, A. S. and Stahnke, L. (2004a). Generation of flavour compounds in fermented sausages—The influence of curing ingredients, *Staphylococcus* starter culture and ripening time. *Meat Science,* **66**, 675–687.

Olesen, P. T. and Stahnke, L. (2003). The influence of precultivation parameters on the catabolism of branched-chain amino acids by *Staphylococcus xylosus* and *Staphylococcus carnosus*. *Food Microbiology,* **20**, 621–629.

Olesen, P. T., Stahnke, L. and Talon, R. (2004b). Effect of ascorbate, nitrate and nitrite on the amount of flavour compounds produced from leucine by *Staphylococcus xylosus* and *Staphylococcus carnosus*. *Meat Science,* **68**, 193–200.

Papamanoli, E., Tzanetakis, N., Litopoulou-Tzanetaki, E. and Kotzekidou, P. (2003). Characterization of lactic acid bacteria isolated from a Greek dry-fermented sausage in respect of their technological and probiotic properties. *Meat Science,* **65**, 859–867.

Pennacchia, C., Ercolini, D., Blaiotta, G., Pepe, O., Mauriello, G. and Villani, F. (2004). Selection of *Lactobacillus* strains from fermented sausages for their potential use as probiotics. *Meat Science,* **67**, 309–317.

Pidcock, K., Heard, G. M. and Henriksson, A. (2002). Application of nontraditional meat starter cultures in production of Hungarian salami. *International Journal of Food Microbiology,* **76**, 75–81.

Ravysts, F., De Vuyst, L. and Leroy, F. (2012). Bacterial diversity and functionalities in food fermentations. *Engineering in Life Sciences,* **12**, 356–367.

Rimaux, T., Rivière, A., Illeghems, K., Weckx, S., De Vuyst, L. and Leroy, F. (2012). Expression of arginine deiminase pathway genes in *Lactobacillus sakei* is strain dependant and is affected by the environmental pH. *Applied and Environmental Microbiology,* **78**, 4874–4883.

Rimaux, T., Vrancken, G., Vuylsteke, B., De Vuyst, L. and Leroy, F. (2011). The pentose moiety of adenosine and inosine is an important energy source for the fermented-meat starter culture *Lactobacillus sakei* CTC 494. *Applied and Environmental Microbiology,* **77**, 6539–6550.

Rosenstein, R., Nerz, C., Biswas, L. et al. (2009). Genome analysis of the meat starter culture bacterium *Staphylococcus carnosus* TM300. *Applied and Environmental Microbiology,* **45**, 811–822.

Sànchez Mainar, M. and Leroy, F. (2015). Process-driven bacterial community dynamics are key to cured meat colour formation by coagulase negative staphylococci via nitrate reductase or nitric oxide synthase activities. *International Journal of Food Microbiology,* **212**, 60–66.

Sànchez Mainar, M., Weckx, S. and Leroy, F. (2014). Coagulase negative staphylococci favour arginine conversion into ornithine despite a widely present potential for nitric oxide synthase activity. *Applied and Environmental Microbiology,* **80**, 7741–7751.

Sapp, A. M., Mogen, A. B., Almand, E. A. et al. (2014). Contribution of the nos-pdt operon to virulence phenotypes in methicillin-sensitive *Staphylococcus aureus*. *PLOS ONE,* **9**(10), e108868. doi:10.1371/journal.pone.0108868.

Sakinc, T., Woznowski, M., Ebsen, M. and Gatermann, S. G. (2005). The surface associated protein of *Staphylococcus saprophyticus* is a lipase. *Infection and Immunity,* **73**, 6419–6428.

Sondergaard, A. K. and Stahnke, L. (2002). Growth and aroma production by *Staphylococcus xylosus, S. carnosus* and *S. equorum:* A comparative study in model systems. *International Journal of Food Microbiology,* **75**, 99–109.

Stahnke, L. H. (2002). Flavour formation in fermented sausage. *In:* Toldrá, F. (ed.) *Research Advances in Quality of Meat and Meat Products*. Trivandrum, India: Research Signpost, pp. 193–223.

Stravopoulou D. A., Borremans, W., De Vuyst, L., De Smet, S. and Leroy, F. (2015). Amino acid conversions by coagulase negative staphylococci in a rich medium: Assessment of inter- and intraspecies heterogeneity. *International Journal of Food Microbiology,* **212**, 34–40.

Sturino, J. M., Rajendran, M. and Altermann, E. (2013). Draft genome sequence of the pediocin-encoding biopreservative and biocontrol strain *Pediococcus acidilactici* D3. *Genome Announcements,* **1**, e00208–e00213.

Talon, R., Leroy-Sétrin, S. and Fadda, S. (2002). Bacterial starters involved in the quality of fermented meat products. *In:* Toldrá, F. (ed.) *Research Advances in Quality of Meat and Meat Products*. Trivandrum, India: Research Signpost, pp. 175–191.

Talon, R., Leroy-Sétrin, S. and Fadda, S. (2004). Dry fermented sausages. *In:* Hui, Y. H., Meunier-Goddik, L., Hansen, A. S. et al. (eds.) *Handbook of Food and Beverage Fermentation Technology*. New York, NY: Marcel Dekker, pp. 397–416.

Talon, R. and Leroy, S. (2014). Fermented meat products and the role of starter cultures. In: Batt, C. A. and Tortorello, M. L. (eds.) *Encyclopedia of Food Microbiology*, vol. 1. Oxford: Elsevier, Academic Press, pp. 870–874.

Talon, R., Walter, D. and Montel, M. C. (2000). Growth and effect of staphylococci and lactic acid bacteria on unsatured free fatty acid. *Meat Science,* **54**, 41–47.

Tjener, K., Stahnke, L. H., Andersen, L. and Martinussen, J. (2004). The pH-unrelated influence of salt, temperature and manganese on aroma formation by *Staphylococcus xylosus* and *Staphylococcus carnosus* in a fermented meat model system. *International Journal of Food Microbiology,* **97**, 31–42.

Työppönen, S., Petäjä, E. and Mattila-Sandholm, T. (2003). Bioprotectives and probiotics for dry sausages. *International Journal of Food Microbiology,* **83**, 233–244.

Verluyten, J., Messens, W. and De Vuyst, L. (2003). The curing agent sodium nitrite, used in the production of fermented sausages, is less inhibiting to the bacteriocin-producing meat starter culture *Lactobacilluscurvatus* LTH 1174 under anaerobic conditions. *Applied and Environmental Microbiology*, 69, 3833–3839.

Vermassen, A., de La Foye, A., Loux, V., Talon, R. and Leroy, S. (2014). Transcriptomic analysis of *Staphylococcus xylosus* in the presence of nitrate and nitrite in meat reveals its response to nitrosative stress. *Frontiers in Microbiology,* **5**, 691, 1–15.

Vermassen, A., Dordet-Frisoni, E., de La Foye, A. et al. (2016). Adaptation of *Staphylococcus xylosus* to nutrients and osmotic stress in a salted meat model. *Frontiers in Microbiology,* **7**, 87, 1–17.

Vignolo, G., Castelano, P. and Fadda, S. (2015). Bioprotective cultures. *In:* Toldrá, F. (ed.) *Handbook of Fermented Meat and Poultry* (2nd Ed.). Chichester, West Sussex, UK: John Wiley & Sons, pp. 129–137.

Villani, F., Sannino, L., Moschetti, G. et al. (1997). Partial characterization of an antagonistic substance produced by *Staphylococcus xylosus* 1E and determination of the effectiveness of the producer strain to inhibit *Listeria monocytogenes* in Italian sausages. *Food Microbiology,* **14**, 555–566.

Wang, Y., Chen, C., Ai, L. et al. (2011). Complete genome sequence of the probiotic *Lactobacillus plantarum* ST-III. *Journal of Bacteriology,* **193**, 313–314.

Zhang, Z. Y., Liu, C., Zhu, Y. Z. et al. (2009). Complete genome sequence of *Lactobacillus plantarum* JDM1. *Journal of Bacteriology,* **191**, 5020–5021.

20

Modified Atmosphere Packaging

Joseph G. Sebranek and Terry A. Houser

CONTENTS

20.1 Introduction 616
20.2 Definitions 617
20.3 History 618
20.4 Purposes 619
20.5 Applications 620
20.6 Gases Used in Map Systems 620
20.6.1 Carbon Dioxide 621
20.6.2 Nitrogen 623
20.6.3 Oxygen 624
20.6.4 Carbon Monoxide 625
20.6.5 Dynamic Headspace Changes in MAP Systems 626
20.7 Product Characteristics Following Map Applications 627
20.7.1 Fresh (Raw) Meat 627
20.7.1.1 High-Oxygen MAP 627
20.7.1.2 High-Carbon-Dioxide MAP 629
20.7.1.3 MAP with Carbon Monoxide 629
20.7.2 Cooked and Cured-and-Cooked Meat Products 629
20.8 Comparisons of Map with VP for Meat and Poultry 630
20.8.1 Vacuum versus MAP for Fresh Meat 630
20.8.2 Vacuum versus MAP Packaging of Cooked and Cured-and-Cooked Meat Products 631
20.8.3 Effects of High-Carbon-Dioxide MAP 632
20.9 Packaging Films and Film Properties 632
20.9.1 Exterior Film Materials 633
20.9.1.1 Nylon 633
20.9.1.2 Polyester 633
20.9.1.3 Polypropylene 633
20.9.2 Barrier Film Materials 633
20.9.2.1 Ethylene Vinyl Alcohol 633
20.9.2.2 Polyvinylidene Chloride 634
20.9.3 Sealant Materials 634
20.9.3.1 Polyethylenes 634
20.9.3.2 Ethylene Vinyl Acetate 634

20.9.4 Rigid Materials 634
20.9.4.1 Polyvinyl Chloride 634
20.9.4.2 Polystyrene 634
20.10 Effects of MAP on Pathogens in Meat and Poultry 635
20.10.1 *Salmonella* spp. 635
20.10.2 *Yersinia enterocolitica* 635
20.10.3 *Escherichia coli* O157:H7 635
20.10.4 *Campylobacter jejuni* 636
20.10.5 *Staphylococcus aureus* 636
20.10.6 *Clostridium botulinium* 636
20.10.7 *Clostridium perfringens* 637
20.10.8 *Bacillus cereus* 637
20.10.9 *Listeria monocytogenes* 637
20.11 MAP as a Component in the Hurdle Concept 638
20.12 AP Components 638
20.13 Leakers and Package Integrity 640
20.14 Regulatory Issues 640
20.15 Conclusions 641
References 642

20.1 Introduction

Modified atmosphere packaging (MAP) for meat and poultry products is a food preservation concept that is far from new. However, the development of plastic films in the 1960s (Siegel, 2010a) made it possible to easily and cheaply enclose meat products in clear films and to incorporate preservative gases with the closed atmosphere to improve product shelf life and appearance. This has allowed the successful commercialization of MAP. Prior to the development of flexible films, carbon dioxide (CO_2) gas was recognized as a preservative agent for meat and poultry for well over 100 years (Rao and Sachindra, 2002), and consequently, use of carbon dioxide gas in MAP has been studied extensively (Brooks, 1933; Clark and Lentz, 1969; Gill and Tan, 1980; McMillin, 2008). Various other gases including nitrogen, oxygen, and carbon monoxide (CO) have been studied more recently as MAP technology for meat and poultry products has continued to develop (Kropf, 2004). The current use of MAP for commercial applications has become commonplace because more and more meat and poultry products are processed in centralized facilities followed by transportation over increasingly long distances. As a result, it has become critical for the industry to increase the shelf life of meat and poultry products as much as possible in order to consistently deliver wholesome, high-quality products to consumers. Consumers demand, and have come to expect, fresh-like, high-quality food products, and the role of packaging systems such as MAP has become increasingly important to meet

consumer expectations. New developments in flexible film properties and new technology for gas handling and packaging equipment have facilitated the development of an increasingly sophisticated body of scientific knowledge about MAP systems.

There are several distinct advantages to MAP technology for meat and poultry products. Use of gases such as carbon dioxide will slow the growth of many microorganisms and, therefore, extend the microbiological shelf life of fresh (raw) meat products. Gases can also be used to slow some of the chemical and biochemical processes that are responsible for product deterioration, and this will also contribute to improved shelf life (Summo et al., 2016; Bao and Ertbjerg, 2015).

One of the recent developments in MAP technology that has contributed to further improvements in shelf life for meat and poultry is the case-ready option for retail packages. Retail-ready products that are packaged in centralized facilities under controlled, highly sanitary conditions will have a reduced number of contaminating microorganisms, and because there is no opportunity for subsequent contamination, bacterial numbers will remain low for a longer period of time. In addition to the advantage of time for transport over longer distances, increased shelf life typically means less wasted products at the retail level because there are less throwaways due to spoilage. MAP also offers a significant opportunity to improve product appearance. Attractive meat color, for example, can be maintained longer and even dramatically improved in some cases, with MAP gases. Package purge (free water inside the package) is reduced by MAP compared with vacuum, and product appearance is improved as a result. Finally, the use of trays and rigid containers to provide a headspace for gases also offers opportunity for attractive visual displays of the products inside the packages.

There are also some potential disadvantages to MAP systems. Packaging costs may be greater than other systems, though a longer product shelf life and less waste can more than pay the difference. Specialized equipment and worker training may also be necessary and, if so, represent increased time and money commitments though, again, the return on these investments is most often positive.

20.2 Definitions

MAP has been defined as a process whereby a perishable product is placed in a barrier film package, air is removed by vacuum or flushing, and the package is filled with a predetermined gas or mixture of gases with a composition different than air, followed by sealing of the package (Kropf, 2004; McMillin, 2008). Simply put, this means that MAP is a

packaging technique that utilizes an atmosphere modified to be different from air. It is important to note that MAP atmospheres for meat and poultry, particularly raw meat and poultry, are dynamic and will change with time. The gas or gas mixture used in the packages will change as a result of product and microbial metabolism, absorption of gases by the product and, in some cases, by limited diffusion of gases through the barrier film. Controlled atmosphere packaging (CAP), on the other hand, is a similar packaging concept but one in which the gas atmosphere is kept relatively constant during the package life. For meat and poultry, this usually means very complete evacuation of the package, very high-barrier packaging film, and a means of maintaining the gas composition, often by utilizing a compound or component that will slowly generate the desired gases to maintain the target composition. Active packaging (AP) is yet another packaging concept in which the package actively modifies conditions during storage. Examples of this concept include packages with oxygen, odor or moisture absorbers, carbon dioxide emitters, and packaging films that release antimicrobial compounds and other substances during storage.

Vacuum packaging (VP), commonly used for fresh, raw meat and poultry, is simply the removal of air prior to sealing the product in a barrier film. However, in reality, for fresh meat this is a form of MAP because muscle and microbial metabolism will utilize residual oxygen to produce carbon dioxide and the net result is a modified atmosphere that achieves significant shelf-life extension due to both decreased oxygen and increased carbon dioxide concentrations. MAP is distinguished from VP by the headspace that allows introduction of a much larger volume of gases to the package, and by elimination of the physical pressure on the product that occurs with vacuum.

20.3 History

The use of carbon dioxide as a preservative for meat was suggested as early as in 1882 and was further developed for long-range shipments of meat from Australia and New Zealand to Great Britain in the 1930s (Dixon and Kell, 1989). By the 1950s, researchers were investigating the relationships between carbon dioxide concentrations, product shelf life, and product quality. It was determined that fresh meat was likely to discolor when high concentrations of carbon dioxide were used, and recommendations of an upper limit of about 20%–25% were common for red meat species (beef, pork, and lamb). Nitrogen was typically added to exclude oxygen in atmospheres that included increased carbon dioxide. In the 1970s and 1980s, the use of oxygen in MAP to achieve attractive meat color was studied, and it was demonstrated that high oxygen levels

(40% or more) would help to overcome the discoloration of high carbon dioxide concentration (Bartkowski et al., 1982). The combination has been shown to achieve an improved color and shelf life but there have been concerns for oxidized odor and taste as a result of the high-oxygen atmosphere (Kropf, 2004; Bao and Ertbjerg, 2015). More recently, use of carbon monoxide for MAP packaging of fresh meat has been commercialized. The U.S. Food and Drug Administration (FDA) approved master-bag packaging with 0.4% carbon monoxide for fresh meat in 2002 (U.S. FDA, 2002), and in 2004, extended this approval to retail case-ready packaging (U.S. FDA, 2004). However, approval of carbon monoxide is not universal around the world with only Australia and New Zealand in addition to the United States allowing its use.

20.4 Purposes

The primary purpose of MAP systems for meat and poultry products is to achieve a longer shelf life in order to meet the demands of distribution over increasingly greater distances. Improved shelf life for distribution can be achieved in two general ways: first, by packaging of large primal cuts in either vacuum or MAP for transportation, followed by fabrication and repackaging at or near the retail location, or second, by MAP packaging of fully case-ready units which are then shipped to the retail location. A variation of the case-ready packaging approach is to use a large master package with MAP technology to enclose several case-ready units which are then removed from the master package for retail display. In each case, shelf life is improved over simple overwrap packaging. Shelf life, however, is not a simple issue. The predominant concern for shelf life is bacterial growth, which is most often the limiting factor. However, an attractive color is also a critical component in the determination of shelf life and products must be capable of providing an attractive color for retail display. For fresh, raw meat, desirable color may be developed and retained by the packaging system during distribution or may be allowed to develop later in display. These two approaches require very different packaging systems in order to achieve the same color end point for fresh meat. For cooked and for cured-and-cooked meat products, packaging is more critical for color preservation than for color development. Consequently, MAP systems for these two types of cooked products are generally similar. In addition to reduced microbial growth and extended color life, shelf life also includes product quality attributes such as odor, flavor, and texture. Minimal change in these attributes from what is typical for freshly harvested or further processed products is an important objective of MAP systems.

Finally, MAP systems have been studied both for concerns about, and contributions to, product safety. There has been concern in the past that suppression of spoilage microorganisms in anaerobic conditions might result in growth of pathogens, especially in case of temperature abuse. More recently, MAP systems have been studied as a means of improving the impact of various other product preservation treatments. This "hurdle concept" would mean that MAP might be most valuable for improving product safety when combined with other antimicrobial treatments.

20.5 Applications

Applications of MAP systems for meat and poultry products include use of a variety of gases, depending on the product being packaged. In addition, gas blends in a variety of ratios are also utilized to meet different product needs. For example, fresh meat primals may be packaged in a blend of carbon dioxide and nitrogen because at least 20%–25% carbon dioxide is necessary to impact bacterial growth and extend shelf life. For retail display of fresh meat, however, MAP packaging may include high levels of oxygen (up to 80%) with carbon dioxide or sometimes nitrogen for the balance. The purpose of oxygen is color development. Carbon monoxide at 0.3%–0.5% may also be included, where permitted, for color development of fresh meat and, in this case, because color is more stable with carbon monoxide, carbon dioxide can be included at greater concentrations to improve microbial inhibition. Nitrogen gas is commonly used at 100% in MAP applications for cooked and cured-and-cooked meat products where the microbial load is very low and exclusion of oxygen is the most critical concern for shelf life. Further, MAP systems can take the form of a master pack (large package overwrap of several smaller packages) or stand-alone small units (typically retail-ready packages) (Siegel, 2010b). The master-pack approach typically utilizes a high-barrier bag for the outer package, which contains the desired atmosphere, and films with relatively high permeability for the smaller units. The case-ready pack approach will use high-barrier films for the final package in order to retain the MAP gas mixture, which is placed inside the package.

20.6 Gases Used in Map Systems

The three most common gases used for MAP are carbon dioxide, nitrogen, and oxygen. All of these are present in the air that is removed from the

package initially, but the ratio of these gases is considerably different in MAP applications compared to the ambient atmosphere. Air is composed of about 0.03% carbon dioxide, 78% nitrogen, and 21% oxygen. In MAP systems, however, ambient air is typically replaced by gas mixtures that are much higher in one or more of these gases.

In addition to these three gases, carbon monoxide at levels of 0.3%–0.5% has been used in some MAP systems for fresh meat because of the unique effects that carbon monoxide has on the formation and stability of an attractive red meat color. The noble gases (helium, argon, xenon, and neon) have also been studied for use in MAP systems because they are very inert and serve well as filler gases. These gases are used in some MAP systems for food products (Mullan and McDowell, 2003; Heinrich et al., 2016). However, there is no scientific advantage to the noble gases over the use of nitrogen for meat and poultry, and there is very little, if any, use of these gases for meat and poultry packaging.

20.6.1 Carbon Dioxide

Carbon dioxide is a colorless gas with a slightly pungent odor (Mullan and McDowell, 2003). The gas dissolves readily in water and will produce carbonic acid (H_2CO_3) in solution (Jakobsen and Bertelsen, 2002), reducing the solution pH. A pH change in meat held under a high-carbon-dioxide atmosphere is typically observed despite the relatively high buffering capacity of meat.

The centerpiece of MAP systems for fresh meat has long been carbon dioxide because of the ability of this gas to inhibit a wide range of microorganisms. Carbon dioxide is most effective for inhibition of gram-negative bacteria that grow rapidly on fresh meat. Consequently, carbon dioxide is considered the primary antimicrobial agent in MAP systems. Carbon dioxide has been shown to increase both the lag phase of growth and the generation time of affected microorganisms, but the mechanism by which carbon dioxide achieves microbial inhibition is not entirely clear. Certainly, the replacement of oxygen by carbon dioxide will help to suppress growth of aerobic organisms. However, it has been most frequently suggested that the primary antimicrobial effect of carbon dioxide is due to its ability to penetrate bacterial membranes and alter the interior pH of cells, thus affecting cellular metabolic processes (Dixon and Kell, 1989). The effect of carbon dioxide on meat pH is well recognized and may, by itself, contribute to the inhibition of microbial growth that has been observed. Even though the amount of carbonic acid formed from carbon dioxide is relatively small (~2%) at the normal pH range of meat, it has been reported that meat pH can decline as much as 0.35 pH units in a carbon dioxide atmosphere (Daniels et al., 1985; Tan and Gill, 1982). In addition to the pH effect, it has also been reported that carbon dioxide inhibited substrate uptake by microbial cells. Substrate limitation is another potential explanation of the inhibitory effect of carbon

dioxide on bacteria (Farber, 1991). Other theories on the antimicrobial action of carbon dioxide include the direct inhibition of enzymes and changes in the basic properties of proteins (Dixon and Kell, 1989). While the mechanism of action of carbon dioxide that results in bacterial inhibition is not entirely understood, it is clear that carbon dioxide is an effective antimicrobial agent.

The effectiveness of carbon dioxide as an antimicrobial agent is usually considered to be a function of the concentration of the gas in the package headspace but several researchers have suggested that the concentration of carbon dioxide in the product may be more indicative of inhibitory effectiveness (Devlieghere et al., 2001; Jakobsen and Bertelsen, 2004). The effectiveness of carbon dioxide atmospheres on bacterial growth has been reported to be greater for products with low initial bacterial contamination (Gill and Tan, 1980; Krause et al., 2003). This may be the result of carbon dioxide extending the lag phase of bacterial growth for a bacteriostatic effect rather than a bacteriocidal effect.

Carbon dioxide is highly soluble in meat and aqueous solutions. The solubility of carbon dioxide in water is about 30 times greater than the solubility of oxygen and about 60 times greater than the solubility of nitrogen (Gill, 1988). Solubility in the water phase means that carbon dioxide dissolves readily in lean meat tissue. Because carbon dioxide has a linear molecular structure (O=C=O) with little polarity, the gas is even more soluble in nonpolar solvents than in water. Consequently, carbon dioxide is also readily soluble in fats, and is more soluble in fats than oxygen or nitrogen. Thus, carbon dioxide is highly soluble in both lean and fat tissues of meat and poultry products. The solubility of carbon dioxide gas in meat is great enough that when a large volume of the gas is used in meat package headspace, the gas concentration in the headspace will decline very significantly and will continue to do so until a product saturation point or equilibrium is reached (Daniels et al., 1985). The amount of gas absorbed can be great enough to cause collapse of the package. Typically, package collapse results in an unattractive appearance and can be so extreme that the package appears to have been sealed under vacuum; consequently, it is recommended that the amount of carbon dioxide used in MAP for fresh meat be applied in the amount that will not collapse the package. Including an inert, filler gas such as nitrogen is effective for preventing package collapse. However, several factors can influence carbon dioxide solubility in meat tissue. For example, fat content, water content, pH, and temperature are product factors that affect carbon dioxide absorption (Gill, 1988; Jakobsen and Bertelsen, 2002; Mullan and McDowell, 2003; Zhao et al., 1995) while gas partial pressure and the ratio of headspace volume-to-meat volume are package factors that are important.

The solubility of carbon dioxide increases with decreasing temperature and it has been noted that the antimicrobial activity of carbon dioxide is considerably greater at temperatures below 10°C, compared with temperatures of 15°C or higher (Devlieghere et al., 2001). The solubility of carbon dioxide in meat has also been reported to change by as much as 35% for

each unit change in pH (Kropf, 2004). The relationships between temperature, gas pressure, gas volume, absorbed carbon dioxide, pH changes, and penetration of microbial cells by the gas are complex, but are likely to be a very important part of the shelf-life extension achieved by carbon dioxide in MAP systems. These factors were studied in a multifactorial experiment to determine the relative impact of each factor on the amounts of carbon dioxide absorbed by ground pork samples (Jakobsen and Bertelsen, 2002). The results of this study suggested that the gas partial pressure and the gas headspace-to-meat volume ratio were the most important factors. If more than 30% carbon dioxide was used, a volume of at least 2 L of gas/kg of meat was necessary to prevent package collapse. For volume ratios greater than 2 L:kg, the partial pressure of carbon dioxide was most predictive of the amount of absorbed gas. The product factors of fat/lean composition, pH, and temperature did not have as great an impact on absorption of carbon dioxide as gas partial pressure and volume.

Package atmospheres that contain relatively low levels of carbon dioxide (less than 30%) seldom result in package collapse because the proportion of total headspace gas absorbed is relatively small (Jakobsen and Bertelsen, 2004). In some cases, carbon dioxide concentrations have been observed to increase in the headspace of packages with less than 30% initially due to generation of carbon dioxide from muscle or microbial metabolism of residual oxygen. Excessive carbon dioxide in package headspace can be an issue in ground meat packages when carbon dioxide is used to chill the ground meat prior to packaging. Excess carbon dioxide "snow" remaining in the meat will volatilize in the package, causing an initial swelling followed by extreme package collapse when the carbon dioxide is slowly absorbed by the meat. It has been observed that products that have absorbed significant amounts of carbon dioxide may show evidence of physical disruption including visible pores and fissures following cooking. The physical disruption appears to result from volatilization of the absorbed gas when heat is applied (Bruce et al., 1996). Sørheim et al. (2004) reported greater cooking losses from ground beef stored in 100% carbon dioxide, and observed fissures in the product following cooking.

20.6.2 Nitrogen

Nitrogen is an inert gas that is colorless, odorless, and tasteless (Mullan and McDowell, 2003). The gas is nonflammable, has a lower density than air, and has a low solubility in water and fat. Nitrogen can affect meat product shelf life indirectly because when nitrogen is used to completely displace oxygen, the atmosphere will not allow growth of aerobic microorganisms. Because aerobic organisms are the fastest growing organisms normally present on fresh meat and poultry, preventing aerobic growth will improve shelf life. However, nitrogen has no direct effect on microbial growth and, consequently, has no impact on anaerobic bacteria. The low solubility of nitrogen

is advantageous for use as a filler gas with carbon dioxide to prevent package collapse that can occur when carbon dioxide is absorbed by the product. Nitrogen gas, usually 100%, is most often used for flush-and-fill packages of cooked, cured meats, particularly sliced items where slice adhesion is to be prevented. In these packages, oxygen must be reduced to 0.5% or less for good cured color stability (Møller et al., 2000). For uncured, cooked products, exclusion of oxygen is critical to suppression of rancidity and flavor losses. It is important to remember that fully cooked products, either cured or uncured, will typically have low microbial numbers and prevention of flavor changes during storage is often more critical to shelf life of these products than microbial inhibition. The use of 100% nitrogen can extend shelf life of the products by preventing the chemical changes and flavor losses induced by exposure to oxygen. Carbon dioxide is seldom used for packaging these products because microbial control has been achieved by other means.

20.6.3 Oxygen

Oxygen is a colorless, odorless gas that has relatively low solubility in water, supports combustion (is explosive), and is very reactive with a wide variety of biological compounds (Mullan and McDowell, 2003). Oxygen is involved in many of the deteriorative reactions in food systems that result in serious losses of quality. These reactions include fat oxidation, rancidity development, browning reactions, and pigment oxidation. The principle role of oxygen in packaging of meat and poultry is for development and maintenance of the cherry-red color that is considered essential to display of fresh meat (Kropf, 2004). The red oxymyoglobin pigment develops readily in normal atmospheric oxygen pressure but an elevated oxygen concentration of 65%–80% in MAP helps to form a deeper layer of oxymyoglobin pigment on the product surface that will extend the time during which the color appears attractive (Siegel, 2010b). However, because oxygen will also promote growth of rapidly proliferating, aerobic microorganisms, oxygen in MAP systems for fresh meat is usually combined with 20%–25% carbon dioxide to achieve improved microbial control (Siegel, 2010b). High-oxygen MAP systems with carbon dioxide will achieve a greater shelf life for fresh meat than conventional (atmospheric) aerobic packages but will not match the shelf life of vacuum-packaged products under similar conditions.

It should be noted that fresh meat is particularly susceptible to discoloration by low levels of oxygen. A partial oxygen pressure in the range of 5–10 mm of mercury (normal atmospheric partial pressure of oxygen is 159.2 mmHg) will rapidly convert the myoglobin pigment in meat to metmyoglobin, which is brown. Further, even very low levels of residual oxygen in MAP packages of fresh meat will result in at least some metmyoglobin. The meat tissue will utilize metmyoglobin reducing capacity to convert metmyoglobin back to myoglobin, but if this occurs, the subsequent reducing capacity is lessened, and the meat color may not be as stable in later display. Because of this, it is recommended

that, for fresh meat MAP systems that exclude oxygen, residual oxygen should not be more than 0.01% (100 parts per million [ppm]) after packaging, and essentially zero within 24 hours following packaging (Solomon, 2004).

