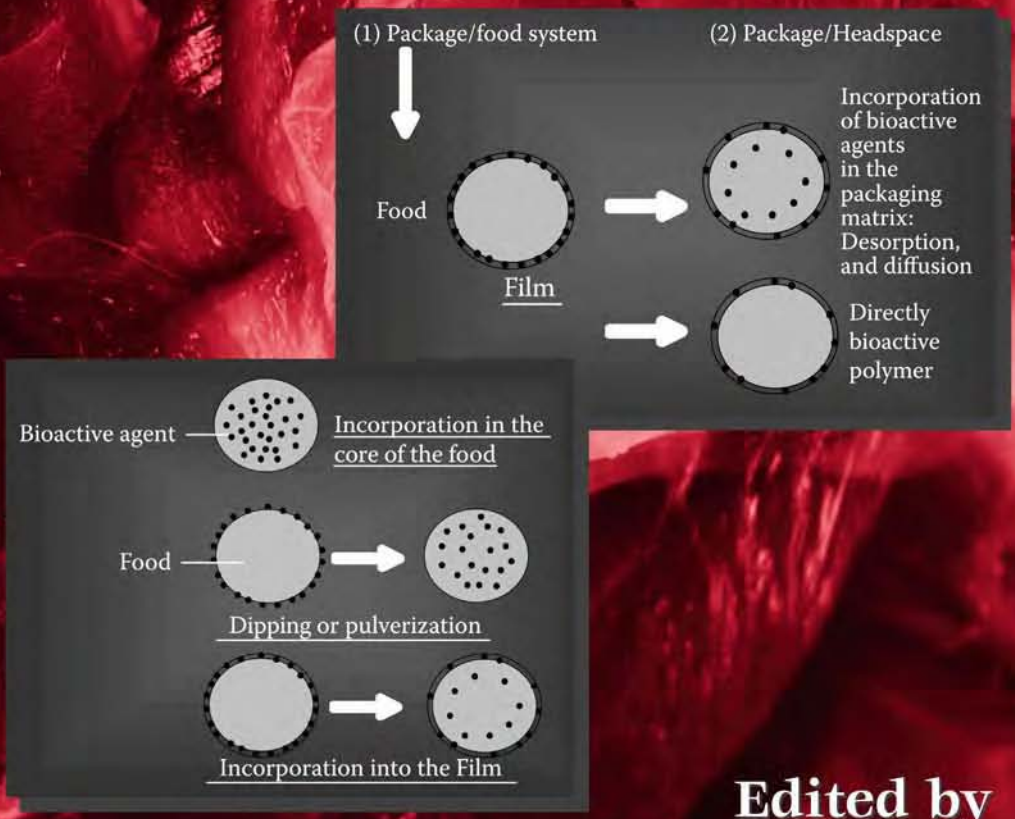
Advanced Technologies for Meat Processing



Edited by
Leo M. L. Nollet
Fidel Toldrá

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Advanced Technologies for Meat Processing

FOOD SCIENCE AND TECHNOLOGY

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Taylor & Francis
Taylor & Francis Group
Boca Raton London New York

A CRC title, part of the Taylor & Francis imprint, a member of the Taylor & Francis Group, the academic division of T&F Informa plc.

Published in 2006 by
CRC Press
Taylor & Francis Group
6000 Broken Sound Parkway NW, Suite 300
Boca Raton, FL 33487-2742

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CRC Press is an imprint of Taylor & Francis Group

No claim to original U.S. Government works
Printed in the United States of America on acid-free paper
10 9 8 7 6 5 4 3 2 1

International Standard Book Number-10: 1-57444-587-1 (Hardcover)
International Standard Book Number-13: 978-1-57444-587-9 (Hardcover)
Library of Congress Card Number 2005024763

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Library of Congress Cataloging-in-Publication Data

Advanced technologies for meat processing / edited by Leo M. L. Nollet and Fidel Toldrá.
p. cm. -- (Food science and technology ; 158)
Includes bibliographical references and index.
ISBN-13: 978-1-57444-587-9 (alk. paper)
ISBN-10: 1-57444-587-1 (alk. paper)
1. Meat. 2. Meat industry and trade. I. Nollet, Leo M. L., 1948- II. Toldrá, Fidel. III. Food science and technology (Taylor & Francis) ; 158

TS1960.A38 2006
664'.9--dc22 2005024763

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Preface

Meat and meat products constitute some of the most important foods in Western societies. However, the area of meat science and technology is not as fully covered as other foods from the point of view of books dealing with such important aspects as quality, analysis, and processing technology. It must be pointed out that the meat industry has incorporated important technological developments in recent years.

The main goal of this book is to provide the reader with recent developments in new technologies for the full meat processing chain. It starts with the production systems through the use of modern biotechnology (chapters 1 and 2); followed by automation in slaughterhouses (chapter 3); rapid nondestructive online detection systems (chapters 4, 5, and 6); the description of new technologies such as decontamination, high-pressure processing, fat reduction, functional meat compounds such as peptides or antioxidants, processing of nitrite-free products, and dry-cured meat products (chapters 7–14). Bacteriocins against meat-borne pathogens and the latest developments in bacterial starters for improved flavor in fermented meats are discussed in chapters 15 and 16. The two remaining chapters (17 and 18) detail recent final product packaging systems.

This book is written by distinguished international contributors with extensive experience and solid reputations. It brings together all the advances in such varied and different technologies as biotechnology, irradiation, high pressure, and active packaging to be applied in different stages of meat processing.

For all their efforts and for sharing their knowledge on these different topics we would like to thank very cordially all contributors of this volume.

Editors

Leo M. L. Nollet is professor of biotechnology at Hogeschool Gent, Ghent, Belgium. The author and coauthor of numerous articles, abstracts, and presentations, Dr. Nollet is also the editor of the three-volume *Handbook of Food Analysis* (Second Edition), *Handbook of Water Analysis*, *Food Analysis by HPLC* (Second Edition) and *Chromatographic Analysis of the Environment* (Third Edition).

His research interests include air and water pollution, liquid chromatography, and applications of different chromatographic techniques in food, water, and environmental parameters analysis.

He earned a master's degree (1973) and a Ph.D. (1978) in biology from the Katholieke Universiteit Leuven, Belgium.

Fidel Toldrá earned a bachelor's degree in chemistry in 1980, a high degree in food technology in 1981, and a Ph.D. in chemistry in 1984. He is research professor and head of the Laboratory of Meat Science at the Instituto de Agroquímica y Tecnología de Alimentos (CSIC), Valencia, Spain. He is also associate professor of food technology at the Polytechnical University of Valencia.

Professor Toldrá has received several awards such as the 2002 International Prize for Meat Science and Technology. He has authored and coauthored many book chapters, research articles, and patents. He has authored one book and coedited nine others. Professor Toldrá is the editor of the journal *Trends in Food Science and Technology,* editor-in-chief of the new journal *Current Nutrition & Food Science,* and a member of the editorial boards of *Meat Science, Food Chemistry,* and *Journal of Muscle Foods*.

His research interests are based on food chemistry and biochemistry, with a special focus on muscle foods. He serves on the Executive Committee of the European Federation of Food Science and Technology and the Scientific Commission on Food Additives of the European Food Safety Authority.

Contributors

D. U. Ahn
Animal Science Department
Iowa State University
Ames, Iowa

Keizo Arihara
Department of Animal Science
Kitasato University
Towada-shi, Japan

Teresa Aymerich
Meat Technology Center
Institute for Food Research and Technology
Monells, Spain

José Manuel Barat
Food Science and Technology Department
Polytechnical University of Valencia
Valencia, Spain

Brian C. Bowker
Food Technology and Safety Laboratory
USDA-ARS
Beltsville, Maryland

Amparo Chiralt
Food Science and Technology Department
Polytechnical University of Valencia
Valencia, Spain

Véronique Coma
Centre de Recherche en Chimie Moléculaire
Université Bordeaux
Bordeaux, France

Eric Dufour
Département Qualité & Economie Alimentaires
ENITA Clermont Ferrand
Lempdes, France

Janet S. Eastridge
Food Technology and Safety Laboratory
USDA-ARS
Beltsville, Maryland

Pedro Fito
Food Science and Technology Department
Polytechnical University of Valencia
Valencia, Spain

Margarita Garriga
Meat Technology Center
Institute for Food Research and Technology
Monells, Spain

Raul Grau
Food Science and Technology Department
Polytechnical University of Valencia
Valencia, Spain

Kjell Ivar Hildrum
Norwegian Food Research Institute
Matforsk, Norway

Terry A. Houser
Department of Animal Science
University of Florida
Gainesville, Florida

Yoshihide Ikeuchi
Department of Bioscience and Biotechnology
Kyushu University
Fukuoka, Japan

Francisco Jiménez-Colmenero
Instituto del Frío (CSIC)
Ciudad Universitaria
Madrid, Spain

Anna Jofré
Meat Technology Center
Institute for Food Research and Technology
Monells, Spain

Ken Kim
Department of Applied Biological Chemistry
Niigata University
Niigata, Japan

E. J. Lee
Animal Science Department
Iowa State University
Ames, Iowa

Mark Loeffen
Mark Loeffen & Associates Ltd.
Hamilton, New Zealand

Sabine Leroy
SRV-UR Microbiologie INRA Theix
Champanelle, France

Martha N. Liu
Food Technology and Safety Laboratory
USDA-ARS
Beltsville, Maryland

Belén Martín
Meat Technology Center
Institute for Food Research and Technology
Monells, Spain

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

Joseph M. Monfort
Meat Technology Center
Institute for Food Research and Technology
Monells, Spain

Tadayuki Nishiumi
Department of Applied Biological Chemistry
Niigata University
Niigata, Japan

Ernest W. Paroczay
Food Technology and Safety Laboratory
USDA-ARS
Beltsville, Maryland

Jitu R. Patel
Food Technology and Safety Laboratory
USDA-ARS
Beltsville, Maryland

Ronald B. Pegg
Department of Applied Microbiology and Food Science
University of Saskatchewan
Saskatoon, SK, Canada

Graham Purnell
Food Refrigeration and Process Engineering Research Centre
University of Bristol
Somerset, UK

Peter Rådström
Applied Microbiology, Lund Institute of Technology
Lund University
Lund, Sweden

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

Jean-Pierre Renou
STIM INRA Theix
Champanelle, France

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

Vegard H. Segtnan
Norwegian Food Research Institute
Matforsk, Norway

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

Manan Sharma
Food Technology and Safety Laboratory
USDA-ARS
Beltsville, Maryland

Morse B. Solomon
Food Technology and Safety Laboratory
USDA-ARS
Beltsville, Maryland

Atsushi Suzuki
Department of Applied Biological Chemistry
Niigata University
Niigata, Japan

Régine Talon
SRV-UR Microbiologie INRA Theix
Champanelle, France

Hiroyuki Tanji
Department of Applied Biological Chemistry
Niigata University
Niigata, Japan

Declan J. Troy
The National Food Centre
Dublin, Republic of Ireland

John L. Williams
Division of Genetics and Genomics
Roslin Institute
Edinburgh, Scotland

Jens Petter Wold
Norwegian Food Research Institute
Matforsk, Norway

Petra Wolffs
Applied Microbiology, Lund Institute of Technology
Lund University
Lund, Sweden

Contents

Chapter 1
Bioengineering of Farm Animals: Meat Quality and Safety 1
Morse B. Solomon, Janet S. Eastridge, and Ernest W. Paroczay

Chapter 2
Gene Technology for Meat Quality 21
John L. Williams

Chapter 3
Automation for the Modern Slaughterhouse 43
Graham Purnell and Mark Loeffen

Chapter 4
Hot-Boning of Meat: A New Perspective 73
Declan J. Troy

Chapter 5
New Spectroscopic Techniques for Online Monitoring of Meat Quality 87
Kjell Ivar Hildrum, Jens Petter Wold, Vegard H. Segtnan, Jean-Pierre Renou, and Eric Dufour

Chapter 6
Real-Time PCR for the Detection of Pathogens in Meat 131
Petra Wolffs and Peter Rådström

Chapter 7
Meat Decontamination by Irradiation 155
D. U. Ahn, E. J. Lee, and A. Mendonca

Chapter 8
Application of High Hydrostatic Pressure to Meat and Meat Processing 193
Atsushi Suzuki, Ken Kim, Hiroyuki Tanji, Tadayuki Nishiumi, and Yoshihide Ikeuchi

Chapter 9
Hydrodynamic Pressure Processing to Improve Meat Quality and Safety 219
Morse B. Solomon, Martha N. Liu, Jitu R. Patel, Brian C. Bowker, and Manan Sharma

Chapter 10
Functional Properties of Bioactive Peptides Derived From Meat Proteins 245
Keizo Arihara

Chapter 11
New Approaches for the Development of Functional Meat Products..... 275
Francisco Jiménez-Colmenero, Milagro Reig, and Fidel Toldrá

Chapter 12
Processing of Nitrite-Free Cured Meats 309
Ronald B. Pegg and Fereidoon Shahidi

Chapter 13
Biochemical Proteolysis Basis for Improved Processing
of Dry-Cured Meats..... 329
Fidel Toldrá

Chapter 14
Vacuum Salting Treatment for the Accelerated Processing
of Dry-Cured Ham..... 353
José M. Barat, Raul Grau, Pedro Fito, and Amparo Chiralt

Chapter 15
The Use of Bacteriocins Against Meat-Borne Pathogens 371
Teresa Aymerich, Margarita Garriga, Anna Jofré, Belén Martín,
and Joseph M. Monfort

Chapter 16
Latest Developments in Meat Bacterial Starters..... 401
Régine Talon and Sabine Leroy

Chapter 17
Modified Atmosphere Packaging 419
Joseph G. Sebranek and Terry A. Houser

Chapter 18
Perspectives for the Active Packaging of Meat Products..... 449
Véronique Coma

Index 473

1 Bioengineering of Farm Animals: Meat Quality and Safety

Morse B. Solomon, Janet S. Eastridge, and Ernest W. Paroczay
Food Technology and Safety Laboratory, USDA*

CONTENTS

1.1 Bovine 3
1.2 Ovine 5
1.3 Caprine 8
1.4 Porcine 8
1.5 Food Safety Implications 13
References 14

A tremendous amount of variation in muscle and meat characteristics exists among and within breeds and species. Conventional science to improve muscle and meat parameters has involved breeding strategies, such as selection of dominant traits or selection of preferred traits by crossbreeding, and the use of endogenous and exogenous growth hormones. Improvements in the quality of food products that enter the market have largely been the result of postharvest intervention strategies. Biotechnology is a more extreme scientific method that offers the potential to improve the quality, yield, and safety of animal products by direct genetic manipulation of livestock. In essence, biotechnology is a new approach to the methods of genetic selection, crossbreeding, or administration of growth hormones in its final result. However, progress in this area is very slow and has a long way to go before having an impact at a commercial usage level.

Biotechnology in animals is primarily achieved by cloning, transgenesis, or transgenesis followed by cloning. Animal cloning is a method used to produce genetically identical copies of a selected animal (i.e., one that possesses high breeding value),

* Mention of brand or firm names does not constitute an endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

and transgenesis is the process of altering an animal's genome by introducing a new, foreign gene (i.e., DNA) not found in the recipient species, or deleting or modifying an endogenous gene with the ultimate goal of producing an animal expressing a beneficial function or superior attribute (e.g., adding a gene that promotes increased muscle growth). A combination of the two methods, transgenic cloning, is the process of producing a clone with donor cells that contain heritable DNA inserted by a molecular biology technique, as used in a transgenic event. A pioneering report by Palmiter et al. (1982) on the accelerated growth of transgenic mice that developed from eggs microinjected with a growth hormone fusion gene started the revolution in biotechnology of animals. Based on this research, many novel uses for biotechnology in animals were envisioned, beginning with enhancement of production-related traits (yield and composition) and expanding into disease resistance strategies and production of biological products (i.e., pharmaceuticals).

Early methods of cloning involved a technology called embryo splitting, but the traits of the resulting clones were unpredictable. Today's method of cloning, somatic (adult) cell nuclear transfer, became established in 1997 with the production of the world's first cloned farm animal, Dolly the sheep (Wilmut, Schnieke, McWhir, Kind, and Campbell 1997), and has since been used for cattle, goats, mice, and pigs. Cloning could be a promising method of restoring endangered or near-extinct species and populations. Production of transgenic animals is carried out by a technique called pronuclear microinjection, reported first in mice (Gordon, Scangos, Plotkin, Barbosa, and Ruddle 1980), and later adapted to rabbits, sheep, and pigs (Hammer et al. 1985). An excellent review on genome modification techniques and applications was published by Wells (2000).

Before 1980, applications for patents on living organisms were denied by the U.S. Patent and Trademark Office (USPTO) because anything found in nature was considered nonpatentable subject matter. However, U.S. scientist Ananda Chakrabarty, who wanted to obtain a patent for a genetically engineered bacterium that consumes oil spills, challenged the USPTO in a case that landed in the U.S. Supreme Court, which in 1980 ruled that patents could be awarded on anything that was human-made. Since then, some 436 transgenic or bioengineered animals have been patented, including 362 mice, 26 rats, 19 rabbits, 17 sheep, 24 pigs, 20 cows, 2 chickens, and 3 dogs (Kittredge 2005). Due to steps specific to transgenic procedures, for instance the DNA construct, its insertion site, and the subsequent expression of the gene construct, animals derived from transgenesis have more potential risks than cloned animals. Based on a National Academy of Sciences (NAS), National Research Council (NRC) report (2002), "Animal Biotechnology: Science-Based Concerns," the U.S. Food and Drug Administration (FDA 2003) announced that meat or dairy products from cloned animals are likely to be safe to eat, but to date has not yet approved these products for human consumption. The NAS report recommended a rigorous and comprehensive evaluation on two key issues: 1) collecting additional information about food composition to be sure that these food products are not different from normal animals, and 2) an evaluation of health status indicators of genetically engineered animals and their progeny. Even if FDA regulatory approval is granted, consumer perceptions of genetically engineered animals as food products would need to be addressed. There is a popular belief that alterations to the normal

genetic makeup triggers the creation of harmful new compounds, or that food products derived from genetically altered animals created in a laboratory are considerably less wholesome and more risky to eat compared to a normal animal raised on a farm. On the other hand, the use of biotechnology in animals to treat infectious diseases or produce new vaccines may be widely accepted. In any event, bioengineered animal products won't be on the market in the foreseeable future: High costs ($20,000–$200,000 each), extremely low efficiency rate (< 1% for livestock, < 4% for mice), and the several-year investment of time needed to generate these animals and progeny need to be overcome. The low efficiency of the process can be attributed to three factors: embryo survival, gene integration rate, and gene expression. The majority of original genetic engineering research reports focus on developing faster growing animals.

In the U.S., bioengineered foods are regulated by three agencies: the U.S. Department of Agriculture (USDA), FDA and Environmental Protection Agency (EPA). The USDA has oversight for meat and poultry, whereas seafood regulation falls under the FDA. The FDA Center for Veterinary Medicine (CVM) also regulates transgenic animals because any drug or biological material created through transgenesis is considered a drug and has to undergo the same scrutiny to demonstrate safety and effectiveness (Lewis 2001). The EPA has responsibility for pesticides that are genetically engineered into plants. In the mid-1980s, federal policy declared that biotechnologically derived products would be evaluated under the same laws and regulatory authorities used to review comparable products produced without biotechnology. As stated on the FDA Web site, the CVM has asked companies not to introduce animal clones, their progeny, or their food products into the human or animal food supply until there is sufficient scientific information available on the direct evaluation of safety.

1.1 BOVINE

Information in this area is very limited and highly desired by federal agencies that regulate food safety issues. There have been some studies evaluating the meat of animals cloned from embryonic cells (Gerken, Tatum, Morgan, and Smith 1995; Harris et al. 1997; Diles et al. 1999). Those results, however, do not correspond with products from animals cloned from adult somatic cells. This is because embryonic animal clones are produced from blastomeres of fertilized embryos at a very early stage of development, and thus embryonic clones may undergo little gene reprogramming during their development. Consequently, they would not serve well as scientific evidence for assessing the food safety risks of somatic cloned food animals. A few reports that provide data on the composition of meat and dairy products derived from adult somatic cell clones indicate that these products are equivalent to those of normal animals. The first report on the chemical composition of bovine meat arising from genetic engineering was in cloned cattle (Takahashi and Ito 2004). In meat samples derived from cloned and noncloned Japanese Black cattle at the age of 27 to 28 months, data were collected for proximate analysis (water, protein, lipids, and ash) as well as fatty acids, amino acids, and cholesterol. The results of this study showed that the nutritional properties of meat from cloned cattle are similar to those of noncloned animals, and were within recommended values of

Japanese Dietetic Information guidelines. Also, based on the marbling score, the meat quality score of the cloned cattle in this study graded high (Class 4) according to the Japanese Meat Grading Standard (ranging from Class 1 [poor] to Class 5 [premium]). No other carcass characteristics were discussed in this report.

A comprehensive study designed specifically to provide scientific data desired by U.S. regulatory agencies on the safety issue of the composition of meat and milk from animal cloning was recently published (Tian et al. 2005). All animals were subjected to the same diet and management protocols. The study analyzed more than 100 parameters that compared the composition of meat and milk from beef and dairy cattle derived from cloning to those of genetic- and breed-matched control animals from conventional reproduction. The beef cattle in this study were slaughtered at 26 months of age and also examined for meat quality and carcass composition. A cross-section between the sixth and seventh rib of the left side dressed carcass was inspected according to Japan Meat Grading Association guidelines. Additional parameters of the carcass analyzed were organ or body part weights, and total proportion of muscle and fat tissue to carcass weight. The histopathology of seven organs was examined for appearance of abnormalities. Six muscles (*Infraspinatus, Longissimus thoracis, Latissimus dorsi, Adductor, Biceps femoris*, and *Semitendinosus*) were removed from the carcass and measured for percentages of moisture, crude protein, and crude fat. Sampling from these muscles for muscle fiber type profiling, however, was not performed. The fatty acid profile of five major fat tissues (s.c. fat, intra- and intermuscular fats, celom fat, and kidney leaf fat) and the amino acid composition of the *Longissimus thoracis* muscle were also determined. Out of the more than 100 parameters examined, a significant difference was observed in 12 parameters for the paired comparisons (clone vs. genetic comparator and clone vs. breed comparator). Among these 12 parameters, 8 were related to the amount of fat or fatty acids in the meat or fat. The other four parameters found different between clones and comparators were yield score, the proportion of *Longissimus thoracis* muscle to body weight, the muscle moisture, and the amount of crude protein in the *Semitendinosus* muscle, and all fell within the normal range of industry standards. Therefore, none of these parameters would be cause for concern to product safety.

The mechanisms of regulation of muscle development, differentiation, and growth are numerous and complex. Meeting the challenge of optimizing the efficiency of muscle growth and meat quality requires a thorough understanding of these processes in the different meat-producing species. Application of biotechnology for livestock and meat production potentially will improve the economics of production, reduce environmental impact of production, improve pathogen resistance, improve meat quality and nutritional content, and allow production of novel products for the food, agricultural, and biomedical industries.

In a recent article, Wall et al. (2005) reported on the success of genetically enhanced cows with lysostaphin to resist intramammary *Staphylococcus aureus* (mastitis) infection. Mastitis is the most consequential disease in dairy cattle and costs the U.S. dairy industry billions of dollars annually. Their findings indicated that genetic engineering of animals can provide a viable tool for enhancing resistance to disease, thus improving the well-being of livestock.

1.2 OVINE

Although the first mammalian species to be cloned using a differentiated cell (Wilmut et al. 1997) was ovine, continued development of cloning technology in this species has been in support of conserving endangered species (Loi et al. 2001; Ryder 2002). About 5% to 10% of cloned sheep embryos result in offspring, but not all are healthy. Several groups have attempted transgenic introduction of growth hormone genes in sheep, but none have resulted in commercially useful transgenic animals. Growth-promoting transgenes in sheep was first accomplished by Hammer et al. (1985), followed by Rexroad et al. (1989, 1991), where gene constructs inserted into the sheep produced a 10 to 20 times elevation of plasma growth hormone level. Growth rates were similar to control sheep early in life, but after 15 to 17 weeks of life, the overexpression of growth hormone was cited by Ward et al. (1989) and Rexroad et al. (1989) to be responsible for reduced growth rate and shortened life span. Ward et al. (1990) summarized their studies with transgenic sheep, noting reduced carcass fat, elevated metabolic rate and heat production, skeletal abnormalities, and impaired survival due to the unregulated production of growth hormone in the transgenic sheep unless an all-ovine construct was used.

The pattern of expression of the various growth hormone (GH) and growth-hormone releasing factor (GRF) transgenes in sheep could not be predicted (Murray and Rexroad 1991), as circulating levels of growth hormone and IGF-I levels did not correlate to expression of the transgenes. Transgenic sheep that were nonexpressing had transgenic progeny that also failed to express the transgene (Murray and Rexroad 1991). Transgenic lambs that expressed either GH or GRF had growth rates similar to nontransgenic controls even though the transgenic lambs had elevated plasma levels of IGF-I and insulin. Early literature on transgenic sheep expressing GH indicated similar growth rates and feed efficiency (Rexroad et al. 1989) as nontransgenic controls; however, all transgenic sheep displayed pathologies and shortened life span. Further, transgenic sheep expressing GH were noted to have significantly reduced amounts of body and perirenal fat (Ward et al. 1990; Nancarrow et al. 1991) and were also susceptible to developing chronically elevated glucose and insulin levels of diabetic conditions.

Progress in overcoming the health problems of GH transgenic sheep was made by switching to an ovine GH gene with ovine metallothionein promoter (Ward and Brown 1998). They encountered no health problems through, at least, the first four years of life, although Ward and Brown (1998) noted increased organ sizes and noticeably reduced carcass fat in the G1 generation. Twenty transgenic lambs of the G2 generation (Ward and Brown 1998) grew significantly faster than controls, with differences detected between rams and ewes. Growth rate of transgenic rams was greater than controls from birth onward, whereas increased growth rate in transgenic ewes was not noted until four months of age. No difference in feed conversion from four to seven months of age was observed between control and transgenic lambs (Ward and Brown 1998). In the G3 generation, Brown and Ward (2000) reported the average difference in body weight between transgenic and controls at 12 months of age was 8% and 19% heavier for rams and ewes, respectively. Their results were

consistent with the increased circulating levels of GH in transgenics compared to controls.