Atmospheric or greater concentration of oxygen results in an attractive red color for fresh red meat, whereas complete elimination of oxygen (i.e., VP) prevents color deterioration so that later exposure to oxygen will allow formation of attractive color. A poorly flushed package, a poorly sealed package, or one that has been subjected to inadequate vacuum is likely to discolor quickly if low levels of oxygen are present. Low levels of oxygen can also be a problem in cooked or cured-and-cooked meats where color fading and rancidity may result. In this case, 0.5% or less oxygen in package atmospheres is recommended. The problems of excess residual oxygen are sometimes solved by using oxygen scavengers or absorbers to react with any residual oxygen that may remain in a package. Packets containing iron powders are most often used for this and can be frequently found in packages of highly oxygen-susceptible products such as dried snack sticks and jerky (Siegel, 2010a). Use of oxygen scavengers also extends into the concept of AP systems where oxygen absorbers and other atmosphere modifiers are used to keep the package atmosphere constant.

20.6.4 Carbon Monoxide

Carbon monoxide is a colorless, odorless, tasteless gas that is flammable and highly reactive (Mullan and McDowell, 2003). Carbon monoxide has low solubility in water but is soluble in some organic solvents. It binds very strongly to blood hemoglobin and muscle myoglobin. The binding to hemoglobin is the basis for concerns about human toxicity and exposure when working with carbon monoxide because the carbon monoxide displaces oxygen on hemoglobin and prevents oxygen transport in the bloodstream. While exposure to high concentrations of carbon monoxide can be fatal, low level exposure is not harmful (Krause et al., 2003). Concern about toxicity and human exposure, however, is one reason why carbon monoxide has not been universally used for meat and poultry packaging. On the other hand, carbon monoxide is not uncommon for treatment of fish, particularly tuna. In this case, carbon monoxide is applied as a single gas or as a component of what is termed "tasteless smoke" to improve the color stability of tuna.

The reaction between carbon monoxide and myoglobin also results in a strong bond between them, similar to that for hemoglobin, and this bond is about 1000-fold stronger than the bond between oxygen and myoglobin (Siegel, 2010b). A beneficial result of this reaction is a stable, bright red meat color that is visibly undistinguishable from the cherry-red meat color produced by oxygen. The color induced by carbon monoxide, however, due to the strong attraction between carbon monoxide and myoglobin, is much more stable than that resulting from oxygen and can last for several weeks

as opposed to several days for oxymyoglobin color. Because of the color stability, there are several commercial applications of low levels of carbon monoxide for MAP packaging. Research has demonstrated that concentrations of 0.3%–0.5% carbon monoxide in MAP are adequate to result in stable, attractive meat color and that at these concentrations, no human hazard exists. The US FDA approved use of 0.4% carbon monoxide for meat packaging in 2002, and categorized carbon monoxide as a Generally Recognized As Safe (GRAS) substance when used as described for MAP (U.S. FDA, 2002).

A distinct advantage of the meat color stability resulting from carbon monoxide in MAP is that discoloration from elevated levels of carbon dioxide is no longer a problem. Consequently, greater concentration of carbon dioxide can be introduced to improve microbial control without affecting color. Commercial packaging applications typically combine carbon monoxide with up to 60% carbon dioxide but research has shown that carbon dioxide at concentrations as high as 99.5% will not result in discoloration when combined with 0.5% carbon monoxide (Krause et al., 2003). However, excessive absorption of carbon dioxide by the meat tissue precludes use of carbon dioxide at such high concentrations.

The highly stable meat color resulting from carbon monoxide, however, does not require continuous exposure to carbon monoxide gas. For example, it has been reported that exposure of beef to 5%–100% carbon monoxide for up to 24 h, followed by VP, resulted in bright red color for several weeks (Jayasingh et al., 2001). This approach would allow exposure of meat cuts to carbon monoxide in a central facility followed by VP for distribution and retail display.

Another approach, which has been utilized for commercial applications of carbon monoxide in the United States (U.S. FDA, 2002), is to package retail fresh meat in a permeable film, then enclose the retail package with a large master package containing a low (0.4%) level of carbon monoxide combined with carbon dioxide (30%) and nitrogen (69.6%). Retail packages are then removed from the master package for retail display.

One of the issues that has been raised relative to use of carbon monoxide for stabilizing meat color is a concern that the color might be too stable, resulting in good color even after microbiological spoilage. However, research has demonstrated that the combined use of low carbon monoxide with high-carbon dioxide levels to suppress bacterial growth and spoilage circumvents this concern (Hunt et al., 2004).

20.6.5 Dynamic Headspace Changes in MAP Systems

Because MAP systems, by definition, are a onetime modification of the package atmosphere during packaging, all subsequent metabolic and chemical activities that occur within the package are likely to change the atmosphere by consuming some gases and emitting others. In the case of fresh meat, muscle respiration is still an active process that consumes residual

oxygen to produce carbon dioxide. Microbial metabolism is also commonly recognized as a source of oxygen depletion and carbon dioxide emission (Jakobsen and Bertelsen, 2002). Other factors that contribute to changes in the gas composition of MAP atmospheres include absorption of gases by the product and permeability of the packaging film (Zhao et al., 1995). Muscle respiration generally is most important during the early stages of package storage while muscle enzyme and metabolic systems are most active (Daniels et al., 1985). Clearly, the amount of carbon dioxide emitted by respiration will be dependent upon the initial amount of oxygen available. Microbial effects on the atmospheric gas composition become more pronounced during the later stages of storage, when growth of microorganisms becomes more extensive. Absorption of gases by the product generally reaches equilibrium early, during the first 12–72 h after packaging (Jakobsen and Bertelsen, 2004). Change in the MAP atmosphere resulting from gas exchange through the package film is usually very slow with high-barrier films, but will be affected by factors such as the partial pressure of gases inside and outside the package, environmental temperature, and package film thickness.

20.7 Product Characteristics Following Map Applications

Because the objectives of MAP systems for various meat and poultry products are different, the use of different gases and gas blends have been developed. Thus, the effects of MAP systems on product characteristics can vary considerably depending on the gases used and the product involved. Fresh meat MAP systems, for example, are vastly different than those for cooked or cured-and-cooked products.

20.7.1 Fresh (Raw) Meat

As discussed earlier, fresh meat MAP systems can range from atmospheres utilizing high oxygen concentrations to those using high carbon dioxide levels. Atmospheres containing high levels of either oxygen or carbon dioxide usually are balanced with nitrogen. Some package atmospheres will also incorporate carbon monoxide.

20.7.1.1 High-Oxygen MAP

Case-ready MAP packages that utilize high oxygen levels for color development of fresh red meat most often utilize a gas blend of about 70%–80% oxygen and 20%–30% carbon dioxide. This combination results in a deeper-than-usual layer of red oxymyoglobin on the product surface for improved

color life and improved shelf life, both of which are increased about three-fold over that of conventional overwrapped aerobic packages (Kropf, 2004; Siegel, 2010b). The color advantages of high-oxygen packaging are generally realized best in those red meats that have a high concentration of muscle pigment.

Comparison of beef packaged in a high-oxygen (80% oxygen: 20% carbon dioxide) atmosphere with beef packaged in a low-oxygen (80% nitrogen: 20% carbon dioxide) atmosphere has demonstrated that the high-oxygen atmosphere can be expected to result in significantly longer color life (Seyfert et al., 2004a). High-oxygen packaging of pork has also been demonstrated to achieve increased color life and consumer acceptability (Buys, 2004). While color and microbial shelf life are usually improved by high-oxygen–carbon dioxide combinations over conventional aerobic packaging, there have been reports of increased lipid oxidation in meat packaged in high-oxygen atmospheres (Spanos et al., 2016). The increased lipid oxidation induced by high-oxygen packaging has been shown to be effectively reduced by the use of antioxidants, particularly rosemary extract. Addition of rosemary extract enhanced lipid stability in beef patties (Sánchez-Escalante et al., 2001; Lund et al., 2007, Brooks et al., 2008) and in ground chicken meat (Keokamnerd et al., 2008), both of which were packaged in 80% oxygen atmospheres. The oxidative environment of high-oxygen packaging has also been observed to result in reduced meat tenderness due to reduced protein hydrolysis during aging and to increased protein cross-linking (Clausen et al., 2009; Bao and Ertbjerg, 2015).

Exposure of beef to high-oxygen atmospheres has been observed to result in "premature browning" when the beef is cooked (Seyfert et al., 2004b). Premature browning occurs when cooked beef turns brown at lower-than-usual cooking temperatures. The result is a well-done appearance in meat that is heated to medium doneness (71.1°C internal) or less. Research has shown that premature browning can occur in ground beef at cooked temperatures as low as 49°C (John et al., 2004). The concern arising from this observation is for microbial safety because many consumers use cooked color as an indicator of the temperature achieved during cooking (doneness). Ground beef patties stored in an 80% oxygen atmosphere have been observed to result in premature browning in nearly 100% of the patties evaluated (John et al., 2004).

Another problem sometimes observed with high-oxygen packaging of bone-in meat cuts is discoloration of bone marrow, sometimes called black bone. The problem results from disruption of red blood cells in the bone marrow during cutting of the bone, followed by exposure to high levels of oxygen. It has been demonstrated that the problem can be avoided by reducing oxygen in the package to less than 100 ppm or by including 0.4% CO in a MAP system (Mancini et al., 2005). The use of an ascorbic acid solution applied to cut bone surfaces has been reported to also prevent bone discoloration (Raines et al., 2006; Grobbel et al., 2006).

20.7.1.2 High-Carbon-Dioxide MAP

Packaging with elevated carbon dioxide is sometimes referred to as a low- or ultralow-oxygen system (Kropf, 2004). This is because, to be capable of good fresh meat color development later when the products are exposed to oxygen, a high-carbon-dioxide atmosphere must exclude as much oxygen as possible. This means that, in addition to adding carbon dioxide at levels of 20%–30% or more to the atmosphere for microbial inhibition, it is also critical to eliminate oxygen as completely as possible. Even though fresh meat will metabolize residual oxygen and thereby remove the oxygen from the atmosphere, this activity will consume some of the inherent reducing capacity of the muscle and subsequent color life is likely to be lessened. Residual oxygen typically ranges from about 0.1% to 1.0% or more in MAP packages and this is enough to impact color longevity in retail displays. Because of this, addition of oxygen absorbers to packages can be advantageous. The use of oxygen absorbers, for example, has been reported to improve the subsequent color stability of pork cuts first stored in a 100% carbon dioxide atmosphere to a level comparable to high-oxygen packaged products (Buys, 2004).

20.7.1.3 MAP with Carbon Monoxide

Fresh meat products packaged with carbon monoxide demonstrate dramatically improved color stability. For example, comparison of ground beef, beef steaks, and pork chops packaged in 0.4% CO/60% CO_2/40% N_2 or 70% O_2/30% CO_2 showed that the carbon monoxide atmosphere extended the color life three to seven days longer than that of the high-oxygen atmosphere (Sørheim et al., 1999). Pork chops packaged in 0.5% CO/70% CO_2/29.5% N_2 were reported to have desirable color after 36 days of storage at 3°C while conventionally overwrapped packages showed color losses after seven days of storage (Krause et al., 2003). Including carbon monoxide in MAP for bone-in meat cuts has been shown to prevent the bone discoloration that often occurs in high-oxygen MAP systems (Mancini et al., 2005).

One of the concerns for carbon monoxide packaging systems has been the potential for color to outlast microbial spoilage. This could be the case considering the length of color life observed in relation to microbial numbers (Sørheim et al., 1999). Because consumers utilize color as an indicator of microbial quality, it is important that sanitation, good manufacturing practices, and additional microbial inhibitors, such as carbon dioxide, be considered when color stability is extended with carbon monoxide.

20.7.2 Cooked and Cured-and-Cooked Meat Products

Because cooked items such as sliced roast beef and turkey, and cured-and-cooked products like sliced ham and dry sausage, are normally low in bacterial numbers, the principle concerns for spoilage during storage are flavor

and color deterioration. Changes in both flavor and color in these products are most often due to chemical oxidation rather than microbial growth, so exclusion of oxygen from the package is the principle objective for maximizing the shelf life of these products. A study of several factors affecting cured ham color in a package atmosphere of 20% carbon dioxide/80% nitrogen suggested that residual oxygen, nitrite concentration, degree of illumination, headspace volume, and film permeability to oxygen were all important for color stability (Møller et al., 2003). However, headspace volume relative to product volume was found to be among the most important because of the effect of volume on total oxygen content in the headspace. A second study of color stability for cured ham with similar MAP atmospheres included a range of headspace:product volume ratios and included low levels (up to 1.5%) of oxygen. Results of this study confirmed the importance of eliminating residual oxygen as much as possible for cured color stability and showed that the absolute oxygen content (the product of volume and concentration) was more important than concentration alone (Nannerup et al., 2004). Although 100% nitrogen is the most common atmosphere used for cured meats, carbon dioxide is included in some commercial applications. However, because fading of cured meat pigment is sensitive to oxygen there has been some question about the effects of carbon dioxide on cured meat color as well. A recent study of the stability of cured meat pigment to both autoxidation and photooxidation in the presence of 5%, 20%, and 30% carbon dioxide atmospheres (Summo et al., 2016) reported that autoxidation was decreased by carbon dioxide, which might have implications for reducing rancidity and off-flavor development in MAP systems. Other researchers have also reported reduced lipid oxidation in the presence of carbon dioxide atmospheres. Photooxidation, however, which, in the presence of oxygen, is the most important reaction contributing to cured meat color losses, was unaffected by carbon dioxide (Møller et al., 2005). Consequently, either 100% nitrogen or carbon dioxide/nitrogen blends can be used for MAP applications involving cooked or cured-and-cooked meat and poultry products.

20.8 Comparisons of Map with VP for Meat and Poultry

20.8.1 Vacuum versus MAP for Fresh Meat

Because VP is used a great deal for meat and poultry products and is, in reality, a form of modified atmosphere, a consideration of VP in relation to MAP is appropriate. VP is a simple process whereby product is packaged "in a high barrier package from which air is removed . . ." (Rao and Sachindra, 2002). As described earlier, for fresh meat, a vacuum package typically becomes a carbon-dioxide-enhanced package because residual oxygen is

converted to carbon dioxide by muscle and microbial respiration. The carbon dioxide concentration in vacuum packages of fresh meat typically reaches 10%–20% of the gases present (Tewari et al., 1999). Much of the antimicrobial impact of VP on shelf life of fresh meat has been credited to the production of carbon dioxide rather than to the removal of oxygen. For cooked or cured-and-cooked meats, there is very little formation of carbon dioxide after packaging because respiration is not active. In this case, VP is used because the exclusion of oxygen for control of chemical changes is the most critical factor for shelf life of these products. Microbial growth is less of a concern as long as microbial recontamination after cooking is restricted.

VP of fresh meat has typically been restricted to distribution of wholesale cuts because the dark purple color of vacuum-packaged red meat is unacceptable for retail display. Color is not as critical for poultry meat depending on the amount of pigment present (white vs. dark poultry meat) because color change is not as great. VP of beef, pork, lamb, and poultry will extend the shelf life of products by several folds over that of aerobic packaging (Siegel, 2010b). Similar to MAP, the actual shelf life achieved for a specific product is very dependent on the meat species, film permeability, initial microbial load, and storage temperature. The two most critical factors for VP are the microbial load at time of packaging and the storage temperature. For MAP, the gas atmosphere must be considered in addition to microbial load and temperature. VP has also been used commercially for storage of beef to achieve "wet aging" for tenderization and flavor development, though MAP has been shown to be equally effective for this application as well (Lee et al., 1996).

In addition to a lack of attractive color, VP for fresh meat typically results in the separation of a small amount of free water from the product. The separated water or purge forms a pool on the product surface beneath the package film, and in corners and creases of the package film. Purge occurs from the physical pressure of the external atmosphere on the surface of the product under vacuum, and results in product weight loss when the package is opened. Purge in vacuum-packaged cooked and cured-and-cooked products is also common, especially those products that have additional water added during manufacturing. The physical pressure of VP on product surfaces is also a disadvantage for sliced products where the pressure can cause slices to stick together. These effects of physical pressure on the product surface can be avoided by the use of MAP systems.

20.8.2 Vacuum versus MAP Packaging of Cooked and Cured-and-Cooked Meat Products

Both VP and MAP are widely used for cooked and cured-and-cooked products. Because exclusion of oxygen is the most important consideration for these products, the two packaging systems can function equally well for preserving color, suppressing microbial spoilage, and extending shelf life (Siegel, 2010a,b). The principle difference between applications of VP or MAP for cooked and

cured-and-cooked products results from the production of purge and adhesion of slices that can result from VP but that are largely avoided by MAP.

20.8.3 Effects of High-Carbon-Dioxide MAP

Because carbon dioxide is the active antimicrobial agent in both VP and MAP, there has been significant interest in utilizing increased concentrations of this gas in MAP systems. This has not been feasible in the past for fresh meat because of the discoloration that occurs at more than about 30% carbon dioxide. However, the research with carbon monoxide in MAP has shown that as much as 99.5% carbon dioxide will not cause discoloration if 0.5% carbon monoxide is included for color stability (Krause et al., 2003), though this concentration typically results in extreme package collapse. Consequently, it has been suggested that increased carbon dioxide concentrations, within the limits that avoid package collapse, could be used for both fresh and cooked cured-and-cooked products to further improve the shelf life.

Because high-carbon-dioxide atmospheres can affect meat pH and result in increased carbon dioxide absorbance by the meat tissue, a variety of quality changes of meat in high-carbon-dioxide atmospheres have been studied. One of the visual observations reported for meat stored in 40% carbon dioxide or more was development of pores between muscle bundles after cooking (Bruce et al., 1996). The pore development was observed for beef packaged in carbon dioxide for three days and for beef repackaged in vacuum for three days after 48 hours in a carbon dioxide atmosphere. It was suggested that the pores were formed by evolution of dissolved carbon dioxide induced by heat during cooking. The development of pores and fissures as well as changes in pH could cause product quality changes, particularly for protein functionality and product texture. It has been reported that when ground beef was stored in atmospheres containing 20%–100% carbon dioxide, reduced cooking yield of 1%–3% resulted (Sørheim et al., 2004).

20.9 Packaging Films and Film Properties

The development of flexible films with a wide array of strength and barrier properties has allowed MAP applications to reach their full potential. Currently available films offer a range of oxygen and moisture barriers, shrinking properties, sealing characteristics, smoke and/or color transfer options, shirred or roll stock, cook-in and retort capability, and a variety of print and color options.

The majority of flexible packaging films used for meat and poultry are based on nylon, polyester, or polypropylene to provide basic strength. Most, if not all, MAP packages consist of multiple layers of several plastic films combined by coextrusion, lamination, or coating treatments to create the

desired blend of film properties for control of moisture and gas permeability and for heat-sealing properties. There are no single films that provide all the properties needed for effective MAP systems. Flexible packaging film materials can be generally categorized as exterior materials, barrier materials, sealants, or rigid materials (Dawson, 2010; Siegel, 2010a). The following discussion briefly addresses properties of some of the basic individual films that might be used in MAP packaging. This is by no means a comprehensive list or discussion and more details can be obtained from the references listed.

20.9.1 Exterior Film Materials

20.9.1.1 Nylon

Nylon or polyamides are widely used for strength and are resistant to puncture, abrasion, and tearing (Mullan and McDowell, 2003; Dawson, 2010; Siegel, 2010a). The toughness of nylon means that it is often used as a component of fresh meat pouches where puncture resistance and strength are important. Nylon is generally difficult to heat seal and will be combined with other films that provide sealing ease. Because of the strength and rigidity, this film is often part of thermoformed base packs for MAP applications.

20.9.1.2 Polyester

Polyesters are condensation polymers, polyethylene terephthalate, with a wide range of properties. These films are heat resistant (Dawson, 2010; Siegel, 2010a) so they may not seal well. Polyester is often used for barrier pouches and lids for tray packs where strength is needed without flexing since they are prone to flex cracking. Polyester can be metallized with aluminum to provide an exceptional barrier to oxygen and may be used this way as part of the package for highly oxygen-sensitive products.

20.9.1.3 Polypropylene

Polypropylene applications for MAP systems are most often found in rigid base trays. This material is a good water vapor barrier but a poor gas barrier so combinations with high gas barrier films are likely (Mullan and McDowell, 2003; Dawson, 2010; Siegel, 2010a). This film has a high heat tolerance but can be heat sealed, though shrinkage when sealing can be an issue.

20.9.2 Barrier Film Materials

20.9.2.1 Ethylene Vinyl Alcohol

This film is generally laminated between two or more films when used for MAP to protect it from moisture. The film has good gas barrier properties when dry but must be protected from moisture to retain its barrier properties

(Mullan and McDowell, 2003; Dawson, 2010; Siegel, 2010a). The film is often coextruded with nylon and other films for fresh meat applications.

20.9.2.2 Polyvinylidene Chloride

Polyvinylidene chloride is a flexible film that is a superior barrier for water vapor, gases, and odors (Mullan and McDowell, 2003; Dawson, 2010; Siegel, 2010a). This film is best known by the trade name of Saran®. The film is heat sealable, highly shrinkable when heated, and commonly used in combined films. Heat-shrink packages may include monolayer films of polyvinylidene chloride but the film is most often used in coextrusions with multiple layers of other flexible film materials.

20.9.3 Sealant Materials

20.9.3.1 Polyethylenes

Polyethylenes are commonly used in multiple layer package films primarily for heat-sealing properties. The film melts at relatively low temperature (~20°C) and welds to itself readily, thus forming effective seals. The film is not a good barrier and requires combination with other films for any significant MAP barrier package (Mullan and McDowell, 2003; Dawson, 2010; Siegel, 2010a).

20.9.3.2 Ethylene Vinyl Acetate

This film is relatively weak but has a low initial heat-sealing temperature as well as a wide window of heat-sealing temperatures, which makes it a very effective sealant. Consequently, ethylene vinyl acetate is used primarily for its heat-sealing properties (Mullan and McDowell, 2003; Dawson, 2010; Siegel, 2010a).

20.9.4 Rigid Materials

20.9.4.1 Polyvinyl Chloride

Polyvinyl chloride is a material that can be softened and formed relatively easily by heat. Consequently, it is often used for thermoformed structures such as base trays (Mullan and McDowell, 2003; Dawson, 2010; Siegel, 2010a). The film is not a good gas barrier so combinations with barrier films are usually necessary for MAP applications.

20.9.4.2 Polystyrene

Polystyrene is relatively low cost with good stiffness. It is not transparent and does not have good moisture or gas barrier properties so other films need to be included to provide necessary oxygen and/or moisture barrier properties (Siegel, 2010a).

20.10 Effects of MAP on Pathogens in Meat and Poultry

Elevated concentrations of carbon dioxide have been shown to have an inhibitory effect on several pathogenic microorganisms. As a result, MAP systems have the potential to increase the safety of meat and poultry products. Because meat and poultry products are normally chilled, psychrotrophic pathogens are of greatest concern. However, temperature abuse can also give rise to those pathogens that grow at warmer temperatures.

There are several pathogens of concern to meat and poultry. These include *Salmonella* spp., *Yersinia enterocolitica*, *Escherichia coli* O157:H7, *Campylobacter jejuni*, *Staphylococcus aureus*, *Clostridium botulinum*, *Clostridium perfringens*, *Bacillus cereus*, and *Listeria monocytogenes*.

20.10.1 *Salmonella* spp.

Salmonella-caused illnesses are classified as infections, meaning that live organisms must be ingested to cause illness. This organism is quite sensitive to heat; consequently most cases of salmonellosis result from undercooked meat, poultry, or eggs (Knabel, 1995; Baer et al., 2013). A review of several studies suggests that high-carbon-dioxide (60% to 100%) MAP can slow the growth of *Salmonella* compared to aerobic packaging but the organism will survive in the carbon dioxide environment and may grow when exposed to more favorable conditions. At best, MAP with high carbon dioxide may offer partial inhibition of *Salmonella* but temperature control and adequate cooking are still required to assure safety, and addition of antimicrobial treatments may be necessary if this organism is a concern (Sukumaran et al., 2016).

20.10.2 *Yersinia enterocolitica*

Y. enterocolitica is a facultative anaerobe and can grow well in the absence of oxygen (Knabel, 1995; Baer et al., 2013). Yersiniosis is an infection frequently resulting from undercooked meat, most often pork. Several studies have reported that high-carbon-dioxide atmospheres will slow the growth of *Y. enterocolitica* but there are reports that this pathogen can grow even under a 100% carbon dioxide atmosphere. Adequate cooking, rather than MAP, provides assurance of safety from this pathogen.

20.10.3 *Escherichia coli* O157:H7

There appears to be relatively little information on the effects of MAP systems on *E. coli* O157:H7 (Rao and Sachindra, 2002). This organism commonly contaminates raw meat, induces illness as an infection, and is easily killed by heat. It appears that *E. coli* O157:H7 is similar to *Salmonella* in that growth

can be slowed by high-carbon-dioxide atmospheres as long as temperature is relatively low but the organism is largely unaffected by MAP gas atmospheres (Heinrich et al., 2016).

20.10.4 *Campylobacter jejuni*

C. jejuni is classified as a microaerophilic organism (Baer et al., 2013) meaning that it prefers a small amount of oxygen but seems to be largely unaffected by carbon dioxide atmospheres. *C. jejuni* causes illnesses as a foodborne infection, meaning that a significant number of live organisms must be ingested to induce illness. The organism is very heat sensitive and does not compete well for growth against other microorganisms (Knabel, 1995). Studies on *C. jejuni* growth under a variety of atmospheres suggest that the absence of oxygen has a larger impact on this organism than the presence of high concentrations of carbon dioxide (Baer et al., 2013). *C. jejuni* is frequently associated with poultry and adequate cooking is the preferred method for assuring product safety.

20.10.5 *Staphylococcus aureus*

S. aureus is a pathogen that causes illness by producing a toxin during growth, resulting in what is considered a food poisoning rather than a food infection. However, because a relatively large number of organisms are needed for detectable levels of the enterotoxin, prevention of growth of this organism is important. There is evidence that high-carbon-dioxide atmospheres inhibit growth of *S. aureus* and, in some cases, reduce cell counts (Baer et al., 2013). It appears that low temperature is very important and may be synergistic with carbon dioxide concentrations to increase cell death rate of *S. aureus*. It may be that the greatly increased solubility of carbon dioxide that has been observed at lower temperatures plays a role in the greater antimicrobial impact on *S. aureus* at reduced temperatures.

20.10.6 *Clostridium botulinium*

C. botulinum is an anaerobic organism that produces a very potent toxin under conditions favorable to growth. Because ingestion of *C. botulinum* toxin frequently results in death, this organism is notorious for potential risk in low-acid foods packaged under anaerobic conditions. Several studies of the effects of MAP atmospheres on *C. botulinum*, especially with fish, have shown that this pathogen is largely unaffected by high-carbon-dioxide or nitrogen atmospheres. Fortunately, the incidence of *C. botulinum* on meat and poultry is typically very low. Further, refrigeration temperatures are normally sufficient to prevent toxin production. In fish products, where *C. botulinum* contamination is high, anaerobic modified atmospheres are not considered safe for refrigerated products (Baer et al., 2013).

20.10.7 *Clostridium perfringens*

C. perfringens also induces illness by producing a toxin, but unlike *C. botulinum,* this food poisoning is seldom fatal. This organism does not appear to be affected by high-carbon-dioxide atmospheres at warm temperatures (above 20°C) but at lower temperatures (5°C or less) high carbon dioxide has been reported to prevent growth (Hardin, 2012). The general consensus seems to be that, similar to *C. botulinum*, preventing contamination and maintaining proper temperature control are more important than MAP for assuring control of this pathogen on meat and poultry.

20.10.8 *Bacillus cereus*

B. cereus is a toxin-producing pathogen that induces a food poisoning illness, but the illness is usually not fatal. While this pathogen is among the less-common causes of foodborne illness from meat and poultry, it also seems to be the pathogen that is most susceptible to the antimicrobial effects of carbon dioxide atmospheres. Several studies have documented that *B. cereus* is inhibited by carbon dioxide even at 25°C (Baer et al., 2013). Carbon dioxide has been reported to be much more effective against *B. cereus* than nitrogen or anaerobic atmospheres.

20.10.9 *Listeria monocytogenes*

L. monocytogenes has become a major concern to the meat and poultry industry because this pathogen can grow at very low refrigerated temperature, and is widespread, very hardy, and very difficult to eradicate from food processing plants. Further, listeriosis is a foodborne infection that can result in a 20%–30% fatality rate in an outbreak (Devlieghere et al., 2001). The heat process used during production of fully cooked (71°C internal) products has been shown to eliminate *L. monocytogenes* from meat and poultry, but because *L. monocytogenes* is a common environmental contaminant, postcooking contamination, particularly during slicing and packaging, is likely. Because fully cooked products are often eaten without further heating, this ready-to-eat (RTE) category of meat and poultry products has been the source of most listeriosis outbreaks (Hardin, 2012; Baer et al., 2013; Heinrich et al., 2016).

There have been conflicting reports on the effects of MAP atmospheres on *L. monocytogenes.* Studies with fresh beef reported that 100% carbon dioxide inhibited *L. monocytogenes* growth at 5°C but not at 10°C (Nissen et al., 2000). Other reports have suggested that carbon dioxide atmospheres will reduce *L. monocytogenes* counts only by about 1.5 log, meaning that additional antimicrobial treatments should be included for adequate control of this pathogen (Saraiva et al., 2016).

20.11 MAP as a Component in the Hurdle Concept

Most microorganisms have been shown to be more resistant to change in a single environmental factor than to change in two or more environmental factors at the same time. As a result, sublethal levels of two or more treatments can often be applied to create a total inhibitory effect or "hurdle" that the organism cannot overcome (Jay, 2000). This "hurdle" concept is frequently credited to Dr. Luther Leistner of Germany who has been a strong proponent of this approach. The hurdle concept is being studied in many applications because it often allows reduced intensity of any one antimicrobial treatment while improving the overall antimicrobial protection.

The use of MAP as one component in the hurdle concept to add to a greater overall inhibition of spoilage or pathogenic microorganisms is very compatible with many other inhibitory treatments. Because MAP exerts potentially inhibitory effects following processing and during storage, it is easy to include MAP as an "add-on" treatment to complement preservatives, thermal processes, high pressure processing, irradiation, and many other antimicrobial treatments. There have been several reports of MAP, as part of a hurdle concept, contributing to increased overall inhibition of microorganisms or improved product quality (Rao and Sachindra, 2002). For example, treatment of poultry carcasses with lactic acid or sorbate solutions combined with MAP increased the inhibition of spoilage and pathogenic microorganisms, respectively (Gammariello et al., 2014). High pressure processing has been demonstrated in multiple studies to be effective (Myers et al., 2013; Lavieri et al., 2014; Lerasle et al., 2014), and the use of bacteriophage in combination with MAP can provide control of pathogens like *Salmonella* (Sukumaran et al., 2016).

Combining a variety of treatments to achieve an adequate microbiological hurdle for safety and to retain product quality at the same time is a complex challenge and will require a great deal of product-specific research to harmonize the effects of product and packaging treatments.

20.12 AP Components

While AP systems are generally considered separately from MAP, there are some elements of AP that are becoming important for improving the effectiveness of MAP. The concept of AP is an interactive packaging system that modifies package conditions according to needs (Ozdemir and Floros, 2004). The AP concept includes a wide variety of potential package adjustment mechanisms including oxygen scavengers, flavor releasers, flavor absorbers, moisture absorbers, time–temperature indicators, and films that contain antimicrobial agents (Siegel, 2010a).

Where AP and MAP are beginning to overlap is in the use of oxygen scavengers. It is clear that for anaerobic MAP systems applied to fresh meat, small amounts of oxygen are very detrimental (Kropf, 2004). It is also clear that it is time consuming to achieve very low oxygen levels during packaging operations because vacuum and/or flush treatments must be extended to remove as much oxygen as possible. In some cases, products may be vacuumized or flushed multiple times. The addition of oxygen scavengers is advantageous, not only because they will remove small amounts of residual oxygen, but also because use of scavengers will permit faster packaging operations and greater production line speed.

Oxygen scavengers are also used in many RTE meat applications where flavor preservation is critical (Ozdemir and Floros, 2004). A good example is dried products such as jerky or sliced pepperoni. These products are usually packaged in a nitrogen atmosphere and will develop rancid flavors easily in the presence of small amounts of oxygen. An oxygen scavenger significantly increases the shelf life of these products from the quality standpoint. The oxygen scavengers are typically added to MAP systems as a small packet of iron powder that is oxidized to iron oxide when oxygen is absorbed (Siegel, 2010a). In the United States, these packets must be labeled as "Do Not Eat" to prevent consumption by children or by accident. Recent developments in packaging have included the incorporation of oxygen scavengers into the packaging film. This alternative solves the potential problems associated with the iron packet on the inside of the package.

A second component of AP systems that may be effective in MAP applications is carbon dioxide emitters. Because high levels of carbon dioxide are important to antimicrobial effects, it might be useful for packaging systems to include the capability to generate more carbon dioxide after a package containing a high amount of carbon dioxide is sealed. However, this concept does not appear to be used a great deal for meat and poultry.

Packaging films that include antimicrobial compounds would be a powerful complement for MAP systems to help control microorganisms and pathogens that tolerate MAP atmospheres. There has been a considerable amount of research on antimicrobial films and coatings including antibiotics and organic acids (Bhatia and Bharti, 2015; Clarke et al., 2016), bacteriophages (Gouvea et al., 2015), spice essential oils (Ahmed et al., 2016), and silver nanoparticles (Azlin-Hasim et al., 2015). In many cases, the properties of the film involved may be modified by the inclusion of the incorporated materials and would need to be taken into consideration for applications of this technology.

A final example of an AP innovation for meat products is the development in 2010 of a nitrite-containing film for fresh meat (Siegel, 2010b). This film, when used to vacuum package fresh meat, releases a very small quantity of nitrite when in contact with meat surfaces, which results in a bright red color from formation of nitric oxide myoglobin on the product surface while under vacuum. The color is visually indistinguishable from the red oxymyo-

globin color of fresh meat in oxygen atmospheres. The nitrite concentration is far too low (less than 10 ppm) to result in cured color when the product is cooked or to have an antimicrobial effect. In this case, the vacuum package provides the microbial control and shelf-life extension (Siegel, 2010b).

20.13 Leakers and Package Integrity

One of the minor disadvantages to MAP technology in comparison to VP for meat and poultry is that leaks and faulty seals are not as visibly obvious. If the integrity of the package and/or the seal is not intact, the gas atmosphere is likely to be lost very quickly and product safety and quality will be compromised, but the shape or form of the package may not change in the case of MAP the way it does with a vacuum package. Because the majority of MAP systems employ a heat-sealing operation, this step is a critical control point in the process and needs to be carefully monitored during production.

There are a variety of testing methods to check the integrity of MAP packages. These include immersion in water, compression of packages, and dye penetration as well as several others. (Smolander et al., 1997; Mullan and McDowell, 2003). Most of these methods are destructive and can be used to measure only selected packages. More recent developments in monitoring systems for package integrity have suggested that leak indicators should be an integral part of every package to be most effective. The use of oxygen sensors, for example, attached to the inside of the lid of packages of cooked chicken patties was reported to detect oxygen levels as low as 0.07%. These sensors were composed of a phase-fluorimetric membrane that was measured for phosphorescence intensity and phase shift (Smiddy et al., 2002).

20.14 Regulatory Issues

The use of carbon dioxide, nitrogen, and oxygen gases for MAP systems is common around the world for meat and poultry and for many other food products. Fish, fruits, vegetables, and dairy products are among the most common foods packaged in MAP. There are relatively few regulatory limits for the use of these three gases, though safety concerns for fish in anaerobic MAP systems have been expressed. Carbon monoxide, on the other hand, has not been permitted in many countries. The most substantial use was in Norway where 0.5% carbon monoxide was approved for use in 1985 and was utilized for over half of the retail packaging of fresh meat (Sørheim et al., 2001). However, because Norway moved to the European Union (EU)

for commodity trade, use of carbon monoxide for red meat packaging in Norway was discontinued in 2004 to comply with EU regulations.

Because of a considerable amount of research demonstrating the advantages of low carbon monoxide MAP, in February 2002, the US FDA approved use of 0.4% carbon monoxide for MAP used as part of a master-pack system (ActiveTech™ by Pactiv Corp. Pactiv LLC, Lake Forest, IL) (U.S. FDA, 2002). This system utilizes retail packages that have an oxygen-permeable film and are enclosed in a master pack with 0.4% carbon monoxide, 30% carbon dioxide, and 69.6% nitrogen. An oxygen scavenger is added to the master pack to eliminate residual oxygen and protect the product color for later retail display. The FDA categorized this use of carbon monoxide as a GRAS procedure. Packaging systems utilizing carbon monoxide for master-pack applications similar to the Pactiv system have been approved for use in Australia and New Zealand as well (Sørheim et al., 2001). The FDA also, in July 2004, approved use of 0.4% carbon monoxide with carbon dioxide and nitrogen for MAP packaging of case-ready beef and pork (U.S. FDA, 2004). This approval was granted on behalf of Precept Foods, LLC, and utilizes the MAP atmosphere for retail packages as opposed to a master pack (Siegel, 2010b).

While carbon dioxide, oxygen, and nitrogen enjoy widespread acceptance as components of MAP systems, carbon monoxide suffers from the perception of environmental toxicity. Even though the risk represented by the concentrations of carbon monoxide used in MAP systems is negligible (Sørheim et al., 2001; U.S. FDA, 2002, 2004), the perception of danger continues to limit potential applications of this gas.

20.15 Conclusions

An extensive amount of research and development has shown that MAP technology offers very significant improvements in preservation and safety of meat and poultry products. The meat industry is utilizing MAP technology more and more as case-ready packaging of fresh meat becomes more widely adopted. Because case-ready packaging is done in centralized locations, the sanitation and temperature control is typically more consistent, and as a result, products not only achieve longer shelf life but are delivered to consumers with a more consistent quality. Utilization of MAP systems for cooked, RTE products has been in place for quite some time but is changing as new ways to use MAP for improved safety are being developed. This is an active area of current research because more information is needed on how MAP interacts with other inhibitory processes and ingredients for control of quality and safety.

It is important to realize that MAP is far from a stand-alone technology for preservation of meat and poultry products. The use of MAP is much more

effective when the packaged product is of high microbial quality and when subsequent temperature is well controlled. For meat and poultry products, the mixture of gases used must be appropriate to the application and, for anaerobic MAP environments, residual oxygen must be removed as completely as possible. Additional considerations include use of appropriate films and materials for necessary barriers or permeability, adequate sealing of packages and monitoring for leakers, and attractive presentation to appeal to consumers. The use of MAP systems for meat and poultry has become widespread and, with the additional research and development currently underway, has great potential for even wider use in the future.

References

Ahmed, J., Hiremath, N. and Jacob, H. (2016). Antimicrobial, rheological, and thermal properties of plasticized polyactide films incorporated with essential oils to inhibit *Staphyloccus aureus* and *Campylobacter jejuni*. *Journal of Food Science,* **81**, E419–E429.

Azlin-Hasim, S. M., Cruz-Romero, C., Morris, M. A., Cummins, E. and Kerry, J. P. (2015). Effects of a combination of antimicrobial silver low density polyethylene nanocomposite films and modified atmosphere packaging on the shelf life of chicken breast filets. *Food Packaging and Shelf Life,* **4**, 26–35.

Baer, A. A., Miller, M. J. and Dilger, A. C. (2013). Pathogens of interest to the pork industry: A review of research on interventions to assure food safety. *Comprehensive Reviews in Food Science and Food Safety,* **12**, 183–217.

Bao, Y. and Ertbjerg, P. (2015). Relationship between oxygen concentration, shear force and protein oxidation in modified atmosphere packaged pork. *Meat Science,* **110**, 174–179.

Bartkowski, L., Dryden, F. D. and Marchello, J. A. (1982). Quality changes of beef steaks stored in controlled gas atmospheres containing high or low levels of oxygen. *Journal of Food Protection,* **45**, 41–45.

Bhatia, S. and Bharti, A. (2015). Evaluating the antimicrobial activity of nisin, lysozyme and ethylenediaminetetraacetate incorporated in starch based active food packaging film. *Journal of Food Science and Technology,* **52**(6), 3504–3512.

Brooks, J. (1933). The effects of carbon dioxide on the color changes or bloom of lean meat. *Journal of the Society of Chemical Industry,* **52**, 17–19.

Brooks, J. C., Alvarado, M., Stephens, T. P. et al. (2008). Spoilage and safety characteristics of ground beef packaged in traditional and modified atmosphere packages. *Journal of Food Protection,* **71**, 293–301.

Bruce, H. L., Wolfe, F. H., Jones, S. D. M. and Price, M. A. (1996). Porosity in cooked beef from controlled atmosphere packaging is caused by rapid CO_2 evolution. *Food Research International,* **29**, 189–193.

Buys, E. M. (2004). Colour changes and consumer acceptability of bulk packaged pork retail cuts stored under O_2, CO_2 and N_2. *Meat Science,* **68**, 641–647.

Clark, D. S. and Lentz, C. P. (1969). Use of carbon dioxide for extending shelf-life of prepackaged beef. *Canadian Institute of Food Science and Technology Journal,* **5**(4), 175–178.

Clarke, D., Molinaro, S., Tyuftin, A., Bolton, D., Fanning, S. and Kerry, J. P. (2016). Incorporation of commercially-derived antimicrobials into gelatin-based films and assessment of their antimicrobial activity and impact on physical film properties. *Food Control,* **64**, 202–211.

Clausen, I., Jakobsen, M., Ertbjerg, P. and Madsen, N. T. (2009). Modified atmosphere packaging affects lipid oxidation myofibrillar fragmentation index and eating quality of beef. *Packaging Technology and Science,* **22**, 85–96.

Daniels, J. A., Krishnamurthi, R. and Rizvi, S. S. H. (1985). A review of effects of carbon dioxide on microbial growth and food quality. *Journal of Food Protection,* **48**, 532–537.

Dawson, P. L. (2010). Packaging. *In:* Owens, C. M., Alvarado, C. Z. and Sams, A. R. (eds.) *Poultry Meat Processing* (2nd Ed.). Boca Raton, FL: CRC Press, pp. 101–123.

Devlieghere, F., Geeraerd, A. H., Versyck, K. J., Vandewaetere, B., Van Impe, J. and Debevere, J. (2001). Growth of *Listeria monocytogenes* in modified atmosphere-packed, cooked meat products: A predictive model. *Food Microbiology,* **18**, 53–66.

Dixon, N. M. and Kell, D. B. (1989). The inhibition by CO_2 of the growth and metabolism of micro-organisms. *Journal of Applied Bacteriology,* **67**, 109–136.

Farber, J. M. (1991). Microbiological aspects of modified-atmosphere packaging—A review. *Journal of Food Protection,* **54**, 58–70.

Gammariello, D., Incoronato, A. L., Conte, A. and Del Nobile, M. A. (2014). Use of sodium lactate and modified atmosphere packaging for extending the shelf life of ready-to-cook fresh meal. *Packaging Technology and Science,* **28**, 101–112.

Gill, C. O. (1988). The solubility of carbon dioxide in meat. *Meat Science,* **22**, 65–71.

Gill, C. O. and Tan, K. H. (1980). Effect of carbon dioxide on growth of meat spoilage bacteria. *Applied and Environmental Microbiology,* **39**, 317–319.

Gouvea, D. M., Mendonca, R. C. S., Soto, M. L. and Cruz, R. S. (2015). Acetate cellulose film with bacteriophages for potential antimicrobial use in food packaging. *LWT—Food Science and Technology,* **63**, 85–91.

Grobbel, J. P., Dikeman, M. E., Yancey, E. J., Smith, J. S., Kropf, D. H. and Milliken, G. A. (2006). Effects of ascorbic acid, rosemary, and Origanox™ in preventing bone marrow discoloration in beef lumbar vertebrae in aerobic and anaerobic packaging systems. *Meat Science,* **72**, 47–56.

Hardin, M. (2012). *Processed Meats Microbiology: Food Safety and Shelf Life Considerations. Meat Processing Technology Series*. Champaign, IL: American Meat Science Association.

Heinrich, V., Zunabovic, M., Nehm, L., Bergmair, J. and Kneifel, W. (2016). Influence of argon modified atmosphere packaging on the growth potential of strains of *Listeria monocytogenes* and *Escherichia coli*. *Food Control,* **59**, 513–523.

Hunt, M. C., Mancini, R. A., Hachmeister, K. A. et al. (2004). Carbon monoxide in modified atmosphere packaging affects color, shelf life and microorganisms of beef steaks and ground beef. *Journal of Food Science,* **45**, 45–52.

Jakobsen, M. and Bertelsen, G. (2002). The use of CO_2 in packaging of fresh meats and its effect on chemical quality changes in the meat: A review. *Journal of Muscle Foods,* **13**, 143–168.

Jakobsen, M. and Bertelsen, G. (2004). Predicting the amount of carbon dioxide absorbed in meat. *Meat Science,* **68**, 603–610.

Jay, J. M. (2000). *Modern Food Microbiology* (6th Ed.). Gaithersburg, MD: Aspen Publishers.

Jayasingh, P., Cornforth, D. P., Carpenter, C. E. and Whittier, D. (2001). Evaluation of carbon monoxide treatment in modified atmosphere packaging or vacuum packaging to increase color stability of fresh beef. *Meat Science,* **59**, 317–324.

John, L., Cornforth, D., Carpenter, D. E., Sørheim, O., Pettee, B. C. and Whittier, D. R. (2004). Comparison of color and thiobarbituric acid values of cooked hamburger patties after storage of fresh beef chubs in modified atmospheres. *Journal of Food Science,* **69**, C608–C614.

Keokamnerd, T., Acton, J. C., Han, I. Y. and Dawson, P. L. (2008). Effect of commercial rosemary oleoresin preparations on ground chicken thigh meat quality packaged in a high-oxygen atmosphere. *Poultry Science,* **87**, 170–179.

Knabel, S. J. (1995). Foodborne illness: Role of home food handling practices. *Food Technology,* **49**(4), 119–131.

Krause, T. R., Sebranek, J. G., Rust, R. E. and Honeyman, M. S. (2003). Use of carbon monoxide packaging for improving the shelf life of pork. *Journal of Food Science,* **68**, 2596–2603.

Kropf, D. H. (2004). Packaging/modified and controlled atmosphere. *In:* Jensen, W., Devine, C. and Dikeman, M. (eds.) *Encyclopedia of Meat Sciences* (1st Ed.). Oxford: Elsevier, pp. 962–969.

Lavieri, N. A., Sebranek, J. G., Cordray, J. C. et al. (2014). Effects of different nitrite concentrations from a vegetable source with and without high hydrostatic pressure on the recovery of *Listeria monocytogenes* on ready-to-eat restructured ham. *Journal of Food Protection,* **77**(5), 781–787.

Lee, M., Sebranek, J. G. and Parrish, F. C., Jr. (1996). Accelerated postmortem aging of beef utilizing electron-beam irradiation and modified atmosphere packaging. *Journal of Food Science,* **61**, 133–136, 141.

Lerasle, M., Federighi, M., Simonin, H. et al. (2014). Combined use of modified atmosphere packaging and high pressure to extend the shelf-life of raw poultry sausage. *Innovative Food Science and Emerging Technologies,* **23**, 54–60.

Lund, M. N., Hviid, M. S. and Skibsted, L. H. (2007). The combined effect of antioxidants and modified atmosphere packaging on protein and lipid oxidation in beef patties during chill storage. *Meat Science,* **76**, 226–233.

Mancini, R. A., Hunt, M. C., Hachmeister, K. A., Kropf, D. H. and Johnson, D. E. (2005). Exclusion of oxygen from modified atmosphere packages limits beef rib and lumber vertebrae marrow discoloration during display and storage. *Meat Science,* **69**, 493–500.

McMillin, K. W. (2008). Where is MAP going? A review and future potential of modified atmosphere packaging for meat. *Meat Science,* **80**(1), 43–65.

Møller, J. K. S., Jakobsen, M., Weber, C. J., Mortinussen, T., Skibsted, L. H. and Bertelsen, G. (2003). Optimization of colour stability of cured ham during packaging and retail display by a multifactorial design. *Meat Science,* **63**, 169–175.

Møller, J. K. S., Jensen, J. S., Olsen, M. B., Skibsted, L. H. and Bertelsen, G. (2000). Effect of residual oxygen on color stability during chill storage of sliced pasteurized ham packaged in modified atmosphere. *Meat Science,* **54**, 399–405.

Møller, J. K. S., Nannerup, L. and Skibsted, L. H. (2005). Effect of carbon dioxide on autoxidation and photooxidation of nitrosylmyoglobin. *Meat Science,* **69**, 71–78.

Mullan, M. and McDowell, D. (2003). Modified atmosphere packaging. *In:* Coles, R., McDowell, D. and Kirwan, M. J. (eds.) *Food Packaging Technology*. Boca Raton, FL: CRC Press, pp. 303–331.

Myers, K., Montoya, D., Cannon, J., Dickson, J. S. and Sebranek, J. G. (2013). The effect of high hydrostatic pressure, sodium nitrite and salt concentration on the growth of *Listeria monocytogenes* on RTE ham and turkey. *Meat Science,* **93**(2), 263–268.

Nannerup, L. D., Jakobsen, M., Van den Berg, F., Jensen, J. S., Møller, J. K. S. and Bertelsen, G. (2004). Optimizing colour quality of modified atmosphere packed sliced meat products by control of critical packaging parameters. *Meat Science,* **68**, 577–585.

Nissen, H., Alvseike, O., Bredholt, S., Holck, A. and Nesbakken, T. (2000). Comparison between the growth of *Yersinia enterocolitica, Listeria monocytogenes, Escherichia coli* O157:H7 and *Salmonella* spp. in ground beef packed by three commercially used packaging techniques. *International Journal of Food Microbiology,* **59**, 211–220.

Ozdemir, M. and Floros, J. D. (2004). Active food packaging technologies. *Critical Reviews in Food Science and Nutrition,* **44**, 185–193.

Raines, C. R., Dikeman, M. E., Grobbel, J. P. and Yancey, E. J. (2006). Effects of ascorbic acid and Origanox™ in different packaging systems to prevent pork lumbar vertebrae discoloration. *Meat Science,* **74**, 267–271.

Rao, D. N. and Sachindra, N. M. (2002). Modified atmosphere and vacuum packaging of meat and poultry products. *Food Reviews International,* **18**, 263–293.

Sánchez-Escalante, A., Djenane, D., Torrescano, G., Beltrán, J. A. and Roncalés, P. (2001). The effects of ascorbic acid, taurine, carnosine and rosemary powder on colour and lipid stability of beef patties packaged in modified atmosphere. *Meat Science,* **58**, 421–429.

Saraiva, C., Fontes, M. C., Patarata, L., Martins, C., Cadavez, V. and Gonzales-Barron, U. (2016). Modelling the kinetics of *Listeria monocytogenes* in refrigerated fresh beef under different packaging atmospheres. *LWT—Food Science and Technology,* **66**, 664–671.

Seyfert, M., Hunt, M. C., Mancini, R., Hachmeister, K. A., Kropf, D. H. and Unruh, J. A. (2004a). Accelerated chilling and modified atmosphere packaging affect colour and colour stability of injection-enhanced beef round muscles. *Meat Science*, 68, 209–219.

Seyfert, M., Mancini, R. A. and Hunt, M. C. (2004b). Internal premature browning in cooked ground beef patties from high-oxygen modified-atmosphere packaging. *Journal of Food Science,* **69**, C721–C725.

Siegel, D. G. (2010a). *Packaging for Processed Meats. Meat Processing Technology Series*. Champaign, IL: American Meat Science Association.

Siegel, D. G. (2010b). *Retail Packaging for Fresh Meats. Meat Processing Technology Series*. Champaign, IL: American Meat Science Association.

Smiddy, M., Papkovskaia, N., Papkovsky, D. B. and Kerry, J. D. (2002). Use of oxygen sensors for the non-destructive measurement of the oxygen content in modified atmosphere and vacuum packs of cooked chicken patties; impact of oxygen content on lipid oxidation. *Food Research International,* **35**, 577–584.

Smolander, M., Hurme, E. and Ahvenainen, R. (1997). Leak indicators for modified atmosphere packages. *Trends in Food Science and Technology,* **8**, 101–106.

Solomon, J. (2004). Eliminating oxygen. *Meat and Poultry,* **50**(9), 38–41.

Sørheim, O., Nissen, H., Aune, T. and Nesbakken, T. (2001). Use of carbon monoxide in retail meat packaging. In *Proceeding of the Reciprocal Meat Conference*. Savoy, Illinois. American Meat Science Association, 47–51.

Sørheim, O., Nissen, H. and Nesbakken, T. (1999). The storage life of beef and pork packaged in an atmosphere with low carbon monoxide and high carbon dioxide. *Meat Science,* **52**, 157–164.

Sørheim, O., Ofstad, R. and Lea, P. (2004). Effects of carbon dioxide on yield, texture and microstructure of cooked ground beef. *Meat Science,* **67**, 231–236.

Spanos, D., Torngren, M. A., Christensen, M. and Baron, C. (2016). Effect of oxygen level on the oxidative stability of two different retail pork products stored using modified atmosphere packaging (MAP). *Meat Science,* **113**, 162–169.

Sukumaran, A. J., Nannapaneni, R., Kiess, A. and Sharma, C. S. (2016). Reduction of Salmonella on chicken breast fillets stored under aerobic or modified atmosphere packaging by the application of lytic bacteriophage preparation SalmoFresh™. *Poultry Science,* **95**, 668–675.

Summo, C., Pasqualone, A., Paradiso, V. M., Centomani, I., Centoducati, G. and Caponio, F. (2016). Lipid degradation and sensory characteristics of ripened sausages packed in modified atmosphere at different carbon dioxide concentrations. *Journal of the Science of Food and Agriculture,* **96**, 262–270.

Tan, K. H. and Gill, C. O. (1982). Physiological basis of CO_2 inhibition of a meat spoilage bacterium, *Pseudomonas flourescens*. *Meat Science,* **7**, 9–17.

Tewari, G., Jayas, D. S. and Holley, R. A. (1999). Centralized packaging of retail meat cuts: A review. *Journal of Food Protection,* **62**, 418–425.

U.S. FDA. (2002). Food and Drug Administration. Agency Response Letter GRAS Notice No. GRN 000083.

U.S. FDA. (2004). Food and Drug Administration. Agency Response Letter GRAS Notice No. GRN 000143.

Zhao, Y., Wells, J. H. and McMillin, K. W. (1995). Dynamic changes of headspace gases in CO_2 and N_2 packaged fresh beef. *Journal of Food Science,* **60**, 571–575, 591.

21

Nanotechnology-Based Packaging Materials for Fresh and Processed Meats

Brandon Guild, Hans Bernard Tee, and Loong-Tak Lim

CONTENTS

21.1 Nanotechnology in Food 647
21.1.1 Spoilage Reactions in Fresh and Processed Meat 649
21.2 Nanotechnology-Based Meat Packaging Systems 650
21.2.1 Enhanced Barrier Using Nanoclay 651
21.2.2 Application of Nanoclay Composites in Meat Packaging 653
21.2.3 Antimicrobial Active Packaging 654
21.2.3.1 Antimicrobial Ions 658
21.2.3.2 Bacteriophage 661
21.2.3.3 Antimicrobial Proteins and Peptides 663
21.2.4 Intelligent Packaging 664
21.2.4.1 Colorimetric Indicators 667
21.2.4.2 Biosensors 670
21.3 Nanostructured Edible Films and Coatings 671
21.4 Societal Implications 675
21.4.1 Potential Risks 676
21.4.2 Regulation 678
21.4.3 Public Acceptance 679
21.5 Conclusion 679
References 680

21.1 Nanotechnology in Food

The meat industry comprises a large segment of the food industry in many developed countries, significantly contributing to the gross domestic product (Barbut, 2015). Meat consumption is increasing around the world due to rising income levels and increased population growth. The United Nations Population Fund estimates the global population will reach 9 billion in the mid-2040s (UNDESA, 2015; Population Division, 2015). In developing countries, people tend to shift from a plant-based diet to a diet higher in meat as

the society becomes more affluent (Barbut, 2015). As the demand for meat increases, there is a need to develop packaging technology that will meet the requirements of the modern meat processing industry, fulfill changing consumer preferences, and at the same time, address environmental and societal issues.

Nanotechnology is a rapidly growing field, which encompasses scientific and engineering phenomena observed at the nanoscale. By and large, nanomaterials are referring to those that have at least one dimension ranging between 1 and less than 100 nm. Growing research interest and advancement in nanotechnology can be seen across many industries, such as automobiles, biomedical, and electronics. At nanoscale, properties such as melting point, fluorescence, electrical conductivity, magnetic permeability, chemical reactivity, and so on, change dramatically as compared to the larger bulk counterparts (Gupta, 2014). Due to recent advances in manufacturing technologies and atomic microscopy, it is now possible to manipulate and study nanomaterials more efficiently than ever, greatly facilitated by the commercial exploitation of nanomaterials for various applications.

In the food industry, nanotechnologies have been applied in the formulation of various products and packaging materials. For example, nanotechnology has been applied in the development of controlled release systems for flavors and antimicrobials in foods through nanoencapsulation (Figure 21.1). Applying nanotechnology in the manufacturing of food packaging can result in composite structures with enhanced material properties (Lim, 2014). By virtue of their large modulus and crystalline structure, dispersing of nanoparticles within a thermoplastic matrix can form composite materials with an increased tortuous path for gas diffusion, resulting in enhanced barrier properties. Surface modification of these rigid fillers for optimal dispersion in the polymer can also result in packaging structures with substantial mechanical reinforcement, providing potential opportunities for package lightweighting. Other means of improving protection is through the application of surface coatings containing nanomaterials on conventional packaging materials, for improving barrier properties. Figure 21.2 shows how nanomaterials can be incorporated in packaging material. For some pre-

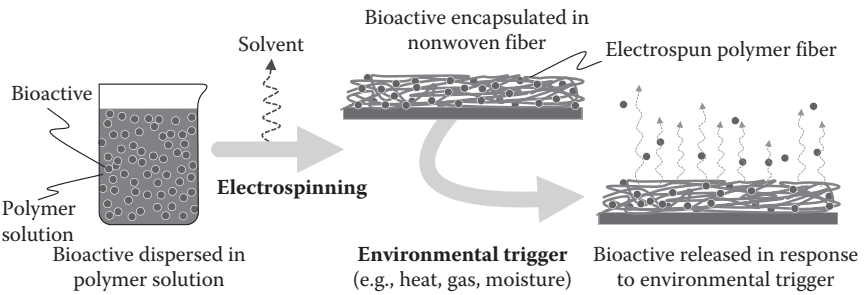


FIGURE 21.1
Schematic of the activated release of a bioactive agent from electrospun nonwoven membrane.