Piper, Bell, Ward, and Brown (2001) evaluated the effects of an ovine GH transgene on lamb growth and wool production performance using 62 transgenic Merino sheep. The G4 transgenic lambs were from a single transgenic founder ram and were compared to 46 sibling controls. Preweaning body weights were similar for transgenic and controls, but began to diverge and were significantly different from seven months of age onward. Transgenic lambs were about 15% larger than controls at 12 months of age and had very low amounts of subcutaneous fat. Major wool production traits, greasy fleece weight and mean fiber diameter, were not different from controls.

Adams, Briegel, and Ward (2002) also examined the effects of a transgene encoding ovine GH and an ovine metallothionein promoter in progeny of 69 Merino and 49 Poll Dorset lambs from ewes inseminated by G4 transgenic rams heterozygous for the gene construct. As seen in earlier research using mouse-derived GH transgenes, the effects of the ovine construct varied according to active expression of the transgene. The transgene failed to be expressed in some progeny (Adams et al. 2002) despite positive status for the transgene. The ovine GH produced negligible health problems, similar to that reported by Ward and Brown (1998). Among progeny with active transgene expression, plasma GH levels were twice those of controls. Those sheep also grew faster to heavier weights and were leaner, but had higher parasite fecal egg counts compared to nontransgenic sheep. Females at 18 months of age had decreased *Longissimus* muscle depth compared to males. Adams et al. (2002) concluded that phenotypic effects of genetic manipulation of sheep may depend on age, breed, and sex of the animal and that modification to the fusion genes is required to meet the species-specific requirements to enhance expression in transgenic sheep while maintaining the long-term health status.

Callipyge sheep have muscle fiber hypertrophy determined by a paternally inherited polar overdominance allele (Cockett et al. 1994) that is a result of a single base change (Freking et al. 2002; Freking, Smith, and Leymaster 2004). This naturally occurring mutation that alters muscle phenotype in sheep was described by Jackson and Greene (1993) and Cockett et al. (1994), and since has been the subject of much research. The callipyge phenotype is a posttranslational effect (Charlier et al. 2001) in which the dam's normal allele suppresses synthesis of at least four proteins that form muscle tissue. The phenotype is characterized by hypertrophy in certain muscles (viz., *Longissimus thoracis et lumborum* [LTL], *Gluteus medius, Semimembranosus, Semitendinosus, Adductor, Quadriceps femoris, Biceps femoris* [BF] and *Triceps brachii*), whereas other muscles (*Infraspinatus* [IS] and *Supraspinatus* [SS]), are unaffected. The hypertrophy is caused by increased size of the fast-twitch fibers rather than increased fiber numbers (Carpenter, Rice, Cockett, and Snowder 1996). Lorenzen et al. (1997) measured an elevated protein to DNA ratio in callipyge LTL and BF but not in IS and SS. Fractional protein accretion rate did not differ among those muscles, and protein synthesis rate was decreased by 22% in callipyge LTL and by 16% in callipyge BF muscles. Because the protein degradation rate was also decreased by 35% in callipyge compared to controls, Lorenzen et al. (1997) concluded that callipyge-induced muscle hypertrophy was due to decreased muscle protein degradation.

Reduced tenderness in callipyge was also related to higher calpastatin (Goodson, Miller, and Savell 2001; Freking et al. 1999; Koohmaraie, Shackelford, Wheeler, Lonergan, and Doumit 1995) and m-calpain activities (Koohmaraie et al. 1995) compared to control sheep. Otani et al. (2004) presented evidence in mice that overexpression of calpastatin contributes to muscle hypertrophy, although this has not been investigated in relation to the callipyge phenotype.

Busboom et al. (1994) indicated that callipyge lambs had less monounsaturated and more polyunsaturated fatty acids than controls. Muscle hypertrophy in callipyge sheep was also at the expense of adipose tissue (Rule, Moss, Snowder, and Cockett 2002), possibly from a decrease in differentiation of adipocytes. Rule et al. (2002) measured lower lipogenic enzyme activities in adipose tissues of heterozygous callipyge lambs compared to controls but were unable to relate these differences to insulin or IGF-I levels. The callipyge locus has been mapped to a chromosome segment that carries four genes that are preferentially expressed in skeletal muscle and are subject to parental imprinting, namely, Delta-like 1 (DLK1), gene-trap locus 2 (GTL2), paternal expressed gene 11 (PEG11), and maternal expressed gene 8 (MEG8). The same conserved order was found on human and mouse chromosomes. The causative mutation for callipyge is a single base transition from A to G in the intergene region between DLK1 and GLT2 (Bidwell et al. 2004). Charlier et al. (2001) demonstrated the unique very abundant expression of DLK1 (involved in adipogenesis) and PEG11 (unknown function) in callipyge sheep; however, they were not able to explain how the overexpression of these genes was related to muscle hypertrophy. They suggested that the callipyge mutation does not alter the imprinting of DLK1 or PEG11, but modifies the activity of a common regulatory element that could be an enhancer or silencer. Bidwell et al. (2004) similarly detected elevated DLK1 and PEG11 in muscles of lambs with the callipyge allele and named them as candidate genes responsible for the skeletal muscle hypertrophy. PEG11 was 200 times higher in heterozygous and 13 times higher in homozygous callipyge sheep than in controls. Freking et al. (2004) discussed expression profiles and imprint status of genes near the mutated region of the callipyge locus. Markers for polymorphic genes that control fat and lean, such as thyroglobulin, or the callipyge gene, could be used for making genetic selection improvements in animals (Sillence 2004).

The apparent advantages of higher carcass yield, increased lean, and reduced fat content of callipyge sheep would benefit the meat industry except for the associated toughness in the hypertrophied muscles. In contrast to minimal tenderness improvement using antemortem techniques to control growth rate, size, or fatness level (Duckett, Snowder, and Cockett 2000) or treatment with dietary vitamin D_3 (Wiegand, Parrish, Morrical, and Huff-Lonergan 2001), some success at improving tenderness of meat from callipyge has been accomplished by various postmortem treatments. Tenderness was improved slightly by electrical stimulation (Kerth, Cain, Jackson, Ramsey, and Miller 1999). Other postmortem treatments effective for improving tenderness in callipyge include prerigor freezing prior to aging (Duckett, Klein, Dodson, and Snowder 1998), calcium chloride injection (Koohmaraie, Shackelford, and Wheeler 1998), hydrodynamic pressure treatment (Solomon 1999), and extended aging to 48 days (Kuber et al. 2003). The higher calpastatin level responsible for the hypertrophy of callipyge lambs (Freking et al. 1999; Goodson et al. 2001;

Koohmaraie et al. 1995) is often cited as contributing to the lower tenderness of the meat because calpastatin interferes with the normal postmortem proteolysis during aging, particularly the breakdown of troponin-T (Wiegand et al. 2001). The lack of tenderness associated with the callipyge gene must be addressed before the economic advantages can be realized.

1.3 CAPRINE

Prior to the first transgenic goat, Fehilly, Willadsen, and Tucker (1984) produced an interspecies chimera between sheep and goat, the geep. Today, cloning (Behboodi et al. 2004) and embryo splitting (Oppenheim, Moyer, Bondurant, Rowe, and Anderson 2000) are employed as the most rapid means of highly focused initial expansion of a transgenic herd. This approach combines the two techniques by first creating the transgenic goat with the desired traits. Cloning is then used to create replicas of the transgenic animal. Goats have cloning efficiency of 3% to 7%. The benefits of cloned and transgenic goats are accelerated genetic improvements in production of hair, meat, and milk; however, the production of products in goat milk for the pharmaceutical industry is the most widely used application of this technology.

Goats, rabbits, and flies are often employed for recombinant protein production because mice do not efficiently scale up, transgenic cattle take too long to prepare, plants produce pollen that drifts in the wind, and chickens have problems with long-term stability of germ-line expression as well as carrying viruses and new strains of flu (Anonymous 2004). Goats, then, are the animal of choice for biomedical and industrial bioreactors for the production of protein therapeutics for the health care and agro-biotech industries (Baldassarre, Wang, Keefer, Lazaris, and Karatzas 2004; Goldman, Kadulin, and Razin 2002; Ko et al. 2000; Nicholls 2004; Tulsi 2004). Transgenic goats require much less capital investment, are more efficient than manufacturing systems using cell culture (Tulsi 2004), and are easier to scale up production. Published literature lacks information regarding the amount of hair, milk, or meat produced using transgenic goats. The products produced through transgenic goats primarily are pharmaceutical and are regulated by the FDA.

1.4 PORCINE

Among major livestock species, the pig was last to be cloned (Betthauser et al. 2000; Onishi et al. 2000; Polejaeva et al. 2000). There appears to be more interest in transgenesis and cloning of pigs as a model for studying human diseases, such as osteoporosis and diabetes, and for donor organs for xenotransplantation rather than for improving meat production. Pigs, due to their vast numbers and similar organ size and function to humans, are desirable for xenotransplantation. Hyperacute rejection of xenotransplanted organs was a major concern until Prather, Hawley, Carter, Lai, and Greenstein (2003) accomplished genetic modification of the $\alpha(1,3)$-galactosyltransferase gene prior to nuclear transfer cloning. Nuclear transfer cloning efficiency rates for swine average between 1% and 6% of embryos. This and other issues need to be solved with this technology. Cloned pigs appear to have inadequate

immune systems (Carroll, Carter, Korte, Dowd, and Prather 2004), display behavioral variations (Archer, Friend, Piedrahita, Nevill, and Walker 2003), and could transmit viruses (van der Laan et al. 2000). In contrast, Carter et al. (2002) used green fluorescent protein transgene then cloned pigs to evaluate phenotype and health status. They declared that cloned pigs can be normal and without impaired immune systems.

Approximately 40% of the red meat consumed worldwide comes from pigs (Food and Agriculture Organization of the United Nations 2004), and pork consumption has increased consistently with increasing world population. Continued improvements in pork production, therefore, are needed to meet future demands for red meat. Research in genomics is one avenue to increase production efficiency. Selection of pigs based on the ryanodyne receptor (RyR) gene, muscle regulatory factor (MRF) gene family, hormones, or other potential candidate genes affecting growth and fattening traits is needed to increase production. Quantitative trait loci (QTL) evaluation of factors associated with meat quality and growth are underway; however, in pigs, some quality traits are polygenic (Krzecio, Kocwin-Podsiada, et al. 2004), requiring evaluation of their interactions.

QTL analysis of factors affecting tenderness and juiciness of pork were mapped to chromosome 2, and based on that location, the calpastatin (CAST) gene was considered a likely candidate (Ciobanu et al. 2004). One of three CAST haplotypes identified using a restriction enzyme (viz., Ras1) was found to be associated with the investigated traits and might serve as a marker for selection and breeding. Meat quality traits in pigs negative for the halothane sensitivity ryanodyne receptor (RyR1) and RN- alleles were evaluated for interactions with CAST (Krzecio, Kury, Kocwin-Podsiada, and Monin 2004). For stress-resistant RyR1 pigs, CAST polymorphisms using Rsa1 restriction enzyme (CAST/Rsa1) were identified as AA, AB, and BB genotypes. These were found to affect water holding capacity (WHC), drip loss, and water and protein content of muscle. CAST/Rsa1 AA genotype pigs had lower WHC, lower drip loss at 96 hours, less moisture, and higher protein content in muscle compared to the BB genotype. Stress-resistant pigs (homozygous and heterozygous RyR1 resistant genotype) had highly significant lactate level measured by pH at 35 and 45 minutes postmortem and on reflectance values. Homozygous stress-resistant pigs produced the most desirable quality traits. The interaction of CAST/Rsa1 and RyR1 was significant for *Longissimus lumborum* muscle pH at 45 minutes postmortem and drip loss at 48 hours; however, no interactions were detected for carcass lean (Krzecio, Kocwin-Podsiada, et al. 2004; Krzecio, Kury, et al. 2004) or cooking yield. That CAST and RyR1 would interact is not surprising because calpastatin is an endogenous inhibitor of calcium-dependent cysteine proteases, the calpains, and a mutation in RyR1 is partly responsible for disturbed regulation of intracellular Ca^{2+} in pig skeletal muscle (Kuryl, Krzecio, Kocwin-Podsiadla and Monin 2004). These studies indicate that quality of meat should be considered not only by each individual genotype, but also by interactions with other genes.

Polymorphisms of the CAST gene and their association between genotypes at the porcine locus myostatin (MSTN) growth differentiation factor 8 were considered by Klosowska et al. (2005). Mutations in the MSTN gene are responsible for extreme muscle hypertrophy, or double muscling, in several breeds of cattle. Myostatin is important for controlling development of muscle fibers and is considered a negative

regulator of muscle growth (McPherron, Lawler, and Lee 1997). Because calpain activity is required for myoblast fusion and cell proliferation and growth, it might also affect the number of skeletal muscle fibers. The fusion of myoblasts to form fibers is accompanied by a dramatic change in the calpain/calpastatin ratio. Overexpression of calpastatin, an endogenous calpain inhibitor, in transgenic mice resulted in substantially increased muscle tissue (Otani et al. 2004). Klosowska et al. (2005) analyzed the interaction of MSTN and CAST in Piétrain × (Polish Large White × Polish Landrace) crossbred pigs and the Stamboek line of Dutch Large White × Dutch Landrace pigs. The MSTN genotypes identified using the Taq1 restriction enzyme were CC or CT, and CAST/Rsa1 genotypes were identified as EE, EF, or FF. Klosowska et al. (2005) reported that 79.5% of the Stamboek line was characterized as MSTN/Taq1 CC genotype. Interestingly, the FF genotype of CAST/Rsa1 was not detected in the Piétrain crossbred pigs. Muscle fiber size and type distributions were not affected by the MSTN genotypes although there were breed differences. Piétrain crosses had larger mean fiber diameters in all fiber types compared to Stamboek pigs. Proportion of fiber types in a bundle was higher for slow-twitch oxidative (SO) and lower for fast-twitch glycolytic (FG) fibers in Piétrain crossbred pigs compared to Stamboek pigs. Of multiple deletions or substitutions identified for MSTN, only one results in muscle hypertrophy seen in double muscle cattle and in mice. The C to T replacement in the MSTN gene does not result in an amino acid substitution (Stratil and Kopecny 1999), thus, it is probable that this genotype has no effect on the myostatin function in pigs. Muscle fiber diameters and number of fibers per unit area were not different for CAST genotypes in Piétrain cross pigs, whereas the CAST genotype had an effect in the Stamboek line. In all fiber types, fiber diameters were larger in the CAST EE and EF genotypes and smallest in FF. Loin eye area of EE genotype also was significantly larger than for EF or FF genotypes. Because of the missing FF genotype in Piétrain cross pigs, the interaction of CAST and MSTN could not be assessed.

The peroxisome proliferator-activated receptor-gamma coactivator-1 (PPARGC1 or PGC-1α) gene was investigated by Kunej et al. (2005) as a potential candidate gene affecting fattening traits and pork meat quality. This gene has a single nucleotide substitution at position 1378 within the central region of PGC-1α on chromosome 8 and occurs predominantly in Western pig breeds, whereas the conserved gene occurred in 92.6% (± 4.8%) in Chinese pig breeds. These findings were associated with marked differences in fat and lean tissue depositions in Western and Chinese pig breeds. Bayesian analysis indicated that these two groups of pigs had diverged at this locus during genetic evolution of breeds. PGC-1α is a transcriptional coactivator of many nuclear hormone receptors involved in lipid metabolism and adipocyte differentiation. In humans, PGC-1α is associated with abdominal and subcutaneous fat, and PGC-1α is expressed in skeletal muscle to a greater extent in lean than in obese individuals. It can be increased in skeletal muscle by calorie restriction. Insulin-sensitive glucose transporter (GLUT4; also called SLC2A4) also is regulated by PGC-1α and was investigated as a candidate gene for meat quality traits by Grindflek, Holzbauer, Plastow, and Rothschild (2002). GLUT4 is located on porcine chromosome 12 and plays a role in muscle and adipose tissue glucose metabolism and has unique muscle and fat expression.

In transgenic mice overexpressing calpastatin, fat content was greatly reduced and GLUT4 concentration was elevated more than three times (Otani et al. 2004). Otani et al. (2004) suggested that because calpain can degrade GLUT4, inhibition of calpain also diminished GLUT4 degradation, resulting in increased muscle growth. Grindflek et al. (2002) utilized approximately 1,700 pigs from U.S. and Norwegian commercial pig lines to determine any association of GLUT4 to meat quality. Significant associations were found for GLUT4 and drip loss, marbling, and loin depth in some U.S. lines, although association of GLUT4 polymorphisms to quality traits were not consistent across lines. No significant associations were detected for any meat quality traits in the Norwegian pig population. Among reasons given for the weak associations, Grindflek et al. (2002) suggested that linkage disequilibria or interactions with other genes might cause interference.

The transgenic Enviro™ pig was created (Forsberg 2002) to be better able to digest cereal grains by utilizing the enzyme phytase. Transgenic pigs producing phytase in their saliva (Golovan et al. 2001) were able to digest 90% to 100% of the phosphorus in their diets compared to 50% in control pigs. This transgenesis would eliminate the need to supplement pig diets with phosphorus and would reduce the amount of phosphorus in their manure by about 60%. This translates to greatly reduced phosphorus concentration in manure, which would have a positive environmental impact. Phytase can be added to pig feed, but ultimately, the transgenic pig could be more cost-effective, according to Forsberg (2002). In anticipation of marketing meat from the Enviro pig, the Medical and Related Links to Agricultural Network for Development and Innovation with Guelph (MaRS LANDING) consortium in Guelph, Canada had performed extensive analysis of the meat and found it to be indistinguishable from ordinary pork (Dove 2005). Similar efforts to improve the digestibility of feeds, and hence, feed efficiency, are underway in poultry and aquaculture. Dietary cellulose and xylan digestion in poultry is by microbial fermentation in the hind gut, a relatively inefficient process. Transgenesis to express bacterial cellulase enzymes in poultry and aquaculture species could improve digestion of plant polysaccharides, increasing feed efficiency similar to that demonstrated in the mouse (Hall et al. 1993).

Transgenic pigs expressing a plant gene, spinach desaturase, for the synthesis of essential polyunsaturated fatty acids (PUFA), linoleic and linolenic acids, have been produced (Saeki et al. 2004), marking the first time that a plant gene has been functionally expressed in mammalian tissue. This transgenesis could result in significant improvement in pork quality beneficial to human health. Saeki et al. (2004) detected levels of linoleic acid in adipocytes about 10 times higher in transgenic than in control pigs. Niemann (2004) suggested that modifying the fatty acid composition of products from domestic animals might make this technology more appealing to the public. High levels of dietary PUFA were shown to improve processing and increase PUFA in pork muscle. Earlier work with transgenic pigs and with injected porcine somatotropin also led to reduced levels of saturated fatty acids in pork (Pursel and Solomon 1993; Solomon, Pursel, and Mitchell 2002).

Many reports have documented the effects on growth of pigs receiving additional GH by exogenous administration or endogenously through transgenesis (Pursel et al. 1988; Pursel and Rexroad 1993; Pursel et al. 1997; Solomon, Pursel, Paroczay, and Bolt 1994; Vize et al. 1998; Wieghart et al. 1988). Transgenic pigs expressing

IGF-I, a regulator of growth hormone, have been described in detail (Mitchell and Pursel 2003; Pursel et al. 2004; Pursel, Mitchell, Wall, Coleman, and Schwartz 2001; Pursel, Mitchell, Wall, Solomon, et al. 2001; Solomon et al. 2002). Pursel et al. (2004) summarized the advances made in pigs expressing a skeletal α-actin-hIGF-I transgene; namely, the expression of IGF-I in skeletal muscles gradually improved body composition in transgenic pigs without major effects on growth performance. Lean tissue accretion rates were significantly higher (30.3% and 31.6%), and fat accretion rates were 20.7% and 23.7% lower in transgenic gilts and boars, respectively, compared to controls. Body fat, bone, and lean tissue measurements by dual-energy X-ray absorptiometry confirmed that transgenic pigs had less fat and bone but higher lean tissue amount than control pigs.

Dietary conjugated linolenic acid (CLA) and IGF-I transgene (TG) had little or no effect on pork quality (Solomon et al. 2002; Eastridge, Solomon, Pursel, Mitchell, and Arguello 2001). Carcass weight of IGF-I TG pigs was less than non-TG controls; however, TG pigs had a 16% larger loin eye area, 26% to 28% reduced backfat thickness, and 21% less carcass fat. Dietary CLA acted synergistically with the IGF-I TG in reducing backfat thickness. Muscle pH at 45 minutes (pH_{45}) was lower ($p < .01$) in TG than non-TG (6.0 vs. 6.1) pigs, and dietary CLA resulted in significantly higher pH_{45} than for pigs fed control diets (6.1 vs. 6.0). At 24 hours, muscle pH was not different, averaging pH 5.6 for all carcasses. Neither gene status nor dietary CLA affected drip/purge loss during 21-day refrigerated storage in vacuum package, pork chop cooking yield, or thiobarbituric reactive substances measured in vacuum-packaged loins stored for 5 days and 21 days fresh and 6 months frozen. In pigs receiving the control diet, pork chop tenderness was improved significantly (i.e., lower shear force values) in IGF-I TG compared to non-TG (5.3 vs. 7.0 kgf) pigs. Dietary CLA improved tenderness in non-TG pigs equivalent to tenderness of TG pigs. Wiegand, Parrish, Swan, Larsen, and Baas (2001) detected no effects of CLA supplementation of swine diets on sensory attributes, although, it improved meat color, marbling, and firmness. Bee (2001) detected no effect of CLA on pig growth performance, carcass lean, or fat deposition, but there was a marked effect on fatty acid profiles. Saturated fatty acids, palmitic and stearic, were increased significantly, whereas monounsaturated linoleic and polyunsaturated arachidonic acids were reduced. Activity of lipogenic enzymes *in vitro* was not altered by the dietary CLA suggesting that lipogenesis was not affected by CLA (Bee 2001).

The shelf life of pork loin samples from IGF-I TG pigs with or without dietary CLA was not different from non-TG pigs (Nedoluha, Solomon, Pursel, and Mitchell 2001a). Aerobic plate counts of TG pork samples stored in retail or vacuum packages were similar to non-TG samples throughout 21 days of refrigerated storage. Ground pork from IGF-I TG pigs, with or without dietary CLA, that was inoculated with *Listeria innocua*, a nonpathogenic bacteria used as a model for *L. monocytogenes*, *E. coli* O157:H7, *Salmonella typhimurium*, and *Yersinia enterocolitica* and stored for 14 days at 7°C showed that meat from IGF-I TG pigs may be less supportive of growth of foodborne pathogens than non-TG meat (Nedoluha, Solomon, Pursel, and Mitchell 2001b). Growth of *L. innocua, E. coli, S. typhimurium,* and *Y. enterocolitica* was lower in meat from TG compared to non-TG pigs. There was no effect of dietary CLA on *Y. enterocolitica* and *E. coli*; however, *L. innocua* and *S. typhimurium* growth

was slightly higher in meat from pigs receiving CLA. More studies are needed to confirm these results.

Directing IGF-I expression specifically to skeletal muscle appeared to overcome the problems encountered with GH transgenics or with daily injections of exogenous IGF-I (Pursel et al. 2004) and clearly had a major impact on carcass composition. Piétrain pigs have 5% to 10% more meat than comparable pigs of other breeds (Houba and te Pas 2004), although, the muscle hypertrophy phenotype in Piétrain pigs is not as strongly expressed as the double-muscle condition in cattle or callipyge in sheep. The mechanism of Piétrain pig hypertrophy is still unknown; however, it might be associated with changes to the calpastatin gene. Klosowska et al. (2005) did not detect a calpastatin (CAST) polymorphism FF genotype in Piétrain cross-bred pigs. Pigs with the FF CAST genotype had smaller muscle fiber diameters compared to the EE and EF phenotypes. Linking the CAST genotype with phenotype to meat quality would benefit the meat industry, especially in pigs. The relationship between genotype at the CAST and MSTN loci to phenotype remains to be elucidated.

1.5 FOOD SAFETY IMPLICATIONS

The NRC (2002), at the request of the FDA, conducted an independent evaluation of foods from cloned animals and concluded that meat from clones and other products was safe. Based on these findings, the FDA (2003) announced that it would consider two issues: Are the animals themselves healthy, and are the products nutritionally indistinguishable from those produced by noncloned animals? After evaluating more than 100 parameters for meat and milk composition, U.S. and Japanese researchers (Tian et al. 2005) declared there were no statistical differences in these products from two Japanese Black beef and four Holstein dairy cattle clones compared to matched controls (20 beef and four dairy cattle). Walsh and Norman (2004) and Norman and Walsh (2004) also reported no differences in composition of milk from cloned cows. Few data are available on the consequence of consuming products from cloned animals. Guillén et al. (1999) evaluated consumption of transgenic tilapia by healthy human volunteers over 5 days. No differences in clinical or biochemical parameters measured were detected between those who consumed the transgenic and nontransgenic fish. Guillén et al. (1999) suggested that GH would be degraded under the ordinary acidic and enzymatic conditions during digestion in the human stomach, thus posing no effect due to consumption of the transgenic fish. Tomé, Dubarry, and Fromentin (2004) presented data from a preliminary 3-week study in which rats were fed cow's milk and meat from cloned animals. No differences between the control and cloned products were detected for food intake, body weight gain, body composition, and fasting insulin at the end of 3 weeks. Specific antimilk and meat protein immunoglobulin subtype analysis also revealed no differences between control and cloned-animal-derived products. There appeared to be no major difference in the nutritional value of milk and meat from cloned animals compared to controls. Tomé et al. (2004) cautioned that it might require a longer consumption time to confirm these observations. Technically, the introduction of novel proteins in genetically modified foods could elicit an allergic reaction (Poulsen 2004); however, there is no single test to predict allergenicity. In pigs fed transgenic plant protein

in the form of Roundup Ready soybean meal, Jennings et al. (2003) could not detect any fragment of the transgenic plant DNA nor fragment of the transgenic protein in the muscle tissue.

To date, livestock producers have honored a voluntary prohibition on requesting approval for bioengineered meat products in the United States. CBS News (2003) reported that a livestock company has made a request to Health Canada to sell meat from cloned animals but that Health Canada was still exploring the risks associated with cloned animals. The Japanese Ministry of Health, Labour and Welfare (Betterhumans 2003) concluded after a 3-year study that meat and milk products from cloned animals are safe for humans. At least 40 Japanese facilities raise cloned cattle but are prohibited under a voluntary ban from marketing the meat and milk.

REFERENCES

Adams, N. R., J. R. Briegel, and K. A. Ward. 2002. The impact of a transgene for ovine growth hormone on the performance of two breeds of sheep. *J. Anim. Sci.* 80:2325–2333.

Anonymous. 2004. Down on the pharm. *Economist Tech. Quar.* 372:37–38.

Archer, G. S., T. H. Friend, J. Piedrahita, C. H. Nevill, and S. Walker. 2003. Behavioral variation among cloned pigs. *App. Anim. Beh. Sc.* 82:151–161.

Baldassarre, H., B. Wang, C. L. Keefer, A. Lazaris, and C. N. Karatzas. 2004. State of the art in the production of transgenic goats. *Repro. Fertil. Dev.* 16:465–470.

Bee, G. 2001. Dietary conjugated linoleic acids affect tissue lipid composition but not de novo lipogenesis in finishing pigs. *Anim. Res.* 50:383–399.

Behboodi, E., E. Memili, D. T. Melican, M. M. Destrempes, S. A. Overton, J. L. Williams, P. A. Flanagan, R. E. Butler, H. Liem, L. H. Chen, H. M. Meade, W. G. Gavin, and Y. Echelard. 2004. Viable transgenic goats derived from skin cells. *Transgene. Res.* BW2118:1–10.

Betterhumans. 2003, April 21. Meat and milk from cloned animals is safe, says Japanese government. http://betterhumans.com/Print/index.aspx?ArticleID=2003-04-21-3.

Betthauser, J., E. Forsberg, M. Augenstein, L. Childs, K. Eilertsen, J. Enos, T. Forsythe, P. Golueke, G. Jurgella, R. Koppang, T. Lesmeister, K. Mallon, G. Mell, P. Misica, M. Pace, M. Pfister-Genskow, N. Strelchenko, G. Yoelker, S. Watt, S. Thompson, and M. Bishop. 2000. Production of cloned pigs from *in vitro* systems. *Nat. Biotech.* 18:1055–1059.

Bidwell, C. A., L. N. Kramer, A. C. Perkins, T. S. Hadfield, D. E. Moody, and N. E. Cockett. 2004. Expression of PEG11 and PEG11AS transcripts in normal and callipyge sheep. *BMC Bio.* 2:17–27.

Brown, B. W., and K. A. Ward. 2000. Abstract #19:22. *14th Int. Congr. Anim. Reprod.* 14:250

Busboom, J. R., W. F. Hendrix, C. T. Gaskins, J. D. Cronrath, L. E. Jeremiah, and L. L. Gibson. 1994. Cutability, fatty acid profiles and palatability of callipyge and normal lambs. *J. Anim. Sci.* 72:Suppl. 1, 60.

Carpenter, C. E., O. D. Rice, N. E. Cockett, and G. D. Snowder. 1996. Histology and composition of muscles from normal and callipyge lambs. *J. Anim. Sci.* 74:388–393.

Carroll, J., B. Carter, S. Korte, S. Dowd, and R. Prather. 2005. The acute-phase response of cloned pigs following an immune challenge. *J. Anim. Sci.* 83:Suppl. 2, 11.

Carter, D. B., L. Lai, K. W. Park, M. Samuel, J. C. Lattimer, K. R. Jordan, D. M. Estes, C. Besch-Williford, and R. S. Prather. 2002. Phenotyping of transgenic cloned piglets. *Cloning and Stem Cells*. 4:131–145.

CBS. 2003. Company wants to sell "cloned" meat in Canada. http://www.cbc.ca/stories/print/2003/10/13/Consumers/clonedmeat-030113.

Charlier, C., K. Segers, L. Karim, T. Shay, G. Gyapay, N. Cockett, and M. Georges. 2001. The callipyge mutation enhances the expression of coregulated imprinted genes in cis without affecting their imprinting status. *Nature Genet*. 27:367–369.

Ciobanu, D. C., J. W. M. Bastiaanseni, S. M. Lonergan, H. Thomsen, J. C. M. Dekkers, G. S. Plastow, and M. F. Rothschild. 2004. New alleles in calpastatin gene are associated with meat quality traits in pigs. *J. Anim. Sci*. 82:2829–2839.

Cockett, N. E., S. P. Jackson, T. L. Shay, D. M. Nielsen, S. S. Moore, M. R. Steele, W. Barendse, G. D. Green, and M. Georges. 1994. Chromosomal localization of the callipyge gene in sheep (*Ovis aries*) using bovine DNA markers. *Proc. Nat. Acad. Sci. USA*. 91:3019–3023.

Diles, J. J. B., R. D. Green, H. H. Shepard, G. L. Mathiews, L. J. Hughes, and M. F. Miller. 1999. Relationships between body measurements obtained on yearling brangus bulls and measures of carcass merit obtained from their steer clone-mates. *Prof. Anim. Scientist*. 12:244–249.

Dove, A. W. 2005. Clone on the range: What animal biotech is bringing to the table. *Nat. Biotechnol*. 23:305–310.

Duckett, S. K., T. A. Klein, M. V. Dodson, and G. D. Snowder. 1998. Tenderness of normal and callipyge lamb aged fresh or after freezing. *Meat Sci*. 49:19–26.

Duckett, S. K., G. D. Snowder, and N. E. Cockett. 2000. Effect of the callipyge gene on muscle growth, calpastatin activity, and tenderness of three muscles across the growth curve. *J. Anim. Sci*. 78:2836–2841.

Eastridge, J. S., M. B. Solomon, V. G. Pursel, A. D. Mitchell, and A. Arguello. 2001. Dietary conjugated linoleic acid and IGF-I transgene effects on pork quality. *J. Anim. Sci*. 79:Suppl. 1, 20.

FAO. 2004. *The state of agricultural commodity markets*. Geneva: Food and Agriculture Organization of the United Nations.

FDA. 2003, October 31. Executive summary of the assessment of safety of animal cloning. http://www.fda.gov/bbs/topics/news/2003/new00968.html

Fehilly, C. B., S. M. Willadsen, and E. M. Tucker. 1984. Interspecific chimaerism between sheep and goat. *Nature*. 307:634–636.

Forsberg, C. 2002. Economics and marketing of transgenic animals. In *Biotech in the barnyard: Implications of genetically engineered animals: Proceedings of The Pew Initiative on Food and Biotechnology Workshop, Dallas, TX, Sept. 24–25*, 19–20. Washington, DC: The Pew Initiative.

Freking, B. A., J. W. Keele, S. D. Shackelford, T. L. Wheeler, M. Koohmaraie, M. K. Nielsen, and K. A. Leymaster. 1999. Evaluation of the ovine callipyge locus: III. Genotypic effects on meat quality traits. *J. Anim. Sci*. 77:2336–2344.

Freking, B. A., S. K. Murphy, A. A. Wylie, S. J. Rhodes, J. W. Keele, K. A. Leymaster, R. L. Jirtle, and T. P. L. Smith. 2002. Identification of the single base change causing the callipyge muscle hypertrophy phenotype, the only known example of polar overdominance in mammals. *Genome Res*. 12:1496–1506.

Freking, B. A., T. P. L. Smith, and K. A. Leymaster. 2004. The callipyge mutation for sheep muscular hypertrophy—Genetics, physiology and meat quality. In *Muscle development of livestock animals: Physiology, genetics and meat quality*, ed. M. F. W. te Pas, M. E. Everts, and H. P. Haagsman, 317–342. Wallingford, UK: CABI.

Gerken, C. L., J. D. Tatum, J. B. Morgan, and G. C. Smith. 1995. Use of genetically identical (clone) steers to determine the effects of estrogenic and androgenic implants on beef quality and palatability characteristics. *J. Anim. Sci.* 73:3317–3324.

Goldman, I. L., S. G. Kadulin, and S. V. Razin. 2002. Transgenic goats in the world pharmaceutical industry of the 21st century. *Russian J. Gene.* 38:1–14.

Golovan, S. P., R. G. Meidinger, A. Ajakaiye, M. Cottrill, M. Z. Wiederkehr, D. J. Barney, C. Plante, J. W. Pollard, M. Z. Fan, M. A. Hayes, J. Laursen, J. P. Hjorth, R. R. Hacker, J. P. Phillips, and C. W. Forsberg. 2001. Pigs expressing salivary phytase produce low-phosphorus manure. *Nat. Biotechnol.* 19:741–745.

Goodson, K. J., R. K. Miller, and J. W. Savell. 2001. Carcass traits, muscle characteristics, and palatability attributes of lambs expressing the callipyge phenotype. *Meat Sci.* 58:381–387.

Gordon, J. W., G. A. Scangos, D. J. Plotkin, J. A. Barbosa, and F. H. Ruddle. 1980. Genetic transformation of mouse embryos by microinjection of purified DNA. *Proc. Natl. Acad. Sci. USA.* 77:7380–7384.

Grindflek, E., R. Holzbauer, G. Plastow, and M. F. Rothschild. 2002. Mapping and investigation of the porcine major insulin sensitive glucose transport (SLC2A4/GLUT4) gene as a candidate gene for meat quality and carcass traits. *J. Anim. Breed. Gene.* 119:47–55.

Guillén, I., J. Berlanga, C. M. Valenzuela, A. Morales, J. Toledo, M. P. Estrada, P. Puentes, O. Hayes, and J. de la Fuente. 1999. Safety evaluation of transgenic tilapia with accelerated growth. *Marine Biotech.* 1:2–14.

Hall, J., S. Ali, M. A. Surani, G. P. Hazlewood, A. J. Clark, J. Simons, B. H. Hirst, and H. J. Gilbert. 1993. Manipulation of the repertoire of digestive enzymes secreted into the gastrointestinal tract of transgenic mice. *Bio/Tech.* 11:376–379.

Hammer, R. E., V. G. Pursel, C. E. Rexroad, Jr., R. J. Wall, D. J. Bolt, K. M. Ebert, R. D. Palmiter, and R. L. Brinster. 1985. Production of transgenic rabbits, sheep and pigs by microinjection. *Nature,* 315:680–683.

Harris, J. J., D. K. Lunt, S. B. Smith, W. L. Mies, D. S. Hale, M. Koohmaraie, and J. W. Savell. 1997. Live animal performance, carcass traits, and meat palatability of calf- and yearling-fed cloned steers. *J. Anim. Sci.* 75:986–992.

Houba, P. H. J., and M. F. W. te Pas. 2004. The muscle regulatory factors gene family in relation to meat production. In *Muscle development of livestock animals: Physiology, genetics and meat quality,* ed. M. F. W. te Pas, M. E. Everts, and H. P. Haagsman, 201–224. Wallingford, UK: CABI.

Jackson, S. P., and R. D. Green. 1993. Muscle trait inheritance, growth performance and feed efficiency of sheep exhibiting a muscle hypertrophy phenotype. *J. Anim. Sci.* 71:Suppl. 1, 14–18.

Jennings, J. C., D. C. Kolwyck, S. B. Kays, A. J. Whetsell, J. B. Surber, G. L. Cromwell, R. P. Lirette, and K. C. Glenn. 2003. Determining whether transgenic and endogenous plant DNA and transgenic protein are detectable in muscle from swine fed Roundup Ready soybean meal. *J. Anim. Sci.* 81:1447–1455.

Kerth, C. R., T. L. Cain, S. P. Jackson, C. B. Ramsey, and M. F. Miller. 1999. Electrical stimulation effects on tenderness of five muscles from Hampshire × Rambouillet crossbred lambs with the callipyge phenotype. *J. Anim. Sci.* 77:2951–2955.

Kittredge, C. 2005. A question of chimeras. *The Scientist.* 19:54–55.

Klosowska, D., J. Kury, G. Elminowska-Wenda, W. Kapelanski, K. Walasik, M. Pierzchaa, D. Cieslak, and J. Bogucka. 2005. An association between genotypes at the porcine loci MSTN (GDF8) and CAST and microstructural characteristics of m. longissimus lumborum: A preliminary study. *Archiv. Tierzucht.* 48:50–59.

Ko, J. H., C.-S. Lee, K. H. Kim, M.-G. Pang, J. S. Koo, N. Fang, D.-B. Koo, K. B. Oh, W.-S. Youn, G. D. Zheng, J. S. Park, S. J. Kim, Y.-M. Han, I. Y. Choi, J. Lim, S. T. Shin, S. W. Jin, K.-K. Lee, and O. J. Yoo. 2000. Production of biologically active human granulocyte colony stimulating factor in the milk of transgenic goat. *Transgenic Res.* 9:215–222.

Koohmaraie, M., S. D. Shackelford, and T. L. Wheeler. 1998. Effects of prerigor freezing and calcium chloride injection on the tenderness of callipyge longissimus. *J. Anim. Sci.* 76:1427–1432.

Koohmaraie, M., S. D. Shackelford, T. L. Wheeler, S. M. Lonergan, and M. E. Doumit. 1995. A muscle hypertrophy condition in lamb (callipyge): Characterization of effects on muscle growth and meat quality traits. *J. Anim. Sci.* 73:3596–3607.

Krzecio, E., M. Kocwin-Podsiada, J. Kury, K. Antosik, A. Zybert, H. Sieczkowska, E. Pospiech, A. Yczynski, and B. Grzes. 2004. An association between genotype at the CAST locus (calpastatin) and meat quality traits in porkers free of RYR1 SUP T allele. *Anim. Sci. Papers Rep.* 22:489–496.

Krzecio, E., J. Kury, M. Kocwin-Podsiada, and G. Monin. 2004. The influence of CAST/RsaI and RYR1 genotypes and their interactions on selected meat quality parametres in three groups of four-breed fatteners with different meat content of carcass. *Anim. Sci. Papers Rep.* 22:469–478.

Kuber, P. S., S. K. Duckett, J. R. Busboom, G. D. Snowder, M. V. Dodson, J. L. Vierck, and J. F. Bailey. 2003. Measuring the effects of phenotype and mechanical restraint on proteolytic degradation and rigor shortening in callipyge lamb longissimus dorsi muscle during extended aging. *Meat Sci.* 63:325–331.

Kunej, T., X.-L. Wu, T. M. Berlic, J. J. Michal, Z. Jiang, and P. Dovx. 2005. Frequency distribution of a Cys430Ser polymorphism in peroxisome proliferators-activated receptor-gamma coactivator-1 (PPARGC1) gene sequence in Chinese and Western pig breeds. *J. Anim. Breeding and Gen.* 1:7–11.

Kuryl, J., E. Krzecio, M. Kocwin-Podsiada, and G. Monin. 2004. The influence of CAST and RYR1 genes polymorphism and their interactions on selected quality parametres in four-breed fatteners. *Anim. Sci. Papers Rep.* 22:479–488.

Lewis, C. 2001, January–February. A new kind of fish story: The coming of biotechnology animals. FDA Consumer. http://www.cfsan.fda.gov/~dms/fdbiofish.html

Loi, P., G. Ptak, B. Barboni, J. Fulka, Jr., P. Cappai, and M. Clinton. 2001. Genetic rescue of an endangered mammal by cross-species nuclear transfer using post-mortem somatic cells. *Nat. Biotech.* 19:962–964.

Lorenzen, C. L., M. L. Fiorotto, F. Jahoor, H. C. Freetly, S. D. Shackelford, T. L. Wheeler, J. W. Savell, and M. Koohmaraie. 1997. Determination of the relative roles of muscle protein synthesis and protein degradation in callipyge-induced muscle hypertrophy. In *Proceedings 50th Reciprocal Meat Conference, June 29–July 2,* 175. Savoy, IL: American Meat Science Association.

McPherron, A. C., A. M. Lawler, and S. J. Lee. 1997. Regulation of skeletal muscle mass in mice by a new TGF-ß super family member. *Nature,* 387:83–90.

Mitchell, A. D., and V. G. Pursel. 2003. Efficiency of energy deposition and body composition of control and IGF-I transgenic pigs. In *Progress in research on energy and protein metabolism,* ed. W. B. Souffrant, and C. C. Metges. EAAP Scientific Series, 109:61–64.

Murray, J. D., and C. E. Rexroad, Jr. 1991. The development of sheep expressing growth promoting transgenes. *NABC Report,* 3:251–263.

Nancarrow, C. D., J. T. Marshall, J. L. Clarkson, J. D. Murray, R. M. Millard, C. M. Shanahan, P. C. Wynn, and K. A. Ward. 1991. Expression and physiology of performance regulating genes in transgenic sheep. *J. Reprod. Fert.* 43:Suppl., 277–291.

Nedoluha, P. C., M. B. Solomon, V. G. Pursel, and A. D. Mitchell. 2001a. Microbial growth in transgenic pork. *J. Food Prot.* 64:Suppl. A, 81–82.

Nedoluha, P. C., M. B. Solomon, V. G. Pursel, and A. D. Mitchell. 2001b. Pathogen growth in transgenic pork. BARC Poster Day Abstract Book. Beltsville, MD: Agricultural Research Service.

Nicholls, H. 2004. Milking goats for malaria vaccine. *Drug Discovery Today.* 9:248.

Niemann, H. 2004. Transgenic pigs expressing plant genes. *Proc. Nat. Acad. Sci.* 101:7211–7212.

Norman, H. D., and W. K. Walsh. 2004. Performance of dairy cattle clones and evaluation of their milk composition. *Cloning and Stem Cells.* 6:157–164.

NRC. 2002. *Animal biotechnology: Science-based concerns.* Washington, DC: National Academies Press.

Onishi, A., M. Iwamoto, T. Akita, S. Mikawa, K. Takeda, T. Awata, H. Hanada, and A. C. Perry. 2000. Pig cloning by microinjection of fetal fibroblast nuclei. *Science,* 289:1188–1190.

Oppenheim, S. M., A. L. Moyer, R. H. Bondurant, J. D. Rowe, and G. B. Anderson. 2000. Successful pregnancy in goats carrying their genetically identical conceptus. *Theriogen.* 54:629–639.

Otani, K., D.-H. Han, E. L. Ford, P. M. Garcia-Roves, H. Ye, Y. Horikawa, G. I. Bell, J. O. Holloszy, and K. S. Polonsky. 2004. Calpain system regulates muscle mass and glucose transporter GLUT4 turnover. *J. Biol. Chem.* 278:20915–20920.

Palmiter, R. D., R. L. Brinster, R. E. Hammer, M. E. Trumbauer, M. G. Rosenfeld, N. C. Brinbert, and R. M. Evans. 1982. Dramatic growth of mice that develop from eggs microinjected with metallothionein-growth hormone fusion genes. *Nature,* 300:611–615.

Piper, L. R., A. M. Bell, K. A. Ward, and B. W. Brown. 2001. Effect of ovine growth hormone transgenesis on performance of merino sheep at pasture: 1. Growth and wool traits to 12 months of age. *Proc. Assoc. Adv. Anim. Breed. Genet.* 14:257–260.

Polejaeva, I. A., S.-H. Chen, T. D. Vaught, R. L. Page, J. Mullins, S. Ball, Y. Dal, J. Boone, S. Walker, D. L. Ayares, A. Colman, and K. H. S. Campbell. 2000. Cloned pigs produced by nuclear transfer from adult somatic cells. *Nature.* 407:86–90.

Poulsen, L. K. 2004. Allergy assessment of foods or ingredients derived from biotechnology, gene-modified organisms or novel foods. *Molecular Nutrition Food Res.* 48:413–423.

Prather, R. S., R. J. Hawley, D. B. Carter, L. Lai, and J. L. Greenstein. 2003. Transgenic swine for biomedicine and agriculture. *Theriogen.* 59:115–123.

Pursel, V. G., R. G. Campbell, K. F. Miller, R. R. Behringer, R. D. Palmiter, and R. L. Brinster. 1988. Growth potential of transgenic pigs expressing a bovine growth hormone gene. *J. Anim. Sci.* 66:Suppl. 1, 267.

Pursel, V. G., A. D. Mitchell, G. Bee, T. H. Elsasser, J. P. McMurtry, R. J. Wall, M. E. Coleman, and R. J. Schwartz. 2004. Growth and tissue accretion rates of swine expressing an Insulin-like Growth Factor I transgene. *Anim. Biotech.* 15:33–45.

Pursel, V. G., A. D. Mitchell, R. J. Wall, M. E. Coleman, and R. J. Schwartz. 2001. Effect of an IGF-I transgene on tissue accretion rates in pigs. *J. Anim. Sci.* 79:Suppl. 1, 29.

Pursel, V. G., A. D. Mitchell, R. J. Wall, M. B. Solomon, M. E. Coleman, and R. J. Schwartz. 2001. Transgenic research to enhance growth and lean carcass composition in swine. In *Molecular farming,* ed. J. P. Toutant and E. Balazs, 77–86. Paris: INRA Editions.

Pursel, V. G., and C. E. Rexroad, Jr.. 1993. Status of research with transgenic farm animals. *J. Anim. Sci.* 71:Suppl. 3, 10–19.

Pursel, V. G., and M. B. Solomon. 1993. Alteration of carcass composition in transgenic swine. *Food Rev. Int.* 9:423–439.

Pursel, V. G., R. J. Wall, M. B. Solomon, B. J. Bolt, J. D. Murray, and K. A. Ward. 1997. Transfer of an ovine metallothionein-ovine growth hormone fusion gene into swine. *J. Anim. Sci.* 75:2208–2214.

Rexroad, Jr., C. E., R. E. Hammer, D. J. Boh, K. E. Mayo, L. A. Frohman, R. D. Palmiter, and R. L. Brinster. 1989. Production of transgenic sheep with growth-regulating genes. *Mol. Reprod. Dev.* 1:164–169.

Rexroad, Jr., C. E., K. Mayo, D. J. Bolt, T. H. Elsasser, K. F. Miller, R. R. Behringer, R. D. Palmiter, and R. L. Brinster. 1991. Transferrin- and albumin-directed expression of growth-related peptides in transgenic sheep. *J. Anim. Sci.* 69:2995–3004.

Rule, D. C., G. E. Moss, G. D. Snowder, and N. E. Cockett. 2002. Adipose tissue lipogenic enzyme activity, serum IGF-I, and IGF-binding proteins in the callipyge lamb. *Sheep Goat Res. J.* 17:39–46.

Ryder, O. A. 2002. Cloning advances and challenges for conservation. *Trends Biotech.* 20:231–232.

Saeki, K., K. Matsumoto, M. Kinoshita, I. Suzuki, Y. Tasaka, K. Kano, Y. Taguchi, K. Mikami, M. Hirabayashi, N. Kashiwazaki, Y. Hosoi, N. Murata, and A. Iritani. 2004. Functional expression of a Δ12 fatty acid desaturase gene from spinach in transgenic pigs. *Proc. Nat. Acad. Sci.* 101:6361–6366.

Sillence, M. N. 2004. Technologies for the control of fat and lean deposition in livestock. *Veterinary J.* 167:242–257.

Solomon, M. B. 1999. The callipyge phenomenon: Tenderness intervention methods. *J. Anim. Sci.* 77:Suppl. 2, 238–242.

Solomon, M. B., V. G. Pursel, and A. D. Mitchell. 2002. Biotechnology for meat quality enhancement. In *Research advances in the quality of meat and meat products,* ed. F. Toldrá, 17–31. Kerala, India: Research Signpost.

Solomon, M. B., V. G. Pursel, E. W. Paroczay, and D. J. Bolt. 1994. Lipid composition of carcass tissue from transgenic pigs expressing a bovine growth hormone gene. *J. Anim. Sci.* 72:1242–1246.

Stratil, A., and M. Kopecny. 1999. Genomic organization, sequence and polymorphism of the porcine myostatin (GDF8; MSTN) gene. *Anim. Gene.* 30:468–469.

Takahashi, S., and Y. Ito. 2004. Evaluation of meat products from cloned cattle: Biological and biochemical properties. *Cloning and Stem Cells,* 6:165–171.

Tian, X. C., C. Kubota, K. Sakashita, Y. Izaike, R. Okano, N. Tabara, C. Curchoe, L. Jacob, Y. Zhang, S. Smith, C. Bormann, J. Xu, M. Sato, S. Andrew, and X. Yang. 2005. Meat and milk compositions of bovine clones. *Proc. Nat. Acad. Sci.* 102:6261–6266.

Tomé, D., M. Dubarry, and G. Fromentin. 2004. Nutritional value of milk and meat products derived from cloning. *Cloning and Stem Cells.* 6:172–177.

Tulsi, B. 2004. Bugs punch the clock as next protein manufacturers. *Drug Discovery Development.* 7:56–62.

van der Laan, L. J. W., C. Lockey, B. C. Griffeth, F. S. Frasler, C. A. Wilson, D. E. Onlons, B. J. Hering, Z. Long, E. Otto, B. E. Torbett, and D. R. Salomon. 2000. Infection by porcine endogenous retrovirus after islet xenotransplantation in SCID mice. *Nature.* 407:90–94.

Vize, P. D., A. E. Michalska, R. Ashman, B. Lloyd, B. A. Stone, P. Quinn, J. R. E. Wells, and R. F. Seamark. 1998. Introduction of a porcine growth hormone fusion gene into transgenic pigs promotes growth. *J. Cell Sci.* 90:295–300.

Wall, R. J., A. M. Powell, M. J. Paape, D. E. Kerr, D. D. Bannerman, V. G. Pursel, K. D. Wells, N. Talbot, and H. W. Hawk. 2005. Genetically enhanced cows resist intramammary *Staphylococcus aureus* infection. *Nature Biotech*. 23:445–451.

Walsh, M. K., and H. D. Norman. 2004. Performance of dairy cattle clones and evaluation of their milk composition. *Cloning and Stem Cells*. 6:157–164.

Ward, K. A., and B. W. Brown. 1998. The production of transgenic domestic livestock: Successes, failures and the need for nuclear transfer. *Reprod. Fertil. Dev.* 10:659–665.

Ward, K. A, C. D. Nancarrow, C. R. Byrne, C. M. Shanahan, J. D. Murray, Z. Leish, C. Townrow, N. W. Rigby, B. W. Wilson, and C. Hunt. 1990. The potential of transgenic animals for improved agricultural productivity. *OIE Revue Scientifique et Technique*. 9:847–864.

Ward, K. A., C. D. Nancarrow, J. D. Murray, P. C. Wynn, P. Speck, and J. R. S. Hales. 1989. The physiological consequences of growth hormone fusion gene expression in transgenic sheep. *J. Cell. Biochem.* Suppl. 13B:164.