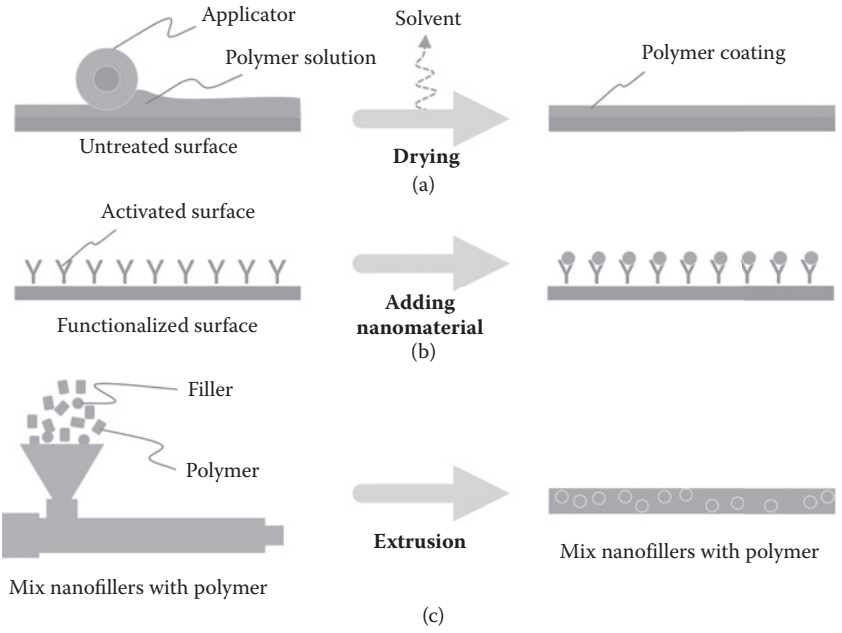


FIGURE 21.2
Incorporation of nanomaterials by (a) coating, (b) surface functionalization, and (c) adding as a filler.

mium products, "intelligent" packaging features that enhance product traceability and those capable of communicating with consumers on actual product freshness will become more integral to meat package design. These meat-packaging advancements will increase the efficiency of supply chain management, improve food safety, reduce waste, and enhance brand and product protection.

In order to leverage nanotechnology for food packaging applications, developing an adequate regulatory framework will also be important for assessing the potential risks and benefits involved. This chapter discusses how nanotechnology can be used to improve the functions of meat packaging, opportunities for using nanomaterials in enhancing material properties of meat packaging, and reviews selected examples in intelligent and edible packaging. In Section 21.4, the societal implications of using nanomaterials in meat packaging are discussed.

21.1.1 Spoilage Reactions in Fresh and Processed Meat

Color and microbiological count are the main factors that dictate the quality of meat products. Color is typically regarded as the first limiting factor in product shelf life. The red color of fresh meat produced via the oxygenation of deoxymyoglobin is a desirable trait for consumers shopping for fresh meat

at retail. For cured and processed meats, the pink-red color is fixed by the conversion of myoglobin to nitrosomyoglobin in the presence of nitric oxide from nitrate. In both cases, inadequate packaging design or inappropriate storage temperature can compromise meat color. For fresh meat products, insufficient oxygen ingress will not allow for sufficient "blooming" of red coloration and may be perceived by consumers as expired product. In cured and processed meats, excess oxygen ingress can lead to microbial activity and discoloration due to the growth of spoilage microorganisms. The microflora of fresh and processed meat is diverse. Pathogens associated with meat include *Campylobacter* spp., *Clostridium botulinum*, verotoxigenic *Escherichia coli*, *Salmonella* spp., *Shigella* spp., *Listeria monocytogenes*, and *Yersinia enterocolitica* (Gill and Gill, 2010). At chill temperatures, *Pseudomonas* and *Lactobacillus* strains that thrive under cold conditions can outcompete other microbes in aerobic and anaerobic conditions, respectively. *Pseudomonas* is most commonly implicated in the formation of the slimy, sticky appearance of old meat. When glucose in meat tissue is used up, *Pseudomonas* begins to degrade protein and metabolize amino acids, resulting in the formation of ammonia and other offensive volatiles. For meats packaged under modified atmosphere, the absence of O_2 inhibits the growth of *Pseudomonas* and other aerobic strains, giving way to a microflora dominated by anaerobes and facultative anaerobes such as *Bronchothrix thermosphacta*, Enterobacteriaceae, *Lactobacillus*, and other lactic acid bacteria (Robertson, 2013).

21.2 Nanotechnology-Based Meat Packaging Systems

Typical packaging format for meat products are presented in Figure 21.3. The simplest package involves wrapping the meat product in flexible materials, such as laminated kraft paper, for temporary containment of meat products sold at retailers and butcher shops to facilitate consumer's transportation. To enhance protection and facilitate product sales on the shelf, meat products

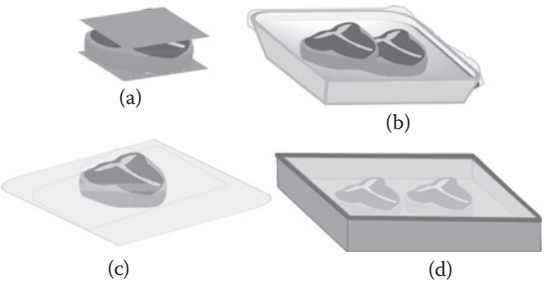


FIGURE 21.3
Illustrations of meat packaging: (a) kraft/butcher paper, (b) expanded polystyrene foam tray with stretch film, (c) vacuum skin package, and (d) modified atmosphere packaging.

are often packaged in thermoformed plastic containers or foamed trays that are overwrapped with stretched film. To further extend the shelf life, more complicated packaging systems such as vacuum packaging and modified atmospheric packaging (MAP) are being used, in conjunction with refrigeration or freezing. In each of these scenarios, the use of nanotechnology can lead to novel packaging designs that improve meat product quality.

21.2.1 Enhanced Barrier Using Nanoclay

Nanoclays, the most common type being montmorillonite (MMT), are layered silicates. The building block of MMT structure consists of two tetrahedral sheets of silica that sandwich a central octahedral sheet of aluminum, iron, magnesium, or lithium hydroxides. Particles of MMT and other nanoclays such as bentonite and kaolinite are available commercially. The use of nanoclay or MMT can allow for an enhanced barrier of meat packaging material when modified and dispersed in a compatible polymer, such as polyethylene (PE), to create a nanocomposite packaging film for meat products (Busolo and Lagaron, 2012). Polymer–clay nanocomposites can improve mechanical, barrier, and physical properties, and thermal stability of polymer and biopolymer composite films due to the high surface area to volume ratio (Lim, 2014). Understanding the factors that affect the physical properties of nanocomposites is important in order to fully exploit nanomaterials for food packaging applications. Of great importance are nanoparticle–matrix and particle–particle interactions (Pukanski and Fekete, 1999). For instance, when 1 or 3 wt% MMT is incorporated into chitosan (a deacetylated derivative of chitin), water vapor permeability of MMT–chitosan composite film can be reduced by about 40% or 45%, respectively (Abdollahi et al., 2012). Tensile strength of the composite film created by these researchers increased significantly through the incorporation of MMT into chitosan up to 3 wt%, but adding more MMT beyond this point does not further improve the mechanical properties of the composite due to MMT particle aggregation. Improved mechanical properties can be achieved through surface modification of nanoparticles that increases interfacial interaction between the nanomaterial filler and matrix, as well as results in uniform dispersion of the fillers to form a coherent polymer network to optimize load transfer between the polymer matrix and the nanofillers (Islam et al., 2013). Other reinforcing fillers for enhancing the material properties of packaging include carbon nanotubes as well as nanoparticles of silver, titanium oxide, silica, nanocalcium carbonate, hydroxyapatite, and nanocellulose crystals.

The use of MMT and other fillers in packaging thermoplastics can enhance barrier performance, which is important in preserving the shelf life of processed meat products, particularly cooked ready-to-eat (RTE) products that are susceptible to color change and spoilage due to O_2 ingress. For fresh meat products, it is important to have the optimal O_2 concentration to maintain color and quality during the limited shelf life. In this regard, nanomaterials

can be used to modify the barrier of a film or packaging material to have the necessary O_2, CO_2, and water vapor flow to ensure microbial growth and light-inducted photooxidation is minimized.

To enhance the material properties of packaging composite structure, the filler particles must be dispersed homogeneously in the polymer matrix by (1) in situ polymerization, (2) solution casting, or (3) melt processing. For in situ polymerization, the layered silicates are expanded with the monomers and the galleries of the silicate layers are being intercalated with the polymer chains as they grow during polymerization. By contrast, in solution casting, the polymer is dissolved in a solvent to form a solution, the nanomaterial added, and the resulting mixture is cast onto a solid surface and the solvent is allowed to evaporate, forming a solidified film. Melt processing is more commonly used in the industry, where nanofillers are dispersed in the molten polymer during extrusion or compounding processes, followed by cooling to solidify the polymer. Regardless of the method used, modification of the filler is often needed to promote its interaction with the polymer. For MMT, one of the strategies used to ensure its homogeneous distribution in thermoplastic is to modify the layered silicate with an organic surfactant. Native MMT is relatively more hydrophilic than typical thermoplastic polymers used in food packaging applications. To ensure the compatibility of the dispersed and continuous phases, organo-modified MMT is often used, where the clay is modified by exchanging the sodium, lithium, calcium cations within the gallery of the layered silicates with organic surfactants, such as trimethyl stearyl ammonium, octadecylamine, and methyl dihydroxyethyl hydrogenated tallow ammonium (Singala et al., 2012; Sengwa and Choudhary, 2012). The exchange of cations within MMT with surfactants allows polymer chains or monomers to intercalate into the galleries in the MMT. The enhanced mechanical strength and barrier properties of MMT nanocomposite, as compared with the neat polymer, can be attributed to the MMT's physical anchorage effects on the polymer molecules, which limit the segmental chain mobility. Moreover, by applying adequate shear during melt processing, the expanded MMT can be exfoliated into separate silicate layers, the dispersion of which in a polymer matrix can increase the tortuous path of permeant molecules moving through the composite structure (Figure 21.4). Another study observed the effect four different surfactants had on their ability to modify MMT clay. Field-emission scanning electron microscopy (SEM) results showed that the size of the dispersed poly(butylene succinate-co-adipate) (PBSA) phase was reduced with the addition of organoclay and the extent of this size reduction was dependent on interlayer spacing of the clay, in addition to enthalpic interaction between the clay surface and the poly(lactic acid) (PLA)–PBSA polymer blend (Ojijo et al., 2011). Dispersion of silicate layers in the blend matrix was also characterized by x-ray diffraction. An improved adhesion between the continuous polymer phases and the fine morphology of the dispersed phase contributed to the improvement in the mechanical and thermal properties of the final composites (Ojijo et al., 2011).

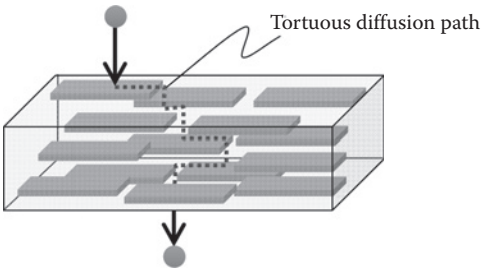


FIGURE 21.4
Torturous diffusion path of a permeant molecule through a nanocomposite.

21.2.2 Application of Nanoclay Composites in Meat Packaging

Picouet et al. (2014) investigated the use of nanoclay to modify a polyamide 6 (PA6)-multilayer film in vacuum-packed meat. The films were prepared via A/B/C/B/A structure. The outer layers (A) were composed of low-density polyethylene (LDPE) (LDPE Dow 410E), while the interlayer (C) was composed of either commercial nanoclay modified polyamide or a standard PA6 (Ultramid B36LN, BASF, Ludwigshafen, Germany). B layers were adhesives. The prototype films were prepared via cast film coextrusion at 38 rpm for the LDPE, 50 rpm for the polyamide, and 15 rpm for the adhesive. Vacuum-aging of meat in the multilayer polyamide nanocomposite took place for 7, 14, and 21 days at 2 ± 2°C in darkness. After each aging time, the 100 mm pieces of beef loin were cut into four slices in 20 mm thick, packed in MAP, and displayed using the same conditions as the nonaged meat slices, which were in a display case with 12 h exposure to fluorescent light and 12 h exposure to darkness. It was found that reinforced PA6 films reduced the oxygen transmission rate by over 50%, as compared with the neat PA6 films at an equivalent thickness (Picouet et al., 2014).

Beatrice et al. (2010) reported enhanced barrier and mechanical properties of PA6 nanocomposites with organo-modified exfoliated MMT. They observed improved tensile mechanical properties with the addition of 5 wt% modified MMT in the PA6 nanocomposite film prepared by first compounding PA6 with nanomaterial twin-screw extrusion, then processed into films by extrusion-blowing (Beatrice et al., 2010). Water vapor and oxygen permeability rates of the nanocomposites containing 3% or 5% MMT were lower than the neat PA6 (Beatrice et al., 2010). In another study, Mohammad and Abolghasemi (2011) observed that O_2 gas permeability through PA6 nanocomposite films decreased significantly by the addition of 3 wt% of modified sodium MMT clay.

The rising popularity of nanoclay fillers for food applications can be attributed to the relatively low cost of the raw materials and improvements to the O_2 barrier that can be made for films for which the barrier would be other-

wise low. Concerning the costs of using MMT, though the cost of many MMT nanoclays can be high on a per-kilo basis, a relatively small amount, often less than 5 wt% in a film formulation, is required to achieve the desired effect. The wt% of MMT used in the formulation also substitutes a given amount of polymer of known cost, and the benefits to product shelf life imparted by using the MMT may further help to offset the cost increase. Future work should continue on evaluating the efficacy of MMT composite materials for modified atmosphere meat packaging under typical storage conditions encountered during distribution. Enhanced barrier and mechanical properties of nanocomposite over the neat polymer imply that less packaging material could be used in meat packaging through the incorporation of nanoclays in the package structure, providing opportunities for weight reduction and cost savings. Commercially available nanoclay-based polymer films that may be advantageous for meat packaging are the Imperm® and Durethan® film series by Nanocor© and Lanxess©, respectively. These nanoclay-based polymers are transparent with improved stiffness and oxygen-scavenging ability (Pradhan et al., 2015). Imperm is made up of nylon and nanoclay with oxygen-scavenging properties. Durethan is a polyamide-based film which can be used as synthetic sausage casings (Flanagan and Singh, 2006). In contrast to the many benefits to performance imparted by MMT, one area of concern for clay-based nanocomposites is the potential migration and toxicity of the dispersed nanomaterials—an important but seldom studied issue. These risks are addressed in Section 21.4.2 of this chapter.

21.2.3 Antimicrobial Active Packaging

Over the past 10–15 years, many studies incorporated antimicrobials and antioxidants such as organic acids, essential oils, and natural extracts into a variety of polymers to create active packaging for food. Active packaging is defined as "packaging in which subsidiary constituents have been deliberately included in or on either the packing material or the package headspace to enhance the performance of the package system" (Robertson, 2013). Recent advances in this area have been focusing on exploiting the unique properties of nanomaterials. This section reviews some of the recent developments in nano- and micromaterial-enabled active packaging systems.

Thymol is a natural extract of thyme known to have antimicrobial effects on pathogens and spoilage organisms in meat products, as well as provide antioxidant effects (Fratianni et al., 2010; Jayasena and Jo, 2013). The nanocomposite can be used as a carrier for gradual release of thymol from the composite matrix over time, allowing for enhanced preservation of foods for extended time. Elucidating the release kinetic will be important when deploying such compounds in meat products to inhibit certain strains of spoilage and pathogenic microorganisms, especially under typical refrigerated distribution and storage conditions. Further research in developing food-grade solvent for the preparation of antimicrobial composites will be essential to ensure commercial viability.

In another study, MMT nanoclay and polyphenols extracted from brewery waste were incorporated into ethylene vinyl acetate (EVA) and LDPE films (Barbosa-Pereira et al., 2014). In beer production, surplus polyphenols cause excess haze and cloudiness. The removal of these polyphenols will improve the stability and shelf life of beers. Barbosa-Pereira et al. (2014) developed a method to remove the polyphenols using polyvinylpolypyrrolidone resin (PVPP) in a clarification step, producing a PVPP "sludge" with a high content of phenolic compounds such as flavonols, hydroxycinnamic, and hydroxybenzoic acids including gallic acid, caffeic acid, p-coumaric acid, and ferulic acid. Briefly, in their approach, the sludge from beer filtration was acidified to pH 1.5 with HCl (37%), followed by extraction of polyphenols using ethyl acetate (1:2 ratio) by stirring for 30 min at room temperature. Following phase separation, the organic portion was collected via decanting and evaporated to dryness at 40°C under vacuum. These researchers added the dry phenolic extract to organo-modified MMT clay (Nanobioter® AE 21) at 20 wt% level to create functionalized MMT. The functionalized nanoclays were then incorporated into EVA and LDPE films during extrusion, or the dry polyphenol powder was added directly into the polymer matrix of films during extrusion by dry compounding using a microcompounder (Haake MiniLab II Micro Compounder, Thermo Scientific, Germany) with a corotating twin-screw extruder operating at a speed of 150 rpm at 150°C for 6 min. The antimicrobial activity of the active films in this study was assessed by the ASTM E 2149-01 standard test method. EVA film with 6 wt% of free, unfunctionalized phenol extract was effective against *E. coli* after incubation for 48 h and throughout the entire incubation time for *Staphylococcus aureus*. All EVA-active films with MMT-functionalized phenolic extract showed antimicrobial activity against *S. aureus* during incubation. Considering the overall bioactive migration and antimicrobial effects, EVA films appeared to be the more suitable type of film for incorporating the MMT-factionalized phenolic extract and provided more favorable antimicrobial and antioxidative results than LDPE in this work (Barbosa-Pereira et al., 2014). An intriguing finding from this study was that the inclusion of the functionalized nanoclays improved the effectiveness of the active packaging film, even though they contained lower amounts of natural extract (Barbosa-Pereira et al., 2014). These studies suggest there is an advantage in functionalizing MMT with low molecular weight antimicrobials such as phenolic compounds, and that nanoclay functionalization may facilitate their controlled release into in vitro systems.

Halloysite (HT) is a two-layered aluminosilicate nanotube with internal diameter of 20–50 nm and length up to 10 mm (Lvov et al., 2015). These nanotubes are cost-effective, widely available, durable, and possess high levels of mechanical strength and biocompatibility. They are of particular research interest due to the chemistry of the inner and outer surface of these nanotubes, and the possibilities of loading antimicrobial agents inside their core, thereby designing carrier systems to control their release for meat packaging

applications. Recent research in this area has shown the ability of HT nanotubes to be used for controlled released bioactives and antimicrobials in food packaging systems (Boelter and Brandelli, 2016; Tornuk et al., 2015). The effectiveness of active packaging films of linear low-density polyethylene (LLDPE) with thymol-loaded HT for controlling discoloration, lipid oxidation, and *E. coli* O157:H7 growth in fermented sausage and fresh beef was investigated by Tornuk et al. (2015). Fresh beef color was maintained up to four days by active use of the nanocomposite films, while a bacteriostatic/bactericidal effect on *E. coli* O157:H7 in fresh beef was reported during a seven-day storage period (Tornuk et al., 2015). Reduced lipid oxidation for fermented sausage vacuum-packaged in active nanocomposite films was observed as well.

In a study by Boelter and Brandelli (2016), liposomes prepared with either soybean lecithin or Phospholipon® 90G (a pure phosphatidylcholine stabilized with 0.1% ascorbyl palmitate supplied by Lipoid GMBH, Germany), with particle sizes ranging from 124 to 178 nm, showed that the Phospholipon liposomes displayed greater stability than those prepared with soybean lecithin. The researchers further loaded Phospholipon liposomes with 1.0 mg/mL nisin into HT and dispersed into casein or gelatin at 0.5 g/L of HT to form composite films that had a smooth surface, but increase in roughness with increasing liposomes and HT contents. Both gelatin and casein nanocomposite films exhibited antimicrobial activity against *L. monocytogenes* in zone of inhibition tests performed on Brain–Heart Infusion agar plates previously inoculated with a swab submerged in a suspension of *L. monocytogenes* ATCC 7644 (Boelter and Brandelli, 2016). In another study, starch/HT/nisin nanocomposite films were developed as active antimicrobial packaging and tested on skimmed milk agar and on cheese (Meira et al., 2016). SEM of composite films revealed that all samples were homogeneous, but that aggregates appeared on film surfaces when a higher amount of nisin was added. Moreover, the addition of nisin decreased thermal stability of nanocomposites as compared with starch/HT films without nisin. The antimicrobial activity was tested against *L. monocytogenes*, *C. perfringens,* and *S. aureus* in skimmed milk agar and on Minas Frescal cheese surface previously inoculated with *L. monocytogenes*. Inhibition toward all the organisms tested was seen in skim milk agar, and during storage of cheese over 14 days, antimicrobial nanocomposite films with 2 g/100 g nisin significantly reduced the initial counts of the bacterium after four-day storage (Meira et al., 2016). For films with 6 g/100 g nisin, significant decrease in *L. monocytogenes* population was observed after day 1, with no bacteria detected at the 4-, 7- and 14-day sample points indicating a bactericidal effect from the applications of the active nanocomposites. While much of the research on HT application on meat systems demonstrates the ability of HT to permit controlled release of a bioactive into a meat product, as well as improve some mechanical properties such as tensile strength in starch films (Meira et al., 2016), more work needs to be done on a broader variety of meat products, as well as coupling studies with sensory evaluation of the product and nanotoxicity studies.

Studies have shown that composite packaging film loaded with MMT or HT and bioactives from botanicals may be promising in direct contact applications, such as for wrapping or skin packaging of fresh meats. Future studies on elucidating the mechanism of release of bioactives, stability of these compounds during storage, as well as more in-depth investigation of the nanocomposite films on meat systems under typical distribution conditions will be useful to demonstrate the antimicrobial efficacy and commercial viability. A summary of select works in antimicrobial active packaging appears in Table 21.1.

TABLE 21.1

Summary of Antimicrobial Active Packaging Systems Relevant to Meat Systems

Nanomaterials	Bioactive (If Added)	Packaging Material(s)	Meat Product Application	Effects	References
MMT nanoclay	n/a	PA6	Beef loins	Enhanced shelf life Lowered thickness of packaging material	Picouet et al. (2014)
MMT	n/a	PA6	Not tested	Reduced WVTR and O_2 permeability	Beatrice et al. (2010)
Silver (Ag) nanoparticles and **zinc oxide (ZnO)**	n/a	LDPE	Chicken breasts	Inhibited *Escherichia coli, Pseudomonas aeruginosa, Listeria monocytogenes*	Panea et al. (2014)
Ag nanoparticles	n/a	PLA	Growth media	Inhibited *E. coli, Salmonella, L. monocytogenes*	Munteanu et al. (2014)
Ag nanoparticles	n/a	Microcrystalline cellulose	Growth media	Inhibition of *Bacillus stearothermophilus*	Vivekanandhan et al. (2012)

(*Continued*)

TABLE 21.1 (*Continued*)

Summary of Antimicrobial Active Packaging Systems Relevant to Meat Systems

Nanomaterials	Bioactive (If Added)	Packaging Material(s)	Meat Product Application	Effects	References
O_2Block® (modified nanoclay)	Thymol essential oil	PCL	Growth media	Nanoclay increased solubility of thymol and enabled controlled release	Sanchez-Garcia et al. (2008)
MMT nanoclay	Polyphenol extract from beer retentate	EVA and LDPE films	Beef	Reduced *Staphylococcus aureus* growth Reduced oxidation and color changes	Barbosa-Pereira et al. (2014)
Halloysite nanoclay	Thymol essential oil	LLDPE	Fermented sausage and fresh beef	Reduced lipid oxidation in fermented sausage Bacteriostatic against *E.coli* in fresh beef over 7-day shelf life	Tornuk et al. (2015)
Encapsulated bacteriophage		Alginate and pectin microspheres; phospholipids	None	Reduced sensitivity to low pH Prolonged storage times	Dini et al. (2012) and Singla et al. (2016)

EVA, ethylene vinyl acetate; LDPE, low-density polyethylene; LLDPE, linear low-density polyethylene; MMT, montmorillonite; PA6, polyamide 6; PCL, polycaprolactone; PLA, poly(lactic acid); WVTR, water vapor transmission rate.

21.2.3.1 Antimicrobial Ions

One of the most investigated antimicrobial ions in recent literature is the silver nanoparticle (AgNP) with particle size ranging from 1 to 100 nm. While AgNPs are often described as "silver," some are composed of silver oxide, in addition to the silver element. AgNPs of different shapes can be produced

depending on the synthesis processes, but sphere, octagon, and thin sheet are common. AgNPs have been investigated in depth for their antimicrobial properties. Reasons why AgNPs are of notable interest as an antimicrobial are their high thermal stability, sustainable bactericidal activity, and broad spectrum efficacy against bacteria, viruses, and fungi (Dehnavi et al., 2012). Also, from a safety perspective, the toxicity of silver to human is fairly low. A study assessing the acute toxicity of AgNPs indicated the LD_{50} of colloidal AgNPs is greater than 5000 mg/kg body weight (Maneewattanapinyo et al., 2011). Another study observed the cytotoxicity of ZnO nanoparticles and AgNPs in human epithelial colorectal adenocarcinoma (Caco-2) cells. It was determined that ZnO nanoparticles possessed higher cytotoxicity than AgNPs when applied in the concentration range, and that the LD_{50} of ZnO NPs in Caco-2 cells is 0.431 mg/L. In comparison, the amount of nano silver used in many packaging materials is approximately 0.05 mg/m^3 (Travan et al., 2009; Vivekanandhan et al., 2012).

To facilitate end-use deployment, AgNP is often temporarily encapsulated in carrier matrices that allow for controlled release of the ions to achieve optimal antimicrobial efficacy. For example, electrospun PLA nanofibers incorporating AgNP and vitamin E may be used in fruit and juice packaging due to large surface area of nanofibers, which allows for the diffusion of AgNP and antioxidant onto the surface of these products (Munteanu et al., 2014). Conceivably, a similar approach may be feasible for fresh meats, which have a high moisture content that facilitates the diffusion of these active agents to inhibit the proliferation of microorganisms. The mode of antimicrobial action exhibited by AgNP is depicted in Figure 21.5.

AgNP has been encapsulated in chitosan gels, PE, polyvinyl acetate, and cellulose films by various researchers. Combining AgNP and chitosan films can result in packaging materials with synergistic antimicrobial effects. Travan

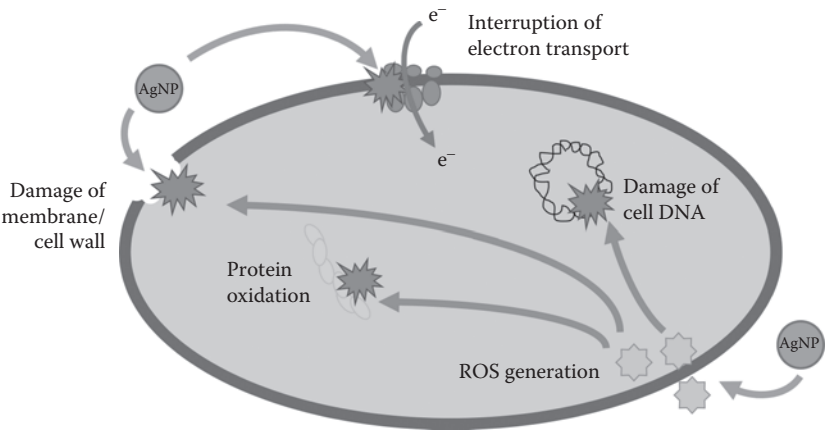


FIGURE 21.5
Possible modes of antimicrobial activity for nanosilver.

et al. (2009) developed a novel method to coat AgNP particles in chitosan and encapsulate them in an alginate hydrogel (Travan et al., 2009). The results suggested that the gels could prevent AgNP against aggregation, providing antibacterial activity against *E. coli, S. aureus,* and *Pseudomonas aeruginosa* without exhibiting toxicity to mammalian cells (Travan et al., 2009). Potara et al. (2011) also showed that chitosan-coated AgNP exhibited antimicrobial activity against two strains of *S. aureus* when incorporated into Mueller Hinton agar. These findings may be of interest to meat processors, as these pathogens can often be a source of foodborne illness from meat products.

Recently, a study reported one interesting approach to encapsulate AgNP ions by impregnating them into microcrystalline cellulose, followed by the inclusion in PLA polymer to produce antimicrobial (AM) films (Vivekanandhan et al., 2012). Here, the researchers used a curry leaf extract-mediated bioreduction process to reduce silver nitrate. The silver ions formed were nucleated as AgNP on dispersed fragments of microcrystalline cellulose (MCC). The AgNP-impregnated MCC was washed with deionized water and dried in an oven at 55°C overnight. The powder was then dispersed in solvent-casted PLA films that demonstrated antimicrobial properties against *Bacillus stearothermophilus* via Charm disk assay. Transmission electron microscopy and x-ray diffraction, respectively, confirmed the formation of spherical AgNP and a face-centered cubic lattice crystalline structure within the MCC network (Vivekanandhan et al., 2012). In another approach, researchers have exploited corona discharge as a pretreatment for PE and LDPE films to activate their surfaces prior to attaching AgNP (Sundaram et al., 2013; Dehnavi et al., 2012). Using surface activation methods, or mediated release methods from inside the film, effective active packaging films may be created for packaging meat in vacuum-sealed packages for either fresh or processed meats.

Besides AgNP, other inorganic nanoparticles such as zinc oxide and titanium dioxide have also been exploited for the development of antimicrobial active packaging for meat products. In a study, both silver and zinc oxide nanoparticles were added to LDPE, at 5% or 10% (w/w) levels to packaging prepared using a JSW J85 ELII electrical injection molding machine, using a clamping force of 85 t and a screw diameter of 35 mm to prepare composite structures for the packaging of chicken meats (Panea et al., 2014). Freshly skinned and deboned chicken breasts were packaged in neat LDPE, 5 wt% ZnO + AgNP, or 10 wt% ZnO + AgNP, and flushed with a gas mixture of 70% O_2, 20% CO_2, and 10% N_2. Samples were stored and evaluated up to 21 days. The researchers reported that adding ZnO + AgNPs to LDPE packaging has an antimicrobial effect against Enterobacteriaceae at 10 and 15 days of storage, and that the amount of AgNP and ZnO migration from packaging was well within the level prescribed by the European Union (Panea et al., 2014). These results are encouraging for meat processors in extending the shelf life of highly perishable meat products, as well as packaging converters in developing commercially viable antimicrobial packaging films by direct incorporation of inorganic nanoparticles into polymers via melt processing.