Wells, K. D. 2000. Genome modification for meat science: Techniques and applications. *Proc. 53rd Annual Reciprocal Meat Conf. Ohio State Univ,* 87–93.

Wiegand, B. R., F. C. Parrish, Jr., D. G. Morrical, and E. Huff-Lonergan. 2001. Feeding high levels of vitamin D3 does not improve tenderness of callipyge lamb loin chops. *J. Anim. Sci*. 79:2086–2091.

Wiegand, B. R., F. C. Parrish, Jr., J. E. Swan, S. T. Larsen, and T. J. Baas. 2001. Conjugated linoleic acid improves feed efficiency, decreases subcutaneous fat, and improves certain aspects of meat quality in stress-genotype pigs. *J. Anim. Sci*. 79:2187–2195.

Wieghart, M., J. Hoover, S. H. Choe, M. M. McGrane, F. M. Rottman, R. W. Hanson, and T. E. Wagner. 1988. Genetic engineering of livestock—Transgenic pigs containing a chimeric bovine growth hormone (PEPCK/bGH) gene. *J. Anim. Sci*. 66:Suppl. 1, 266.

Wilmut, I., A. E. Schnieke, J. McWhir, A. J. Kind, and K. H. S. Campbell. 1997. Viable offspring derived from fetal and adult mammalian cells. *Nature,* 385:810–813.

2 Gene Technology for Meat Quality

John L. Williams
Division of Genetics and Genomics, Roslin Institute

CONTENTS

2.1 Background 22
2.2 Genetic Selection 23
2.3 Genetic Control of Meat Quality Traits 24
2.4 Locating the Genes Controlling Variations in Traits 25
2.4.1 QTL Mapping 26
2.4.2 Genetic Markers 26
2.4.3 Genome Maps 28
2.4.4 Mapping Populations 28
2.4.5 Beef Quality QTL 29
2.4.6 Surprising Findings From Diverse Cross Populations in Pigs 30
2.5 Finding the Trait Genes 31
2.5.1 Double Muscling in Cattle 32
2.5.2 Gene for Carcass Composition in Pigs 33
2.6 Breed Improvement Using Gene Markers 33
2.7 Gene Expression Patterns and Meat Quality 34
2.7.1 Genetic Variations 35
2.7.2 Detecting Environmental Effects 36
2.8 Combining Mapping and Expression Studies to Identify the Important Pathways 36
2.9 Traceability and Safety 37
2.10 Conclusion 38
References 39

Meat quality can be defined in terms of composition, consumer appreciation, and safety. Each of these criteria are influenced by environmental and genetics factors. In recent years there have been major advances in knowledge about the organization of genomes of many species, including the major livestock species. This knowledge has provided methods and resources to investigate the genetic control of commercially important traits, including meat quality. In addition methods have been developed to

simultaneously explore the expression of large numbers of genes. Together these "genomics" approaches will provide information to assist the selection of animals with the best genotypes for particular production needs and help to develop diets best suited to producing meat with desired characteristics. At present the work is in its infancy. Although genome mapping approaches have localized some of the genes controlling important aspects of meat quality to regions on chromosomes—quantitative trait loci—few of the genes have themselves been identified. For those that have, there have been some unexpected findings such as variations in major genes responsible for large phenotypic differences in one breed being associated with little or no phenotype difference in another. Also, breeds with an extreme phenotype in a particular trait might carry less extreme alleles than a breed with a less extreme phenotype. At the level of the genome, functional polymorphisms might occur at considerable distances from the genes thought to control the phenotypic difference. The current explosion of information available on the genomes of many species, including livestock, arsing from genome sequencing projects will allow the functioning of the genome to be investigated in greater detail. In the short term this information will be used to enhance phenotypic selection programs, but will, in due course, allow selection strategies for the improvement of multiple difficult-to-measure traits to be developed.

2.1 BACKGROUND

Meat quality can be defined in terms of consumer appreciation of texture and flavor, and safety, which includes both the health implications of composition (e.g., polyunsaturated vs. saturated fat) and microbiological contamination. These quality factors can be influenced by environmental factors such as feeding and management of the animals during their growth, and by postslaughter handling and processing. In addition the genetic makeup of the individual will influence many aspects of quality. Molecular biological methods could be used to improve meat quality through genetic improvement and by defining the response of meat composition to environmental factors. Safety aspects could also be improved by the application of molecular techniques to individual identification, for tracking meat products and the detection of harmful bacterial contamination on carcasses and processed meat.

Over the last decade studies in many species have led to rapid advances in understanding of the structure of the genome and the regulation of gene expression. Following the publication of the human genome sequence (Lander et al. 2001), the technology for large-scale, high-throughput analysis of DNA sequences and gene expression has become widely available and the costs have rapidly decreased. The first draft of the bovine sequence was released in October 2004, with a full sequence predicted for 2006. Along with the genome sequence, information will be available on several hundreds of thousands of variations (polymorphisms) between the genomes of individuals. A genome sequencing project for pigs is only just starting, but given the now rapid rate of sequencing entire genomes, the pig sequence is likely to be available in 2007. In addition to genomic sequence, the sequences of very many expressed sequences are already available for cattle and pigs in publicly accessible databases. Thus the technology and resources that are being applied to human genetic research are now becoming available to researchers working with

livestock, and will facilitate the identification of the genes involved in variations of commercially relevant traits. Information on polymorphisms within these genes could then be used to enhance selection programs, or to develop improved management strategies.

The DNA sequence and gene expression information, collectively known as *genomics,* can be applied to livestock research for the improvement of several areas of meat quality and safety. Identifying differences in the DNA sequence of individuals, polymorphisms, controlling variations in phenotypes such as composition or toughness could be achieved using a genome mapping approach. This knowledge could then be applied in marker-assisted selection programs to select the individuals carrying beneficial alleles for desired traits. DNA polymorphisms can also be used to identify individuals and track the meat products from those individuals through the production chain, with high confidence. This level of traceability would allow the origin of meat to be assured, for example, for guaranteeing meat produced from animals raised in specific management systems or diets, and in the case of a disease outbreak, identifying the meat from particular animals with certainty. A new area of research that has been opened up through the explosion in genomic information is the examination of changes in gene expression. The information on expressed sequences has enabled micro-arrays to be developed that can be used to interrogate the expression levels of many thousands of genes simultaneously. The impact of environmental factors might be detectable as differences in expression of particular genes, which might in turn be related to differences in meat quality. Identifying genes with a level of expression that might be altered in particular circumstances provides the possibility of developing tests for animals raised in defined environments, or predicting meat quality based on expression of particular genes. These applications are discussed in more detail in the relevant sections that follow with a focus on beef, but with reference to pork production as well. As a final example of the application of genomics, DNA testing could be applied to the detection of bacterial contamination on meat products and the differentiation of harmful from benign strains: This application is not discussed here and the reader is referred to chapter 6 of this book.

2.2 GENETIC SELECTION

Genetic improvement in livestock is achieved through selective breeding, whereby individuals with superior characteristics in particular traits are used to breed the next generation. This approach has brought about spectacular improvements in some traits, such as milk yield in dairy cattle, and growth rates in beef breeds. However, to practice selective breeding the traits to be selected must be recorded in the breeding populations. In commercial populations the measurements that can be made, and hence the traits that are routinely recorded, are by necessity very simple. Only limited attempts have been made to select on difficult-to-measure traits, for obvious reasons: high cost or imprecise measurements. This is partly because the definition of traits associated with, for example, quality or health, is subjective unless detailed and complex measurements are taken, which are difficult to apply in large populations. In addition, until now, market forces have driven selection on cost and hence quantity, rather than on quality

(e.g., composition traits). However, consumer pressure is now demanding high-quality livestock products that are safe, produced from animals farmed in sustainable, environmentally and welfare-friendly systems. Selection criteria are therefore likely to shift from quantity to quality, efficiency of production, and health traits.

Breed improvement in cattle has been enhanced by artificial insemination, which has allowed individual "superior" sires to produce large numbers of progeny. Where the trait of interest is sex limited (e.g., milk production), or can only be determined postslaughter (e.g., meat quality or composition), progeny test schemes allow the genetic quality of sires for the trait to be estimated. This approach uses trait records from daughters or sons of test sires to calculate their genetic merit. The high-merit sires are then used for breeding. The development of sophisticated statistical methods to analyze progeny test data to identify sires that are above average for the desired traits has maximized the genetic gain that can be achieved. Commercial progeny test schemes have resulted in milk yields of the Holstein breed being nearly doubled over the past 40 years. However, the collection of data on milk yield and milk composition is relatively easy in a commercial setting compared with measuring meat quality traits. Hence until now there has been little or no selection for improved meat quality, and no attempts have been made to change meat composition through breeding.

2.3 GENETIC CONTROL OF MEAT QUALITY TRAITS

For progeny selection to be effective a relatively large number of sires have to be tested. However, it takes a long time to breed, raise, and slaughter the animals, then to measure meat quality traits. Before progeny test results can be used for selection for quality traits, many of the sires used will have died or become genetically obsolete. In addition, progeny testing is very expensive. Therefore tools that can be used to identify potentially superior animals at an early stage would be valuable for improving the genetics of animals to produce high-quality meat. In slow-growing or late-maturing species, juvenile predictors of adult performance can be used to speed up selection and reduce costs (Meuwissen 1998). Such predictors would allow earlier selection of breeding stock, before many of the rearing costs had been incurred. However, until now the reliability of juvenile predictors has often been low. The use of molecular markers potentially offers a way to select breeding animals at an early age—indeed as embryos; to select for a wide range of traits; and to enhance reliability in predicting the mature phenotype of the individual.

Many factors affect the quality and composition of meat. These include environmental variables, such as the way animals are fed, age at slaughter, and so on. In addition, handling of the animals preslaughter and stress responses postslaughter affect the maturation of the meat. Thus, many improvements in quality could be achieved by optimizing management practices at these points in the production chain. Nevertheless, in addition to the environmental and processing influences on meat quality, there are undoubtedly genetic factors that affect meat quality, such as fatty acid composition, fat distribution, muscle fiber type, and so on. These meat-quality-associated variables in muscle composition show heritabilities of up to 0.35 (Wheeler, Cundiff, Shackelford, and Koohmaraie 2004); in other words 35% of the variation is under genetic control. Significant differences in sensory appreciation and composition

of meat are found among different breeds, which also indicates the role of genetics in controlling variations in meat quality traits. Analysis of the genetic makeup of breeds has shown that although there is genetic variation within breeds, this is small compared with the variation found between breeds (e.g., Blott, Williams, and Haley 1999). Thus, the genetic makeup of the animal is likely to affect aspects of meat quality, which therefore could be improved by genetic selection. This selection would be most effectively achieved using molecular markers for the traits. However, until now few of the genes controlling variability in meat quality and composition have been identified, and specifically, few functional variations in the genes that control the phenotypic differences are known.

Molecular genetic approaches can be used to identify genes that control variation in phenotypes. Armed with this information, it should be possible to select improved stock for a wide variety of traits on the basis of their genetic makeup. If simple phenotype-guided selection is used in isolation, there are inevitably conflicting choices when considering the diverse range of traits that are important at different levels of the production chain. There are likely to be some traits that are obligatorily in conflict; that is, alleles of a particular gene could be beneficial for one trait but have negative effects on another. When the genes controlling different traits are close together on a chromosome it might appear that there is only one locus having an effect on both traits, as alleles at closely linked genetic loci will generally be inherited together. Nevertheless, even with very closely linked genes, there is the possibility of recombination between them. Knowing the alleles at particular genetic loci will allow direct selection choices to be made by identifying individuals who carry the most beneficial combination of alleles. Therefore, in theory at least, a strategy to select simultaneously for improved performance in a number of traits could be developed using genetic markers, even when at the phenotypic level the improvement in some of the traits might seem to be in conflict. If applied with care, the use of molecular information in selection programs has the potential to increase productivity, enhance environmental adaptation, and maintain genetic diversity.

2.4 LOCATING THE GENES CONTROLLING VARIATIONS IN TRAITS

The first task is to understand the genetic control of the traits of interest, and then to identify the genes involved, so that this information can be applied in selection programs. One approach to identifying the genes controlling a particular trait is to use information on the physiology of the trait to identify the biochemical pathways involved, and hence identify *candidate* genes that might be involved. This information can be coupled with patterns of expression among tissues to facilitate cloning of the genes most likely to affect the trait. Polymorphisms in these candidate genes are then studied in the context of variations in the trait to identify whether they play a role in controlling the observed variation. This approach clearly requires a good *a priori* knowledge of the trait and the underlying physiology. However, even with good knowledge of the trait, important genes are likely to be missed, as many might not be obviously involved in the known physiology. Therefore a two-step approach

is generally used to identify genes controlling a particular trait: Initially the chromosomal location of the gene is identified, using a linkage mapping approach, and then information on the chromosomal location of the gene is used as the starting point to identify the gene itself and ultimately find the functional polymorphism. Most traits that are important for livestock production, such as feed efficiency, disease resistance, growth rate, or muscle composition, are not under the control of a single gene, but are controlled by several genes that have an additive effect. Such traits have a continuous distribution and are referred to as *quantitative traits*. Loci controlling them are termed *quantitative trait loci* (QTL).

2.4.1 QTL Mapping

A linkage mapping approach is used to map the genes controlling quantitative traits to regions on chromosomes. This approach for identifying the genetic loci controlling a trait requires access to families, which are segregating for the trait of interest. The inheritance of chromosomal regions is tracked in these segregating families using DNA markers, and this information is then correlated with measurements characterizing the variations in the trait. Statistical methods to correlate the trait and marker information are then used to localize the trait genes. Therefore, to map trait genes, there are two requirements, families and markers, as discussed later.

The initial mapping step defines the chromosomal location of the QTL through flanking DNA markers. These linked markers are in themselves useful, as they can be used to enhance selection programs by identifying animals that carry the favorable allele at the QTL, which can be for breeding. This process is called marker-assisted selection (MAS; Kashi, Hallerman, and Soller 1990). However, as these markers are likely to be at a significant genetic distance from the gene controlling the trait, there is the possibility of recombination occurring between the marker and the trait gene. Thus, to use MAS, it is first necessary to determine the *phase* of the markers; that is, which alleles at each of the markers as linked to the favorable or unfavorable alleles at the trait gene. Determining the phase of markers has to be done within a family by recording the phenotype of individuals in the family and relating this information to the genotype at the linked markers. However, the phase of flanking markers is likely to be different in different families and can change over generations through recombination. Thus the phase of marker alleles in relation to alleles at the trait gene has to be frequently reconfirmed. This is both time-consuming and not particularly efficient as the information obtained to confirm the phase of the markers could also be used directly for selecting the superior individuals for the trait. In contrast to using linked markers for a QTL, knowledge of the functional mutation in the trait gene can be used directly in the population, without first having to determine the phase, and so represents a more effective tool for enhancing selection. Nevertheless, the first step in identifying the trait gene is currently a linkage mapping approach.

2.4.2 Genetic Markers

Genetic markers used in gene mapping studies must follow a mendelian pattern of inheritance, be readily assayed, and have a reasonable number of alleles at relatively

even frequencies. The first widely used as a genetic marker was the restriction fragment length polymorphism (RFLP). Bacterial restriction enzymes have highly specific recognition sequences, which are typically four to six base pairs long. Variations in genomic sequence can create or destroy these recognition sites and hence differences in the length of DNA fragments generated following digestion with the restriction enzyme. Originally the DNA fragments arising from a particular region of the genome, following restriction enzyme digestion, were detected using radioactive probes. However, the development of the polymerase chain reaction (PCR) technique has revolutionized molecular genetics by providing sufficient DNA for analysis directly by electrophoresis without the need for radioactivity. RFLPs are now detected by amplifying the target region by PCR. Then cutting the PCR product with the restriction enzyme followed by electrophoresis and direct staining of the DNA is used to assay whether the fragment has been cut. Specific functional polymorphisms are detected in DNA-based tests as PCR-RFLPs, but this type of marker is too cumbersome for use in mapping studies to detect QTL where a large number of markers are required to cover the genome.

Until very recently the most frequently used marker type was the microsatellite locus. These loci typically have 5 to 20 copies of a short sequence motif, of two to four base pairs in length, repeated in tandem. The number of repeats at a particular locus occasionally changes because of errors introduced during DNA replication. This relatively high-level mutation rate leads to a large number of alleles being found at the population level for microsatellite loci. The number of alleles at a locus is approximately proportional to the number of repeat units. The large number of alleles at microsatellite loci and their amenability to PCR amplification make them excellent markers for use in genetic studies (see later).

The amount of genome sequence information from livestock species that is available in the public domain is rapidly increasing. The majority of this sequence is from the ends of large-fragment genomic bacterial artificial chromosome (BAC) clones, and emerging from genome sequencing projects (see below). Alignment of sequence from the same genomic region from different individuals allows polymorphic sites to be identified. The types of polymorphism found fall into two classes: insertions or deletions of DNA sequence (indels), or changes to the nucleotide sequence, often at individual bases. Single nucleotide polymorphisms (SNPs) are much more frequent than indels in the genome and occur at high frequency in both noncoding regions and coding regions of the genome. Current estimates from genome sequencing projects indicate that an SNP occurs at about 200 base pair intervals, on average. Thus there are potentially many millions of SNPs in a genome. SNPs within coding regions might have no effect on the protein encoded by the gene (silent polymorphisms) or might result in a change in an amino acid. The latter are most likely to be the polymorphisms responsible for differences in the function of the protein and hence are directly responsible for variations in traits. However, examples have been found where the functional polymorphisms associated with particular traits occur in noncoding intergenic regions (see IGF2 later).

A project to sequence the bovine genome started in 2003 and the first draft sequence with three-fold coverage of the genome was made publicly available in November 2004. A project to sequence the pig genome is currently being planned.

As with the human project, the bovine genome sequencing project is identifying large numbers of SNPs across the entire genome. The immediate objective is to characterize and confirm 20,000 of these SNPs with a view to producing a validated set of 200,000 SNPs over the course of the project. This number of markers coupled with improved genotyping technology will change the way that genes controlling traits can be identified.

SNP polymorphisms have advantages over other marker types, insofar as they can be detected by methods other than electrophoresis, which is slow and difficult to automate. Following the discovery of many hundreds of thousands of SNPs from the human sequencing project (Lander et al. 2001), automated assays have been developed using, for example, fluorescence or mass spectroscopy, to genotype SNPs. It is now possible to rapidly genotype hundreds to thousands of individuals for several thousand SNP markers in a few hours. It is anticipated that in the future genome mapping studies will use SNPs, instead of the microsatellite-based markers. The very high density of SNP markers that will be available will make it possible to carry out association studies at a population level by identifying the regions of DNA that are inherited in *linkage disequilibrium* with the trait gene. Until now the relatively low density of markers (typically around 150 to 200) used in QTL mapping studies has required using families in which there are still large regions of linkage disequilibrium to be able to detect the association between the markers and the traits.

2.4.3 Genome Maps

For genetic markers to be useful in gene mapping studies their location in the genome must be known. This allows markers to be selected covering the whole genome evenly, or to be concentrated on targeted regions. The relation between the markers also has to be known to localize the QTL identified to chromosomal regions. Over the past decade, genetic and physical maps have been developed for the genomes of all the major domestic species. Two types of genome maps exist: genetic and physical maps. Genetic maps are created by determining the linkage between markers from their inheritance in families (Barendse et al. 1997; Bishop et al. 1994; Georges et al. 1995). These genetic maps were initially composed predominantly from microsatellite markers, but more recently genes and expressed sequence tags (ESTs) have been added to the maps. The genetic map of the cow now contains more than 3,800 markers (Ihara et al. 2004). These genetic maps have been used to select markers distributed across the whole genome to track inheritance of regions of chromosomes through generations in families segregating for the traits of interest in QTL mapping studies.

2.4.4 Mapping Populations

The second requirement to map QTLs, in addition to the genetic markers, is families segregating and recorded for the traits of interest. Unfortunately, the range of traits routinely recorded in commercial populations is very limited, and through necessity has focused on simple traits, such as growth rates and milk yields. An additional consideration is that traits are often sex specific, and although selection is applied

most strongly in the males, because of artificial insemination (AI), the trait is often expressed in the females. In the case of meat quality accurate measurements of the traits can only be done postslaughter, and thus cannot be made in the animals intended for breeding.

The structure of the commercial dairy population includes a large number of half-sib cows produced by AI from a limited number of elite bulls. This population structure is particularly appropriate for mapping QTL. The genetic contribution of bulls to milk production traits can be determined with high accuracy by measuring the phenotypes of their daughters. Bulls with high breeding value are then used extensively through AI to improve the dairy cow population. The sons of bulls with high breeding values are in turn used as AI sires to produce a large number of daughters. Georges et al. (1995) used the U.S. Holstein population to map QTL involved in milk yield. Five QTL for dairy-associated traits were identified, many of which have been confirmed independently in subsequent studies in other populations (e.g., Kuhn, Freyer, Weikard, Goldammer, and Schwerin 1999; Wiener, Maclean, Williams, and Woolliams 2000).

The way bulls are selected in the beef industry is different from that in the dairy industry. Beef is produced from a large number of breeds, which have not been under as intense selection as the dairy breeds and the Holstein in particular. In some countries there is some systematic recording of beef production-related traits in the live animal, such as growth, fat, and conformation traits to select the bulls for breeding. These data provide limited opportunities for mapping QTL for simple beef production traits. However, until now there has been little direct recording of meat quality traits that have been related to particular commercial breeding bulls. Thus, the majority of information on QTL controlling beef quality traits comes from specifically bred "resource" herds. These herds are kept under standardized management, which provides the opportunity to record the more difficult-to-measure traits, which would be impossible using commercial herds. Several of the studies investigating meat quality have used resource herds created by crossbreeding *B. taurus* and *B. indicus* breeds, in which there is known to be a very large difference in meat quality traits, particularly toughness. QTL for several beef associated traits have been localized using these extreme crosses for intramuscular fat or marbling a muscle mass, meat texture, and so on (e.g., Stone, Keele, Shackelford, Kappes, and Koohmaraie 1999), but the value of these QTL in pure-bred populations has yet to be demonstrated.

2.4.5 Beef Quality QTL

Many different studies have identified QTL regions that control different aspects of beef quality. However, it is difficult to compare results among studies, as experimental measurements are often very different. Nonetheless, where QTL positions for similar traits are coincident, or are close together, on the same chromosome from independent studies of similar traits, there is increased confidence in the existence of the particular QTL. The most important traits for consumer-defined quality are probably intramuscular fat and toughness or texture. Several studies have reported a QTL on cattle chromosome 2 for marbling, as an indicator of the amount of

intramuscular fat. In a population segregating for a mutation in the myostatin gene, which is located on chromosome 2 and is associated with the double muscling phenotype, variations in fat were attributed directly to the double muscling mutation in this gene (Casas et al. 1998). However, in other studies, which did not include breeds known to carry mutations in the myostatin gene, QTL for fat deposition were also localized on chromosome 2, although to a different position on the chromosome (e.g., MacNeil and Grosz 2002; Stone et al. 1999). QTL for marbling score have also been reported on 11 other bovine chromosomes. Although many of these might be real in the particular populations, some are likely to be false results, or highly specific to the population studies, and so not of general value in commercial herds. Nevertheless, marbling QTL on chromosome 3 (Casas et al. 2001; Casas et al. 2003; Casas, Keele, Shackelford, Koohmaraie, and Stone 2004), chromosome 5 (Casas et al. 2003; Stone et al. 1999), chromosome 10 (Casas et al. 2001; Casas et al. 2003), and chromosome 27 (Casas et al 2001; Casas et al. 2003; Casas et al. 2000) have been identified in independent studies and in different populations, lending support to the general importance of these QTL regions. Tenderness or texture can be measured by force required to cut a piece of meat. Several laboratory techniques have been developed to measure shear force mimicking a biting action, in theory replicating the appreciation of the texture of meat while chewing. The most common method of measuring shear force is the Warner-Bratzler method (WBSF). As with marbling score, several QTL have been identified for WBSF including chromosomes 5, 9, 15, and 20 (Casas et al. 2001; Casas et al. 2003; Casas et al. 2004; Keele, Shackelford, Kappes, Koohmaraie, and Stone 1999); however, only chromosome 29 has been found to have a WBSF QTL detected in different populations (Casas et al. 2003; Casas et al. 2000).

2.4.6 Surprising Findings From Diverse Cross Populations in Pigs

In Europe, consumers demand meat that is low in fat, whereas meat with a high fat content has a high value in Asia. Selection of stock suited to particular markets has produced breeds with widely divergent characteristics. European cattle breeds, such as the Belgian Blue, Charolais, Limousin, and so on, have been selected for rapid growth and good feed conversion efficiency. They also produce lean carcasses, but this currently desirable characteristic came about possibly by chance. In contrast, in Japan, selection of Wagu cattle has been focused on developing a breed that has exceptionally high levels of intramuscular fat for the high-value home market. Similar divergent selection criteria have been applied in pig production. In response to consumer demand, pig breeds used extensively in Europe have been selected for lean growth, whereas Meishan pigs from China lay down large quantities of fat.

Crossbred resource populations have been created to localize QTL for carcass composition in pigs, using as founders breeds of pig with extreme phenotypes. These populations have been produced from wild boar, and phenotypically extreme breeds such as the Chinese Meishan, crossed to European commercial breeds such as the Large White and Landrace pigs. QTL for carcass and fertility traits have been

identified in these populations (e.g., Nagamine, Haley, Sewalem, and Visscher 2003; Rattink et al. 2000).

These divergent crossbred pig populations provide an excellent opportunity to explore the genetic control of lean versus fat growth. A two-generation crossbred resource population established from the Large White and Meishan pigs was used to identify several QTLs associated with carcass fat. One QTL, on chromosome 7, had a particularly large effect, accounting for about a 30% difference in back fat thickness (de Koning et al. 1999). The surprising finding, however, was that the allele associated with lean growth originated from the phenotypically fat Meishan breed.

This example demonstrates that the most beneficial allele for a particular trait might not be present in population showing the desirable phenotype for the trait. This could be because of founder effects—that is, that the most favourable allele simply was not present in the individuals initially used to create the breeds—or that it might have been lost through genetic drift. Alternatively the favorable allele might have a deleterious effect on another trait or is associated with undesirable characteristics, and so is under negative selection.

2.5 FINDING THE TRAIT GENES

There are now a large number of QTLs identified for production traits in livestock (e.g., see *http://bovineqtl.tamu.edu/* and *http://www.animalgenome.org/QTLdb/*). However, so far, few trait genes, and specifically the functional mutations within these genes, have been identified. The identification of the trait genes starting from the chromosomal location is not an easy task. Initial low-resolution QTL mapping studies typically localize a QTL within a 20 centi-Morgan (cM) interval, which equates to 20 Mb DNA or 1/150th of the genome. This amount of the genome could contain 200 or more genes. Thus it is either necessary to refine the map position before trying to identify the specific gene that controls the trait, or other information to select genes within the region likely to have an effect on the trait has to be used.

Linkage mapping relies on recombination to determine the order of the markers in relation to the trait genes on the chromosome. To fine map a QTL, the *linkage disequilibrium* flanking the QTL has to be reduced; that is, the piece of chromosome inherited together with the trait gene has to be broken down by recombination. A large number of individuals are required to find those with recombination occurring within the QTL region, so fine mapping of a QTL requires access to large multi-generation families. Depending on the information available on population structures it might be possible to use distantly related individuals whose common ancestor is several generations back. Over successive generations recombination will reduce the amount of the ancestral genome inherited with the trait gene, therefore identifying regions of the genome associated with the trait in different branches of an extended family that are *identical by descent* (IBD). This can refine the location of a QTL to a much smaller chromosomal region than the original QTL mapping study (see Anderson and Georges 2004). Meta-analysis of data from different mapping populations can also be used to refine the map location. By examining the marker haplotypes

defining the QTL region in the different populations, it might be possible to reduce the limits of linkage disequilibrium between markers and the trait.

Once the QTL region has been fine mapped, two approaches can then be adopted for identifying the trait gene. The most popular and successful approach so far has been to identify "positional candidate" genes, or genes mapped to particular chromosomal regions that are known to have a biological function that putatively affects the trait. The search for positional candidate genes is helped by the finding that extended chromosome segments are conserved between different species; that is, containing the same complement of genes (Chowdhary, Frönicke, Gustavsson, and Scherthan 1996; Hayes 1995; Solinas-Toldo, Lengauer, and Fries 1995). Examination of the equivalent regions across species, together with information on known functions of the genes in controlling phenotypes, can then provide candidate genes, which can then be tested to see if they are involved in the trait of interest.

In the absence of a candidate gene for the trait of interest, or when the candidate genes that are identified prove not to include the trait gene, it is then necessary to clone and sequence the region to obtain information on the genes and variations present in the genome within the QTL region. Usually a large number of variations will be identified within the sequence obtained, so it is then necessary to compare the sequence between individuals that show differences in their phenotypes and identify animals with the appropriate recombination within the sequenced region to associate a specific genetic variation with differences observed in the trait.

2.5.1 Double Muscling in Cattle

The gene controlling double muscling in cattle was the first trait gene to be identified starting with information on its chromosomal location. Double muscling occurs in several European beef breeds and is characterized by muscular hypertrophy and hyperplasia, and reduced intramuscular fat (Ménissier 1982). In beef terms, double-muscled animals produce carcasses that are superior in the choice cuts of meat and are exceptionally lean. The most extreme form of double muscling is found in the Belgian Blue breed where the trait behaves as if it is controlled by a single major gene. A research population of cattle, created by crossing double-muscled Belgian Blue cattle to a noncarrier breed was used to map the double muscling gene to a region on bovine chromosome 2 (Charlier et al. 1995). A candidate gene (GDF-8) for the trait within this region was identified from work in mice on the transforming growth factor (TGFβ) family of genes showing that it had an effect on muscle development. Transgenic mice in which expression of this gene was knocked out developed hypermuscularity similar to the double muscling phenotype in cattle (McPherron, Lawler, and Lee 1997). The GDF-8 gene product was found to be a negative regulator of muscle growth and was therefore called myostatin. Belgian Blue cattle that showed the double muscling phenotype were found to have an 11 base pair deletion within the coding region of this gene (Grobet et al. 1997). Double-muscled cattle in other breeds were also found with mutations in the coding region of their myostatin gene, lending support to this being the gene controlling the double-muscled phenotype.

2.5.2 Gene for Carcass Composition in Pigs

A meta-analysis of several divergent pig resource populations, in which a QTL for muscle and fat depth had been localized on chromosome 2, was able to fine map the likely position of the underlying gene to a region including the insulin-like growth factor 2 (IGF2) gene. Sequencing across the IGF2 locus of animals carrying different alleles at the QTL identified 258 polymorphisms. These polymorphisms could be assigned to haplotype clusters that were assigned to either European or Chinese origin (Van Laere et al. 2003). Correlating these haplotype blocks with variations measured in the trait identified a single SNP, a G to A transition within intron 3 that did not follow the predicted pattern of European or Chinese origin and appeared to be the causative mutation, or quantitative trait nucleotide (QTN). This QTN occurs in a region of DNA that can be methylated as a result of imprinting (inactivation of one of the parental chromosomes), and is thought to be at a binding site for a protein that regulates gene expression. This allele, associated with lean meat, has been strongly selected for in European pig breeds.

The IGF2 QTN controlling fatness is an interesting example of a genetic variation in a noncoding region of the genome that has a large effect on a production trait. Although not in a coding region, this QTN is likely to be within a region of DNA that regulates gene expression. This is not the only example of a noncoding polymorphism that has a large effect on a meat production trait. The Callipyge phenotype in sheep, which is associated with increased muscling, has also been mapped to an imprinted, noncoding region of the sheep genome (Freking et al. 2002).

2.6 BREED IMPROVEMENT USING GENE MARKERS

As discussed previously, breed improvement, until now, has been achieved through phenotypic selection focused on easily measured traits. Over the last four decades the approaches to selection have been refined and trait measurements made on the individual have been replaced by calculated breeding values that make use of all the available information on the genetic merit of the individual, including information from relatives: parents, progeny, and siblings. However, many of the economically important traits, and certainly those involved in variation in meat quality, are difficult to measure and are quantitative in nature. The phenotypic variations in these traits were originally thought to result from the interactions among many genes, each having a small effect on the phenotype—the infinitesimal model (see Flint and Mott 2001, for a review). If this were the case, it was thought that identifying the genes controlling a quantitative trait would be impossible. Fortunately, as demonstrated by the QTL examples given earlier, for at least some economically important traits it seems that, although there might be many genes involved, there are usually a small number of major genes that control a reasonable amount of the observed variation. The contribution of these genes can be readily incorporated into selection programs by adding the information on the alleles carried by an individual to its breeding value calculated from phenotypic measurements. In this way progress could be made in improving both difficult-to-measure traits as well as the easily measured traits that are currently used.

The rate of improvement in the meat quality traits will be governed by the amount of variation that is genetically controlled, and the proportion of this variation that is explained by the genes included in the selection criteria. Unfortunately for both beef and pork, meat quality traits have only fairly low to moderate heritability, explaining perhaps between 10% and 30% of the variation (e.g., Burrow, Moore, Johnston, Barendse, and Bindon 2001). However, some traits that have a well-defined biological basis, that affect specific aspects of meat quality, and have a much higher genetic component controlling the observed variation; for example, the size and number of fibers in particular muscles, which will affect lean muscle development (Rehfeldt, Fiedler, Dietl, and Ender 2000). Indeed the myostatin gene that, as discussed earlier, is associated with double muscling in several breeds of cattle, has been shown to have a major influence in regulating muscle fiber size, type, and number (Rehfeldt et al. 2005). Although the mutation in the myostatin gene controls a major part of the double muscling phenotype in the Belgian Blue breed, in other breeds (e.g., the South Devon) the same mutation has a more limited effect, and in some individuals apparently no influence on the phenotype (Wiener, Smith, Lewis, Woolliams, and Williams 2002). Therefore care should be exercised in extrapolating information obtained in one population for use in another. It is likely that even for a major gene, the effect on the phenotype might be dependent on other *modifier* genes in the genetic background. Thus before genetic markers are adopted as the prime selection criterion, the phenotypic effect should be verified in the population under selection.

Eventually sufficient information will be accumulated to define the biochemical pathways that control particular traits and phenotypes. It will then be possible to select for improvement on several criteria and multiple genetic loci, each of which are involved in the development of the desired phenotype. To identify these pathways QTL mapping and individual trait gene identification is just the first step. Several approaches will be required to examine the factors involved in regulating meat quality parameters. One route to identifying particular biochemical or developmental pathways that are involved in the meat quality traits will be to examine the expression patterns of genes and identify those that are coregulated during particular developmental processes, and are associated with specific nutritional status or with particular phenotypes.

2.7 GENE EXPRESSION PATTERNS AND MEAT QUALITY

A major technological advance in genomics has been the development of approaches for the large-scale analysis of gene expression. The availability of cDNA, or expressed sequence information, has provided the resources to construct, first macro-arrays and more recently micro-arrays. These are arrays of many thousands of either cDNAs coding for specific genes or oligo-nucleotide probes representing fragments of the cDNA sequences, printed and immobilized onto a solid matrix. These arrays can be used to explore the relative expression of genes in samples of RNA prepared from different tissue samples. Using this array technology the expression of a very large number of genes can be compared between samples, for example, of tissues from animals with different phenotypes or in different physiological states.

In the context of meat improvement, the variations in muscle development and biochemistry are affected both by genetics and the environment. The latter might impact the genes that are expressed at different stages of growth and maturation, and hence the final composition and quality of meat produced. Knowledge of the genes involved in variations in composition and structure of the muscle will aid selection for animals that produce better quality meat. In addition, understanding the expression of these genes will also help to improve management strategies and animal diets to optimize particular qualities in the meat. The expression profiles of genes within the muscle could potentially be used as predictors of different aspects of meat quality, such as tenderness or fat composition, or to confirm the management and feeding used in the production of the meat.

Initially, studies of gene expression in bovine muscle were undertaken using gene probes from humans. This is not ideal, as species-specific variations could produce erroneous results. More recently macro- and micro-arrays have been developed for cattle, and sets of cDNA clones and oligo-nucleotides have been identified for constructing expression arrays. Recently micro-arrays have become commercially available for cattle (e.g., from Qiagen Ltd. [USA] and Afymetrix [USA]). Arguably these are generalized arrays with sets of cDNA probes that have not been optimized for examining expression in muscle or adipose tissues, so further development of the array sets will be necessary to carry out studies on particular tissues.

2.7.1 Genetic Variations

Until now there have been few studies to examine the gene expression profiles in muscles from cattle that are known to produce meat with different qualities. The selection for high growth favors the muscle with lower oxidative metabolism and therefore there is some indication of the physiological pathways that could be the subject of fruitful investigation. However, the targeting of specific genes and biochemical pathways at the outset could mean that important genes whose regulation is critical to changes in muscle structure or composition are overlooked. Studies carried out by INRA in France have compared expression patterns of more than 1,300 genes in two muscles from cattle selected for high and low growth potential using an array constructed from human cDNAs. This work identified 34 genes with different levels of expression between the genetic types (Casser-Malek et al. 2003). Many of the genes identified with differential expression were associated with muscle structure (e.g., titin) or cell regulation (e.g., thyroid hormone receptor). A further study using a smaller array, which was constructed from 480 bovine cDNAs, identified four genes with significantly different expression levels between the genetically divergent high- and low-growth lines. The high-growth bulls had high levels of expression of myosin binding protein H, but lower expression of the troponin T slow isoform.

With the recent availability of the more extensive species-specific cDNA and oligonucleotide arrays, it will be possible to carry out more detailed studies of the difference in expression in animals with genetically controlled variations. In designing these studies it will be necessary to consider the tissues, cell types, and developmental stages examined. In biological terms it is important to identify the regulatory pathways that

give rise to the differences between muscles. Differences in expression of genes in these pathways are likely to be stage and cell-type specific. Such information could be important for selection of individuals for breeding, or to match specific genetic types of animal to particular production goals or management systems. However, to develop predictors of quality or indicators of management requirements, gene expression variations that are a result of earlier developmental events might be adequate.

2.7.2 Detecting Environmental Effects

The biochemical composition and structure of muscle can be influenced by nutrition and physiological factors. Even *in utero* the diet of the mother can influence the development of muscle by regulating the number and size of the myoblasts present, possibly through the nutritional modulation of hormone synthesis or metabolism (Dauncey, White, Burton, and Katsumata 2001). Gene expression profiling, using micro-arrays, has shown that there are two periods during development in which there are large changes in the pattern of gene expression; these are at around 6 months of gestation and at parturition. During early growth, changing the components in the diet and energy availability can modify muscle characteristics. The type of diet (e.g., hay vs. grain) can change the muscle type, with grain favoring the development of oxidative over glycocytic muscle fibers (Listrat et al. 1999). Feed restriction followed by provision of surplus feed will result in a growth check followed by a period of compensatory growth, which also results in a change in muscle fiber type and structure that might affect meat quality (Brandstetter, Picard, and Geay 1998). Until now the expression of only a small number of genes has been examined in relation to diet or nutritional status of the animals. The use of micro-arrays to investigate variations in expression resulting from different diets might indicate nutritional regimens that could be used to promote particular muscle qualities. Examination of the gene expression profiles at slaughter could also be developed into a tool to verify the diet used in the production system.

2.8 COMBINING MAPPING AND EXPRESSION STUDIES TO IDENTIFY THE IMPORTANT PATHWAYS

The information content obtained from mapping and expression studies is different, but complementary. Genes in which there are polymorphisms identified from mapping studies are not necessarily those that will be differentially expressed. The polymorphic gene might, for example, be a receptor that regulates another gene with a role in controlling the phenotype. Specific polymorphisms in the receptor might not affect its expression, but could mean that an excess of ligand is required to trigger activity, or conversely that a particular pathway is constitutively activated. This will have a downstream effect on the expression of other genes and could impact developmental pathways or metabolic processes. Identifying genes with functional polymorphisms will allow the pathways involved to be identified and explored. Knowledge of differential expression associated with genetically controlled phenotypic variations will also allow physiological or metabolic pathways to be identified. The

differentially expressed genes might in themselves have no polymorphic differences that could be readily assayed, and their differential expression might be the result of polymorphism in a gene at another point in the pathway. It is therefore important that information on putative quantitative trait genes and expression variations associated with phenotypic differences is combined. In some cases the combined data will confirm the regulatory or physiological pathways involved in the trait and the role of the quantitative trait gene. This knowledge might also provide information on pleiotropic effects of the quantitative trait gene; that is, where the gene has an effect on more than one pathway and hence on different traits. In this case, specific alleles might have a positive effect on one trait and negative effects on another. This information could be taken into account in devising MAS programs.

2.9 TRACEABILITY AND SAFETY

Current animal tracking procedures are paper based and associate ear tags with individuals. These systems are open to fraud and loss of tags and are only robust for live animals, as the paper-based traceability ends when animals are slaughtered and the ear tags linking the individual animal to the paper documentation are separated. The ability to track animals and meat products throughout the production chain is essential to maintain the confidence of the consumer and to protect the producer of quality products from fraud. Where particular production systems or breed types are specified in a retail chain it is important to be able to physically verify the identity of the carcasses using a procedure that allows tracking back from meat product to the animal at the farm. The problems associated with paper-based systems were illustrated when attempts were made to trace the origin of the bovine spongiform encephalopathy (BSE) case discovered in the United States in 2004. Although a Canadian ear tag was linked to the animal with BSE, all animals slaughtered at the same time as the BSE case had to be DNA typed along with their putative relatives to confirm the identity of the carcass once the ear tag had been removed. Using DNA markers it was possible to confirm that the case had originated from a particular farm in Canada using samples from living relatives. However, had tissue or DNA samples or a DNA profile been available with the paper records, it would have been much easier to confirm the origin of the individual animal and identify the carcass directly by matching the DNA profile from the BSE suspect with the sample taken when the animal was originally registered. There are some companies that are now offering commercial systems for taking and storing samples from livestock that can be used to track and verify the identity of the animal and meat from that individual by matching DNA profiles, including Genetic Solutions (Australia), Eurofins (France), and IdentiGEN (Ireland).

Currently these tracing systems are based on microsatellite markers (see earlier). These markers are highly polymorphic, with many alleles segregating in populations, and are therefore highly informative. Thus, relatively few microsatellite markers are required to uniquely identify an individual; for example, 12 markers with four alleles can have a probability of unrelated individuals having matching genotypes of one in a million or more, depending on allele frequencies (Williams, Usha, Urquhart, and Kilroy 1997). However, detection of microsatellite markers uses gel electrophoresis,

which is labor intensive, and hence expensive. In addition there are inherent problems of standardization using this type of marker. Experience has shown that allele calling for microsatellite markers is not consistent among laboratories. Thus, these are not ideal markers for a robust animal tracking system.

Genome sequencing projects typically identify many thousands of DNA polymorphisms, including insertions, deletions, and duplications. However, by far the most common sequence variation is the SNP, as previously discussed. There are over a million SNPs identified across the human genome and the target for the bovine genome sequencing project is to identify and verify 20,000 SNPs during 2005. SNP markers have the advantage that they can be genotyped by methods that do not rely on electrophoresis, such as primer extension and mass spectroscopy. These approaches are potentially rapid and amenable to automation. Thus costs for genotyping are significantly lower than costs for microsatellite markers. The disadvantage of SNPs is that they are dialleleic and so more markers are required to provide the same information as good microsatellite loci; typically four times as many SNPs are required than microsatellite markers. However, as genotyping costs fall with improvements in technology and through competition, it is envisaged that a large panel of SNP markers will be developed that will be able to deliver unambiguous individual identity verification at low cost, and will provide a robust technology for managing animal identification through the production chain. In addition, this panel of markers may be able to determine the breed of origin of meat samples.

2.10 CONCLUSION

Knowledge of the genes controlling quality-associated traits will allow direct selection for favorable alleles at these genes. In the first instance, this could be done by MAS using markers linked to the gene involved in the trait, within families where the allelic associations between markers and traits have been determined. However, ultimately, knowing the functional allelic variation within the trait gene will allow more efficient selection strategies to be devised at the population level. There are several advantages of using markers in selection programs, rather than relying on phenotype-based selection. These include a more rapid prediction of the phenotype and hence earlier selection of breeding stock, associated with a reduction in costs of maintaining animals. In addition, more accurate selection should be possible for individual traits, with the possibility of maximizing simultaneous selection for several traits. Such selection could even compensate for apparent pleiotropic effects. Where current selection suggests that progress in one trait might have a negative impact on another important trait, knowledge of the genes controlling the traits might suggest ways of improving both simultaneously.

The major barrier to identifying the genetic factors controlling variations in meat quality is the lack of well-characterized populations in which quality traits are recorded. Such populations would allow the proportion of the phenotypic variation that is under environmental and genetic control to be determined and allow gene-mapping approaches to locate QTL controlling the traits. Trait-recorded populations are then required to fine map the QTL and test candidate genes to ascertain their effect on the phenotype. As discussed earlier, even a gene with a major effect on a phenotype in one population might be associated with little phenotypic variation in

another and can depend on the genetic background. It is therefore important to carry out studies in a diverse range of genetically different types and in different environments. Further information on gene interactions could come from gene expression studies. Gene expression micro-arrays have now been produced for the majority of livestock species, allowing the expression patterns of many thousands of genes to be assayed simultaneously. Building up information on patterns of gene expression in different tissues and species could reveal coregulated physiological pathways that are currently unknown. These data will add to the gene mapping information to elucidate the genetic control of meat quality and other traits. Examining expression of genes in individuals raised on different diets and that are in different physiological states could provide information to optimize management regimens, and at slaughter to monitor the management that has been used.

In addition to human, mouse, rat, and dog, the sequence of the bovine genome will soon be available. Comparison of sequence information for coding and noncoding regions across species, in conjunction with gene mapping and expression studies, will identify functionally significant variations in genes and will also help to identify regulatory elements in noncoding regions, such as the variation responsible for fat deposition controlled by IGF2 in pigs or the Callipyge phenotype in sheep. With this increasing information, selection and management of livestock could be refined and breeders provided with the tools to rapidly respond to changing market demands for meat products with different qualities.

REFERENCES

Anderson, L., and M. Georges. 2004. Domestic animal genomic: Deciphering the genetics of complex traits. *Nature Rev. Gen.* 5:202–212.

Barendse, W., D. Vaiman, S. Kemp, Y. Sugimoto, S. Armitage, J. L. Williams, et al. 1997. A medium density genetic linkage map of the bovine genome. *Mammalian Genome.* 8:21–28.

Bishop, M. D., S. M. Kappes, J. W. Keele, R. T. Stone, S. L. F. Sunden, G. A. Hawkins, S. S. Toldo, R. Fries, M. D. Grosz, J. Y. Yoo, and C. W. Beattie. 1994. A genetic linkage map for cattle. *Genetics.* 136:619–639.

Blott, S. C., J. L. Williams, and C. S. Haley. 1999. Discriminating between cattle breeds using genetic markers. *Heredity.* 6:613–619.

Brandstetter, A. M., B. Picard, and Y. Geay. 1998. Muscle fibre characteristics in four muscles of growing bulls: II. Effect of castration and feeding level. *Livestock Prod. Sci.* 53:25–26.

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

Casas, E., J. W. Keele, S. D. Shackelford, M. Koohmaraie, T. S. Sonstegard, T. P. L. Smith, S. M. Kappes, and R. T. Stone. 1998. Association of the muscle hypertrophy locus with carcass traits in beef cattle. *J. of Anim. Sci.* 76:468–473.

Casas, E., J. W. Keele, S. D. Shackelford, M. Koohmaraie, and R. T. Stone. 2004. Identification of quantitative trait loci for growth and carcass composition in cattle. *Anim. Gen.* 35:2–6.

Casas, E., S. D. Shackelford, J. W. Keele, M. Koohmaraie, T. P. L. Smith, and R. T. Stone. 2003. Detection of quantitative trait loci for growth and carcass composition in cattle. *J. of Anim. Sci.* 81:2976–2983.

Casas, E., S. D. Shackelford, J. W. Keele, R. T. Stone, S. M. Kappes, and M. Koohmaraie. 2000. Quantitative trait loci affecting growth and carcass composition of cattle segregating alternate forms of myostatin. *J. of Anim. Sci.* 78:560–569.

Casas, E., R. T. Stone, J. W. Keele, S. D. Shackelford, S. M. Kappes, and M. Koohmaraie. 2001. A comprehensive search for quantitative trait loci affecting growth and carcass composition of cattle segregating alternative forms of the myostatin gene. *J. of Anim. Sci.* 79:854–860.

Casser-Malek, I., K. Sundre, A. Listrat, Y. Ueda, C. Jurie, Y. Briand, M. Briand, B. Meunier, C. Leroux, V. Amarger, D. Delourme, G. Rennard, B. Picard, P. Martin, and J. F. Hocquette. 2003. *Integrated approach combining genetics genomics and muscle biology to manage beef quality.* York, UK: British Society of Animal Science.

Charlier, C., W. Coppieters, F. Farnir, L. Grobet, P. L. Leroy, C. Michaux, M. Mni, A. Schwers, P. Vanmanshoven, R. Hanset, and M. Georges, M. 1995. The mh gene causing double-muscling in cattle maps to bovine Chromosome 2. *Mammalian Genome*. 6:788–792.

Chowdhary, B. P., L. Frönicke, I. Gustavsson, and H. Scherthan. 1996. Comparative analysis of the cattle and human genomes: Detection of ZOO-FISH and gene mapping-based chromosomal homologies. *Mammalian Genome*. 7:297–302.

Dauncey, M. J., P. White, K. A. Burton, and M. Katsumata. 2001. Nutrition-hormone receptor-gene interactions: Implications for the development of disease. *Proc. of the Nut. Soc.* 60:63–72.

de Koning, D. J., L. L. G. Janss, A. P. Rattink, P. A. M. van Oers, B. J. de Vries, M. A. M. Groenen, J. J. van der Poel, P. N. de Groot, E. W. Brascamp, and J. A. M. van Arendonk. 1999. Detection of quantitative trait loci for backfat thickness and intramuscular fat content in pigs (*Sus scrofa*). *Genetics*. 152:1679–1690.

Flint, J., and R. Mott. 2001. Finding the molecular basis of quantitative traits: Successes and pitfalls. *Nature Rev. Gen.* 2:437–445.

Freking, B. A., S. K. Murphy, A. A. Wylie, S. J. Rhodes, J. W. Keele, K. A. Leymaster, R. L. Jirtle, and T. P. L. Smith. 2002. Identification of the single base change causing the callipyge muscular hypertrophy phenotype, the only known example of polar over dominance in mammals. *Genome Res.* 12:1496–1506.

Georges, M., D. Nielsen, M. Mackinnon, A. Mishra, R. Okimoto, A. T. Pasquino, L. S. Sargeant, A. Sorensen, M. R. Steele, X. Zhao, J. E. Womack, and I. Hoeschele. 1995. Mapping quantitative trait loci controlling milk production in dairy cattle by exploiting progeny testing. *Genetics*. 139:907–920.

Grobet, L., L. J. R. Martin, D. Poncelet, D. Pirottin, B. Brouwers, J. Riquet, A. Schoeberlein, S. Dunner, F. Ménissier, J. Massabanda, R. Fries, R. Hanset, and M. Georges. 1997. A deletion in the bovine myostatin gene causes the double-muscled phenotype in cattle. *Nature Genetics*. 17:71–74.

Hayes, H. 1995. Chromosome painting with human chromosome-specific DNA libraries reveals the extent and distribution of conserved segments in bovine chromosomes. *Cytogen. and Cell Gen.* 71:168–174.

Ihara, N., A. Takasuga, K. Mizoshita, H. Takeda, M. Sugimoto, Y. Mizoguchi, T. H. T. Itoh, T. Watanabe, K. M. Reed, W. M. Snelling, K. M. Kappes, . C. W. Beattie, G. L. Bennett, and Y. Sugimoto. 2004. A comprehensive genetic map of the cattle genome based on 3802 Microsatellites. *Genome Res.* 14:1987–1998.

Kashi, Y., E. Hallerman, and M. Soller. 1990. Marker-assisted selection of candidate bulls for progeny testing programs. *Anim. Prod.* 51:63–74.

Keele, J. W., S. D. Shackelford, S. M. Kappes, M. Koohmaraie, and R. T. Stone. 1999. A region on bovine chromosome 15 influences beef longissimus tenderness in steers. *J. of Anim. Sci.* 77:1364–1371.

Kuhn, C. H., G. Freyer, R. Weikard, T. Goldammer, and M. Schwerin. 1999. Detection of QTL for milk production traits in cattle by application of a specifically developed marker map of BTA6. *Anim. Gen.* 30:333–340.