21.2.3.2 Bacteriophage

Besides organic and inorganic antimicrobial species, bacteriophages have also been investigated by researchers for AM packaging of food. For example, the Listex™ P100 bacteriophage, which is considered as Generally Recognized as Safe (GRAS) by the Generally Recognized as Safe (GRAS) by the United States Food and Drug Administration (USFDA), specifically targets and lyses cells of pathogenic *L. monocytogenes* (USFDA, 2007). The phage has been tested on RTE cooked turkey and roast beef products (Chibeu et al., 2013). These researchers found topical application of a ListexP100 cocktail of 107 PFU/cm^2 to be effective in reducing counts of *L. monocytogenes* by 2.1 $\log_{10}$ CFU/cm^2 for cooked turkey and 1.7 $\log_{10}$ CFU/cm^2 for roast beef, compared to untreated meat samples. Listex also exhibits listericidal effect on fresh sausages (Rossi et al., 2011), fresh salmon fillets (Soni et al., 2010), and fresh cut fruit and fruit juice (Oliveira et al., 2014). Other approved phage preparations that may be applied to meat products include Salmonellex™ (Micreos; Wageningen, the Netherlands) targeting *Salmonella* spp., as well as SalmoFresh™ and ListoShield™ (Intralytix; Baltimore, Maryland), targeting *Salmonella* spp. and *Listeria* spp. bacteria, respectively.

To extend the use of bacteriophage in packaging for food, several challenges must be addressed to maintain phage stability and activity (Figure 21.6). First, the phage must be oriented or incorporated in the packaging matrix such that it can diffuse to the contacting food and bind with the target pathogens. Second, the stability of the phage in elevated temperature and pressure conditions typically encountered during food processing needs to be investigated, especially for products that are subjected to thermal processes (e.g., retort, pasteurization) and high hydrostatic pressure processing for RTE processed meat products. Thermal stability of the phage is genetic and strain type dependent. For instance, some mutants of the T7 phage cannot replicate at temperatures above 40°C (Kumar et al., 2001). Loss of phage DNA from

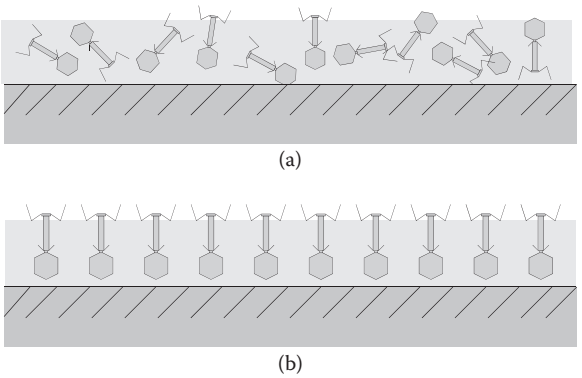


FIGURE 21.6
Bacteriophages aligned (a) randomly and (b) aligned with tail facing away from the packaging surface.

capsids and phage head and tail disintegration can also occur in some phages during prolonged exposure near 80°C (Marvig et al., 2011). In a study by Black et al. (2010), coliphages—T_4, MS2, Qβ, λ *imm*434, λ cI 857, and λ cI 857A—were used as surrogates for Hepatitis A and Aichi viruses for high-pressure treatment of an oyster slurry. They reported that the MS2 phage could survive treatment at 60°C at 500 MPa for 5 min, while other surrogate phages displayed resistance up to 250 and 400 MPa (Black et al., 2010).

High pathogen specificity and the great antimicrobial efficiency of phages make bacteriophages a desirable means for controlling pathogen growth in meat products. While this area has received considerable growing research and commercial interests, several hurdles remain to be tackled. From an end-use standpoint, long-term stability of the phage in the packaging material is important to ensure its AM efficacy. This is of concern considering that food packaging materials are usually being stored for a prolonged period in typical commercial settings. A few methods have been investigated as a means of prolonging phage stability. To increase storage stability, bacteriophages selective for *S. aureus* (strains 9563 and 8588, NCIMB, respectively) and *P. aeruginosa* (strain 217M) have been freeze-dried from SM buffer with or without the addition of gelatin (Puapermpoonsiri et al., 2010). The lytic activity of freeze-dried phage was compared against control samples stored in a tris-buffer (1 M tris–HCl, 0.1 M NaCl, 8 mM $MgSO_4$, 0.1 g/L gelatin, pH 7.5) with and without gelatin and sucrose or poly(ethylene glycol) (PEG) 6000. Phage samples were stored at 4°C and lytic activity was tested up to 30 days. The freeze-dried formulations proved more stable than for bacteriophage encapsulated in PEG matrix (Puapermpoonsiri et al., 2010). Besides freeze-drying, other encapsulation methods involving different biopolymer matrices and preparation technologies may also be useful in enhancing the stability of the bacteriophage. For example, alginate or pectin biopolymers have been utilized to encapsulate CA933P phage (lytic for Enterohemorrhagic *E. coli* [EHEC] serotypes of *E. coli*) in microspheres. Microspheres of emulsified pectin displayed good encapsulation efficiency and protection against acidity, with phages remaining active after 30 min exposure at pH 1.6, and also protected the phage from pepsin activity (4.2 mg/mL). On the other hand, free phages were completely inactivated at pH 1.6 or with pepsin (0.5 mg/mL) after 10 min of exposure (Dini et al., 2012). Another study developed a bacteriophage encapsulation system utilizing liposomal phospholipid bilayers to ensure efficient phage delivery to the site of infection by multidrug-resistant strains of bacteria (Singla et al., 2016). In this study, liposome prepared using soy phosphatidylcholine with Tween 80 and protamine sulfate was most stable at 4°C, having insignificant reduction (0.096 log PFU/mL) in the number of entrapped phages during the entire nine-week storage period. The phage liposomes were found to be less stable at room temperature and highly unstable at 37°C with 1.16 log PFU/mL reduction as compared to the initial number of entrapped phages after nine weeks for phages stored at room temperature (Singla et al., 2016).

Comparisons have also been made between storage of bacteriophage in dry versus wet/moist environments. It was observed that when wet microspheres of 0.8% alginate–5% whey protein, containing Felix O1, were stored at 4°C, the phage remained viable for up to six weeks (Tang et al., 2013). This is comparable to results obtained for chitosan-coated alginate microspheres used to encapsulate bacteriophage for oral delivery in another report (Ma et al., 2008). Tang et al. (2013) also tested the activity of Felix-O1 bacteriophages that were immersed in maltodextrin, drained, and then dried at 22°C for 30 h in a laminar flow hood. For air-dried phage, storage at 4°C showed better stability than those at room temperature. Moreover, 100% survival was observed for over two weeks (Tang et al., 2013). In another study, the antimicrobial efficacy and stability of a phage in edible films made from whey protein isolate was characterized using an *E. coli* growth inhibition assay and plaque-forming unit determination (Vonasek et al., 2014). The researchers reported that whey protein isolate films stabilized phages during storage on a light-exposed laboratory bench at 22°C, and in dark, 4°C conditions without significant loss in phage infectivity for greater than one month.

These studies are relevant to meat processors and packaging designers to demonstrate the encapsulation technologies available to enhance the stability of bacteriophages, in order to ensure that they survive the thermal and pressure treatment processes essential to achieve the required lethality on the target pathogens. This will give meat processors the flexibility to add an approved bacteriophage as an in-process treatment and as an additional microbial growth hurdle prior to packaging of finished product.

21.2.3.3 Antimicrobial Proteins and Peptides

Lysins, also known as endolysins or murein hydrolases, are enzymes produced by bacteriophages for the purpose of disrupting a host's cell wall during the final stage of the lytic cycle. Lysins are highly evolved enzymes that are able to target one of the five bonds in peptidoglycan (murein), the main component of bacterial cell walls. This allows the release of progeny virions from the lysed cell. These enzymes are being investigated for their use as biocontrol agents in the food industry due to their effectiveness and specificity (Mahony et al., 2011; Garcia et al., 2010a). Lysins may prove a preferable biocontrol agent over antibiotics, the latter being susceptible to the evolution of bacterial resistance in target strains. Lysins have shown to have a synergetic effect with nisin on reducing *S. aureus* growth in pasteurized milk (Garcia et al., 2010b). Future studies should determine if synergistic effects can also be demonstrated in fresh or RTE meat products.

Nisin is a polycyclic antibacterial peptide produced by the bacterium *Lactococcus lactis* that is used as a food preservative. In foods, nisin has primarily been applied to meats and cheeses to control growth of Gram-positive spoilage and pathogenic bacteria. It is common to use nisin in foods at levels ranging from ~1 to 25 ppm, depending on the food type and regulation in the

jurisdiction of production. The effect of nisin and ethylenediaminetetraacetic acid (EDTA) treatments on the shelf life of fresh chicken meat stored using MAP at 4°C has been evaluated (Economou et al., 2009). Chicken meat was subjected to nine different antimicrobial treatment combinations: N1 (AM-free control sample), N2 (500 IU/g; no EDTA added), N3 (1500 IU/g; no EDTA added), N4 (500 IU/g; 10 mM EDTA), N5 (1500 IU/g; 10 mM EDTA), N6 (500 IU/g 50 mM EDTA), N7 (1500 IU/g; 50 mM EDTA), N8 (10 mM EDTA; no nisin added), and N9 (50 mM EDTA; no nisin added). In each treatment, the chicken breast samples were dipped in one of the test solutions, packaged in MAP with a 65%:30%:5% CO_2:N_2:O_2 gas mixture, and stored at 4°C for up to 24 days. Plating stomached chicken samples during the study period showed that N3, N4, N5, N6, and N7 decreased populations of mesophilic bacteria, *B. thermosphacta*, lactic acid bacteria, and Enterobacteriaceae. Sensory analysis suggested chicken was best preserved under treatments N6 and N7, maintaining acceptable odor attributes up to 24 and 20 days of storage, respectively (Economou et al., 2009). In another study, the effect of nisin combined with different packaging treatments on *L. monocytogenes* growth in RTE meat (turkey bologna) was evaluated (Naas et al., 2013). Here, bologna was inoculated with *L. monocytogenes* and exposed to 100% CO_2, air, or vacuum MAP, each with and without nisin. *L. monocytogenes* counts were 1–2 log CFU/cm^2 lower for each treatment with nisin. 100% CO_2 packaging prevented outgrowth throughout 42 days of storage whereas a 1–2-log increase in populations during storage was observed for air and vacuum packed. Reportedly, nisin (500 IU/mL) combined with 100% CO_2 was the most effective in preventing the growth of *L. monocytogenes* on bologna during 42 days of refrigerated storage (Naas et al., 2013). More recently, another study has focused on assessing the bacterial communities in beef burgers stored in nisin-coated LLDPE packaging, and highlighted the effectiveness of this strategy in prolonging beef burger shelf life (Ferrocino et al., 2016). In this study, the researchers evaluated beef from two different production lots. Control samples showed a significantly higher abundance of bacteria taxa sensitive to nisin, such as *Kocuria rhizophila, Staphylococcus xylosus, Leuconostoc carnosum*, and *Carnobacterium divergens,* as compared to control samples. However, the nisin-treated batch exhibited a significantly lower amount of nisin-sensitive bacteria found on treated samples (Ferrocino et al., 2016). These nisin studies demonstrate the effectiveness of this antimicrobial under different meat packaging configurations, which is promising for inhibiting the growth of both spoilage and pathogenic bacteria.

21.2.4 Intelligent Packaging

The U.S. Center for Disease Control (CDC) estimates that 9.4 million cases of foodborne illnesses are caused by 31 major pathogens in the United States each year (Scallan et al., 2011). Most cases in the United States have been attributed to produce (46%) while meat and poultry are the second most common sources at 26% (Painter et al., 2013). To help manufactures and consumers

identify contaminated products, intelligent packaging with enhanced communicative functions that provide indication on food quality and safety of the food can be useful for reducing the spread of foodborne illness, protecting brand name, and avoiding financial strain of lawsuits. As fresh or processed meats approach their end of shelf life, they will deteriorate and develop off odors, discoloration, and other physical and sensory cues that result in product rejection by consumers. However, many packaged products are isolated from the environment and as a result, consumers must rely on "best before" or "sell-by" dates to accept or reject the products. Unfortunately, these printed dates are often determined conservatively by meat processors based on a "worst-case" scenario and typical conditions encountered by the product during storage and transportation. Thus, a deviation from these assumed conditions, such as unexpected thermal abuses during product distribution and postprocessing contamination, will overestimate the shelf life, potentially resulting in product rejection, or worse still, foodborne illness. To overcome these hurdles, an intelligent packaging approach has been explored by researchers. By definition, intelligent packaging in food is equipped with internal or external indicators that provide information about the quality or history of the product, hence enhancing the communication aspect of the product (Lim, 2014). Selected intelligent examples relevant to the packaging of meats are presented below. Select intelligent and biosensing packaging systems and their tested or potential application in meat products is summarized in Table 21.2.

TABLE 21.2

Summary of Intelligent and Biosensing Packaging Systems Cited with Tested or Potential Application in Meat Systems

Nanomaterials	Packaging Material(s)	Meat Products	Effects	Reference
Semiconductor nanocrystals	Polyethylene	Uncooked bacon	Detect O_2 to assure package seal integrity	Mills (2005)
Carbon-coated copper nanoparticles	n/a	Not tested	RH detection due to optical shifting; 50 nm change for 1% change in RH	Luechinger et al. (2007)
Nanocubic ZnO/SnO_2	Sputter-coated glass substrate	Not tested	RH detection via luminescence change in the presence of different RH levels	Md Sin et al. (2013)

(*Continued*)

TABLE 21.2 (*Continued*)

Summary of Intelligence and Biosensing Packaging Systems Cited with Tested or Potential Application in Meat Systems

Nanomaterials	Packaging Material(s)	Meat Products	Effects	Reference
TiO_2 nanoparticles	PEO with glycerol, MB, and nano-TiO_2	Not tested	Electrospun indicator more sensitive to RH change than solvent cast nanocomposite	Mihindukulasuriya and Lim (2013)
Nanofibrils of perylene-based fluorophores	Deposition on glass substrate	Not tested	Millisecond response time for vapor sensing Color change from red to blue	Che et al. (2008)
SnO_2 nanoparticles + ZnO microrod composite	Sensor material coated on Al_2O_3 rods placed inside test jars containing fish	TMA detection in fish	Good sensitivity to TMA	Zhang and Zhang (2008)
Fe_3O_4 nanoparticles bioconjugated to streptavin	(n/a—Biosensor)	*E.coli* O157:H7 in ground beef	Detected as low as 1.2×10^3 cells of *E. coli* in ground beef	Varshney et al. (2007)
Anti-*Salmonella* antibody gold nanoparticles	(n/a—Biosensor)	*Salmonella* on growth media	Bacteria bound to antibody-coated nanoparticles exposed to near-infrared radiation, photothermal lysis observed	Wang et al. (2010)
Silver-coated PVA immobilized on mica sheets	(n/a—Biosensor)	Tested on chicken rinse water	Rapid identification of *Salmonella* Typhimurium, *E. coli*, and *L. innocua* via surface-enhanced Raman scattering	Sundaram et al. (2013)

MB, methylene blue; PEO, poly(ethylene oxide); PVA, polyvinyl alcohol; RH, relative humidity; TMA, trimethylamine.

21.2.4.1 Colorimetric Indicators

One of the potential applications of intelligent packaging is in meat products packaged in MAP systems, which will benefit from continuous monitoring of the headspace gas composition during distribution. Nanomaterial-based indicators that provide visibly interpretable information have been developed by Mills and coworkers for relative humidity (RH) and oxygen detections. Humidity detection is of importance in meat packaging because excess moisture in meat packaging can accelerate spoilage in dried meats such as jerky and for this reason, desiccants are often included in packaging to enhance quality and shelf life. The reversible RH indicator ink is based on methylene blue (MB) and urea (Mills et al., 2010). In the work done by Mills and coworkers, it was also found that when MB is encapsulated within a polymer, such as hydroxyethyl cellulose with an excess of urea, the dye appears white under ambient or dry conditions yet is rapidly and reversibly rendered blue colored when exposed to RH values >85% (Mills et al., 2010). The oxygen indicator involved a reaction between nanosized TiO_2 or SiO_2 with a redox MB dye (Lawrie et al., 2013; Mills, 2005). To create the indicator, TiO_2 particles, with diameter ~5 nm, were prepared via hydrothermal treatment of titanium isopropoxide in the presence of tartaric acid. The indicator ink was composed of a redox dye (MB), a colloidal semiconductor photocatalyst (TiO_2), and a sacrificial electron donor (tartaric acid). The ink was inkjet-printed on a polyester film, resulting in a colorimetric oxygen-indicating film with a thickness of ~63 nm. Upon exposure to three minutes UVA light (4 mW/cm^2), the indicator was photobleached to a white/light blue color where MB was reduced to leucomethylene blue. The photobleached film recovers its blue color in approximately 12 h under ambient conditions (~21°C, ~60% RH, 21% O_2), but remained bleached in the absence of oxygen. The rate of recovery increased linearly with increasing O_2 concentration, and also RH and temperature dependent. The process of color loss and recovery of the O_2 indicator as part of a meat package can be seen in Figure 21.7.

CO_2 is one of the gases commonly deployed in MAP. Typically, CO_2 concentration in MAP of meat products ranges from 20% to 100% for the purpose of inhibiting the proliferation of microorganisms, especially the Gram-negative bacteria such as Pseudomonads and psychrotrophs, but is less effective against the lactic acid bacteria. To ensure the efficacy of the elevated CO_2 MAP for meat, it is desirable to continuously monitor the in-package CO_2 level. Zhang and Lim (2016) developed a printing methodology, based on bubble-jet printing technology, for printing colorimetric CO_2 indicators. In their studies, pH dyes (M-cresol purple and/or phenol red), along with a phase transfer agent (tetrabutylammonium hydroxide) were dissolved in ethylcellulose solution using a 1:2 ethanol:1-butanol solvent. Different formulations of inks were printed on paper or plastic substrates using a commercially available thermal inkjet printer, resuling in colorimetric indicators that were sensitive to CO_2 in the absence of moisture (Zhang and Lim, 2016). Mills and Skinner (2010)

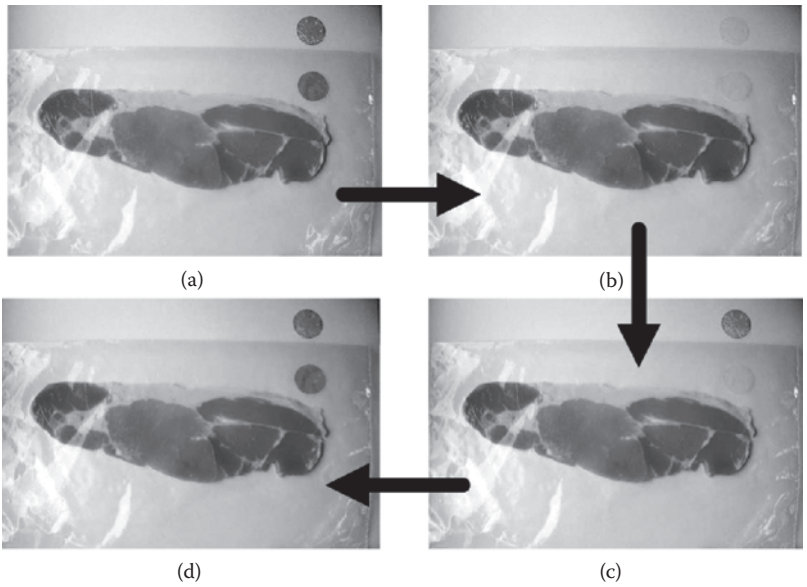


FIGURE 21.7
Photographs of O_2 indicators based on UV-activated TiO_2 nanoparticles and methylene blue dye, showing that one indicator was placed inside of a food package flushed with CO_2 and one placed outside. (a) Package freshly sealed and both indicators are blue. (b) Indicators immediately after photobleaching with UVA irradiation. (c) Indicator outside of the package returned to blue color, whereas the indicator inside the oxygen-free atmosphere package remained white. (d) Package was opened—influx of oxygen caused the indicator to change back to blue color. This oxygen indicator could be used to easily and noninvasively detect the presence of leaks in packaged products throughout distribution. (Reproduced from Mills (2005 with permission of The Royal Society of Chemistry.)

investigated similar solvent- and water-based indicator inks sensitive to CO_2 that can be applied to ceramic, paper, and metal substrates using a felt-tipped pen. These researchers applied the ink to a variety of different surfaces including ceramics, paper, and aluminum foil. Additionally, they found the solvent-based CO_2 indicator to be more sensitive toward CO_2 than its water-based counterpart, but it had a short shelf life, while increasing the amount of plasticizer in both water and solvent formulations helped to improve indicator response and recovery time (Mills and Skinner, 2010).

Other works have shown RH can also be detected using carbon-coated copper nanoparticles with silicon surfactant (Luechinger et al., 2007), or nanocubic ZnO/SnO_2 film-based detectors (Md Sin et al., 2013). Luechinger et al. (2007) prepared porous metal films for humidity detection from copper nanoparticles coated with 2–3 nm of carbon, followed by amphiphilic surfactant. The unique preparation of the copper coating resulted in sensitivity to water or its vapor with optical shifts in the visible light range of up to 50 nm for a 1% change in RH. In a study by Md Sin et al. (2013), a humidity sensor was pre-

pared using nano ZnO/SnO_2 prepared via sol–gel and immersion method and deposited on sputtered ZnO-film-coated glass substrate. The emission bands were apparent in the thin ZnO-film sensor at ultraviolet (UV) emission with low intensity at 408 nm and in the broad visible region dominating the peak emission around 600 nm. They also found that the sensitivity of the sensor was dependent on the porosity of materials in the detector, which agreed with the findings of Zhang and Zhang (2008), who used SnO_2 nanoparticles supplemented with 5–15 wt% ZnO microrods to detect trimethylamine.

A UV-activated oxygen indicator has been prepared by dispersing TiO_2 nanoparticles, glycerol, and MB in poly(ethylene oxide) solution using aqueous ethanol as a solvent (Mihindukulasuriya and Lim, 2013). The solution was either electrospun or solvent cast, producing oxygen-sensitive fibrous membrane and continuous cast film, respectively. SEM assessment of fibers showed the diameter of the electrospun fibers varied from about 350–1000 nm. The electrospun indicators were four to five times more sensitive to UV irradiation as compared to continuous film indicators prepared by casting. This was mainly due to morphological differences between the two preparation methods, and a much greater surface area on electrospun indicators for color-changing reactions to take place. It was also discovered that increasing ethanol concentration of the electrospinning solvent enhanced the sensitivity of the indicator to UV irradiation (Mihindukulasuriya and Lim, 2013).

The production of gaseous amines, due to the growth of spoilage bacteria, is one of the hallmarks of meat deterioration. The growth of *Pseudomonas* on meat results in the degradation of proteins, forming ammonia and other foul-smelling amines. The detection of these alkaline volatiles can be made at the parts per trillion level using fluorescence quenching of nanofibrils of perylene-based fluorophores (Che et al., 2008). Upon deposition onto a substrate, via a technique such as electrospinning, the resulting porous nonwoven made up of entangled nanofibers allowed for maximal exposure to the gaseous analyte molecules. This substrate permits rapid diffusion of the molecules through the film coating and increased adsorption and accumulation of gas molecules within a porous matrix. Compared to the sensors prepared by Luechinger et al. (2007) and Md Sin et al. (2013), the sensor developed by Che et al. (2008) was relatively simpler for chemical detection. At the ppm level, another study detected volatile trimethylamine from fish during spoilage with composites consisting of SiO_2 nanoparticles and TiO_2 microrods (Zhang and Zhang, 2008). By doping SiO_2 nanoparticles with 5–15 wt% ZnO microrods, the researchers observed enhanced gas-sensing properties, as measured by the change in the ratio of the resistance in air to that in a sample of the sensor.

In MAP packaged meats, the growth of facultative anaerobes such as *B. thermosphacta*, Enterobacteriaceae, *Lactobacillus*, and other lactic acid bacteria is undesirable, and their metabolic activity may alter the gas composition initially flushed into the product headspace during packaging. Indicators and sensors capable of detecting a change in headspace gas composition are worthy of investigation for real-time monitoring of freshness of meat. However, many of the

indicator/sensor innovations mentioned above have yet to be applied in commercial food systems. Further research is needed to elucidate if they are robust enough for the detection of spoilage volatiles in meat packaging during actual end-use conditions. Issues such as stability and accuracy of the indicator/sensor, as well as the effects of temperature and RH effects, will need to be addressed.

21.2.4.2 Biosensors

Besides detecting gaseous compounds indicative of product freshness, other strategies are based on detecting the presence of specific microorganisms by using biosensors. These sensors take advantage of antibodies that bind specifically to antigens present on the pathogens of interest. However, food is a complex system consisting of many components that can interfere with the antibody–antigen interaction. Therefore, the target microorganism's antigens will need to be somehow isolated from the food matrices and surrounding environment, in sufficient quantities, to ensure adequate signal-to-noise ratios for optimal detection. To this end, various techniques have been used. For example, immunomagnetic separation (IMS) has been used by researchers to increase the amount of the analyte. Here, nanomagnetic particles are functionalized with selected antibodies, onto which the antigens from the pathogens attached. A magnetic force is then applied to selectively separate the target analyte from the food matrix before detection. On the basis of this principle, Varshney and coworkers extended the IMS concept and developed a method to detect *E. coli O157:H7*. They applied an interdigitated array microelectrode-based impedance biosensor in combination with antibody-conjugated nanoparticles to detect the presence of the microorganism (Varshney et al., 2005, 2007). Rather than measuring electrical signals, other indicators rely on color changes as a means for detection. Rapid colorimetric identification of *Salmonella* bacteria by using bioconjugated oval-shaped gold nanoparticles has also been demonstrated (Wang et al., 2010). These researchers were able to detect *Salmonella* Typhimurium with an excellent detection limit (10^4 bacteria/mL). When bacteria bound to the conjugated gold nanoparticles and these were exposed to near-infrared radiation, a reduction in bacterial cell viability was observed due to photothermal lysis. Different concentrations of bound *Salmonella* resulted in a unique color change, ranging from black at 10 CFU/mL, to dark blue at 50 CFU/mL, light blue at 10^3 CFU/mL, and pink at 10^4 CFU/mL (Wang et al., 2010). Other studies have demonstrated that silver-coated PVA immobilized on mica sheets can be used as a biosensor in the rapid identification of *S. enterica ser.* Typimurium, *E. coli, Listeria innocua,* and *S. aureus*, isolated from chicken rinse water (Sundaram et al., 2013). These researchers used silver ion-encapsulated PVA deposited on a mica sheet to use as substrate in surface-enhanced Raman scattering (SERS) assessment. Cultures of *S.* Typhimurium, *E. coli, S. aureus* and *L. innocua* were isolated from chicken rinse water and suspended in 10 mL of sterile deionized water. Approximately 5 µL of the bacterial suspensions was placed on

the coated mica sheet detector and exposed to 785 nm HeNe laser excitation; spectral data was recorded between 400 and 1800 cm^{-1} for 15 different spots on the detector strip for each bacterial sample. While many of these direct microbial detection methods are intended for rapid detection of microorganisms in meat prior to packaging, attempts have been made to integrate them into packaging technology with a few modifications. For instance, either of the biosensors in Wang et al. (2010) or Sundaram et al. (2013) could be included as a small strip underneath product packaged fresh in a Styrofoam tray or MAP. To permit pathogen detection, workers at meat plants would have to be equipped with the appropriate detection equipment to read packaging prior to shipping and/or upon receiving. The major constraint for such an indicator system is that the components used for the manufacturing of the indicator must be nontoxic and approved for food contact applications. Also, antigen-binding to food-grade nanoparticles should couple with a reaction that results in color change to provide freshness indication that can be visually detected by the consumer/manufacturer. The choice of color may need to be considered when designing the indicator, as certain consumers may be affected by color blindness. Because the food industry, in general, is sensitive to the cost of packaging materials, the deployment of a freshness indicator will need to be justified on the basis of how the technology can help in enhancing product safety and increasing brand name protection.

21.3 Nanostructured Edible Films and Coatings

The formation of edible films for processed meat products can be achieved either by wet or dry processing. Wet processed films are produced from polymers with good film-forming ability, such as long-chain polysaccharides or proteins. Here, the polymers must be dissolved and dispersed in a food-grade solvent (e.g., water, alcohol, or mixture of both) to form film-forming solution. Other additives (e.g., plasticizers, antimicrobial agents, colors, flavors, and fillers) are often added to enhance the functionalities of the film. Adjustment of pH and/or heating the film-forming solutions may be done to facilitate dispersion of polymer in the solvent to increase chain–chain interactions essential for the formation of coherent film structure. The film-forming solution is then casted or extruded to obtain free-standing films. Alternatively, the film-forming solutions can also be applied directly onto the surface of food to form coating via methods such as dipping, spraying, and brushing followed by drying. On the other hand, dry processing of an edible film does not involve solvent dispersion, but rather relies on the thermoplastic characteristics of the polymer. Here, the polymer is compression molded or extruded above its glass transition or melting temperatures, along with the additive components, to form a continuous film (Pommet et al., 2005; Liu et al., 2006). Because of the elevated temperature involved, the dry process may not be suitable for active components that are heat labile.

To produce a coherent protein film, globular proteins must be denatured by disrupting the disulfide bonds, as well as hydrogen and hydrophobic bonding interactions inherently present in native protein molecules, often through heating, the addition of denaturant, and adjusting the pH of the protein solution away from the isoelectric point. The main purpose of the denaturation process is to unfold the protein, allowing the intermolecular chain interaction to occur, which is essential for forming a coherent and strong film. Edible protein films are generally good gas and oxygen barriers at low RH. This gas barrier property may be used to prevent oxidative rancidity and enzymatic browning, and minimize flavor loss in processed meat products. Types of edible proteins that are well established for coating on meat products are collagen, corn zein, wheat gluten, and soy. Zein is a prolamine protein from corn that has been applied as a nanomaterial for active packaging purposes (Moomand and Lim, 2014; Fernandez et al., 2009). Enhancing the barrier of corn and whey protein films by incorporating TiO_2 or SiO_2 has also been demonstrated (Kadam et al., 2014).