Lander, E. S., L. M. Linton, B. Birren, C. Nusbaum, M. C. Zody, et al. 2001. Initial sequencing and analysis of the human genome. *Nature.* 40:9860–9921.

Listrat, A., N. Rakadjiyski, C. Jurie, B. Picard, C. Touraille, and Y. Geay. 1999. Effect of the type of diet on muscle characteristics and meat palatability of growing Salers bulls. *Meat Sci.* 53:115–124.

MacNeil, M. D., and M. D. Grosz. 2002. Genome-wide scans for QTL affecting carcass traits in Hereford x composite double backcross populations. *J. Anim. Sci.* 80:2316–2324.

McPherron, A. C., A. M. Lawler, and S.-J. Lee (1997). Regulation of skeletal muscle mass in mice by a new TGF-b superfamily member. *Nature.* 387:83–90.

Ménissier, F. 1982. General survey of the effect of double muscling on cattle performance. In *Muscle hypertrophy of genetic origin and its use to improve beef production,* ed. J. W. B. King and F. Ménissier, 437–449. London: Martinus Nijhoff.

Meuwissen, T. H. E. 1998. Optimizing pure line breeding strategies utilizing reproductive technologies. *J. of Dairy Sci.* 81:Suppl. 2, 47–54.

Nagamine, Y., C. S. Haley, A. Sewalem, and P. M. Visscher. 2003. Quantitative trait loci variation for growth and obesity between and within lines of pigs (*Sus scrofa*). *Gen.* 164:629–635.

Rattink, A. P., D. J. De Koning, M. Faivre, B. Harlizius, J. A. M. van Arendonk, and A. M. Groenen. 2000. Fine mapping and imprinting analysis for fatness trait QTLs in pigs. *Mamm. Genome.* 11:656–661.

Rehfeldt, C., I. Fiedler, G. Dietl, and K. Ender. 2000. Myogenesis and postnatal skeletal muscle cell growth as influenced by selection. *Livestock Prod. Sci.* 66:177–188.

Rehfeldt, C., G. Ott, D. E. Gerrard, L. Varga, W. Schlote, J. L. Williams, and L. Bünger. 2005. Effects of the compact mutant myostatin allele $Mstn^{Cmpt\text{-}dl1Abc}$ introgressed into a high growth mouse line on skeletal muscle cellularity. *J. of Musc. Res. and Cell Motility.* 26:103–112.

Solinas-Toldo, S., C. Lengauer, and R. Fries. 1995. Comparative genome map of human and cattle. *Genomics.* 27:489–596.

Stone, R. T., J. W. Keele, S. D. Shackelford, S. M. Kappes, and M. Koohmaraie. 1999. A primary screen of the bovine genome for quantitative trait loci affecting carcass and growth traits. *J. of Anim. Sci.* 77:1379–1384.

Van Laere, S.-A., M. Nguyen, M. Braunschweig, C. Nezer, C. Collette, L. Moreau, A. L. Archibald, C. S. Haley, N. Buys, M. Tally, G. Andersson, M. Georges, and L. Andersson. 2003. A regulatory mutation in IGF2 causes a major QTL effect on muscle growth in the pig. *Nature.* 425:832–836.

Wheeler, T. L., L. V. Cundiff, S. D. Shackelford, and M. Koohmaraie. 2004. Characterization of biological types of cattle (Cycle VI): Carcass, yield, and longissimus palatability traits. *J. Anim. Sci.* 82:1177–1189.

Wiener, P., I. Maclean, J. L. Williams, and J. A. Woolliams. 2000. Testing for the presence of QTL for milk production traits in new populations. *Anim. Gen.* 31:385–395.

Wiener, P., J. A. Smith, A. M. Lewis, J. A. Woolliams, and J. L. Williams. 2002. Muscle-related traits in cattle: The role of the myostatin gene in the South Devon breed. *Gen. Sel. and Evol.* 34:221–232.

Williams, J. L., A. P. Usha, B. G. D. Urquhart, and M. Kilroy. 1997. Verifying the identity of bovine semen using DNA microsatellite markers. *Vet. Rec.* 140:446–449.

3 Automation for the Modern Slaughterhouse

Graham Purnell
Food Refrigeration and Process Engineering Research Centre, University of Bristol

Mark Loeffen
Mark Loeffen & Associates Ltd.

CONTENTS

3.1 Industrial Drivers for Slaughterhouse Automation 45
3.2 Risks and Pitfalls of Automation 46
3.3 System Components 48
3.4 Generic Meat Automation Systems 50
3.4.1 Automation and Hygiene 50
3.4.2 Automated Grading 50
3.4.3 Automated Chill Rooms 50
3.5 Automation for Pork Carcass Production 50
3.5.1 Pork Killing 51
3.5.2 Pork Dehairing 51
3.5.3 Pork Evisceration 52
3.5.4 Pork Splitting 53
3.5.5 Pork Grading 54
3.5.6 Pork Carcass Break-Up 54
3.5.6.1 Pork Primalization 54
3.5.6.2 Case Example: The ARTEPP Pork Primalization Robot 55
3.5.7 Pork Boning 60
3.6 Automation for Beef Carcass Production 60
3.6.1 Beef Killing 60
3.6.2 Beef Dehiding 61
3.6.3 Beef Evisceration 62
3.6.4 Beef Carcass Splitting 62
3.6.5 Beef Carcass Break-Up 62
3.6.5.1 Beef Primalization Automation 62
3.6.5.2 Beef Boning Automation 63

3.7 Automation for Lamb Carcass Production 63
3.7.1 Sheep Killing 64
3.7.2 Sheep Pelting 64
3.7.3 Sheep Evisceration 65
3.7.4 Sheep Carcass Break-Up 65
3.7.4.1 Sheep Primalization Automation 65
3.7.4.2 Sheep Boning Automation 66
3.8 General Trends 67
3.9 Conclusions 68
3.10 The Future 69
References 69

Tools used in the meat industry range widely, from simple knives wielded by a butcher, to autonomous systems for complex tasks such as evisceration or optimal carcass break-up. Although grammatically the term *automation* can be used interchangeably with the term *mechanization,* it is more common to use mechanization to describe simple powered equipment that has little sensing or adaptation to the task or work piece, and automation to describe more advanced, sensory-guided, adaptive machinery. A number of approaches can be embodied in automation to solve the problems of dealing with product variation in a number of ways. Some use advanced sensing, some use stored knowledge of statistically likely variations, and others modify the process to utilize machinery strengths.

Mechanization includes simple powered devices with little or no sensing, such as the overhead rail to transport carcasses around the slaughterhouse or mechanical splitting saws that remove the need for human effort. Such items are exceptionally useful and vital to the throughput of the modern slaughterhouse, but they are not considered as automation in this chapter.

Justification of automation in manufacturing is a complex process, mainly dependent on the production rate and flexibility required in the process. Although mechanization is suitable for manufacturing industries with consistent products such as automobiles, electronics, and so on, the inherent biological variability in animals requires more sophisticated automation processing solutions. High production rates favor dedicated machinery that tends to be inflexible. High process flexibility can be achieved with human staff but lower throughputs and greater processing variance must be tolerated. Automation using robotic-type devices is suited to medium production rates, as seen in slaughterhouse operations.

Simple automation such as a simple sensor-controlled door or a no-touch tap can make a significant difference with only a small outlay and level of disruption and risk. However, this chapter considers the larger processing systems for major tasks in the pork, lamb, and beef slaughter production line.

Although specific cutting and dressing methods vary throughout the world, the same basic processes, shown in table 3.1, are required to produce pork, beef, and lamb carcasses.

TABLE 3.1
Slaughter Operations

	Pork	Beef	Lamb
Killing	Stunning	Stunning	Stunning
	Shackling	Sticking	Sticking
	Bleeding	Bleeding	Bleeding
Dehairing/skinning	Scalding	Belly hide splitting	Y-cut
	Dehairing	Hide pulling	Defleecing
	Singeing		
	Polishing		
Evisceration	Rectum loosening	Rectum loosening	Rectum loosening
	Belly opening	Belly opening	Belly opening
	Organ removal	Organ removal	Sternum splitting
	Sternum splitting	Sternum splitting	Organ removal
Splitting	Preparation	Backbone splitting	
	Backbone splitting		
		Grading and inspection	
		Chilling	
		Carcass break-up	
		Primalization	
		Boning	
		Trimming	

The majority of these operations are currently performed manually with simple tooling. Automation has much to offer the slaughter industry but significant technical and business hurdles need to be cleared before there can be widespread uptake of technology.

3.1 INDUSTRIAL DRIVERS FOR SLAUGHTERHOUSE AUTOMATION

There are a wide variety of commercial and quality reasons leading many companies to investigate and apply automation to meat production lines. Ultimately all drivers have the same aim: increased profitability. If no profit or long-term benefit is foreseeable, no changes will be implemented. The use of automation in the slaughterhouse in place of human operatives has many potential benefits, which might be tangible, intangible, social, or economic. Many generic drivers are quoted by the meat industry for the introduction of automation, including the following:

- *Difficulties in recruiting staff.* There is a shortage of skilled labor for many of the tasks in the meat industry. The work is typically repetitive, physically intensive, and takes place in an unpleasant environment.
- *Staff safety and welfare.* Removing staff from repetitive, high-concentration tasks leads to greater job satisfaction and reduced risks of accident or repetitive strain injury. Injury occurs to both experienced and trained

staff, illustrating that it is the nature of the work rather than inexperience that causes the danger. Cuts made with high force toward the body, bad knife design, and cold fingers contribute to the poor safety record (North 1991). In an increasingly litigious society the cost of employer liability insurance is an additional concern.

- *Food safety.* The human operative is a major factor in introducing microbial and foreign body contamination. The costs of preserving hygiene with the large numbers of staff present in a normal meat plant increase overall production costs.
- *Production quality.* It is widely accepted that meat cuts best in the range from 2 °C to 5°C. As the temperatures fall, the cut quality improves, but cutting forces increase (Brown, James, and Purnell 2004) to an extent where human strength could be insufficient to maintain production rates. Automation can be used to exert higher forces, maintaining or improving cutting quality and production rates.
- *Product consistency.* Automation typically performs a task more consistently than a human. Boredom, stress, and tiredness are not a problem.
- *Process control.* Automation can make subtle adjustments beyond the skill of a human operative, or be endowed with "superhuman" sensory, recall, reasoning, or other capabilities (e.g., infrared detection, increased strength, X-ray vision, huge memory, etc.). Machines can be designed to operate under conditions in which humans could not perform effectively. This can allow processing in environments beneficial to quality (e.g. sustained low temperatures, aseptic atmospheres, etc.). "Getting things right" reduces waste and increases overall yield.
- *Legislation.* The minimum legal continuous working temperature for a standing, active laborer in the United Kingdom is 10°C (U.K. Factories Act 1961). European Economic Community (EEC) directive 95/23/CE states that during cutting meat, temperatures should not exceed 7°C and the processing rooms should be at a maximum of 12°C. Automation and robotics can work closer to the optimum temperatures than can be legally achieved with human operatives.
- *Traceability.* Traceability is of increasing importance across all food production processes and meat is no exception. Although the sensory information inherently required for automation of many tasks might give the opportunity to collect traceability data as a matter of course, uncontrolled application of automation can adversely affect traceability systems (International Consultative Group on Food Irradiation 1999).

3.2 RISKS AND PITFALLS OF AUTOMATION

In the last decade, many of the technological barriers to automation of meat production have been reduced or removed. Business and commercial factors are now becoming the predominant limiting factor. The automotive industry has been very successful in implementing automated processing. Regular components and a high-value product coupled with relatively low production rates make vehicle production

an ideal process for automation. Despite the product and process differences, some business experiences and observations can be transferred into the meat sector. A longer term, less risk-averse company culture is required, and employees at all levels must be prepared to change. Where automation projects have failed is often in the lack of buy-in throughout the company and lack of awareness of the skills and organizational changes required to support the implementation.

The same organizational risks apply to the food sector, with additional challenges of high product variability and a constricting market structure. The low margin on most meat products reduces the money available for investment and a marketplace dominated by major multiple retailers exacerbates the situation. The majority of labor in the food sector is unskilled, and thus sums saved by manpower substitution are low. Supply, demand, and processing specifications are flexible, seasonal, and regional.

From an automation viewpoint, the complexity of carcass production tasks should not be underestimated. Human staff members are innately dextrous, flexible, and well provided with integrated sensors. The majority of tasks in meat production utilize these inherent abilities. Most automation systems have a limited decision-making ability. Humans are excellent at evaluating situations and acting accordingly. A machine system has a predestined function and correction of only a limited number of possible errors can be incorporated into the design. Any automated system to replicate even a small subset of human abilities can require sophisticated systems integration.

Many current food plants lack the in-house skills to specify and support automation systems. The skills required stretch beyond the engineering function to specify, install, and maintain the system. Management and production staff working alongside the automated systems need to understand the strengths and weaknesses of the equipment and adjust practices accordingly. The entire organization, from cleaners to directors, has to embrace a positive mindset toward the automation of traditionally manual operations. Inappropriate attitudes at any of many levels can cause automation projects to fail.

A traditionally conservative, cash-poor meat industry with low margins has some fundamental financial and attitudinal business challenges in implementing automation systems.

Despite the advances in meat automation progress made in recent years, the greatest technical problem is still that of coping with the natural biological variation in the product. Variable products require variable production strategies and flexible processing methods. This has implications for sensing systems and system elements in contact with the meat such as fixtures, grippers, and cutting tools. Many meat products are relatively delicate and can be damaged by inappropriate handling. These factors tend to exclude direct technology transfer from other industries.

The secondary technical challenge is in equipment longevity and suitability for food production environments. Hygienic and robust systems to resist high-pressure washdown, cold, and condensation can be designed and built, but at additional cost and complexity. This further increases costs for implementation of automation for food production.

Automation technology is still a long way from the general robot-type system capable of replacing people in most food operative situations as envisaged by Khodabandehloo and Clarke (1993).

3.3 SYSTEM COMPONENTS

For a typical meat production task (figure 3.1), the operator uses his or her senses to assess each input product and compares it to the required output product. Then, using his or her acquired experience from previous performance of the task and an appropriate tool, the operator performs the required processing actions (figure 3.2).

Throughout the process, the operator is sensing progress and effort and reacting to changes to complete each action. Similar process steps and requirements are required for an automated system to perform the task (figure 3.3).

Sensors are required to gain information about each individual meat section and monitor progress during the task. A task description is required to interpret the specific information to produce an action plan, and an actuation device is necessary to carry out the process required on the meat section. Various levels of intelligence and feedback are required to accommodate process variation and react to errors.

Data interpretation and control functions are common to automation systems across many industrial sectors. The key developments for automation in the food industry are in the sensing and end effector or tool subsystems that interact directly with the meat.

FIGURE 3.1 Manual meat cutting.

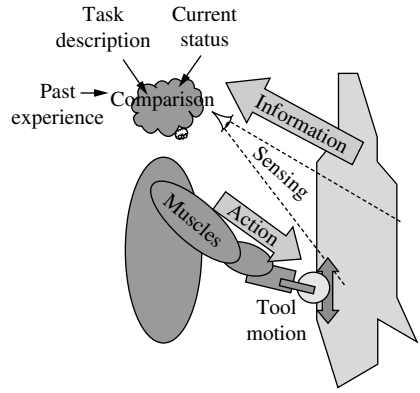


FIGURE 3.2 Information flow.

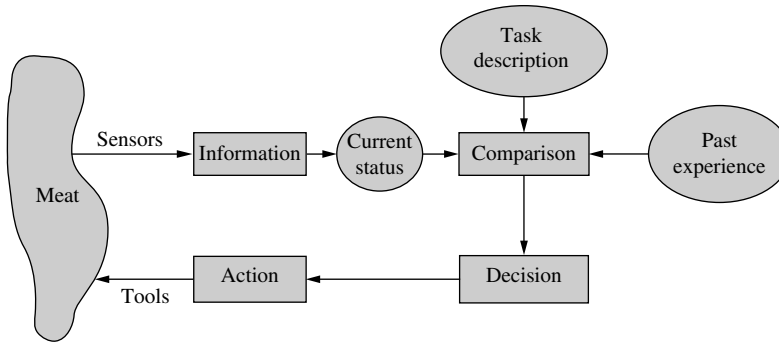


FIGURE 3.3 Generic meat automation machine system schematic.

Cutting and separation are the most common operations in carcass disassembly during the slaughter process. A number of novel cutting methods such as lasers, water jets, and ultrasonic tooling have been investigated for automated meat cutting. However, mechanical blades are the most common method of cutting. These are robust and well-accepted tools, although the underlying science of their cutting action is still to be fully understood (Brown et al. 2004). Water jet cutting has a small niche in cutting planar products such as fish and poultry filets, but is not commonly used in red meat production.

The predominant sensing subsystem used in meat industry automation is machine vision. Many manufacturers have equipment that is suitable and used within the food sector. Image capture devices can be placed remotely from the operating site and thus removed from the rigorous cleansing regimes that have to be endured by equipment in close and direct contact with the meats. Vision is also very applicable to the complex data extraction required to enable intelligent processing of meat products.

3.4 GENERIC MEAT AUTOMATION SYSTEMS

3.4.1 Automation and Hygiene

A key aspect of food safety is hygiene. Automation systems are now becoming available for hand hygiene (Anonymous 1998; Attec 2004). Turnstile access to the plant food areas is only permitted once a controlled handwashing and sanitation process has been performed. These automated measures show more consistent handwashing effects than sink-based washing (Paulson 1993). Improved hand hygiene gives benefits in longer shelf life and improved product safety (Field 2004).

There is a popular opinion that automation, by removing staff from the production process, can improve the microbial condition of the processed meat. A number of studies of specific systems support these suppositions (Clausen 2002; Holder, Corry, and Hinton 1997) but cleanable design of the often-complex machinery is a concern in many cases.

3.4.2 Automated Grading

Automatic grading and classification systems compare an image of each carcass against a standard reference carcass image. This procedure is impartial and removes variation due to individual graders. The captured image can be stored and used for traceability, production management, or process quality audit.

Watkins, Lu, and Chen (1999) projected that switching to an automated poultry inspection would be worth $1.5 billion to $2.5 billion to the U.S. broiler industry over five years, but further testing and more robust equipment were required to realize these benefits. In recent years machine vision meat inspection systems have improved, but there is relatively slow commercial uptake. Although laboratory development systems show the potential for rapid, economic, hygienic, consistent, and objective assessment systems, there are still limitations in the industrial environment (Brosnan and Sun 2002).

3.4.3 Automated Chill Rooms

Certain wavelengths of visible light can reduce shelf life and encourage rancidity of stored chilled meat (Field 2004). Automation to move carcasses in darkened chill rooms could improve product quality through reducing a contamination route from the human operative to the meat and reducing the spoilage organism growth rate. This type of automated carcass loading and unloading system has been commonplace in the New Zealand sheep meat industry for the last 20 to 30 years. It is one possible step toward the "lights-out" fully automated food factory.

3.5 AUTOMATION FOR PORK CARCASS PRODUCTION

Automation of pork production processes has received considerable research and development (R&D) attention in recent years with many systems now on the market. Dutch researchers at TNO (Paardekooper, van der Hoorn, and van Dijk 1994) reported progress on a large pork slaughter automation project called Slaughterline 2000.

They were developing an advanced slaughter line for pigs including stunning, sticking, bleeding, automatic gambrelling, carcass cutting and evisceration, robotic application of grading stamps and EC stamps, automatic head and loin deboning, carcass identification, voice control, and video imaging systems.

The Danish Meat Research Institute (DMRI) has been involved in many key developments and has a stated goal of producing a virtually fully automated pork process line by the end of 2005. Some operations such as shackling, sticking, gambrelling, veterinary inspection, final trimming, and removal or separation of specific organs are not included in the plan (Clausen 2002). This ambitious target can be attempted due to the cooperative and nationally integrated structure of the Danish pork industry, research establishments, and equipment producers. Since 1998 over €40 million has been spent developing pork automation systems (Wiegand 2004).

3.5.1 Pork Killing

After animals are delivered to the slaughter plant they undergo a rest period in the lairage before slaughter. This eases product flow in ensuring there is always a raw product supply for the slaughter line, but it also introduces other problems such as fighting among already stressed animals. Any stress in the live animals is detrimental to finished meat quality. The enforced herding required to move the animals around the lairage increases stress further. An automated lairage in which these movements are performed gently and without human presence was developed in Scandinavia in the early 1990s and is now commercially available (SFK 2004).

There are many systems to convey animals to the stun station, most consisting of V-shaped conveyors to carry the pig to the stun operator. Electrical stunning is carried out by a human operative for animal welfare reasons and due to the complexity of accurately applying stun electrodes to a live, moving animal. However an alternative stunning method in which carbon dioxide (CO_2) is used to render unconsciousness has been automated.

The automated CO_2 stunning units operate like enclosed ferris wheels, with multiple compartments rotating cyclically. About six pigs are herded into each compartment. The compartment then descends into a deep well area filled with CO_2, emerging on the opposite side to pig entry where the compartment tilts, and the animals slide down a chute to the shackling line below. Residence time is typically three minutes, with a set point of 82% CO_2 (Butina 2004). Although this stunning method can be automated, there are some concerns for animal welfare (Grandin 1992).

Once stunned, animals are manually shackled, usually with a chain loop around one hind leg, and hoisted to hang head down. A human operative then makes the throat cut to drain the blood. These shackling and cutting operations are complex and difficult to automate due to the complexity of the operations, the implications for downstream processes if performed incorrectly, and the need to maintain animal welfare.

3.5.2 Pork Dehairing

Operations to dehair the carcass are mechanized and a variety of companies have been producing such equipment for many years.

Once drained of blood, the carcass passes through a sequence of mechanized operations typically consisting of a hot water scald to loosen hairs, and a passage through a dehairing machine where rotating metal-tipped rubber fingers brush most of the hairs from the carcass surface. This is followed by a singeing operation where the carcass passes through a series of gas flames to burn off the remaining fine hairs. Finally it passes through a second burnt hair removal or polishing operation. Some plants wash the carcass at this stage.

These operations avoid the need to adapt to carcass geometries by using techniques that conform to the product shape. Fingers on flexible rubber mounts, gas flames, and water jets can all act on the carcass without detailed knowledge of surface position. This approach allows simpler mechanization to be used for these tasks.

3.5.3 Pork Evisceration

This task is particularly unpleasant and arduous, and there are substantial hygiene implications of mistakes. Work at the DMRI in the early 1990s (figure 3.4) and a later collaboration with SFK-Danfotech has developed automation for pork evisceration (Madsen and Nielsen 2002).

The equipment makes a few simple anatomical measurements that guide the process. Conformation of the flexible carcass to fixed machine trajectories is also used to reduce complexity and hence increase reliability of the system. The equipment consists of a measurement station, followed by a processing station. An evisceration cycle proceeds as follows:

1. The carcass arrives with belly and sternum opened, and the bung released.
2. The carcass is pushed into the evisceration system by the motion of the line conveyor.
3. Measurements are taken and process path start points are determined.
4. The tools move to starting positions.
5. Clamps move in to hold the carcass to the back support.
6. An intestine shovel lifts the organs hanging out from the previously opened carcass. This exposes, and allows access to, the chest cavity at the sternum.
7. Leaf fat brackets enter at the sternum and are opened.
8. Additional thorax arms are inserted to open the carcass at the throat.
9. The intestine shovel is released, allowing the organs to pass down between the leaf lard brackets.
10. Knives built into the leaf brackets cut around the periphery of the diaphragm.
11. A back cutter is then moved into the carcass to penetrate the diaphragm adjacent to the spine.
12. The back cutter is then traversed upward along the spine to sever the connective tissue between the organs and spine in the hind section of the carcass.
13. A tenderloin knife moves downward, releasing the tenderloin from the spine.

14. The tenderloin tool is then placed on the diaphragm with predetermined force to act as a resistance to the leaf fat loosening operations (Steps 15 and 16).
15. The leaf fat brackets move upward inside the pig, passing between the leaf fat and abdominal wall, thus completely detaching the leaf fat.
16. The tenderloin tool continues to progress down into the thoracic cavity ensuring all possible adhesions between the lungs and cavity wall are severed.
17. The thorax arms open the thorax further.
18. The released organs are then pulled forward out of the carcass with a horizontal movement of the tenderloin tool.
19. The clamps are released and the carcass is moved out of the supports.
20. The tools are washed before the next carcass arrives.

Steps 6 through 10 are carried out simultaneously with Steps 11 through 13. This automated evisceration system performs all these operations in 10 seconds, giving a line speed of 360 carcasses per hour. DMRI is currently working on equipment for the subsequent separation and sorting of the organs. Microbial analysis has shown that carcasses automatically eviscerated possess fewer pathogens (*E. coli*) and aerobes than conventionally eviscerated carcasses (Clausen 2002).

3.5.4 Pork Splitting

Automatic carcass splitting has been available for many years. Suppliers such as Stork, SFK, Danfotech, Durand, Automeat, and others sell these systems. These machines have a range of cutting actions and complexities. The basic systems use a simple downward motion of a circular saw through the space where the carcass should be. A higher level of complexity uses a series of rollers to locally position the spine onto the cutting saw.

Back finning is sometimes carried out as part of splitting. This process reduces damage to the eye muscle during the splitting operation by separating it from the dorsal spine "fins" before splitting the carcass. An automated system using a relatively complex arrangement of rotary knives, plain blades, and active rollers has been developed for this task in the Danish pork industry.

Most equipment producers claim an increased accuracy of automatic carcass opening and splitting over human-based splitting operations. However, the experience of some users is that there is still deviation from the precise center line of the carcass. This can cause problems for carcass inspection and subsequent automated systems using the spine as a reference or datum position.

3.5.5 Pork Grading

Automated carcass weighing systems are common on many slaughter lines. There has been much R&D work investigating automatic systems for pork grading. Canadian researchers took an interesting sensing approach using a robot, laminar water jets, and ultrasound (Goldenberg and Seshan 1993). DMRI has developed the Danish

Carcass Classification Centre, and SFK produces an automated grading system called AUTO-FOM (Madsen and Nielsen 2002). Noninvasive machine vision systems that are in development, but some studies show them to be less accurate in predicting saleable yield than existing technology (McClure, Scanga, Belk, and Smith 2003).