The drawback of protein films, such as those derived from wheat gluten and soy, is that they are food allergens to some consumers. Polysaccharide-based edible films do not have this limitation. Polysaccharides that have been widely used for the formation of edible films include pullulan, xanthan, chitosan, starch, and cellulose. Pullulan and xanthan are both derived from microorganisms, while chitosan is a deacetylated derivative of chitin—an abundant polysaccharide found in nature, second only to cellulose. Recent research has shown that chitosan is nontoxic, biodegradable, antimicrobial, and has the ability to chelate cations (Rinaudo, 2006; Srinivasa and Tharanathan, 2007; Aider, 2010). However, poor mechanical and gas barrier properties and weak water resistance limit its application, particularly in applications involving elevated moisture content or RH (Wang et al., 2005; Xu et al., 2006). Therefore, the creation of composite edible films of chitosan with a nanomaterial filler is desirable for preserving meat quality. The incorporation of nanoclay in the range of 1–5 wt% has led to improvement of mechanical properties of chitosan (Lavorgna et al., 2010; Xu et al., 2006), thermal stability (Wang et al., 2005), functional properties (Rhim et al., 2006), and barrier properties (Casariego et al., 2009; Rhim et al., 2006). In another study, potato starch-based nanocomposite films were prepared using microfibrillated cellulose (Dufresne et al., 2000). The researchers reported that the addition of a filler significantly reinforced the starch matrix, increasing tensile modulus by adding up to 50 wt% fraction of potato starch nanocellulose in the finished film. At a high RH, the reinforcing effect diminished due to plasticization effects of the absorbed water. In addition, a higher modulus value was observed for a formulation with less glycerol plasticizer (Dufresne et al., 2000). Similar findings were reported by Moran et al. on nanocellulose reinforced potato starch (Moran et al., 2013). Other modified cellulose polymers that have been used in the area of food packaging include hydroxypropyl methyl cellulose, methyl cellulose, and ethyl cellulose (Lua et al., 2007; Imran et al., 2010; Arnon et al., 2015).

Besides strengthening the physical properties of edible films, other combination of materials may be applied to provide antimicrobial effects for shelf life

extension of meats. One study evaluated the effects of alginate films dispersed with MMT nanoclay and essential oil from marjoram herb on the shelf life of trout fillets inoculated with *L. monocytogenes*. The nanoclay was pretreated by dispersing 3 wt% in distilled water, followed by gradual addition of 1% w/v alginate solution for four hours to form the film-forming solution. Marjoram essential oil and Tween 80 (0.25 g/g of essential oil) were added, followed by homogenization, degassing under vacuum, casting, and finally drying to form composite films (Alboofetileh et al., 2016). Fish samples wrapped in the film with 1% marjoram essential oil significantly delayed the growth of *L. monocytogenes* during the 15-day storage at 4°C, with final counts of approximately 1.2 log CFU/g lower than those observed in the untreated control samples. Total viable and psychrotrophic counts, as well as volatile nitrogen levels in the trout slices during storage were lower as well (Alboofetileh et al., 2016). Chitosan–nanocellulose nanocomposite films have been developed for direct contact application on the surface of ground meat slabs (Dehnad et al., 2014). The researchers observed a lower lactic acid bacterial population on ground meat samples packaged with nanocomposite active packaging compared to control samples which received a nylon film covering. The active packaging treatments had lactic acid bacteria counts that were lower than control by 1.3 and 3.1 log cycles at 3°C and 25°C, respectively, after six days of storage. Considering that cellulose is a renewable feedstock and abundantly available as a food processing byproduct, it is anticipated that composite films with nanocellulose fillers would be a growing area of research potentially making significant impact on food packaging in the future (Khan et al., 2012).

Food-grade coatings can be applied to packaging films that are in contact with meat for various purposes, such as for the delivery of antimicrobial or antioxidative agents, and therefore can be considered as an active packaging system component. This approach is often applied in the delivery of bioactive agent with relatively low volatility, where the mass transport of the compound takes place via diffusion across the packaging–food interface. For instance, a food contact approved active packaging with an inner coating of bioactive agent (rosemary or oregano extract) enhanced the oxidative stability and shelf life of lamb steaks compared to control samples stored under modified atmosphere conditions (Camo et al., 2008). There are several natural antimicrobial compounds such as essential oils and spice extracts which can be effectively used in edible films or coatings against foodborne pathogens found in meat such as *E. coli, Campylobacter jejuni, Salmonella enterica,* and *L. monocytogenes* (Olasupo et al., 2003). Rosemary and oregano essential oils and silver and zinc oxide nanoparticles incorporated into pullulan films prepared with xanthan and locust bean gum were effective against pathogenic microorganisms, such as *S. aureus*, *L. monocytogenes*, *E. coli O157:H7,* and *S.* Typhimurium, all of which may be potential pathogens in various meat products (Morsy et al., 2014). Future work should determine if nanoencapsulation of bioactive agents and the incorporated nanomaterial might benefit the delivery efficiency of the biocontrol agent. Optimization of the amount of edible material applied as a

coating in food through sensory evaluation is essential considering the fact that the applied coating may impact the sensory attributes of the products. A summary of select edible film systems cited herein can be seen in Table 21.3.

TABLE 21.3

Summary of Edible Packaging Systems Cited with Tested or Potential Application in Meat Systems

Nanomaterials	Bioacitve(s) Added	Packaging Material(s)	Food Product(s) Application or Other Testing	Effects	Reference
MMT nanoclay	Marjoram essential oil	Alginate	Trout fillet	Enhanced shelf life Reduced growth of *L. monocytogenes* in vitro and in food system	Alboofetileh et al. (2016)
Halloysite nanoclay	Nisin	Corn starch	Fresh cheese	Inhibition of *L. monocytogenes* on cheese and in vitro	Meira et al. (2016)
Halloysite nanoclay	Nisin	Liposomes in casein and gelatin films	Growth media	Reduced counts of *B. cereus*, *C. perfringens*, and *L. monocytogenes* Liposomes increased surface roughness of films Casein films were thinner, slightly yellow, and more elastic than gelatin films	Boelter and Brandelli (2016)
CNT	AIT	Cellulose	Cooked chicken breasts	Reduction in *Salmonella chloeraesuis* Reduction in oxidation and color changes	Dias et al. (2013)
Zein prolamine nanofibers	B-carotene and fish oil	Zein as encapsulating nanomaterial	Tested for stability outside of food systems	Stabilized against UV–Vis irradiation and heat-induced oxidation	Fernandez et al. (2009) and Moomand and Lim (2014)

(*Continued*)

TABLE 21.3 (*Continued*)

Summary of Edible Packaging Systems Cited with Tested or Potential Application in Meat Systems

Nanomaterials	Bioacitve(s) Added	Packaging Material(s)	Food Product(s) Application or Other Testing	Effects	Reference
Nanocellulose	Not added	Potato–nanostarch nanocomposite	Tested material properties	Increased tensile modulus Reduced reinforcement at elevated RH	Dufresne et al. (2000) and Moranet al. (2013)
Nanocellulose	Chitosan	Chitosan–nanocellulose nanocomposite	Ground meat	Inhibited *S. aureus*, *S. enteritidis*, and *E. coli* Reduced population of lactic acid bacteria in anaerobic packaging environment	Dehnad et al. (2014)
Silver and zinc oxide nanoparticles	Rosemary and oregano essential oils	Pullulan films blended with xanthan and locust bean gum	Growth media and vacuum-packaged raw turkey breast, raw beef (top round), and RTE turkey breast	Reduced counts of *E.coli* O157:H7, *L. monocytogenes*, *S. aureus*, and *S.* Typhimurium. Reduction varied from 1–3 log CFU/g depending on food/microorganisms system	Morsy et al. (2014)

AIT, allyl isothiocyanate; CNT, carbon nanotube; MMT, montmorillonite; RH, relative humidity; RTE, ready-to-eat.

21.4 Societal Implications

The success of nanotechnology-enabled packaging ultimately depends on consumer acceptance of the product, which is affected by its costs, performance, and perceived benefits and risks as compared with existing products (Siegrist et al., 2008; Bieberstein et al., 2013). Considering many consumers are unfamiliar with and/or skeptical of nanotechnology, strategic knowledge

transfer and translation will be beneficial to increase the level of understanding on how nanomaterials can help in enhancing food safety and quality, as well as communicating risks involved under typical end-use conditions. Because nanomaterials tend to exhibit properties different from their bulk counterparts, the onus is on the food manufacturers to ensure that their products are safe. Enforcing safety regulations and guidelines established by governmental regulatory agencies will strengthen consumer confidence in nanotechnology-based packaging materials.

21.4.1 Potential Risks

The main concern with nanotechnology-based food packaging is unintended migration of nanoparticles from the packaging matrix into the food during storage, exposing consumers to these materials (Ramachandraiah et al., 2015; Bumbudsanpharoke and Ko, 2015; Dimitrijevic et al., 2015). Unfortunately, there is a lack of understanding regarding the safety of nanomaterials, as the toxicity of some materials is unpredictable when scaled down to nano dimension. Furthermore, differences in morphology, reactivity, hydrophobicity, and surface functionalization affect the toxicity of nanomaterials, further complicating the characterization and making it difficult to fully understand the mechanisms that make these materials hazardous. As such, extensive research and testing need to be done by governing and health agencies to fully understand these materials before they can be safely used. This is a challenging task considering the rapid development of many new nanomaterial-based products today.

Metal and metal oxide nanoparticles, which are used as antimicrobial agents, have been shown to be cytotoxic *in vitro* as well as in animal studies (Prabhu and Poulose, 2012; Kang et al., 2015). The synthesis method and surface functionalization also affect the toxicity of the nanoparticles (Kwok et al., 2012). It should be noted that these studies are typically acute exposure studies involving doses significantly higher than those observed in migration studies from packaging materials. When performing risk assessments for nanocomposite packaging materials, the following parameters have to be considered: realistic exposure doses from consumer products, the ability of these nanoparticles to be absorbed through the gastrointestinal track, and whether or not the body is able to clear these materials in a reasonable timeframe.

There have also been studies that examined the potential of nanoparticles to diffuse from nanocomposites into food simulants. Schmidt et al. (2011) examined the migration of synthesized Mg–Al layered double hydroxide nanoparticles from PLA films into a food simulant of 95% ethanol. They found that increased nanoparticle loading from 1.8% to 5.5% w/w resulted in an increase in the migration of packaging components into the simulant, but is still able to conform to the total migration limit of 10 mg/dm^2 set

by the European Commission (EC) (Schmidt et al., 2011). Lin et al. (2014) showed that the migration of these nanomaterials from the packaging to the food simulant depends on the compatibility of the nanoparticles with the packaging matrix. It was shown that TiO_2 nanoparticles of around 30 nm in PE films diffuse slower compared to nanoparticles of around 100 nm due to the better incorporation of the smaller nanoparticles into the PE matrix. Studies have shown that the initial loading, time, and temperature are important factors that affect the amount and rate of nanomaterial migration from packaging films (Farhoodi et al., 2014; Lin et al., 2014; Ramachandraiah et al., 2015).

There are, however, limitations to migration studies. Many studies used homogeneous solutions (e.g., 95% or 50% aqueous ethanol and 3% aqueous acetic acid) to simulate food matrices, which are significantly more complex in composition with multiphase components (Schmidt et al., 2011; Lin et al., 2014; Farhoodi et al., 2014). These studies neither consider the potential for these materials to be absorbed by the gastrointestinal track nor whether they simply pass through unchanged. Furthermore, since the amounts of nanoparticles that migrate are very low, sample preparation and analysis methods have to be chosen carefully to ensure the measurements performed are accurate (Bumbudsanpharoke and Ko, 2015).

The release of nanoparticles into the environment at the end of the lifecycle of the nanocomposite packaging is another area of concern. AgNPs, in particular, have gained much attention as their use in consumer products has spread over the recent years. AgNPs possess nonspecific toxicity and could negatively affect bacterial and aquatic populations when released into the environment (Oberdörster et al., 2005; Marambio-Jones and Hoek, 2010; Prabhu and Poulose, 2012). As such, lifecycle analyses for these nanocomposites are needed to ensure they do not have a negative impact on the environment when these packaging materials are ultimately disposed of.

A potential tool that permits evaluation of nanoclay toxicity is environmental biomonitoring using bioassays or plant genetic models. For example, the use of *Allium cepa* (onion) could be applied as a test organism for analysis of compost samples containing nanoclay biocomposites. *Allium cepa* has demonstrated a great potential for environmental monitoring of metals, pesticides, and other complex mixtures (Leme and Marin-Morales, 2009). One study observed the manifestation of disease in the organs of Wistar rats exposed for 90 days to an extract of PLA and MMT-derivative nanocomposite. Tissues were observed using optical microscopy and SEM, while inflammation and oxidative stress biomarkers were determined as well. The subchronic oral exposure over a 90-day period did not produce significant adverse effects in any of the markers studied; however, it is recommended more studies are performed in this area over a longer period to confirm toxicological effects (Maisanaba et al., 2014). Yoshida et al. (2012) showed that nanosilica particles with an external diameter of 70 nm induced cytotoxicity,

reactive oxygen species generation, and DNA damage in rat and human cell lines during acute exposure. Adverse effects were reduced by surface modification with carbonyl and amine groups, and it is speculated that surface modification changed cellular distribution of the nanomaterial, considering it was previously observed that the unmodified and carbonyl-coated nanosilica were taken up equally into cells.

21.4.2 Regulation

Currently, there is no universally accepted definition as to what a nanomaterial is. Typically, agencies and organizations around the world define a nanomaterial as a material having at least one dimension that is less than 100 nm (Amenta et al., 2015; Bumbudsanpharoke and Ko, 2015). However, this definition is inadequate as there may be materials with dimensions larger than 100 nm which exhibit behavior that is different than that of its bulk counterpart (USFDA, 2014).

There has also been difficulty in setting regulations on the use of nanomaterials in food packaging due to lack of data. The toxicity of nanomaterials is affected greatly by both intrinsic (e.g., size, morphology, charge, and functionalization) and extrinsic (e.g., pH, temperature, agglomeration, and matrix in which they are dispersed in) factors, and so there are numerous parameters that need to be studied in order to fully understand the hazards associated with using these materials. The long-term effects of being exposed to these materials also need to be examined (Handy and Shaw, 2007). As a result, a large number of studies are needed to fully understand these hazards, making risk assessment and regulation with reasonable assumptions difficult at this time. A balance that adequately protects consumers from harm without discouraging the industry from further investing in and developing these technologies is required when regulating these materials.

In the United States, jurisdiction on the use of nanocomposite food packaging falls under the Food and Drug Administration (FDA) (Amenta et al., 2015). The FDA does not have a formal definition for nanomaterials, but rather only provides guidelines as to what may be considered as nanomaterials (USFDA, 2014). The FDA recommends preliminary safety assessments of products that may contain nanomaterials on a case-by-case basis, but does not have any specific regulations regarding the use of nanomaterials in products (Chau et al., 2007; Amenta et al., 2015). In the European Union, any chemical or material, including nanomaterials, have to be registered under Regulation (EC) No 1907/2006, called registration, evaluation, authorization, and restriction of chemicals (REACH), before it can be commercially used (Amenta et al., 2015). This approval process ensures that the material hazards are identified and risk management strategies are employed. Moreover, the legislation tends to place the burden of proof of safety on the companies that use these materials (European Council and Parliament, 2006). Specific

to food packaging, the EU also sets limits on amounts of migrants that are allowed to transfer from the packaging material into the food (European Commission, 2009).

21.4.3 Public Acceptance

The public perception of using nanomaterials in the food industry is comparable to the consumer perception on genetically modified foods (Scheufele and Lewenstein 2005; Chau et al., 2007). In general, many consumers are reluctant to embrace these new technologies due to the potential for unknown hazards associated with using them. Consumer confidence can be improved by further research on the safety of these materials and informing consumers of the benefits and risks associated with these products. As more data regarding its safety become available and as consumers are more well informed on this topic, they will see nanotechnology as one of the tools for enhancing the quality and safety of food products, as well as for reducing food waste.

Studies have shown that nanotechnology in food packaging is considered to be more acceptable to consumers compared to incorporating nanotechnology into food products themselves (Siegrist et al., 2007, 2008; Bieberstein et al., 2013). This is likely due to the lower perceived risk of incorporating them into packaging, where they are not directly consumed by its users. As such, the use of nanotechnology-enabled food packaging is likely the first step in introducing this concept to the public for the public to gain consumer confidence for the use of nanotechnology in the food industry.

21.5 Conclusion

Technologies based on exploiting nanomaterials discussed in the chapter can be promising in advancing active and intelligent packaging for meat products, in order to enhance the protective and communication functions of a meat package. More research and development activities in these areas are expected especially to integrate low-cost sensors/indicators fabricated from printing sensors/indicators onto meat packaging structures. Edible films and coatings incorporated with nanomaterials (e.g., nanocellulose) and antimicrobials (e.g., chitosan, nisin, and essential oils) offer unique opportunities to improve physical and antimicrobial properties of meat packages. To achieve full potential in the marketplace, besides being cost-effective, the new nanotechnology-based packaging materials must exhibit benefits that outweigh the perceived risks of these new materials. Governing bodies should institute rules and regulations on the basis of sound science.

References

Abdollahi, M., Rezaei, M. and Farzi, G. (2012). A novel active bionanocomposite film incorporating rosemary essential oil and nanoclay into Chitosan. *Journal of Food Engineering*, **111**(2), 343–350.

Aider, M. (2010). Chitosan application for active bio-based films production and potential in the food industry: Review. *LWT—Food Science and Technology,* **43**(6), 837–842.

Alboofetileh, M., Rezaei, M., Hosseini, H. and Abdollahi, M. (2016). Efficacy of activated alginate-based nanocomposite films to control *L. monocytogenes* and spoilage flora in rainbow trout slice. *Journal of Food Science and Technology,* **53**(1), 521–530.

Amenta, V., Aschberger, K., Arena, M. et al. (2015). Regulatory aspects of nanotechnology in the agri/feed/food sector in EU and non-EU countries. *Regulatory Toxicology and Pharmacology,* **73**(1), 463–476.

Arnon, H., Granit, R., Porat, R. and Poverenov, E. (2015). Development of polysaccharides-based edible coatings for citrus fruits: A layer-by-layer approach. *Food Chemistry,* **166**, 465–472.

Barbosa-Pereira, L., Angulo, I., Lagarón, J. M., Paseiro-Losada, P. and Cruz, J. M. (2014). Development of new active packaging films containing bioactive nanocomposites. *Innovative Food Science and Emerging Technologies,* **26**, 310–318.

Barbut, S. (2015). The Science of Meat and Poultry Processing. Creative Commons Open Source. Available at http://www.poultryandmeatprocessing.com/

Beatrice, C. A. G., Branciforti, M. C., Alves, R. M. V. and Bretas, R. E. S. (2010). Rheological, mechanical, optical, and transport properties of blown films of polyamide 6/residual monomer/montmorillonite nanocomposites. *Journal of Applied Polymer Science*, **116**, 3581–3592.

Bieberstein, A., Roosen, J., Marette, S., Blanchemanche, S. and Vandermoere, F. (2013). Consumer choices for nano-food and nano-packaging in France and Germany. *European Review of Agricultural Economics,* **40**(1), 73–94.

Black, E. P., Cascarino, J., Guan, D. et al. (2010). Coliphage as pressure surrogates for enteric viruses in foods. *Innovative Food Science and Emerging Technologies,* **11**(2), 239–244.

Boelter, J. F. and Brandelli, A. (2016). Innovative bionanocomposite films of edible proteins containing liposome-encapsulated nisin and halloysite nanoclay. *Colloids and Surfaces: Biointerfaces,* **145**, 740–747.

Bumbudsanpharoke, N. and Ko, S. (2015). Nano-food packaging: An overview of market, migration research, and safety regulations. *Journal of Food Science,* **80**(5), R910–R923.

Busolo, M. A. and Lagaron, J. M. (2012). Oxygen scavenging polyolefin nanocomposite films containing an iron modified kaolinite of interest in active food packaging applications. *Innovative Food Science and Emerging Technologies,* **16**, 211–217.

Camo, J., Beltrán, J. A. and Roncalés, P. (2008). Extension of the display life of lamb with an antioxidant active packaging. *Meat Science,* **80**(4), 1086–1091.

Casariego, A., Souza, B. W. S., Cerqueira, M. A. et al. (2009). Chitosan/clay films' properties as affected by biopolymer and clay micro/nanoparticles' concentrations. *Food Hydrocolloids,* **23**(7), 1895–1902.

Chau, C. F., Wu, S. H. and Yen, G. C. (2007). The development of regulations for food nanotechnology. *Trends in Food Science and Technology,* **18**(5), 269–280.

Che, Y., Yang, X., Loser, S. and Zang, L. (2008). Expedient vapor probing of organic amines using fluorescent nanofibers fabricated from an N-type organic semiconductor. *Nano Letters,* **8**(8), 2219–2223.

Chibeu, A., Agius, L., Gao, A., Sabour, P. M., Kropinski, A. M. and Balamurugan, S. (2013). Efficacy of bacteriophage LISTEX™ P100 combined with chemical antimicrobials in reducing *L. Monocytogenes* in cooked turkey and roast beef. *International Journal of Food Microbiology,* **167**(2), 208–214.

Dehnad, D., Mirzaei, H., Emam-Djomeh, Z., Jafari, S. M. and Dadashi, S. (2014). Thermal and antimicrobial properties of chitosan-nanocellulose films for extending shelf life of ground meat. *Carbohydrate Polymers,* **109**, 148–154.

Dehnavi, A. S., Aroujalian, A., Raisi, A. et al. (2012). Preparation and characterization of polyethylene/silver nanocomposite films with antibacterial activity. *Journal of Applied Polymer Science,* **127**(2), 1180–1190.

Dias, M. V., De Fátima, N., Soares, F. et al. (2013). Use of allyl isothiocyanate and carbon nanotubes in an antimicrobial film to package shredded, cooked chicken meat. *Food Chemistry,* **141**(3), 3160–3166.

Dimitrijevic, M., Karabasil, N., Boskovic, M. et al. (2015). Safety aspects of nanotechnology applications in food packaging. *Procedia Food Science,* **5**, 57–60.

Dini, C., Islan, G. A., De Urraza, P. J. and Castro, G. R. (2012). Novel biopolymer matrices for microencapsulation of phages: Enhanced protection against acidity and protease activity. *Macromolecular Bioscience,* **12**(9), 1200–1208.

Dufresne, A., Dupeyre, D. and Vignon, M. (2000). Cellulose microfibrils from potato tuber cells: Processing and characterization of starch–cellulose microfibril composites. *Journal of Applied Polymer Science,* **76**, 2080–2092.

Economou, T., Pournis, N., Ntzimani, A. and Savvaidis, I. N. (2009). Nisin-EDTA treatments and modified atmosphere packaging to increase fresh chicken meat shelf-life. *Food Chemistry,* **114**(4), 1470–1476.

European Commission. (2009). Commission regulation (EC) No. 450/2009 of 29 May 2009 on active and intelligent materials and articles intended to come into contact with food. *Official Journal of European Union*, **L135**.

European Council and Parliament. (2006). Regulation (EC) No 1907/2006 of the European Parliament and of the council of 18 December 2006 concerning the registration, evaluation, authorisation and restriction of chemicals (REACH), establishing a European Chemicals Agency, amending directive 1999. *Official Journal of the European Union*, **L 396**, 1.

Farhoodi, M., Mousavi, S. M., Sotudeh-Gharebagh, R., Emam-Djomeh, Z. and Oromiehie, A. (2014). Migration of aluminum and silicon from PET/clay nanocomposite bottles into acidic food simulant. *Packaging Technology and Science,* **27**(2), 161–168.

Fernandez, A., Torres-Giner, S. and Lagaron, J. M. (2009). Novel route to stabilization of bioactive antioxidants by encapsulation in electrospun fibers of zein prolamine. *Food Hydrocolloids,* **23**(5), 1427–1432.

Ferrocino, I., Greppi, A., Lastoria, A., Rantsiou, K. and Ercolini, D. (2016). Impact of nisin-activated packaging on microbiota of beef burgers during storage. *Applied and Environmental Microbiology,* **82**(2), 1–13.

Flanagan, J. and Singh, H. (2006). Microemulsions: A potential delivery system for bioactives in food. *Critical Reviews in Food Science and Nutrition,* **46**(3), 221–237.

Fratianni, F., De Martino, L., Melone, A., De Feo, V., Coppola, R. and Nazzaro, F. (2010). Preservation of chicken breast meat treated with thyme and balm essential oils. *Journal of Food Science,* **75**(8), M528–M535.

Garcia, P., Martinez, B., Rodriguez, L. and Rodriguez, A. (2010b). Synergy between the phage endolysin LysH5 and nisin to kill *Staphylococcus aureus* in pasteurized milk. *International Journal of Food Microbiology,* **141**(3), 151–155.

Garcia, P., Rodriguez, L., Rodriguez, A. and Martinez, B. (2010a). Food biopreservation: Promising strategies using bacteriocins, bacteriophages and endolysins. *Trends in Food Science and Technology,* **21**(8), 373–382.

Gill, A. O. and Gill, C. O. (2010). Packaging and the shelf life of fresh red and poultry meats. *In:* Robertson, G. L. (ed.) *Food Packaging and Shelf Life: A Practical Guide*. Boca Raton, FL: CRC Press, pp. 259–272.

Gupta, S. R. N. (2014). Advances in molecular nanotechnology from premodern to modern era. *International Journal of Materials Science and Engineering*, **2**(2), 99–106.

Handy, R. D. and Shaw, B. J. (2007). Toxic effects of nanoparticles and nanomaterials: Implications for public health, risk assessment and the public perception of nanotechnology. *Health, Risk and Society,* **9**(2), 125–144.

Imran, M., El-Fahmy, S., Revol-Junelles, A. and Desobry, S. (2010). Cellulose derivative based active coatings: Effects of nisin and plasticizer on physico-chemical and antimicrobial properties of hydroxypropyl methylcellulose films. *Carbohydrate Polymers,* **81**(2), 219–225.

Islam, M. S., Masoodi, R. and Rostami, H. (2013). The effect of nanoparticles percentage on mechanical behavior of silica-epoxy nanocomposites. *Journal of Nanoscience,* **2013**, 1–10.

Jayasena, D. D. and Jo, C. (2013). Essential oils as potential antimicrobial agents in meat and meat products: A review. *Trends in Food Science and Technology,* **34**(2), 96–108.

Kadam, D. M., Wang, C., Wang, S., Grewell, D., Lamsal, B. and Yu, C. (2014). Microstructure and antimicrobial functionality of nano-enhanced protein-based biopolymers. *Transactions of the American Society of Agricultural and Biological Engineers,* **57**(4), 1141–1150.

Kang, T., Guan, R., Song, Y., Lyu, F., Ye, X. and Jiang, H. (2015). Cytotoxicity of zinc oxide nanoparticles and silver nanoparticles in human epithelial colorectal adenocarcinoma cells. *LWT—Food Science and Technology,* **60**(2), 1143–1148.

Khan, A., Huq, T., Khan, R. A., Riedl, B. and Lacroix, M. (2012). Nanocellulose based composites and bioactive agents for food packaging. *Critical Reviews in Food science and Nutrition,* **54**(2), 163–174

Kumar, J. K., Kremsdorf, R., Tabor, S. and Richardson, C. C. (2001). A mutation in the gene-encoding bacteriophage T7 DNA polymerase that renders the phage temperature-sensitive. *Journal of Biological Chemistry,* **276**(49), 46151–46159.

Kwok, K. W. H., Auffan, M., Badireddy, A. R. et al. (2012). Uptake of silver nanoparticles and toxicity to early life stages of Japanese medaka (*Oryzias latipes*): Effect of coating materials. *Aquatic Toxicology,* **120–121**, 59–66.

Lavorgna, M., Piscitelli, F., Mangiacapra, P. and Buonocore, G. G. (2010). Study of the combined effect of both clay and glycerol plasticizer on the properties of chitosan films. *Carbohydrate Polymers,* **82**(2), 291–298.

Lawrie, K., Mills, A. and Hazafy, D. (2013). Simple inkjet-printed, UV-activated oxygen indicator. *Sensors and Actuators B: Chemical,* **176**, 1154–1159.

Leme, D. M. and Marin-Morales, M. A. (2009). *Allium cepa* test in environmental monitoring: A review on its application. *Mutation Research: Reviews in Mutation Research,* **682**(1), 71–81.

Liu L., Kerry J.P. and Kerry, J.F. (2006). Effect of food ingredients and selected lipids on the physical properties of extruded edible films/casings. International Journal of Food Science and Technology, 41, 295–302.

Lim, L.-T. (2014). Application of nano- and microencapsulated materials to food packaging. *In:* Kwak, H.-S. (ed.) *Nano- and Microencapsulation for Foods*. Oxford: Wiley, pp. 301–323.

Lin, Q.-B., Li, H., Zhong, H.-N., Zhao, Q., Xiao, D.-H. and Wang, Z.-W. (2014). Migration of Ti from nano-TiO_2-polyethylene composite packaging into food simulants. *Food Additives and Contaminants: Part A*, **0049**(June 2015), 1–7.

Lua, Y. Y., Cao, X., Rohrs, B. R. and Aldrich, D. S. (2007). Surface characterizations of spin-coated films of ethylcellulose and hydroxypropyl methylcellulose blends. *Langmuir,* **23**(8), 4286–4292.

Luechinger, N., Loher, S., Athanassiou, E. K., Grass, R. N. and Stark, W. J. (2007). Highly sensitive optical detection of humidity on polymer/metal nanoparticle hybrid films. *Langmuir,* **23**(6), 3473–3477.

Lvov, Y. M., Shchukin, D. G. and Price, R. R. (2015). Halloysite clay nanotubes for controlled release of protective agents. *ACS Nano,* **2**(5), 814–820.

Ma, Y., Pacan, J. C., Wang, Q., Xu, Y., Huang, X., Korenevsky, A. and Sabour, P. M. (2008). Microencapsulation of bacteriophage Felix O1 into chitosan- alginate microspheres for oral delivery. *Applied and Environmental Microbiology,* 74(15), 4799–4805.

Mahony, J., McAuliffe, O., Ross, R. P. and van Sinderen, D. (2011). Bacteriophages as biocontrol agents of food pathogens. *Current Opinion in Biotechnology,* **22**(2), 157–163.

Maisanaba, S., Gutiérrez-Praena, D., Puerto, M. et al. (2014). In vivo toxicity evaluation of the migration extract of an organomodified clay-poly(lactic) acid nanocomposite. *Journal of Toxicology and Environmental Health. Part A,* **77**(13), 731–746.

Maneewattanapinyo, P., Banlunara, W., Thammacharoen, C., Ekgasit, S. and Kaewamatawong, T. (2011). An evaluation of acute toxicity of colloidal silver nanoparticles. *Journal of Veterinary Medical Science/Japanese Society of Veterinary Science*, **73**(11), 1417–1423.

Marambio-Jones, C. and Hoek, E. M. V. (2010). A review of the antibacterial effects of silver nanomaterials and potential implications for human health and the environment. *Journal of Nanoparticle Research,* **12**(5), 1531–1551.

Marvig, C. L., Aideh, B., Neve, H., Heller, K. J., Knøchel, S. and Vogensen, F. K. (2011). Heat tolerance of dairy lactococcal c2 phages. *International Dairy Journal,* **21**(8), 556–560.

Md Sin, N. D., Mamat, M. H., Malek, M. F. and Rusop, M. (2013). Fabrication of nanocubic ZnO/SnO_2 film-based humidity sensor with high sensitivity by ultrasonic-assisted solution growth method at different Zn:Sn precursor ratios. *Applied Nanoscience,* **4**(7), 829–838.

Meira, S. M. M., Zehetmeyer, G., Scheibel, J. M., Werner, J. O. and Brandelli, A. (2016). Starch-halloysite nanocomposites containing nisin: Characterization and inhibition of *Listeria monocytogenes* in soft cheese. *LWT—Food Science and Technology,* **68**, 226–234.

Mihindukulasuriya, S. D. F. and Lim, L.-T. (2013). Oxygen detection using UV-activated electrospun poly(ethylene oxide) fibers encapsulated with TiO_2 nanoparticles. *Journal of Materials Science,* **48**(16), 5489–5498.

Mills, A. (2005). Oxygen indicators in food packaging. *Chemical Society Reviews,* **32**(12), 1003–1011.

Mills, A., Grosshans, P. and Hazafy, D. (2010). A novel reversible relative-humidity indicator ink based on methylene blue and urea. *Analyst,* **135**(1), 33–35.

Mills, A. and Skinner, G. a. (2010). Water-based colourimetric optical indicators for the detection of carbon dioxide. *The Analyst,* 135, 1912–1917.

Mohammad, F. and Abolghasemi, M. R. (2011). Oxygen barrier and mechanical properties of masterbatch-based PA6/nanoclay composite films. J*ournal of Applied Polymer Science,* 125, E2–E8.

Moomand, K. and Lim, L.-T. (2014). Oxidative stability of encapsulated fish oil in electrospun zein fibres. *Food Research International,* **62**, 523–532, Elsevier.

Moran, J. I., Vazquez, A. and Cyras, V. P. (2013). Bio-nanocomposites based on derivatized potato starch and cellulose, preparation and characterization. *Journal of Materials Science,* **48**(20), 7196–7203.

Morsy, M. K., Khalaf, H. H., Sharoba, A. M., El-Tanahi, H. H. and Cutter, C. N. (2014). Incorporation of essential oils and nanoparticles in pullulan films to control foodborne pathogens on meat and poultry products. *Journal of Food Science,* **79**(4), M675–M684.

Munteanu, B. S., Aytac, Z., Pricope, G. M., Uyar, T. and Vasile, C. (2014). Polylactic acid (PLA)/Silver-NP/VitaminE bionanocomposite electrospun nanofibers with antibacterial and antioxidant activity. *Journal of Nanoparticle Research,* **16**(10), 1–12.

Naas, H., Martinez-Dawson, R., Han, I. and Dawson, P. (2013). Effect of combining nisin with modified atmosphere packaging on inhibition of *L. monocytogenes* in ready-to-eat turkey bologna. *Poultry Science,* **92**(7), 1930–1935.

Oberdörster, G., Oberdörster, E. and Oberdörster, J. (2005). Nanotoxicology: An emerging discipline evolving from studies of ultrafine particles. *Environmental Health Perspectives,* **113**(7), 823–839.

Ojijo, V., Cele, H. and Sinha Ray, S. (2011). Morphology and properties of polymer composites based on biodegradable polylactide/poly[(butylene succinate)-co-adipate] blend and nanoclay. *Macromolecular Materials and Engineering,* **296**(9), 865–877.

Ojijo, V. and Sinha Ray, S. (2013). Processing strategies in bionanocomposites. *Progress in Polymer Science*, **38**(10–11), 1543–1589.

Olasupo, N. A., Fitzgerald, D. J., Gasson, M. J. and Narbad, A. (2003). Activity of natural antimicrobial compounds against *Escherichia coli* and *Salmonella enterica* serovar Typhimurium. *Letters in Applied Microbiology,* **37**(6), 448–451.

Oliveira, M., Vinas, I., Colas, P., Anguera, M., Usall, J. and Abadias, M. (2014). Effectiveness of a bacteriophage in reducing Listeria monocytogenes on fresh-cut fruits and fruit juices. *Food Microbiology,* **38**, 137–142.

Painter, J. A., Hoekstra, R. M., Ayers, T. et al. (2013). Attribution of foodborne illnesses, hospitalizations, and deaths to food commodities by using outbreak data, United States, 1998–2008. *Emerging Infectious Diseases,* **19**(3), 407–415.

Panea, B., Ripoll, G., González, J., Fernández-Cuello, Á. and Albertí, P. (2014). Effect of nanocomposite packaging containing different proportions of ZnO and Ag on chicken breast meat quality. *Journal of Food Engineering,* **123**, 104–112.

Picouet, P., Fernandez, A., Realini, C. E. and Lloret, E. (2014). Influence of PA6 nanocomposite films on the stability of vacuum-aged beef loins during storage in modified atmospheres. *Meat Science,* **96**(1), 574–580.

Pommet, M., Redl, A., Guilbert, S. and Morel, M. (2005) Intrinsic influence of various plasticizers on functional properties and reactivity of wheat gluten thermoplastic materials. *Journal of Cereal Science,* 42, 81–91.

Potara, M., Jakab, E., Damert, A., Popescu, O., Canpean, V. and Astilean, S. (2011). Synergistic antibacterial activity of chitosan silver nanocomposites on *S. aureus*. *Nanotechnology,* **22**(13), 135101–135110.

Prabhu, S. and Poulose, E. K. (2012). Silver nanoparticles: Mechanism of antimicrobial action, synthesis, medical applications, and toxicity effects. *International Nano Letters,* **2**(1), 32.

Pradhan, N., Singh, S., Ojha, N. et al. (2015). Facets of nanotechnology as seen in food processing, packaging, and preservation industry. *BioMed Research International,* **2015**, 1–17.

Puapermpoonsiri, U., Ford, S. J. and van der Walle, C. F. (2010). Stabilization of bacteriophage during freeze drying. *International Journal of Pharmaceutics,* **389**(1–2), 168–175, Elsevier.

Pukanski, B. and Fekete, E. (1999). Adhesion and surface modification. *Advances in Polymer Science,* **139**, 109–153.

Ramachandraiah, K., Gu Han, S. and Bok Chin, K. (2015). Nanotechnology in meat processing and packaging: Potential applications—A review. *Asian-Australasian Journal of Animal Sciences,* **28**(2), 290–302.

Rhim, J. W., Hong, S. I., Park, H. M. and Ng, P. K. W. (2006). Preparation and characterization of chitosan-based nanocomposite films with antimicrobial activity. *Journal of Agricultural and Food Chemistry,* **54**(16), 5814–5822.

Rinaudo, M. (2006). Chitin and chitosan: Properties and applications. *Progress in Polymer Science,* **31**, 603–632.

Robertson, G. L. (2013). *Food Packaging: Principles and Practices* (3rd Ed.). Boca Raton FL: CRC Press, Taylor and Francis, 703p.

Rossi, L., Aleida, R. C. C., Lopes, L. S., Figueiredo, A. C. L., Ramos, M. P. P. and Almeida, P. F. (2011). Occurrence of *Listeria* spp. in Brazilian fresh sausage and control of *L. monocytogenes* using bacteriophage P100. *Food Control,* **22**(6), 954–958.

Sanchez-Garcia, M. D., Ocio, M. J., Gimenez, E. and Lagaron, J. M. (2008). Novel polycaprolactone nanocomposites containing Thymol of interest in antimicrobial film and coating applications. *Journal of Plastic Film and Sheeting,* 24(3–4), 239–251.

Scallan, E., Hoekstra, R. M., Angulo, F. J. et al. (2011). Foodborne illness acquired in the United States-major pathogens. *Emerging Infectious Diseases,* **17**(1), 7–15.

Scheufele, D. A. and Lewenstein, B. V. (2005). The public and nanotechnology: How citizens make sense of emerging technologies. *Journal of Nanoparticle Research,* **7**(6), 659–667.

Schmidt, B., Katiyar, V., Plackett, D. et al. (2011). Migration of nanosized layered double hydroxide platelets from polylactide nanocomposite films. *Food Additives and Contaminants: Part A,* **28**(7), 956–966.

Sengwa, R. J. and Choudhary, S. (2012). Dielectric properties and structural dynamics of melt compounded hot-pressed poly(ethylene oxide)-organophilic montmorillonite clay nanocomposite films. *Bulletin of Materials Science,* **35**(1), 19–25.

Siegrist, M., Cousin, M. E., Kastenholz, H. and Wiek, A. (2007). Public acceptance of nanotechnology foods and food packaging: The influence of affect and trust. *Appetite,* **49**(2), 459–466.

Siegrist, M., Stampfli, N., Kastenholz, H. and Keller, C. (2008). Perceived risks and perceived benefits of different nanotechnology foods and nanotechnology food packaging. *Appetite,* **51**(2), 283–290.

Singala, K. J., Mungray, A. A. and Mungray, A. K. (2012). Degradation behavior of polypropylene—Organically modified clay nanocomposites. *Industrial and Engineering Chemistry Research,* **51**, 10557–10564.

Singla, S., Harjai, K., Raza, K., Wadhwa, S., Katare, O.P. and Chhibber, S. (2016). Phospholipid vesicles encapsulated bacteriophage: A novel approach to enhance phage biodistribution. *Journal of Virological Methods,* **236**, 68–76.

Soni, K. A., and Nannapaneni, R. (2010). Bacteriophage significantly reduces *Listeria monocytogenes* on raw salmon fillet tissue. *Journal of Food Protection,* **73**(1), 32–38.

Srinivasa, P. C. and Tharanathan, R. N. (2007). Chitin/chitosan—Safe, ecofriendly packaging materials with multiple potential uses. *Food Reviews International,* **23**(1), 53–72.

Sundaram, J., Park, B., Kwon, Y. and Lawrence, K. C. (2013). Surface enhanced Raman scattering (SERS) with biopolymer encapsulated silver nanosubstrates for rapid detection of foodborne pathogens. *International Journal of Food Microbiology,* **167**(1), 67–73.

Tang, Z., Huang, X., Baxi, S., Chambers, J. R., Sabour, P. M. and Wang, Q. (2013). Whey protein improves survival and release characteristics of bacteriophage felix O1 encapsulated in alginate microspheres. *Food Research International,* **52**(2), 460–466.

Tornuk, F., Hancer, M., Sagdic, O. and Yetim, H. (2015). LLDPE based food packaging incorporated with nanoclays grafted with bioactive compounds to extend shelf life of some meat products. *LWT—Food Science and Technology,* **64**(2), 540–546.

Travan, A., Pelillo, C., Donati, I. et al. (2009). Non-cytotoxic silver nanoparticle-polysaccharide nanocomposites with antimicrobial activity. *Biomacromolecules,* **10**(6), 1429–1435. USFDA. 2007. Agency Response Letter GRAS Notice No. GRN 000198.

USFDA. (2014). *Guidance for Industry—Considering whether an FDA-Regulated Product Involves the Application of Nanotechnology*. Silver Springs, MD: US FDA.

Varshney, M., Yang, L., Su, X.-L. and Li, Y. (2005). Magnetic nanoparticle-antibody conjugates for the separation of Escherichia coli O157:H7 in ground beef. *Journal of Food Protection,* 68(9), 1804–1811.

Varshney, M., Li, Y., Srinivasan, B. and Tung, S. (2007). A label-free, microfluidics and interdigitated array microelectrode-based impedance biosensor in combination with nanoparticles immunoseparation for detection of *Escherichia coli* O157:H7 in food samples. *Sensors and Actuators, B: Chemical,* **128**(1), 99–107.

Vivekanandhan, S., Christensen, L., Misra, M. and Amar, K. M. (2012). Green process for impregnation of silver nanoparticles into microcrystalline cellulose and their antimicrobial bionanocomposite films. *Journal of Biomaterials and Nanobiotechnology,* **3**(3), 371–376.

Vonasek, E., Le, P. and Nitin, N. (2014). Encapsulation of bacteriophages in whey protein films for extended storage and release. *Food Hydrocolloids*, **37**, 7–13.

Wang, S. F., Shen, L., Tong, Y. J. et al. (2005). Biopolymer chitosan/montmorillonite nanocomposites: Preparation and characterization. *Polymer Degradation and Stability,* **90**(1), 123–131.

Wang, S., Singh, A. K., Senapati, D., Neely, A., Yu, H. and Ray, P. C. (2010). Rapid colorimetric identification and targeted photothermal lysis of *Salmonella* bacteria by using bioconjugated oval-shaped gold nanoparticles. *Chemistry—A European Journal,* **16**(19), 5600–5606.

Xu, Y., Ren, X. and Hanna, M. A. (2006). Chitosan/clay nanocomposite film preparation and characterization. *Journal of Applied Polymer Science,* **99**(4), 1684–1691.

Zhang, W. H. and De Zhang, W. (2008). Fabrication of SnO_2-ZnO nanocomposite sensor for selective sensing of trimethylamine and the freshness of fishes. *Sensors and Actuators, B: Chemical,* **134**(2), 403–408.

Yoshida, T., Yoshioka, Y., Matsuyama, K., Nakazato, Y., Tochigi, S., Hirai, T., et al. (2012). Surface modification of amorphous nanosilica particles suppresses nanosilica-induced cytotoxicity, ROS generation, and DNA damage in various mammalian cells. *Biochemical and Biophysical Research Communications,* 427(4), 748–752.

Zhang, Y. and Lim, L.-T. (2016). Inkjet-printed CO_2 colorimetric indicators. *Talanta,* **161**, 105–113.



Taylor & Francis
Taylor & Francis Group
http://taylorandfrancis.com

Index

A

ACE inhibitory peptides, *see* Angiotensin I-converting enzyme inhibitory peptides
Acetate, glucose *vs.*, 364–366
Acetic acid, 364
Acousto-optic tunable filter (AOTF), 29–30
Acrylamide, 387
Active packaging (AP) systems, 618, 654
  antimicrobial, *see* Antimicrobial active packaging
  bacteriocins in, 577–579
  components, 638–640
Additives, 216, 238
Aging of meats, 373
AgNP, *see* Silver nanoparticle
β-Agonists, 174
Agricultural Research Service (ARS), 49
Agrobacterium tumefaciens, 4
Alanyl aminopeptidase, 449
Aliphatic hydrocarbons, 323
Allergens, 419–420
Allium cepa, 677
Amino acid(s), 422–423
  catabolism, 604–605
Amortization cost, 289
Analysis of spectral differences (ASD), 44
Analysis of variance (ANOVA) test, 68
Angiotensin I-converting enzyme (ACE) inhibitory peptides, 373, 375–378
Animal by-products, 383
Animal production strategies, *see* Raw meat, animal production strategies for
ANN, *see* Artificial neural networks
ANOVA test, *see* Analysis of variance test
Antihypertensive peptides, 375–378
Antimicrobial(s), 238
  activity, 549
  agents, addition of, 216
  ions, 658–660
  peptides, 382, 384
  properties, 526–528
  proteins, 663–664
Antimicrobial active packaging
  antimicrobial ions, 658–660
  antimicrobial proteins and peptides, 663–664
  bacteriophage, 661–663
  halloysite, 655
  liposomes, 656
  polyphenols, 655
  summary of, 657
  thymol, 654
Antioxidants
  addition of, 217
  properties, 523–525
  and vitamins, 413–414, 424–427
Antioxidative peptides, 378–379
Anti-Stokes Raman scattering, 84
Antithrombotic peptides, 381–382
AOTF, *see* Acousto-optic tunable filter
AP, *see* Active packaging
Aqueous smoke condensates, *see* Liquid smoke flavorings
Area scanning mode, 31
Area under the curve (AUC), 44
ARS, *see* Agricultural Research Service
Artificial neural networks (ANN), 42
Aryl hydrocarbon hydroxylases, 325
Ascorbic acid, 426
ASD, *see* Analysis of spectral differences
Asini Corii Collas, 388
Aspergillus, 377
AUC, *see* Area under the curve
Autoxidation, meat, 630

B

Bacillus cereus, 637
BacJ1, 565
"Background toughness," 268

Backward interval partial least squares (biPLS), 44
Bacteria foodborne infection, qPCR detection
Campylobacter, 127
Listeria monocytogenes, 122–124
Salmonella detection, 119–122
verotoxin-producing *E. coli,* 124–126
Yersinia, 127–129
Bacteria foodborne intoxications, qPCR detection
Clostridium botulinum, 131–132
Staphylococcus aureus, 129–131
Bacterial rapid detection using optical scattering technology (BARDOT), 166
Bacterial starter cultures, 598–599
color, 601–602
flavor formation, 602–606
probiotic, 606–608
with protective, 599–600
texture, 601
Bacteriocins, 238
appearance of resistant strains, 582–585
application of, 563
industrial application and regulatory aspects, 584
interaction with meat components, 579–581
microbial flora, 559
preservation technologies, 575, 576
Bacteriophage, 661–663
Band-interleaved-by-line (BIL) format, 24
Band-interleaved-by-pixel (BIP) format, 24
Band sequential (BSQ) format, 25
BARDOT, *see* Bacterial rapid detection using optical scattering technology
Barrier film materials, 633–634
Batch-pilot equipment, 265
BAX® system real-time polymerase chain reaction assay, 1247
Bayesian classification, 36
"Bay region" dihydrodiol epoxides, 325
Beef, 98–100
high-oxygen packaging of, 628
peptide in, 373, 385
Benchtop Raman devices, 86
Benzo[*a*] pyrene (BaP) content
in charcoal-grilled frankfurters, 324
diffusion coefficients for, 347
methanolic solution, 340
Biacore® Q biosensor, 176
Bifidobacterium, 383
BIL format, *see* Band-interleaved-by-line format
Bioactive peptides, 372, 374, 389, 422
ACE inhibitory peptide, 375–378
antimicrobial peptides, 382
antioxidative peptides, 378–379
antithrombotic peptides, 381–382
from by-products, 383–384
hypocholesterolemic peptides, 381
immunomodulatory peptides, 380–381
opioid peptides, 379–380
Bioinformatics, 390
Biological sensors, 154
BIOPEP database, 390
Biopreservation, 563
Biosensors, 670–671
optical fiber-based, 159
recognition elements in, 155
BIP format, *see* Band-interleaved-by-pixel format
biPLS, *see* Backward interval partial least squares
"Black-smoked," 322
Blending, 519
Blood, by-product of meat industry, 383
Bone detection, in chicken, 52–55
Bone marrow, in meat, 628
Botulinum neurotoxin (BoNT), 131
Bovine spongiform encephalopathy (BSE), 1
Bran, 477
Branched-chain amino acids, 605
Breaded meat products, 495
Breading system, 495
Brine curing, 517–518
Brine injection, 273
Brining, 302–303
Broadband illumination sources, 27
BSQ format, *see* Band sequential format
Butyric acid, 363

C

Calcium-activated factor, 537
Calcium-activated neutral proteinases, 537
Calcium-dependent proteases, 537
Callipyge lamb meat, 272
Calpains, 374, 537
Campylobacter, 127
  C.jejuni, 140–141, 562, 636
CAP, *see* Controlled atmosphere packaging
Capillary electrophoresis (CE), 277
Carbohydrate
  based extenders/proteins, 492
  catabolism, 603
Carbon dioxide, 618, 621–623
Carbon monoxide, 620, 625–626
Carboxypeptidases, 538
Carcinogens, 419
L-Carnitine, 422
Carotenoids, 426
Carrageenan gum, 471
Casecidin, 382
Casein-derived peptides, 380
Casomorphins, 379
Catacondensed PAHs, 320
Cathepsin, 276
Cavitation, 282
CCD, *see* Charge-coupled device
CCMP, *see* Cooked cured meat pigment
CCR, *see* Correct classification rate
cDNA, *see* Complementary DNA
Cell-free culture supernatants (CFS), 565
Cellulose-peelable casing, 340
Charcoal grilling, 324
Charge-coupled device (CCD), 19, 85, 166
Chemical contamination
  electromechanical sensors, 177–182
  optical sensors, 176–177
  veterinary drug residues, 171–175
Chemical sensors, recognition elements in, 155
Chemometric principal component analysis (CPCA), 67
Chemometric techniques, 39
Chicken
  brine injection, 273
  HDP treatment of, 272
  meat, 664
Cholesterol, 417–418
Chopping, 519
Cigarette smoking, 325
Ciprofloxacin, 173
CLAs, *see* Conjugated linoleic acids
Clavicle, 54
CLB, *see* Clenbuterol (CLB)
Clenbuterol (CLB), 175
Clostridium botulinum, 131–132, 561, 571, 636
Clostridium perfringens, 562, 637
clpP, 139
CLSM, *see* Confocal laser scanning microscopy
Clupea harengus, 340
Clustered regularly interspaced short palindromic repeat (CRISPR) technology, 5
CMOS cameras, *see* Complementary metal oxide semiconductor cameras
Cold plasma technology (CPT), 304–308
Collagen, 384, 387, 388
Color
  attributes, fat, 468
  changes, in cooked meat, 212–213
  of meat, 601–602, 649
Colorimetric indicators, 667–670
Complementary DNA (cDNA), 358
Complementary metal oxide semiconductor (CMOS) cameras, 26, 30
"Compton effect," 202
"Compton electron," 202
Computer vision, 19
Conducting organic polymers (CPs), 169
Confocal laser scanning microscopy (CLSM), 278, 279
Conjugated linoleic acids (CLAs), 361
Controlled atmosphere packaging (CAP), 618
Cooked cured meat pigment (CCMP), 519
Cooked meat
  color changes in, 212–213
  emulsions, fat in, 467
  products, 567–571, 629–630

vacuum *vs*. MAP packaging, 631–632
Correct classification rate (CCR), 43
Cost analysis of HDP technology, 286
equipment, 287–289
packaging, 286
quantifying meat benefits, 289
CPCA, *see* Chemometric principal component analysis
CPs, *see* Conducting organic polymers
CPT, *see* Cold plasma technology
Cq, *see* Quantification cycle
Crossline-markers, 52
Cross-validation, 43
Ct, *see* Threshold cycle
Cured-and-cooked meat products, 567–571, 629–630
vacuum *vs*. MAP packaging, 631–632

D

Decontamination of meat, 300–301
Denaturation process, 672
Designing meat products, 516
DHA, *see* Docosahexaenoic acid
Dibenzo[*a*,*h*]pyrene (DBa,hP), 325
Dietary fiber, 423, 424
Dietary salt intake, 444
Differential gene expression, 358
Digestible oligosaccharides (DOs), 478
Dipeptidylpeptidases (DPP), 536, 537
DNA bases detection strategies, 7, 10
polymerase chain reaction-based methods, 10–11
sequencing, 11–13
DNA isolation, 114
Docosahexaenoic acid (DHA), 464, 465
Dos, *see* Digestible oligosaccharides
DPP, *see* Dipeptidylpeptidases
Drip loss, 71
Dry-cured ham, 450, 451
data analysis, 550–552
peptidomics, applications of, 545–550
protein mass fingerprint approach, 544
from traditional proteomics to peptidomics, 545
Dry-cured meats, proteolysis in, 542
Dry curing method, 516–517
Dry-fermented sausages, 451
Durethan®, 654

E

Edible coatings, 671–674
Edible films, nanostructured, 671–674
Eicosapentaenoic acid (EPA), 464, 465
EIS, *see* Electrochemical impedance spectroscopy
Electric nose, 57
Electrochemical biosensors, 167
Electrochemical impedance spectroscopy (EIS), 168
Electrochemical sensors, 167–168
Electromagnetic waves, 21
Electromechanical sensors, 167–168, 177–182
Electron beam, 203
Electronic nose-based sensors, 169–170
Emerging technologies
cold plasma technology, 304–308
light-based technologies, 308–310
pulsed electric field, 303–304
ultrasound, *see* Ultrasound technology
EMSC, *see* Extended multiplicative signal correction
Emulsified lipid gel, 488
Emulsified meat products, 465, 466, 492–493
heat treatment, 468
Emulsion gels, 491
Endolysins, *see* Lysins
Endopeptidases, 373, 537, 540–541
Enterobacteriaceae family, 572
Enterococci, use of, 574
Enterococcus strains, 574
Enterotoxigenic strains, 561
Enzymatic amplification, of DNA, 8
Enzyme-linked immunosorbent assay (ELISA), 9
EPA, *see* Eicosapentaenoic acid
Ergosterol, 324
Escherichia coli, 561
E.coli O157:H7, 284, 285, 635–636, 656
Esters, 606
Ethylene vinyl acetate (EVA), 634, 655
Ethylene vinyl alcohol, 633–634

European Food Safety Agency (EFSA), 3
European Union, 678
Exopeptidases, 537–538, 541–543
Explosives, in food industry, 263
Extended multiplicative signal correction (EMSC), 39
Exterior film materials, 633

F

"Farmhouse smoked," 322
Fat, 417–418
  content, 59
  deposition model, 365
  and fatty acid profile, 420–422
  mimetics, 471
  substitutes, 471
Fat consumption, health implications, 462–463
  mono and polyunsaturated fatty acids, 464–465
  saturated fatty acids, 463–464
Fat reduction, in processed meats, 462
  challenges of, 492–495
  as fat source, 465–466
  flavor and taste, 467
  insoluble fibers, 474–478
  lipid reformulation using vegetable oils, 483–491
  mono and polyunsaturated fatty acids, 464–465
  perception of satiety, 469
  physicochemical properties, 467–468
  saturated fatty acids, 463–464
  soluble fibers, 478–483
  strategies for, 469–491
  texture, 466–467
  use of structured lipids, 488–491
Fat replacers
  effects on meat products, 479–481
  gums, 471–472
  nonprotein extenders, 471
  protein-based, 473–474
  starch derivatives, 472–473
Fatty acids
  fat and, 420–422
  ώ-3 fatty acids, 361
  oxidation, 605–606
  in pigs, 408–411
  in ruminants, 412
  synthesis
    conjugated linoleic acids as regulators, 361–364
    de novo, 365
    primary precursor for, 364
Feed forward neural network, 42
Fermentation
  of by-product, 384
  of meats, 374
Fermented foods
  production of, 597
  purpose, 598
Fermented meat products, 465, 493–494
Fermented sausages, 468, 493
Fiber-optic approach, 157
Fibers, as functional ingredients, 474–483
Fickian laws of diffusion, 335, 339
Fish
  chemical compositions, 58–60
  differentiation and classification, 62
  freshness, 57–58
  microbial spoilage, 61–62
  parasites and defects, 60–61
Fisher's linear discriminant (FLD), 67
Flat-field correction, 32–33
Flavor, 467
  characteristics, 525–526
  formation, 602
    amino acid catabolism, 604–605
    carbohydrate catabolism, 603
    esters, 606
    fatty acid oxidation, 605–606
    lipid hydrolysis, 605
    protein hydrolysis, 603–604
FLD, *see* Fisher's linear discriminant
Flexible packaging film, 632–634
  barrier materials, 633–634
  exterior materials, 633
  sealant materials, 634
Food and Drug Administration (FDA), 678
Foodborne botulism, 131
Foodborne diseases, 157
Food contamination, 159
Food-grade coatings, 673
Food irradiation, 199–204

Food packaging applications, nanotechnology for, 648
Food safety, 281
Food Safety and Inspection Service (FSIS), 48
Foods for specified health use (FOSHU), 404
Formulating, meat products, 515–516
FOSHU, *see* Foods for specified health use
FOSs, *see* Fructooligosaccharides
Fourier transform infrared (FTIR) spectroscopy, 164–165
Free-radical oxidation, 210
Free solution conjugate electrophoresis (FSCE), 542
Fresh beef, color, 656
Fresh meat, 273
  carbon monoxide, 619
  case-ready packaging, 641
  high hydrostatic pressure on, 247–257
  MAP systems, 627–629
  nitrite-containing film, 639
  nitrogen, 623
  vacuum packaging, 631
  vacuum *vs*. MAP, 630–631
Fructooligosaccharides (FOSs), 478, 479
FSAs, *see* saturated fatty acids
FSCE, *see* Free solution conjugate electrophoresis
FSIS, *see* Food Safety and Inspection Service
FTIR spectroscopy, *see* Fourier transform infrared spectroscopy
Functionality modification, 274–276
Functional meat products
  allergens, 419–420
  antioxidants and vitamins, 424–427
  definition of, 404
  development of, 415, 416
  fat and cholesterol, 417–418
  fatty acid profile, 420–422
  meat and, 405–429
  minerals, 427
  nitrite, 419
  other compounds, 427–429
  prebiotics and probiotics, 423–424
  proteins, peptides, and amino acids, 422–423
  raw meat, *see* Raw meat, animal production strategies for
  safety and scientific criteria, 428
  sodium and phosphate, 418–419
Fungal contamination detection, sensors for, 160, 161
Furanones, 387
Furans, 387