3.5.6 Pork Carcass Break-Up

3.5.6.1 Pork Primalization

After primary chilling, pork carcasses are commonly cut into smaller "primal" sections that are then further subdivided into retail joints, boned out, or processed into a wide variety of products. Various dedicated automation solutions exist for boning and preparing individual primals, but of major importance are automation systems to produce primals. Because of relative price differences among primals, accurate control of cut paths is vital to optimize overall carcass value.

Early work on robotic systems for pork primalization was performed in Western Australia (Clarke 1985). The system comprised a computer-controlled pork carcass break-up machine that automatically broke down a full carcass into eight pieces in less than 30 seconds.

Automated cutting systems that separate a pork half carcass into fore, middle, and hind sections were developed in Europe by DMRI and partners in the early 1990s. The commercialized equipment is produced by Attec in Denmark and Itec in Germany (Clausen 2002; Folkman 1995). The tenderloins, head, and forefeet are removed as preparatory operations, then carcasses hanging on a standard gambrel are pulled across a conveyer belt and the hind feet are cut off. This releases them from the gambrel onto the conveyor. At a second station each carcass side is moved against datum surfaces and the length between the pubic bone and the foreleg is measured. This measurement is used to position circular saws further down the line to anatomically derived cut positions for that carcass side. A second machine is available for the longitudinal cut to separate the belly from the loin.

Recently a robotic solution that performs all cuts in a single system has become available. The Advanced Technology for Efficient Pork Production (ARTEPP) system was developed as part of a European Union (EU) funded project (European Commission 1998) and is commercially available from BANSS in Germany and Attec in Denmark. The equipment has been patented and is arguably the most advanced robotic meat production system available to date. Because of the need for accurate cut placement, compliance of the carcass is not used and each cutting path is specifically adapted to the individual carcass being processed. The development of this system is examined in section 3.5.6.2. Significant interaction among various expert organizations in cutting blade design, machine vision, robots, systems integration, and meat production were required for the project to be successful.

3.5.6.2 Case Example: The ARTEPP Pork Primalization Robot

In initial R&D studies, model-based machine vision analysis was used to determine cut paths for a fixtured pork carcass. A purpose-built Cartesian food grade robot then wielded a pneumatic cutting tool to make the cuts required (figure 3.5).

Although successful cutting was demonstrated, several factors limited the industrial exploitation potential of the system. The gantry-based Cartesian robot was very large, did not withstand the rigors of the food production environment, and spare parts and engineering support were not readily available. The pneumatic cutting tool was prone to stalling at high cutting duty, thus limiting the possible cutting rates. The cut path generator using a model-based machine vision approach, although elegant and robust, was computationally intensive, relatively slow, and relied on a model that would require tuning for the pig supply to each plant where the system was installed. Although satisfactory for R&D equipment, system cleanability and hygiene were poor for commercial use.

Two different cutting schemes (see table 3.2) were selected for process development targets.

The prime approach for the final system was to use off-the-shelf equipment to avoid development time and costs within the project, and to produce a system with

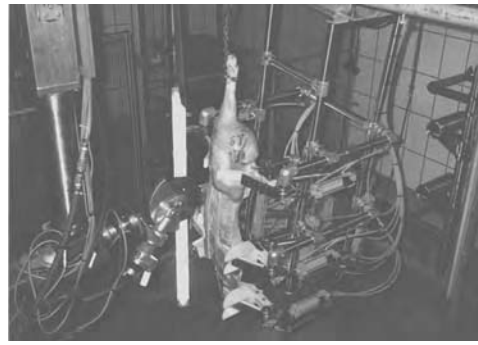


FIGURE 3.4 Initial pork primalization system.

TABLE 3.2
Hot and Cold Butchery

	Cold Butchery	Hot Butchery
Location	Norway, typical of northern Europe	Spain, typical of southern Europe
Temperature	Chilled carcass sides (3°–5°C) about 24 hours postslaughter	Warm carcass sides (25°–30°C) about 15 minutes postslaughter
Support	Hook behind hind Achilles tendon	Looped chain around hind leg
Orientation	Random	Split side toward robot
Speed	225 sides per hour	900 sides per hour
Cuts	3–6 cuts per side (seasonal variation)	1 or 2 cuts per side (conformation dependent)

readily available spare parts and engineering support. The system architecture is shown in figure 3.6.

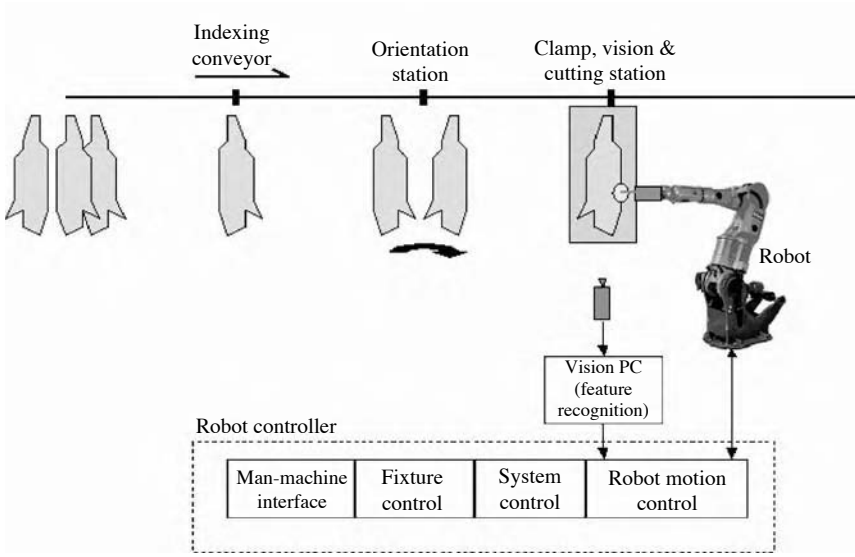


FIGURE 3.5 ARTEPP system architecture.

A six-axis KUKA KR125 anthropomorphic robot arm was used as the central component. The multiple input/output and communications facilities of the robot system controller were also used as the cell supervisory system.

The robot was enclosed in a food grade cover for cleanability. Dry air was ducted into and from the cover to maintain a positive pressure to do the following:

- Keep the cover away from robot joints and trapping points.
- Reduce the condensation effects of operating a heat-producing machine in a cold, moisture-laden environment.
- Prevent ingress of any food materials.

This washdown robot is available as a standard product from BANSS Maschinenfabrik, Germany.

The development of the cutting tool for the ARTEPP system illustrates some of the fundamental differences between human and machine performance of the same task. A pneumatic powered cutting tool as used by a human butcher would stall when used at robot speeds due to the higher cutting forces generated. The humans' strength limit regulates the cutting process, as the cutting forces build up; the human slows, allowing the tool to make the cut. With a higher strength robot making the cut, the separation made by the pneumatic tool did not keep pace with the rate at which the robot was moving the tool along the path. Using a more powerful three-phase electric saw and developing a special blade for high-speed cutting of meat and bone reduced these difficulties. The blade design is currently the subject of a patent application, but it produces lower cutting forces, higher quality cut surfaces, and less bone dust than standard saw blades. This gives a lower yield loss and better

hygiene. The cutter unit has a guard fitted to reduce the amount of bone dust and meat debris sprayed around as the cutter moves. This eases the cleaning of the cell, as most waste material is contained at the cutter.

Carcass transport and handling requirements vary considerably among plants and butchery styles. Separate approaches were taken for the hot and cold butchery examples. However, common factors in the handling and fixturing subsystems were required for the remainder of the system to be standard:

- Carcass sides must be presented split side showing to the vision system on a contrasting (blue) background.
- The fixturing must resist the cutting forces for the cuts performed.
- The side must be in a known position before the start of each cut.
- The fixture must accommodate the full size range of carcass sides
- The position of all carcass sides within the ARTEPP system must be known.

As long as these transport requirements are met, the remaining ARTEPP subsystems can be used with almost no modification. This modular approach eases application of the automation to the differing production processes seen from plant to plant.

For the cold butchery system, three to six cuts per carcass are required at 225 sides per hour. Incoming pork sides are orientated by rubbing bars to align the split plane to the overhead rail. At the orientation station, use is made of a previous processing line feature in that the hook through the Achilles tendon always faces the split plane. An inductive sensor detects the hook and the side is rotated if the split plane is not facing the vision system side of the rail. The side indexes on to the cutting station where adaptive gripper fingers grasp the side and prevent lateral motion, and a fixture board moves in from behind to clamp the side against the fingers and partially support the carcass side at an angle of 10 degrees. Shaped features on the fixture board aid lifting and side location; they also resist cutting forces. Once clamped, vision processing and cutting takes place, the clamps are released, and the side is ejected from the system. An indexing overhead conveyer drives the carcass through the ARTEPP cell and inductive sensors detecting the gambrels track carcass position within the cell. This approach is secure and necessary for the relatively high-force cuts made at the cold butchery plant. However, it is only possible because of the relatively slow line speeds.

For the hot butchery system, one or two cuts per carcass are required at 900 sides per hour. Here higher line speeds but fewer cuts per carcass require cutting to take place as the carcass moves along the overhead line. Because each carcass requires fewer cuts, less clamping for cut force reaction is required, which in turn enables a higher line speed to be achieved. A previous process on the production line ensures pork sides are always facing split side toward the vision side of the ARTEPP system. The overhead rail carries each side onto an inclined support conveyor synchronized with line speed. The rail and conveyor carry each side past the vision sensing and cutting stations at a fixed speed. The image processing is performed as the side travels to the cutting station. Because the image capture time is short, a moving carcass does not affect the carcass appraisal. At the cutting station,

the robot motion, support conveyor, and overhead line are all synchronized, rendering the carcass effectively stationary. The fixturing and handling for hot butchery is less secure than that for cold butchery, but given the low cut forces and low number of cuts required, this has proven satisfactory.

For analysis of the cold butchery system, 110 sides were cut either manually or by the ARTEPP robotic system. Cuts from both methods were compared with the optimal definition of the cut. For manual butchery, cut placement was nominally within 20 mm of the correct location and 89% of manually cut carcasses had cut accuracy of better than ±5mm. The ARTEPP system performed to better than ±5 mm for 97% of cuts (figure 3.7).

The automated system cut more evenly and cuts were more anatomically accurate than with manual cutting. The placement of the high-force H-bone cut, and the angle of the ham cut, were substantially more consistent than with manual cutting (figure 3.8).

The ARTEPP demonstration production system clearly illustrated an ability to exceed human performance at pork carcass cutting. The system can tirelessly produce consistent, anatomically accurate cuts. However, the most important commercial

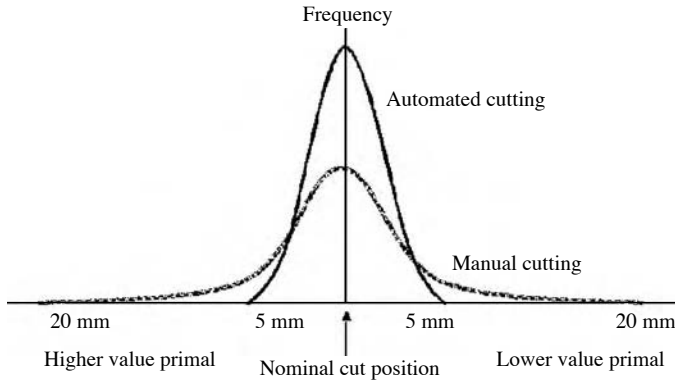


FIGURE 3.6 Robot vs. human cutting performance.

FIGURE 3.7 Carcass sides cut with ARTEPP system.

FIGURE 3.8 Production ARTEPP system.

feature of the automated primal cutting system is that it can be used to finely adjust cuts in response to seasonal and market price fluctuations. Shifting cuts to favor high-value primals (moving "automation" peak to the left in figure 3.7) can result in significant value improvements for each carcass. Other cost benefits connected with not having to find, train, and retain human staff for the task are a bonus.

The latest system (figure 3.9) has been used online for a full year in a Norwegian cold butchery plant doing the work of three staff with a 3% yield improvement. The equipment costs are calculated to be paid back in less than 18 months.

3.5.7 Pork Boning

One of the main advantages of the ARTEPP pork primal cutting system is the ability to control precise cut trajectory on the loin–belly separation cut. Robotic technology has also been used to bring this benefit to separating pork flank ribs from the pork belly (Anonymous 2000). The system uses a machine vision system to assess the size and shape of an incoming belly. The three-dimensional data are used to calculate the cutting path. A Fanuc M710 robot equipped with a curved, double-edged Denver knife executes the path, pulling the shaped knife through the belly in the prespecified trajectory. The robotic cell includes automatic tool changeover and can select from eight different knives. When not in use, knives are sterilized as part of the production process. This system can process 1,400 bellies an hour, equivalent to a six-man crew.

Final trimming and manpower requirements are reduced and the yield is optimized over both the belly and flank rib set.

Automation and dedicated machinery for boning out of specific pork sections are commercially available or under development in many parts of the world. Much of this work has been led by DMRI and its commercial partners, and includes boning equipment for fore-ends and hind legs, and combined boning and trimming equipment for belly and loins (Madsen and Nielsen 2002).

3.6 AUTOMATION FOR BEEF CARCASS PRODUCTION

Beef butchery processes are particularly arduous because of the size and weight of cattle carcasses. Although automated systems could reduce the physical nature of the tasks, beef slaughter automation has received relatively little automation R&D effort compared to lamb and pork.

The key challenge for automation is the large variation seen in cattle. Slaughter animals can be from a wide variety of breeds, ages, and types (bull, steer, heifer, cow, etc.), ranging in weight from 200 kg to 1,000 kg. The variations seen in other carcass types are substantially less.

Mechanized processing aids guided by human staff have been in existence for many years, but the Fututech Australian R&D program (White 1994) sought to develop the world's first truly automated beef processing line. The system was developed through to a commercial prototype stage and designed for a minimum processing rate of 60 carcasses per hour. The system included a large number of automated or semiautomated modules that performed the majority of the slaughter tasks. These modules included rectum clearing and bagging, aitch bone cutting, head removal, brisket cutting, evisceration, and tail cutting.

3.6.1 Beef Killing

The stunning and sticking processes are ergonomically difficult and any errors have far-reaching effects on all downstream processes. Food Science Australia, a joint venture organization of CSIRO and the state of Victoria, is investigating automatic systems for these tasks (Food Science Australia 2004). This system is based largely on the Fututech module. The work is driven by cultural, animal husbandry, and occupational health and safety considerations. A machine vision system is used to determine correct stun and sticking locations.

The Fututech slaughter module separated one animal from a group of cattle using a moving floor conveyor that transferred the animal to a moving conveyor between the animal's legs as the floor dropped away (White 1994). Two bails captured the neck and applied an electrical current to stun the animal. The electrical pathway was then altered to effect a spinal inactivation.

A pneumatically powered knife with oscillating blades was used to enter the thoracic cavity and sever the aorta. Horns were also removed at this stage using hydraulic cutters.

3.6.2 Beef Dehiding

The first task of beef dehiding is to cut the hide from the crotch to the neck. This is a demanding task due to the length and consistency of the cut required. The cut required is typically 2 m or more in length, must be along the center line of the carcass, and must sever only the skin. Industrial Research Ltd. (IRL), based in New Zealand, has developed automation for this task (Templer, Osborn, Nanu, Blenkinsopp, and Freidrich 2002.). The profile of the belly is detected with an infrared laser distance sensor, and this information is processed to form a smooth trajectory for the cutting tool. The purpose-designed tool consists of a guidance spike mounted tangentially to a rotating circular knife. The spike protects the underlying meat from cutting damage and serves as an anvil to improve the cutting efficiency. The tool is moved by a purpose-built robot to place the spike between the skin and meat and then follow the previously determined path to sever the hide along the belly. The system has been proven in a slaughterhouse in Nebraska, successfully cutting many thousands of carcasses. Although the initial development work used a purpose-built robot, during commercialization of the work, plans are to use an off-the-shelf food grade KUKA robot.

Before this cut is made on feedlot cattle, there are often large "dags" or deposits on the skin that must be removed. In 2000, Food Science Australia staff developed a hand-held dedagging tool. A recent MLA project (2005) sought to automate this process using a robot. The project was not successful due to problems restraining the carcass while the robot was operating.

After the skin-opening cut is made, the hide is removed or pulled. Mechanical pulling arms supply the majority of the effort, but a human butcher is required to make specific preparatory cuts, attach the pulling mechanism, and make assisting cuts during the pulling operation.

The Fututech system used bed-dressing for hide removal where the carcass was resting on its back (White 1994). After appropriate manual hide preparation the carcass was suspended from four hooks, one in each hock, while remaining in the supine position. The hide was removed automatically using a three-stage process starting after the application of clamps to the hide. The first stage involved pulling the hide downward, the second separated the hide from the back fat using a blunt knife, and the third pulled the hide over the head and off the carcass.

3.6.3 Beef Evisceration

Once the cattle hide has been detached, the abdominal cavity is opened and the organs removed. Part of the opening process involves sawing the sternum bone to gain full access to the chest cavity. IRL have also been working toward automating this beef task (Templer, Nicolle, Nanu, Osborn, and Blenkinsopp 2000). Through projects running over a number of years, the team has demonstrated first static, then line-synchronized brisket sawing. Using the same robot and guidance system as the belly hide-opening system, a reciprocating bone saw was moved down the center line of the sternum. The tool was similar to, but more powerful than, a manually manipulated brisket saw. However, when implemented on the production line, the

equipment did not perform satisfactorily, as a large number of the carcasses were damaged in the previous dehiding process. This resulted in a twisted carcass at the sternum saw station. The automation was unable to cope with straightening the carcass and completing the cut in the nine-second cycle time available. Future plans involve using another KUKA robot for this task.

The Fututech system used an automated system comprising a paddle that was pushed against the spine and pulled down the carcass to peel the viscera from the abdominal cavity and push it into the viscera tray for sorting (White 1994).

3.6.4 Beef Carcass Splitting

Automation for beef splitting was among the first examples of mechanization in the slaughterhouse. Many equipment manufacturers now include beef splitting machines in their product range. Although this equipment eliminates the arduous manual process, many users of the equipment are still dissatisfied with its performance in terms of accuracy of splitting down the center of the spinal column and the hygiene aspects associated with deposition of bone dust and other detritus on edible surfaces of the carcass.

The Fututech system included a module that automatically split a beef carcass into two sides using a guided bandsaw (White 1994).

More recent work by Food Science Australia funded by Meat & Livestock Australia (2005) uses a robot to guide a band saw. The sensing system for finding the vertebrae is based on ultrasound, which has difficulties on some carcasses with voids caused by the hide puller. Work is currently being undertaken to solve this problem.

3.6.5 Beef Carcass Break-Up

3.6.5.1 Beef Primalization Automation

Mechanical boning aids exerting pulling forces while a human butcher makes key separation cuts as required have been used for many years. Although not at the forefront of automation technologies, these human augmentation systems have enabled higher throughputs with less physical effort for the same number of staff than using traditional individual cutting tables (Field 2004).

French researchers developed a prototype robotic system for subdividing beef forequarters (Damez and Sale 1994). The system was relatively slow because major sensing and trajectory planning problems had to be solved. The prototype worked but was never developed into a commercial system.

An ambitious beef sectioning system was proposed by the Texas beef group in a patent issued in 1993 (O'Brien, and Malloy 1993). A chilled eviscerated carcass is mounted horizontally on an automatic guided vehicle and appraised using X-rays, three-dimensional machine vision, and ultrasonic sensing. The results of the inspection are used to generate cutting paths to enable the carcass to be cut into optimal primal sections. A robot is used to effect this separation with high-pressure water, abrasive, and air jets. Flesh is cut with the water jet while the air jets keep the severed meat clear of the cutting area. The abrasive jet is invoked when cuts are to be made

through bone. This is a particularly high-tech proposal in a patent and it is not known whether the ideas were ever incorporated into a practicable system.

3.6.5.2 Beef Boning Automation

As with many other meat types, specialized automation systems for specific meat sections are under development or in production. A beef rib deboning system has been designed and manufactured by Food Science Australia. This machine will automatically strip the meat from a beef rib set in 21 seconds (Food Science Australia 2004). Longdell (1996) described other beef deboning machines for heads, loins, and forequarters. All systems improve carcass yield, but the levels of automation are low, with most systems providing mechanical forces with and without shaped blades (Trow and Ng 1994). A human butcher is required to operate the equipment and assist cutting in a similar manner to the primalization pulling arms mentioned earlier.

A vision-guided, force-feedback-controlled beef deboning research system has been constructed at the University of Bristol (Purnell, Maddock, and Khodabandehloo 1993). Although based in the laboratory, the work demonstrated the technical feasibility of sensory-guided robotic deboning, but further R&D would be required to bring the concept to commercial reality. The technique made an initial two-dimensional visual assessment of the meat joint, and sought to match that current meat section to a database of previous experience. If a match was found, the previous cut paths were replayed for the current meat section; if no match was found then force feedback from the boning blade was used to guide the robot along the bone and in doing so create another experience example to augment the database. This process showed promise for the two-dimensional deboning of beef forelimb taken as the example process. However these initial concepts would need to be extended substantially to produce a fully automated beef boning line for commercial use.

3.7 AUTOMATION FOR LAMB CARCASS PRODUCTION

Lamb and sheep farming and meat production play a major part in the economies of New Zealand and Australia. Not surprisingly, the majority of automation for these carcass types has originated in these regions.

Notwithstanding the comments on mechanization at the beginning of this chapter, researchers at the Meat Industry Research Institute of New Zealand (MIRINZ) have developed a series of machines for sheep processing that use minimal sensing or adaptation to the task or work piece. However, by rearranging the various tasks in the slaughter chain and by redeploying some labor to act as the "sensing" or adaptation element, relatively simple machines have been developed and commercialized with considerable success.

In the early 1980s researchers at MIRINZ developed an improved manual dressing system, later called the inverted dressing system because the carcass spent most of its time hung from the front feet (inverted when compared to a traditional sheep chain). With this simple change, the manning for an average sheep processing chain

was reduced from 45 butchers to 36 to 40 butchers achieving a throughput of 3,200 carcasses per shift (Annan 1982).

By 1990 a typical sheep chain making use of all available technology developed by MIRINZ over the previous 10 years required only 26 butchers (Authier 1990). This is almost half of the manning required for the traditional manual sheep chain of 10 years earlier.

3.7.1 Sheep Killing

A prototype automated sheep stunning machine was developed in the early 1980s (Richardson 1982). This machine was quickly commercialized and is now available from companies like Millers Mechanical (2004).

For a variety of reasons including ritual slaughter, automated sheep sticking systems have not been successfully developed to date.

3.7.2 Sheep Pelting

The sheep dehiding or pelting process is extremely complex and traditionally used 30% of the labor force on a sheep dressing chain (Longdill 1984). Early attempts to automate this process were reasonably successful although the machinery was complex (Robertson 1980). Researchers at MIRINZ developed a rotary pelting machine that automated the majority of the pelting process. The machine was physically large and operated on a rotary turret principle to achieve the required throughput. Commercial versions of this machine were installed in a number of sheep processing plants in New Zealand during the 1980s. Rotary turret dehiding machines have been superseded by the MIRINZ shoulder fleecer and final puller technologies described later.

Sheep dehiding generally starts at the head. One of the early operations, where initial incisions are made on the forelegs and chest, is called the Y-cut. Researchers at Industrial Research Ltd (IRL) developed a robot to perform the Y-cut (Taylor 1993). This robot was extensively tested in meat plants until it could operate reliably at chain speeds. Once proven, the robot was commercialized and is now operating in several meat plants in New Zealand.

Two small machines were developed by MIRINZ to automate the processes of wide-to-narrow transfer and front foot removal. Although these tasks are not strictly part of the dehiding operation, they are typically performed during the hide removal process.

Brisket clearing is another pelting subprocess that was automated by researchers from MIRINZ. Most slaughter staff considered this task physically difficult. This is an example of a human operator being assisted by a relatively simple machine. The operator performs all the sensing and delicate positioning operations, which are not physically difficult, but the machine is powered and can reliably and repeatedly perform the difficult and physically demanding tasks without tiring or becoming injured.

A machine called the shoulder puller has automated the heavy work associated with clearing the forequarter. This machine was released commercially in 1985

(Longdill 1984) with many units installed around the world. This technology is another example of the MIRINZ design team using a combination of skilled labor for sensing and positioning with machines that perform the heavy work.

The final in the series of pelting machines developed by MIRINZ is the final puller. This machine is deceptively simple in its operation although its design and setup are the keys to its successful operation. This machine was also released commercially in 1985 and has been installed extensively on lamb chains around the world.

3.7.3 Sheep Evisceration

Researchers at MIRINZ developed a mechanical sheep evisceration system in the late 1980s (Authier 1990). The system comprised a brisket and belly cutter, an eviscerator, and an offal-handling system. The system was evaluated in an Australian sheep processing plant and offered to several companies for commercialization (Authier 1994). The system is still awaiting commercialization. Later trials using a variant of the Y-cutting robot were aimed at opening the brisket and the belly in a more conventional chain configuration. This technology was also never commercialized.

3.7.4 Sheep Carcass Break-Up

Until the 1970s, most of the sheep and lamb traded internationally was in the form of frozen whole carcasses. After that time, carcasses began to be progressively broken down into a series of cuts, initially frozen and later chilled and vacuum packed. The main items of technology in a lamb boning room in the early days were band saws and packaging machines.

3.7.4.1 Sheep Primalization Automation

From 1998 to 2001 staff at AgResearch developed a machine for cutting lamb carcasses into primals (Meat New Zealand 2004). The machine was to produce clean, square cuts and hygienic handling of primals to improve product yield and shelf life from subsequent processing. A prototype machine was to be available during 2000 and further enhanced the following year by automatically locating bones within carcasses. There are no records of the machine ever being commercialized.

3.7.4.2 Sheep Boning Automation

The first research on automated sheep boning began at MIRINZ in the early 1980s as part of the mechanical boning project. Mechanical boning was designed to remove whole tissue meat from the bones as intact muscle as opposed to mechanical separation (meat recovery) that recovered meat from bones by crushing them under pressure (Roberts 1984).

The key technology developed in this project was the frame boner that removed the soft sides from the frame of the mutton carcass. Other work was targeted at automated fat trimming and leg deboning.

A follow-up project developed a commercial prototype of the frame boner (Wickham 1988). It was a fully automated machine comprising four main components: the load station, pedestal and carcass support, linear drive and boning head, and the control system.