G

Gadus morhua, 60
Galactomannans, 472
Gamma rays, 202
Gaseous amines production, 669
Gastrointestinal proteolysis, 373
Gaussian maximum likelihood (GML), 61
Gel from emulsion, formation of, 490
GenBank, 358
Generally recognized as safe (GRAS), 473, 570, 584
Genetic algorithms (GAs), 44
Genetically modified (GM) farm animals
  authorization process, 3
  clustered regularly interspaced short palindromic repeat system, 5
  detection options, 7
  DNA bases detection strategies, 10–13
  DNA level modification, 5
  field use of, 3–4
  microinjection, 4
  protein-based detection strategies, 7–10
  protein level modification, 6
  quantitative polymerase chain reaction methods, 13–14
  quantitative protein methods, 13
  traceability process, 1
Genetically modified organisms (GMOs), 2
Genetics, for animal fats reduction, 407–408
GLGCM method, *see* Gray-level gradient co-occurrence matrix method
Glucose, *vs*. acetate, 364–366
Glycine, 387
Glycosylated peptides, 383

gmk gene, 139
GML, *see* Gaussian maximum likelihood
Goat placenta, 384
Gram-negative bacteria, 582
Gram-negative pathogens, 567
GRAS, *see* Generally recognized as safe
Grass-fed beef, 356
Gray-level gradient co-occurrence matrix (GLGCM) method, 58
Grilling, 324
Guar gum, 472
Gums, 471–472

H

Halloysite (HT), 655
Halogen lamps, 27
HDL, *see* Highdensity lipoprotein
HDP, *see* Hydrodynamic pressure processing
Heat-treated pork, 94–95
Hemoglobin, 384
Hemolytic uremic syndrome (HUS), 561
Hemorphins, 380
Hemorrhagic colitis, 561
HHP, *see* High hydrostatic pressure
HI, *see* Hyperspectral imaging
Highdensity lipoprotein (HDL), 464
High hydrostatic pressure (HHP)
  and bacteriocins, 575–577
  consumer acceptance of, 244
  limitations of, 247–257
  Listeria monocytogenes inactivation, 230–232
  microbial inactivation during, 230, 232–241
  multipulsed treatment, 238–239
  quality attributes, effect on, 241
Highly unsaturated omega-3 fatty acids (HUFAs), 360, 361
High-performance liquid chromatography (HPLC), 18, 329
High-pressure processing (HPP)
  hydrodynamic pressure processing *vs*., 260
  for structural modification, 274
  for tenderness improvement, 269
HILIC, *see* Hydrophilic interaction liquid chromatography
Housekeeping genes, 117
HPLC, *see* High-performance liquid chromatography
HPP, *see* High-pressure processing
HT, *see* Halloysite
HUFAs, *see* Highly unsaturated omega-3 fatty acids
Human foodborne viruses, 141
Humidity detection, in meat packaging, 667
Hurdle concept, 239–240, 638
Hydrodynamic pressure processing (HDP), 260
  bacterial inactivation, 283
  cost analysis of, 286–291, 289–291
  improving meat safety, 281–285
  modification of functionality, 274–276
  molecular and structural effects, 276–280
  shock wave technology, *see* Shock wave technology
  tenderness, effect on, 269, 270, 271, 272
  vs. high-pressure processing, 260
Hydrodyne process, 263, 284
Hydrophilic interaction liquid chromatography (HILIC), 542
Hydrophilic peptides, 604
Hypercube, for salmon fillet, 23
Hyperspectral imaging (HI), 166
  acquisition, 24–26
  basic principles, 20–22
  data structure, 22–23
  fluorescence/reflectance imaging, 53
  image sensing modes, 23–24
  instruments
    area detectors, 30
    illumination unit, 27–28
    system calibration, 30–33
    wavelength dispersion devices, 28–30
  system calibration
    flat-field correction, 32–33
    spatial calibration, 31
    spectral calibration, 31–32
Hypocholesterolemic peptides, 381

I

IAC, *see* Internal amplification control
IARC, *see* Working Group of International Agency for Research on Cancer
IEC, *see* Ion exchange chromatography
Image segmentation, 33–34
  spectral image segmentation, 36
  thresholding and morphological processing, 34–36
Image sensing modes, 23–24
IMF, *see* Intramuscular fat
Immunological protein detection methods, 9
Immunomagnetic separation (IMS), 670
Immunomodulatory peptides, 380–381
Impedance biosensors, 168
Imperm®, 654
IMS, *see* Immunomagnetic separation
IN, *see* Inulin
Inelastic scattering, 84
Ingenuity Pathway Analysis (IPA), 359
Injected meat product, 274
Inoculation, of meat products, 563
Insoluble fibers, 474–478
Instrumentation, of hyperspectral imaging, 26
Intelligence packaging
  biosensors, 670–671
  colorimetric indicators, 667–670
Interactance mode, 24
Internal amplification control (IAC), 118
Interval partial least squares (iPLS), 44
Intramuscular fat (IMF), 356
  adipocytes, 364, 365
  deposition, 357
  glucose *vs*. acetate, as precursors, 364–366
Inulin (IN), 478, 479, 480
Ion exchange chromatography (IEC), 542
Iota carrageenan, 472
IPA, *see* Ingenuity Pathway Analysis
iPLS, *see* Interval partial least squares
Irradiated meat
  color changes, mechanism of, 213–215
  consumer acceptance of, 215–216
Irradiated raw meat, color changes in, 212–213
Irradiation dose, 205
Isracidin, 382

K

Kappa carrageenan, 472

L

β-Lactam antibiotics, 172
Lactic acid, 216
Lactic acid bacteria (LAB), 540, 563, 598, 600
Lactobacillus acidophilus, 607
Lactobacillus casei, 607
Lactobacillus curvatus, 378, 598, 599, 603, 604, 608
Lactobacillus curvatus CRL705, 566
Lactobacillus gasseri, 607
Lactobacillus paracasei, 607
Lactobacillus plantarum, 598, 599, 603, 604, 608
Lactobacillus sakei, 378, 565, 573, 598, 599, 603, 604
Lactococcus lactis, 663
α-Lactorphin, 379–380
Lamb, 95–98
Lasers, 27
Lauric acid, 463
LCTF, *see* Liquid crystal tunable filter
LDA, *see* Linear discrimination analysis
LDPE, *see* Low-density polyethylene
Lean animals, 356
Lean meats
  fat content in, 465
  fat reduction and selection, 470
Least squares-support vector machine (LS-SVM) model, 58
LEDs technology, *see* Light-emitting diodes technology
Leucine, 363
Ligand-forming compounds, 213
Light-based technologies, 308–310
  applications, 310
  efficacy of, factors affecting, 309
  UV light, 310

Light-emitting diodes (LEDs) technology, 27
Light-scattering sensors, 166
Linear discrimination analysis (LDA), 41
Linear low-density polyethylene (LLDPE), 656
Line scanning method, 24, 31
Lipid hydrolysis, 605
Lipidomics, 358
Lipid oxidation, 209–210, 628
  of meat and meat products, 241–242
Lipid peroxidation, 425
Lipid reformulation, using vegetable oils, 483–491, 484
Lipogenesis, 361
Lipolysis, 493, 605
Lipopolysaccharide (LPS), 582
Liquid crystal tunable filter (LCTF), 29–30
Liquid media, 335–339
Liquid smoke flavorings (LSFs), 324, 326–328
Listeria innocua, 568
Listeria monocytogenes, 637, 673
  in beef frankfurters, 284, 285
  in foodborne illness, 122–124, 560
  growth of, 382
  inhibition of, 565, 570, 577
  RT-qPCR evaluation, 139–140
LLDPE, *see* Linear low-density polyethylene
Locust gum, 472
Long-chain fatty acids, 360–364
Longissimus thoracis, 279, 280
Low-density polyethylene (LDPE), 329, 331
  liquid media and, 335–339
Low-energy electromagnetic radiations, 202
Low-salt foods
  dry-fermented sausages, 451
  manufacturing strategies for, 445–446
Low-temperature long-time treatment (LTLT), 273
LS-SVM model, *see* Least squares-support vector machine model
LTLT, *see* Low-temperature long-time treatment
Lutein, 426
Lycopene, 426
Lysins, 663

M

Machine learning methods, 41
Maillard reaction
  Amadori compound and, 385–386
  of amino compounds and reducing sugars, 386
  and meat, 386–387
Maillard reaction products (MRPs), 372
  bioactivities, from peptides, 387–388
  in cooked meat, 386
  of enzymatic hydrolysates, 387
  volatile, 388–389
MALDI-ToF MS, *see* Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry
Maltodextrin, 473
MAP, *see* Modified atmospheric packaging
Marbling, 64
Marinated meat products, 494
Mass spectrometry (MS), 10
Master-pack approach, 620
Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-ToF MS), 544
Meat components/additives, 579–581
Meat composition, 205
Meat contamination monitoring
  chemical contamination, 170–182
  microbial contamination, 157–191
Meat decontamination, by irradiation
  control of quality changes, 216–218
  food irradiation, 199–204
  microbial decontamination, 204–208
Meat emulsions, 492
Meat matrix, 465
Meat packaging systems, nanotechnology-based
  antimicrobial active packaging, *see* Antimicrobial active packaging

enhanced barrier using nanoclay, 651–652
intelligence packaging, *see* Intelligence packaging
nanoclay composites in, 653–654
nanostructured edible films and coatings, 671–674
packaging format, 650
societal implications, 675–679
Meat products, as fat source, 465–466
Meat protein-derived peptides, 372–375, 389
aging of meats, 373
bioactivities of, *see* Bioactive peptides
enzymatic treatment, 374–375
fermentation of meats, 374
gastrointestinal proteolysis, 373
Meat quality/safety assessment
fish, 56–62
poultry carcass, *see* Poultry carcass
red meats, 62–72
Meat tenderization, 64, 375
biological interventions for, 268
brine injection, 273
chemical interventions for, 269
effect of ultrasound, 301–302
hydrodynamic pressure processing, 269–272
low-temperature long-time treatment, 273
Meat tissue, 580
Melanoidins, 386
Melt processing, 652
Metabolomics, 358
Metal oxide nanoparticles, 676
Metal–oxide–semiconductor fieldeffect transistors (MOSFETs), 169
Metal-oxide-semiconductors (MOSs), 169, 170
Metal–oxide–semiconductors (MOSs), 169
MFD, *see* Milk fat depression
Microbial contamination
electrochemical and electromechanical sensors, 167–168
electronic nose-based sensors, 169–170
fourier transform infrared spectroscopy, 164–165
light-scattering sensors, 166
optical fibers and microarrays, 159–163
sensors for, 160, 161
Microbial inactivation, 232–241, 306
Microbial metabolism, 627
Microbial proteolytical system
endopeptidases, 540–541
exopeptidases, 541–543
Micrococcus luteus, 384
Microcrystalline cellulose (MCC), 660
Microencapsulation, 607
Microinjection, 4
microRNA, 359
Milk caseins, hydrolysates of, 380
Milk clotting, 381
Milk fat depression (MFD), 362
Minerals, 414–416, 427
MLR, *see* Multiple linear regression
Modified atmosphere packaging (MAP), 306, 616
active packaging components, 638–640
applications of, 620
barrier film materials, 633–634
carbon monoxide, 629
component in hurdle concept, 638
cured-and-cooked meat products, 629–630
definitions, 617–618
dynamic headspace changes, 626–627
exterior film materials, 633
fresh chicken meat, 664
gases used in, 620–627
high-oxygen, 627–628
intelligent packaging, 667
leakers and package integrity, 640
packaged meats, 669
on pathogens effects, 635–637
purpose of, 619–620
regulatory issues, 640–641
rigid materials, 634
sealant materials, 634
vacuum packaging *vs*., 630–631
Modified atmospheric packaging (MAP), 306, 651
Monounsaturated fatty acids (MUFAs), 464–465
Montmorillonite (MMT), 651, 652, 654

Mosaic principal component analysis (MPCA), 67
MOSs, *see* Metal-oxide-semiconductors
MPCA, *see* Mosaic principal component analysis
MpHHP, *see* Multipulsed high hydrostatic pressure
MRPs, *see* Maillard reaction products
MSC, *see* Multiplicative scatter correction
MUFAs, *see* Monounsaturated fatty acids
Multiple linear regression (MLR), 42
Multiple-needle pumping, 518
Multiplicative scatter correction (MSC), 37
Multipulsed high hydrostatic pressure (MpHHP), 238–239
Multivariate analysis
  classification, 39–41
  model evaluation, 43–44
  regression, 41–42
Multivariate regression, 41–42
Murein hydrolases, *see* Lysins
Muscle proteolytic system
  endopeptidases, 537
  exopeptidases, 537–538
Musculus longissimus dorsi, 63
Musculus semimembranosus, 281
Myofibrillar proteins, 492
Myristic acid, 463

N

NAFLD, *see* Nonalcoholic fatty liver disease
Nanoclay, 651
  composites, in meat packaging, 653–654
  enhanced barrier using, 651–652
  toxicity, 677
Nanocomposite films, 673
Nanoencapsulation, 648
Nanomaterial implementation, for enhanced sensing performance, 156–157
Nanomaterials
  incorporation of, 649
  public perception of, 679
  regulation, 678–679
  toxicity of, 678
Nanoparticles, toxicity of, 676
Nanotechnology
  in food industry, 647–650
  in packaging food, 679
NASH, *see* Nonalcoholic steato hepatitis
Native granular, 482
NDOs, *see* Nondigestible oligosaccharides
Near-infrared spectroscopy (NIRS), 19
Negative polymerase chain reaction control, 118
NIRS, *see* Near-infrared spectroscopy
Nisin, 238, 564, 663
Nitrite, 419, 519
Nitrite curing, 519–528
Nitrite/nitrate, 601
Nitrogen, 618, 623–624
Nitrogen gas, 620
Nitrogen oxide (NO), 601
N-nitrosamines, 515
nlpD, 139
Nonalcoholic fatty liver disease (NAFLD), 360
Nonalcoholic steatohepatitis (NASH), 360
Nondigestible oligosaccharides (NDOs), 478
Nonglycosylated peptides, 383
Nonprotein extenders, 471
Nontemplate polymerase chain reaction control, 118
Nontoxic nonhemagglutinin (NTNH) gene, 131
Novel sensors, 156
NTNH gene, *see* Nontoxic nonhemagglutinin gene
Nutrigenomics, 355, 389
  animal agriculture and, 356
  conjugated linoleic acids, 361
  differential gene expression, 358
  experimental approaches, 360
  glucose *vs*. acetate, 364–366
  long-chain fatty acids as, 360–364
  reductionism, 357–359
Nutrition Facts Label, 453
Nylon, 633

O

Ochratoxin A (OTA), 132, 170
Ochratoxin A-producing penicillia, 142
Octapeptide, 385
Off-odor production, in irradiated meat, 210
Oleic acid, 464
Oligopeptides, 373
Oligosaccharides, 382, 424
OMFs, *see* Oscillating magnetic fields
Omics technologies, 390
Opioid peptides, 379–380
Optical fibers, 159–163
Optical microarrays, 159–163, 163
Optical sensors, 176–177
  latest trends in, 166–167
Optical waveguides, 157
Organic selenium, 415
ORP, *see* Oxidation-reduction potential
Oscillating magnetic fields (OMFs), 297, 304
OTA, *see* Ochratoxin A
Oxidation of lipids, 306
Oxidation-reduction potential (ORP), 214
Oxygen, 624–625
Oxygen scavengers, 635, 639

P

Package integrity, 640
Packaging films, 632–634, 639
PAH4 content, 329, 347
Palmitic acid, 463
Partial least squares analysis (PLSA), 67
Partial least squares-discriminant analysis (PLS-DA), 41
Partial least squares regression (PLSR), 41, 164, 168
Pathogen detection techniques., 158
Pathogens associated with meat, 650
Pattern recognition, 39, 41
PCA, *see* Principal component analysis
PCCMP, *see* Powdered cooked cured meat pigment
PCR, *see* Principle component regression
PD, *see* Polydextrose
Pediocin, 570, 572
Pediococcus, 603
PEF, *see* Pulsed electric field
Pentocin 31-1, 565
Peptidase, 276
Peptides, 371, 422–423, 604
  bioactive, *see* Bioactive peptides
  generation, from meat proteins, 372–375
  Maillard reaction and, 385–389
  with sensory properties, 385
Peptidomic approach, 552
Pericondensed PAHs, 320
Peroxidation, 605
PGP principle, *see* Prism-grating-prism principle
Phosphate, 418–419
Phospholipon liposomes, 656
Photodecomposition, 340–342
Photons, 202
Photooxidation, meat, 630
Physical sensors, 154
Physicochemical processes, 335–339
Physicochemical properties, of fat, 467–468
Plant stanol, 427
Plant sterols, 427
PLSA, *see* Partial least squares analysis
PLS-DA, *see* Partial least squares-discriminant analysis
PLSR, *see* Partial least squares regression
PLS regression, *see* Partial least squares regression
PMF, *see* Protein mass fingerprint
Point scanning method, 24
Polyamides, 633
Polycyclic aromatic hydrocarbons (PAHs), 344
  characterization of, 320–321
  content in processed meats, 339–348
  cooking of smoked sausages, 342–344
  diffusion coefficients of, 332
  formation of, 321–324
  international normalization, 326–328
  in liquid media and low-density polyethylene, 335–339
  in liquid smoke flavorings, 329
  on living organisms, 325–326
  in roasted duck, 344
  in smoked meats, 328–329

and smoked sausages, 344–348
Polydextrose (PD), 478, 480, 481, 482
Polyelectrolyte films, electrogeneration of, 182
Polyester, 633
Polyethylenes, 634
Polymerase chain reaction (PCR), 10
Polymer–clay nanocomposites, 651
Polyphenols, 655
Polypropylene, 633
Polysaccharides, 672
Polystyrene, 634
Polyunsaturated fatty acids (PUFAs), 360, 361, 408, 409, 464–465
Polyvinyl chloride, 634
Polyvinylidene chloride, 634
Polyvinylpolypyrrolidone resin (PVPP), 655
Pork
back fat, 492
decontamination of, 306
heat-treated pork, 94–95
meat, hydrodynamic pressure processing of, 272
oxygen packaging, 628
peptide in, 373
postrigor pork, 93
prerigor pork, 88–92
Positive polymerase chain reaction control, 118
Postharvest interventions, 198
Postrigor pork, 93
Poultry carcass, 48
contamination detection, 49–52
microbiological spoilage detection, 55–56
tumor and bone detection, 52–55
Poultry meat, 411
Powdered cooked cured meat pigment (PCCMP), 521
PrB, *see* Proteinase B
Prebiotic peptides, 382–383
Prebiotics, 423–424
Premature browning, beef, 628
Prerigor pork, 88–92
Preservation technologies, bacteriocins and
active packaging systems, 577–579
high hydrostatic pressure, 575–577
Principal component analysis (PCA), 36, 37, 39–40, 94, 103
score plot, 551
Principle component regression (PCR), 42
Prism-grating-prism (PGP) principle, 28
Probiotics, 423–424
Propionic acid, 364
Proteases, 374
Proteasome complex, 537
Protein, 422–423
denaturation, 282
as emulsifiers, 490
film, 672
modification of, 274–276
Proteinase B (PrB), 541
Protein-based detection strategies, 7–10
enzyme linked immunosorbent assay, 9
flow strip, 8–9
mass spectrometry, 10
Protein-based fat replacers, 473–474
Protein mass fingerprint (PMF), 544
Proteolytic enzymes for meat tenderization, 375
Proteomics, 390, 545
Pseudomonas, 650
PUFAs, *see* Polyunsaturated fatty acids
Pulsed electric field (PEF), 303–304
Purge, vacuum packaging, 631
Purified bacteriocins, 563
Pushbroom method, 24
hyperspectral imaging system, 26
Pyrazines, 387

Q

QCMs, *see* Quartz crystal microbalances
Qflex® kit, 176
QIAamp DNA Mini kit (QIAGEN), 115
QIM, *see* Quality index method
QIS, *see* Quality index scores
qPCR, *see* Quantitative polymerase chain reaction
QTH lamps, *see* Quartz–tungsten–halogen lamps
Quality index method (QIM), 56
Quality index scores (QIS), 58
Quantification cycle (Cq), 116

Quantitative polymerase chain reaction (qPCR)
bacteria foodborne infection, *see* Bacteria foodborne infection, qPCR detection
bacteria foodborne intoxications, 129–132
considerations for, 116–117
controls of, 118
Quantitative regression, 43
Quartz crystal microbalances (QCMs), 168
Quartz–tungsten–halogen (QTH) lamps, 27
Quasi-nutrigenomic, 363
Quinolones, 173

R

Radial basis function (RBF) kernel, 42
"Radiation absorbed dose," 204
Radiation destruction, of microorganisms, 205–207
Radionuclides, 202, 203
Raman spectra of pork chops, 101
Raman spectroscopic devices, 84, 86–87
Random forests (RF), 41
Raw hyperspectral image, 22
Raw meat, animal production strategies for
fatty acids in pigs, 408–411
fatty acids in ruminants, 412
genetics for reduction, 407–408
minerals, 414–416
products, 564–567
vitamins and antioxidants, 413–414
RBF kernel, *see* Radial basis function kernel
Reactive gas species (ROS), 304
Real-time polymerase chain reaction
principles and characteristics of, 114
quantitative polymerase chain reaction for, 119
reverse transcription quantitative polymerase chain reaction, 134
Receiver operator characteristics (ROC) curves, 44
Red meats
chemical compositions, 67–69
microbiological properties, 69–70
sensory attributes, 64–67
technological attributes, 70–72
Reductionism to global gene expression, 357–359
Reflectance calibration, 32
Reflectance spectra, 49
Reformulated meat products, 467
Relative humidity (RH), 667
Residual predictive deviation (RPD), 43
Resistant starch (RS), 478, 481, 482–483
Resistant strains, appearance of, 582–585
Restructured injected meat products, 494
Restructured meat products, 466, 482
Retrovirus-mediated gene transfer, 4
Reuterin, 245, 571
Reversed-phase high-performance liquid chromatography (RP-HPLC), 278, 542
Reverse transcriptase-polymerase chain reaction (RT-PCR), 359
Reverse transcription quantitative polymerase chain reaction (RT-qPCR) tool
Campylobacter jejuni, 140–141
Listeria monocytogenes, 139–140
ochratoxin A-producing penicillia, 142
Salmonella, 135–139
shiga toxin-producing *E. coli*, 140
Staphylococcus aureus, 141
viruses, 141–142
RF, *see* Random forests
Ribosomal RNA (rRNA), 117
Rice bran, 477
Rice starch, 473
RMSEC, *see* Root mean square error of calibration
RMSECV, *see* Root mean square error of cross-validation
RMSEP, *see* Root mean square error of prediction
ROC curves, *see* Receiver operator characteristics (ROC) curves
Root mean square error of calibration (RMSEC), 43
Root mean square error of cross-validation (RMSECV), 43

Root mean square error of prediction (RMSEP), 43
ROS, *see* Reactive gas species
Rotatable disk, 29
RPD, *see* Residual predictive deviation
RP-HPLC, *see* Reversed-phase high-performance liquid chromatography
rpoS, 139
rRNA, *see* Ribosomal RNA
RS, *see* Resistant starch
RT-PCR, *see* Reverse transcriptase-polymerase chain reaction
RT-qPCR, *see* Reverse transcription quantitative polymerase chain reaction
Ruminants, fatty acids in, 412
Rupture effect, 261

S

SA, *see* Simulated annealing
Safety assurance, 260
Salmonella spp., 119–122, 135–139, 560, 635
  S.typhimurium, 284, 285, 565, 670
Salmon fillet, hypercube for, 23
Salt reduction in processed meats
  crystal size and shape of, 446
  enzyme inhibition, 448–449
  and labeling, regulations, 453
  masking agents, 446
  physical properties, 447–448
  preservation properties, 452
  raised blood pressure, 444
  sensory effects, 449–452
  sodium reduction, 445
Sample principal component analysis (SPCA), 67
Satiety, 469
Saturated fatty acids (SFAs), 463–464
Sausages, 342–344
Savory peptides, 385
Savory taste-enhancing peptide, 385
Scanning electron microscopy (SEM), 278, 280
SCFA, *see* Short-chain fatty acid
SDS-PAGE, *see* Sodium dodecyl sulfate-polyacrylamide gel electrophoresis
Sealant materials, 634
SEC, *see* Size-exclusion chromatography
Selenium, 415
SEM, *see* Scanning electron microscopy
Semipurified bacteriocins, 563
Sensor electrode coating, 182
Sensory inspection, 57
Sensory properties, peptides with, 385
Sensory tenderness score, 273
SEP, *see* Standard error of prediction
SFAs, *see* Saturated fatty acids
Shiga toxin-producing *E. coli*, 140
Shock wave technology
  cost analysis of, 287, 289–291
  damages in packaging material, 288
  development of equipment, 263–267
  generation of shock waves, 261
  historical perspective of, 263
  principle of, 262
Short-chain fatty acid (SCFA), 363, 364, 605
Signal-to-noise ratio (SNR), 29
Silicon-based optical thinfilm biosensor, 163
Silver nanoparticle (AgNP), 658, 659, 677
Simulated annealing (SA), 44
Single-pulsed high hydrostatic pressure (spHHP), 238
Single-ring aromatic hydrocarbons, 323
Size-exclusion chromatography (SEC), 542
Slice shear force (SSF), 65
Smoked meat
  polycyclic aromatic hydrocarbons in, 326–328
    occurrence of, 328–329
    reduction of, 339–340
  products, 321–324
Smoothing, 37
SNR, *see* Signal-to-noise ratio
SNV, *see* Standard normal variate
Sodium, 418–419
  reduction, 445
  replacement, 445–446
  selenite, 415
Sodium chloride, 448
Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), 276, 277, 542

Sodium hypophosphite, 527
Soluble fibers, 478–483
Sourness-suppressing peptides, 385
Soy proteins, 423
Spatial calibration, 31
Spatial scanning methods, 25
SPCA, *see* Sample principal component analysis
Spectral calibration, 31–32
Spectral classifier, 53
Spectral image segmentation, 36
Spectral preprocessing, 37–39
Spectral variables, 44
Spectrum derivatives, 44
spHHP, *see* Single-pulsed high hydrostatic pressure
Spoilage reactions, in meat, 649–650
SPR transduction, *see* Surface plasmon resonance transduction
SSF, *see* Slice shear force
Standard error of calibration (SEC), 43
Standard error of prediction (SEP), 43
Standard normal variate (SNV), 37
Staphylococcal foodborne poisoning, 129
Staphylococcus aureus, 129–131, 141, 561, 636
Staphylococcus carnosus, 599, 601, 603, 606
Staphylococcus equorum, 599, 605, 606
Staphylococcus xylosus, 599, 601, 603, 606
Starch derivatives, 472–473
Stepwise regression, 44
Stokes Raman scattering, 84
Strip test, 8
Structured lipids, use of, 488–491
Sulfonamides, 173
Support vector machines (SVMs), 36, 41
Support vector regression (SVR), 42
Surface plasmon resonance (SPR) transduction, 176
SVMs, *see* Support vector machines
SVR, *see* Support vector regression
SYBR Green chemistry, 116
Synthetic peptide, 385

T

Tailor designing nitrite-free meat products, 528–529
TaqMan® hydrolysis probes, 116
Taste, 467
TBARS, *see* Thiobarbituric acid reactive substances
Tender Class System (TCS), 264
Tenderness, *see* Meat tenderization
Texture, 601
  change of meat, 215
  fat, 466–467
  of muscle foods, 241
TFs, *see* Transcription factors
Thermo-labile nature of protein meat systems, 246
Thiobarbituric acid reactive substances (TBARS), 209, 306, 384
Three-dimensional (3D) hyperspectral cube, 22
Threshold cycle (Ct), 116
Thresholding, 34–36
Thymol, 654
Time of curing, in dry-cured ham, 550
TnT degradation, *see* Troponin T degradation
Torturous diffusion path, of nanocomposite, 653
Total viable count (TVC), 18
Toxic equivalency factors, 326
Toxigenic molds, quantitative polymerase chain reaction for, 132–142
TPP, *see* Tripeptidylpeptidases
Transcription factors (TFs), 355, 360, 362
Trans-fatty acids intake, 463
Transmission electron microscopy (TEM), 278, 286
Transmission grating, 28
Transmittance mode, 24
Trichinella spiralis, 284
Tripeptidylpeptidases (TPP), 537
Troponin T (TnT) degradation, 277
Tumbling/massaging, of pickle-injected meat cuts, 518
Tumor detection, in chicken, 52–55
Tunable light sources, 27
Turkey breast, hydrodynamic pressure processing of, 272
TVC, *see* Total viable count
Two-dimensional gel electrophoresis (2-DGE), 542

U

Ultralow-oxygen system, 629
Ultrasound technology, 298
  decontamination of meat, 300–301
  meat brining, 302–303
  meat tenderization, 301–302
Umami taste-enhancing peptides, 385
Underwater electrical discharge, 264
United Nations Population Fund, 647
Up-to-date meat processing procedures, 320
UV irradiation, 669

V

Vacuum packaging (VP), 217, 618
  modified atmosphere packaging *vs.*, 630–632
Valorphin, 380
Venous thromboembolism, 381
Verotoxin-producing *E. coli* (VTEC), 124
Veterinary drug residues, 171
  antibiotics, 172–174
  hormones acting as growth promoters, 174–175
Visual identification, of genetically modified animals, 7
Vitamin E, 413, 425
Vitamins, 413–414, 424–427
Volatile organic compound (VOC), 169
VP, *see* Vacuum packaging
VTEC, *see* Verotoxin-producing *E. coli*

W

Warner–Bratzler shear force (WBSF), 272
Water emulsion, formation of oil and, 490
Water holding capacity (WHC), 447, 448
Wavelength calibration, 31
Wavelength dispersion devices
  filter wheels, 29
  imaging spectrographs, 28–29
  tunable filters, 29–30
Wavelengths, selection of important, 44–45
WBSF, *see* Warner–Bratzler shear force
Whiskbroom method, 24
Wild/direct smoking, 322
Wishbone, 54
Working Group of International Agency for Research on Cancer (IARC), 326
World Health Organization (WHO), 463, 474

X

X-prolyl-dipeptidylpeptidase, 541
X-rays, 202, 204

Y

Yersinia, 127–129
  Y.enterocolitica, 115, 172, 562, 635
Yersiniosis, 635

Z

Zein, 672
Zero-current devices, 167
Zero-order first-class Bessel function, 331