The boning process consisted of the following steps:

1. The load station lifted the carcass off the rail, removed the gambrel, and loaded the carcass onto the carcass support.
2. The pedestal rotated the carcass support about the horizontal axis to present it to the boning head.
3. The linear drive cleared the pelvis by grasping and pulling the rear legs on the upward stroke.
4. On the downward stroke, a combination of rotating knives, flexible disks, ploughs, and a moving wire separated the soft meat sides from the skeletal frame.
5. The skeletal frame was ejected at the pedestal during rotation of the carcass support.

A programmable logic controller and range of sensors controlled the entire machine. The production rate was estimated at 190 carcasses per hour with a payback period of less than one year. However, this machine was never commercialized.

The frame boner laid the groundwork for a very successful second-generation boning machine (Wickham 1990). The machine consisted of a loin support mounted on a carriage that could move horizontally. The loin support located and gripped a loin saddle. The carriage then transported the loin through a set of fixed knives followed by a set of semirigid plastic ploughs. The frame for the knives and ploughs could move vertically to partially accommodate different loin sizes. The machine was tried out in New Zealand and commercially released in 1989.

The second commercial machine to come out of this program was the chine and feather bone removal machine (Ng 1992). This machine removed the chine (vertebrae) and feather bones from a loin saddle leaving the rib bones in place. The chine and feather bone machine was evaluated in 1991 and commercially released in 1992.

In 1992 MIRINZ (Wickham 1992) announced that three further boning machines were under development, namely the rib frenching machine, the shoulder fleecing machine, and the shoulder boning machine. Wickham also outlined plans for a fully automated sheep boning system.

In 1994 MIRINZ (Ng 1994) described the latest developments in shoulder fleecing and rack frenching. Industry trials of the shoulder fleecer were concluded that year and the rack frenching machine was reported as ready for industry trials. The operation of each machine was described in some detail. Neither of these machines were ever commercialized.

That same year Macpro (Roberts 1994) announced funding from the Meat Research Corporation of Australia to develop three machines almost ready for industry trials including a trunk boning machine for mutton, a forequarter fleecing machine for lamb processing, and a shoulder boning machine for both lamb and mutton.

To date, the trunk boning machine and a leg boning machine have been commercialized (Macpro 2005). In the trunk boning machine, after manual loading, the trunk is conveyed away from the operator. Two blades clear the meat from the vertebrae approximately 50 mm either side of the center line. The fleecing blades sweep around the ribs to separate the meat while a second set of knives simultaneously clears tissue from the neck. The leg boning machine tunnel-bones either chilled or prerigor mutton legs. The leg is placed vertically between the boning chucks. The two chucks move toward each other, boning the leg using a scraping and cutting action until the two chucks meet. The bone is finally ejected through the lower chuck. The patella remains in the meat after the boning process.

Macpro has developed several other machines that are currently ready for commercialization or industry trials. These machines include a shoulder boning machine similar in operation to the leg boning machine for the round shoulder bones, reducing the manual skill requirement for scapula and pelvis boning, and a lamb spinal removal machine. The current popularity of lamb and mutton shanks has created a market for a boning machine to remove only one round bone, either the femur or humerus.

Scott Automation, in association with meat processor PPCS, has developed a robotic system for boning lamb legs (Templer 2004). A KUKA robot has been fitted with a boning knife incorporating force feedback, allowing the robot to guide the knife along the bones of the lamb leg.

3.8 GENERAL TRENDS

Despite the wide range of slaughter automation systems, a number of general trends are common across a number of projects.

Initially many meat automation research projects developed bespoke robots for their particular task (Maddock, Purnell, and Khodabandehloo 1989; Taylor and Templar 1997; Templer et al. 2002; Wadie et al. 1995). In these projects, as the developments neared commercialization, the teams changed direction to use standard industrial robots, protected against the rigors of the food production environment. In conjunction with, and in some cases as a result of, these developments, the robot manufacturers involved have started incorporating food grade robots into their product range. This in turn provides off-shelf tools for other systems integrators and speeds the rate of development of automated slaughterhouse production systems. Coupled with this and the need for engineering support of automatic processing systems in the meat industry, there is now a realization that using standard off-the-shelf components has a great benefit. This renders equipment simple to develop, operate, and maintain (Ranger, Ottley, and Smith 2004). KUKA, ABB, Adept, and Fanuc are among the companies producing off-the-shelf food grade robots.

Despite the benefits of proactive quality measures and production process improvements, many food companies are slow to implement changes (Holt and Henson 2000). Customer demands are the main driver for most companies to implement changes. Buy-in to implementation of automation systems is required at the directorial and employee levels. Middle-level engineers and managers are often the first to spot the opportunity and benefit. However, the company can fail to capitalize

due to lack of support throughout the organization. Food companies that have been successful in introducing automation tend to have good working relationships among all grades of staff and have longer term financial viewpoints.

A modular approach has been proven worthwhile at both process and individual task levels. The DMRI is seeking to automate all pork production tasks through developing a series of modular components, each performing a different task in the slaughter process. This has allowed a number of different projects and partnerships to be established, leading to more flexibility in implementation for both the automation user and supplier. The ARTEPP primal cutting system uses modular subsystems to accommodate variations in plant-specific processes. The transport and carcass delivery subsystem operates independently of the sensing, cut path derivation, and robotic cutter elements. This allows standard subsystems to be used in many installations, with customization only required in a few subsystems, thus reducing costs. This modularity has extended to off-the-shelf food grade robots for future slaughterhouse automation.

Some automation systems have been successful in performing tasks currently not possible for a human operative. A human butcher could not perform the multi-armed cutting and handling operations achieved by evisceration automation. Even the strongest, most skilled human cannot match the consistency and high-force cut accuracy achieved with automated primal cutting. Automation of these types of tasks, unperformable by a human, is often the first to exhibit an acceptable cost–benefit ratio. Currently it is mostly uneconomic to replace a slaughterhouse operative with automation unless the automation yields addition benefits.

3.9 CONCLUSIONS

In the last decade automation technology has advanced to a stage where automatic performance of skilled meat processing tasks can now be countenanced. Much R&D has been carried out around the world and many projects are ongoing. The fruits of these efforts are beginning to manifest themselves as technically and economically feasible commercial systems.

- Although it is now technically feasible to automate nearly any task on the slaughter line, commercial factors are limiting the uptake of automation technology.
- Some successful projects have demonstrated an improvement over manual labor in terms of speed, consistency, accuracy, and control.
- A modular approach to subsystem design has been successful and increases the amount of off-the-shelf equipment available for use in other tasks.
- Mimicking human action is not always the best approach, as automated equipment can be endowed with capabilities beyond human skills.
- Additional process benefits can be obtained using automation over human operatives. This aids the financial justification.
- The pork industry is the most advanced, with fully automated processing lines expected to be available within the next few years.

3.10 THE FUTURE

Difficulties in staff availability will increase and encourage more organizations to automate simply to maintain throughput. R&D of new automation solutions will continue in mainly isolated projects as illustrated in this chapter. As a result more off-the-shelf automation subsystems will become available, reducing a current barrier of technology cost. These pockets of automation will have significant impacts in small areas in their specific roles, but widespread automation will not occur immediately. Pork slaughter automation is an exception, with a fully automated lines expected to be available in the near future. Adopting a modular approach to both equipment design and production line automation will aid technical development, systems implementation, and economic feasibility.

The economic break-even point for implementing automated slaughter lines will be affected by an increasing cost of not automating coupled with a decreasing cost to automate. As automation levels rise, staff skill levels will rise accordingly. As the pressures imposed by regulatory agencies, distribution channels, media, and customers increase, meat cutting operations in slaughterhouses will be performed by staff with profiles closer to surgeons and skilled automation engineers by their education, training, and working habits than to traditional meat cutters.

REFERENCES

Annan, D. 1982. MIRINZ manual dressing system. *Proc. of the 22nd Meat Ind. Res. Conf. Hamilton, New Zealand,* 37–39.

Anonymous. 1998, January–February. Keeping clean. *Food Qual.* January/February, 1998, 63–65.

Anonymous. 2000. Slaughterhouse slashes costs and pork bellies. *Advanced Manufacturing.* 2:43–44.

Attec. 2004. Inlet control type 23740. http://www.attec.dk/default.asp?Action=Details&Item=203

Authier, J. F. 1990. Mechanical dressing developments. *Proc. of the 26th Meat Ind. Res. Conf. Hamilton, New Zealand,* 225–231.

Authier, J. F. 1994. Lamb evisceration by machine. *Proc. of the 28th Meat Ind. Res. Conf. Auckland, New Zealand,* 187–191.

Brosnan, T., and D.-W. Sun. 2002. Inspection and grading of agricultural and food products by computer vision systems—A review. *Comp. and Elec. in Agri.* 36:193–213.

Brown, T., S. J. James, and G. L. Purnell. 2004. Cutting forces in foods: Experimental measurements. *J. of Food Eng. 70*(2), 165–170.

Butina. 2004. http://www.butina.dk.

Clarke, P. T. 1985. Automatic break up of pork carcasses. In *Agri-Mation 1: Proceedings of the Agri-Mation 1 Conference & Exposition* (ASAE Publication 01-85), 183–189.

Clausen, V. 2002. Automation in the pork industry. In *Proc. Vet. Cong. Helsinki, Finland.* http://www.danskeslagterier.dk/smcms/SF_forside/Videncenter_SF/Publikationer_SF/publikationer_2002SF/Automation/Index.htm?ID=3786.

Damez, J. L., and P. Sale. 1994. Studies on automation of cutting of the forequarter of cattle carcasses (in French). *Viandes et Produits Carnes.* 15:4, 103–107.

FAIR. 1998. FAIR98-3545, Final Technical Report, ARTEPP project. Brussels, Belgium: FAIR Programme Office, European Commission.

Field, M. 2004. Evolution in practice. *New Food*. 2004:41–43.

Folkman, P. 1995. Fully automatic pig carcass cutting plant. *Fleischwirtschaft*. 75:40–43.

Food Science Australia. 2004. www.foodscience.afisc.csiro.au/tis/equipdev

Goldenberg, A. A., and P. A. Seshan. 1993. An approach to automation of pork grading. *Food Res. Int*. 27:191–193.

Grandin, T. 1992, July. Effect of genetics on handling and CO_2 stunning of pigs. *Meat Focus Int*. 124–126 (with May 2000 updates). http://www.grandin.com/humane/meatfocus7-92.html

Holder, J., J. E. L. Corry, and M. H. Hinton. 1997. The microbial status of chicken portions and portioning equipment. *Brit. Poul. Sci*. 38:505–511.

Holt, G., and S. Henson. 2000. Quality assurance management in small meat manufacturers. *Food Control*. 11:319–326.

International Consultative Group on Food Irradiation. 1999. *Safety of poultry meat: From farm to table*. http://www.iaea.org/icgfi/documents/poultrymeat.pdf

Khodabandehloo, K., and P. T. Clarke. 1993. *Robotics for meat, fish and poultry processing*. New York: Academic Press.

Longdell, G. R. 1996. Recent developments in sheep and beef processing in Australasia. *Meat Sci*. 43:165–174.

Longdill, G. R. 1984. Advances in mechanical dressing technology. *Proc. of the 23rd Meat Ind. Res. Conf. Hamilton, New Zealand,* 127–130.

Macpro. 2005. http://www.macpro.co.nz/mutton.html

Maddock, N. A., G. Purnell, and K. Khodabandehloo. 1989. Research in application of robotics to meat cutting. *Proc. of 20th Int. Symp. on Industrial Robots (ISIR). Tokyo, Japan,* 957–963.

Madsen, K. B., and J. U. Nielsen. 2002. Automated meat processing. In *Meat processing: Improving quality*, ed. J. Kerry, J. Kerry, and D. Ledward, 283–296. Cambridge, UK: Woodhead Publishing.

McClure, E. K., J. A. Scanga, K. E. Belk, and G. C. Smith. 2003. Evaluation of the E+V video image analysis system as a predictor of pork carcass yield. *J. Anim. Sci*. 81:1193–1201.

Meat New Zealand. 2004. http://www.meatnz.co.nz/wdbctx/corporate/docs/SECURED_R_AND_D_BRIEFS/R_D_BRIEF_32.PDF2

Millers Mechanical. 2004. http://www.milmech.com/sheep.html

MLA. 2005. http://www.amic.org.au/Technology_MLA.pps

Ng, W. Y. 1992. Advantages on machine boning of lamb. *Proc. of the 27th Meat Ind. Res. Conf. Hamilton, New Zealand,* 322–326.

Ng, W. Y. 1994. Lamb shoulder fleecer and rack frenching machines. *Proc. of the 28th Meat Ind. Res. Conf. Auckland, New Zealand,* 201–208.

North, S. 1991. *Public health and safety aspects. DTI Seminar: Robotics for the meat processing industry.* Bristol, UK: University of Bristol.

O'Brien, W. H., and J. Malloy. 1993. *Method and apparatus for automatically segmenting animal carcasses*. U.S. Patent 5205779.

Paardekooper, E. R. van der Hoorn, and R. van Dijk. 1994. Slaughter line "2000." *Fleischwirtschaft*. 74:133–134, 137–138.

Paulson, D. S. 1993. Variability evaluation of two handwash modalities employed in the food processing industry. *Dairy, Food and Env. Sanitation*. 13:332–335.

Purnell, G., N. Maddock, and K. Khodabandehloo. 1993. Robotic deboning: A fundamental approach to engineering a system. *Proc. of Art. Int. in Food and Agri. Conf. Nîmes, France.*

Ranger, P., G. Ottley, and N. Smith. 2004. The pitfalls of purchasing. *Food Proc*. October, 2004, 20.

Richardson, J. 1982. Developments in automatic stunning. *Proc. of the 22nd Meat Ind. Res. Conf. Hamilton, New Zealand,* 23–26.

Roberts, C. A. 1984. Mechanical boning and trimming of mutton. *Proc. of the 23rd Meat Ind. Res. Conf. Hamilton, New Zealand,* 90–93.

Roberts, C. A. 1994. Mechanization of ovine boning operations. *Proc. of the 28th Meat Ind. Res. Conf. Auckland, New Zealand,* 209–212.

Robertson, A. A. 1980. Mechanical pelting: Introduction. *Proc. of the 21st Meat Ind. Res. Conf. Hamilton, New Zealand,* 24.

SFK. 2004. http://www.SFK.com

Taylor, M. G. 1993. Automated Y-cutting of sheep carcasses. Meat '93: The Australian Meat Industry Research Conference, Gold Coast, Australia.

Taylor, M. G., and R. G. Templar. 1997. A washable robot for meat processing. *Comp. and Elec. in Agri.* 16:113–123.

Templer, R. 2004. Cutting and boning: Robotics and new technology. In *Encyclopedia of Meat Sciences,* eds. W. Jensen, C. Devine, and M. Dikeman, 381–388. London: Elsevier Applied Science.

Templer, R., T. Nicolle, A. Nanu, A. Osborn, and K. Blenkinsopp. 2000, June. New automation techniques for variable products. *Proc. Meat Auto. Cong. (MAC) 2000. Malaga, Spain.*

Templer, R., A. Osborn, A. Nanu, K. Blenkinsopp, and W. Freidrich. 2002. Innovative robotic applications for beef processing. In *Proc. Australasian Conf. on Robotics and Automation (ARAA). Auckland, New Zealand,* 43–47.

Trow, D., and W. Y. Ng. 1994. Beef and pork loin boning. *Proc. of the 28th Meat Ind. Res. Conf. Auckland, New Zealand,* 187–191.

Wadie, I. H. C., N. Maddock, G. L. Purnell, K. Khodabandehloo, A. Crooks, A. Shacklock, and D. West. 1995. Robots for the meat industry. *Industrial Robot.* 22:5, 22–26.

Watkins, B., Y. C. Lu, and Y. R. Chen. 1999. Economic value and cost of automated on-line poultry inspection for the US broiler industry. *Food Control.* 10:69–80.

White, R. M. 1994. Fututech. *Proc. of the 28th Meat Ind. Res. Conf. Auckland, New Zealand,* 165–168.

Wickham, G. 1988. Progress with boning and cutting technology. *Proc. of the 25th Meat Ind. Res. Conf. Hamilton, New Zealand,* 160–164.

Wickham, G. 1990. Developments in mechanical boning and cutting. *Proc. of the 26th Meat Ind. Res. Conf. Hamilton, New Zealand,* 232–237.

Wickham, G. 1992. An overview of boning machinery developments. *Proc. of the 27th Meat Ind. Res. Conf. Hamilton, New Zealand,* 317–321.

Wiegand, B. 2004. Here the slaughter-technology of the future is developed. *Ingeniøren.* 13:12–13. http://www.danskeslagterier.dk/smcms/SF_-_engelsk/News_SF/6350/DMRI_in_the_media/Here_the/Index.htm?ID=6339

4 Hot-Boning of Meat: A New Perspective

Declan J. Troy
The National Food Centre

CONTENTS

4.1 Variability 74
4.2 Variability of Tenderness 74
4.3 Manipulation of Beef Postmortem 75
4.4 Hot-Boning 77
4.4.1 Benefits of Hot-Boning 78
4.4.2 Disadvantages of Hot-Boning 78
4.5 Increasing the Tenderness of Hot-Boned Beef: A New Perspective 79
4.5.1 Results 80
References 83

Quality itself can be defined in a number of ways. A common definition is that it is a measure of traits sought and valued by the consumer. As meat is a complex mixture of constituents making up the micro- and macrostructure of muscle, the term *meat quality* is ambiguous. Hoffman (1990) defined meat quality as the sum of all quality factors of meat in terms of the sensory, nutritive, hygienic, toxicological, and technological properties.

Hygienic and toxicological factors include bacteria, spores, molds, toxins, residues, and so on. Sensory properties include tenderness, color, flavor, odor, and juiciness. Nutritive factors include fat and protein content as well as vitamins, minerals, and biological value. From a meat processing point of view it is important to control and improve these properties. Hygienic and toxicological properties are, for the most part dictated by extrinsic factors and can be controlled by Hazard Analysis and Critical Control Point (HACCP) procedures to reduce risks and rapid tests are available in the factory for constant monitoring. Sensory and technological properties are more difficult to measure but are of utmost importance in terms of perceived quality. However, intrinsic factors are determined by pre- and postslaughter influences. Some of these properties can be improved by technological handling of the carcass, others can only be monitored and the meat selected for particular needs, and others presently can only be measured after cooking, at which time it is too late to rectify any problems.

The meat industry has changed from being production oriented to consumer driven (Dransfield 1992). Consumers have revealed that consistent eating quality is one of the most important attributes of beef (Jeremiah, Tong, and Gibson 1991; Miller, Carr, Ramsey, Crockett, and Hoover 2001). Because of this, there is pressure on the industry to monitor, evaluate, control, and improve these extrinsic properties of meat, which is one of the most inconsistent and diverse foods.

4.1 VARIABILITY

The origin of meat quality variability stems from many sources. Preslaughter factors within a species (beef, pork, lamb) include breed, sex, age at slaughter, feed, handling and environment, type of muscle, and carcass composition. Meat processors need to take into account knowledge of quality differences emanating from such factors. Postslaughter factors involve the biochemical dynamics of the early postmortem period. The biochemical events that occur in muscle after death are well documented. Due to the oxygen supply being depleted after exsanguination, energy metabolism is shifted to the anaerobic pathway, and lactic acid is produced and accumulates in the muscle tissues until nearly all the glycogen is depleted or until the pH fall inactivates the enzymes of glycolysis (Hendrick, Aberle, Forrest, Judge, and Merkel 1994). This early postmortem period affects the overall tenderness of the meat. For example, the rate and extent of pH fall has profound effects on meat quality. The attainment of a low pH in a "warm" environment (due to lack of natural heat dissipation mechanisms as well as heat produced from metabolism) causes denaturation of muscle proteins. Denaturation causes loss of protein solubility, loss of water holding capacity, and reduced pigment color intensity. Hence muscles with a very rapid pH decline will be pale, soft, and exudative. Conversely muscles that maintain a high pH (due to lactic acid production caused by reduced energy stores after slaughter) are dark, firm, and dry.

Another phenomenon related to the early postmortem events is the development of rigor mortis. As adenosine triphosphate (ATP) is depleted, permanent actomyosin cross-bridges form, causing the muscle to contract, resulting in shorter sarcomeres. The muscle becomes more rigid. However, during storage at refrigeration temperatures after the onset of rigor, changes occur that alter meat quality. This period of changes is termed conditioning, aging, or tenderization of beef. These changes include the degradation of structural and myofibrillar proteins.

4.2 VARIABILITY OF TENDERNESS

The most important sensory attribute contributing to beef quality is tenderness (Koohmaraie 1998; Ouali 1990; Warkup, Marie, and Harrington 1995). Jeremiah (1982) reviewed factors influencing consumption, selection, and acceptability of meat purchases and concluded that the most common cause of unacceptability in beef was toughness. Therefore, providing consistently tender beef should be of utmost importance to the industry. However, there remains an unacceptable level of variability in beef tenderness (Maher, Mullen, Moloney, Buckely, and Kerry 1994;

Morgan et al. 1991). The interaction between the variables pH, temperature, and time, during the early postmortem period has an effect on the extent of rigor and rate of tenderization (White, O'Sullivan, Troy, and O'Neill, in press). The biochemical dynamics of the early postmortem period are critical in relation to tenderness. According to Koohmaraie, Kent, Shakelford, Veisteth, and Wheeler (2002), variation in the ultrastructure and biochemistry of meat accounts for the majority of variation of tenderness in muscle postmortem. Therefore much of the variability stems from how the muscles (carcass and cuts) are treated up to the time of rigor. However, the carcass contains a large number of various-sized and localized cuts throughout and muscles will experience quite different prerigor kinetic profiles in terms of pH and temperature, resulting in variation in contraction, proteolysis, calcium release, and denaturation of proteins. For instance, it is known that the rate of glycolysis in the topside is different than the sirloin (O'Halloran, Troy, and Buckley 1997). Therefore these significant commercial cuts should not be treated equally. The result is that muscles and commercial cuts will have different degrees of toughness and tenderness. Thus manipulation of beef in the early postmortem stage might produce the best opportunity to reduce variability.

4.3 MANIPULATION OF BEEF POSTMORTEM

There have been numerous intervention techniques to manipulate the early postmortem period of beef. Postmortem electrical stimulation has received considerable attention as a possible procedure for improving muscle tenderness. The earliest reported use of electricity on meat animals was the killing of turkeys by electric shock by Benjamin Franklin in 1749, which was found to have a tenderizing effect on the meat (Lopez and Herbert 1975). In 1951, Harsham and Deatherage filed a patent for the tenderizing of meat by electrical stimulation. However, its application in industry was not seriously considered until 1973 when its use in the prevention of cold shortening became recognized (Carse 1973). The ability of electrical stimulation to enhance the tenderness of meat has been observed in several studies (Cross 1979; Dransfield, Etherington, and Taylor 1992; Hwang and Thompson 2001; Jeremiah, Martin, and Murray 1985; Olsson, Hertzman, and Tornberg 1994; Rhee and Kim 2001; Savell, Dutson, Smith, and Carpenter 1978). However, some reports conclude that there was no improvement of tenderness by electrical stimulation (Savell, McKeith, and Smith 1981; Unruh, Kastner, Kropf, Dikeman, and Hunt 1986).

There are three known theories regarding the mechanisms by which electrical stimulation tenderizes meat. The first and principal reason for the use of electrical stimulation is the prevention of cold shortening. Electrical stimulation accelerates postmortem glycolysis, so it can be used to lower the pH of muscles to below 6.0 so that rapid refrigeration can be employed without the risk of cold shortening (Carse 1973). The second theory surfaced as electrical stimulation was found to improve tenderness in the absence of cold shortening (Dransfield, Wakefield, and Parkman 1991; Smulders, Eikelenboom, and van Logtestijn 1986). It was proposed that electrical stimulation enhances or accelerates *postmortem* proteolysis (Devine, Wells, Cook, and Payne 2001; Ferguson, Jiang, Hearnshaw, and Rymill 2000). The rapid acidification, brought about by stimulation, is thought to activate the lysosymal

enzymes (Dutson, Smith, and Carpenter 1980; Harsham and Deatherage 1951). It is also suggested that electrical stimulation promotes the activity of calpain 1 as muscles subjected to electrical stimulation have a faster pH drop and a higher concentration of free calcium ions (Dransfield et al. 1992; Utterhaegan, Claeys, and Demeyer 1992). The third possible mechanism by which electrical stimulation enhances tenderization is the physical disruption of muscle fibers that has been detected by the presence of contracture nodes. It has been shown that sarcomeres in the internodal zones were stretched or fractured (Ho, Stromer, and Robson 1996; Savell et al. 1978; Sorinmade, Cross, Ono, and Wergin 1982; Takahashi, Wang, Lochner, and Marsh 1987). Although prevention of cold shortening remains the principal reason for the application of electrical stimulation, a combination of all three mechanisms might contribute to the production of tender beef by this intervention technique.

Apart from tenderness, electrical stimulation has also been reported to improve the quality of beef in terms of resulting in a brighter lean color, improvement in flavor, improvement in marbling and carcass grades, improvement in retail shelf life, and less heat ring development (Savell 1979; Savell and Smith 1979).

There is a general consensus that electrical stimulation is beneficial in terms of quality, but reported results on the effectiveness of electrical stimulation in improving tenderness show a lot of variation. This might be due to the variability of the process of electrical stimulation, such as the time of application postmortem or voltage used. It had been previously considered that high-voltage electrical stimulation was more effective at improving tenderness than low voltage, yet low voltage is safer and hence more attractive (Savell 1979). More recently, it has been found that there is in fact little difference between the tenderness of muscles subjected to high- and low-voltage stimulation (Utterhaegan et al. 1992).

Another factor that could cause inconsistencies between reports is the location of the muscle in the carcass. Chrystall, Devine, and Davey (1980) suggested that muscles not lying directly in the current pathway might not benefit equally from electrical stimulation. Variability in the muscles' response to electrical stimulation could also occur due to differences in muscle fiber type.

Herring, Cassens, and Briskey (1965) demonstrated that muscles subjected to tension were more tender. Aitch-bone or tenderstretch hanging involves hanging the carcass from the eye of the aitch-bone (*obturator foramen*) rather than the traditional Achilles tendon. This hanging method has a positive effect on tenderness as it increases the tension of the longissimus and most major muscles in the leg. In a consumer assessment of tenderstretched loin steaks, consumers judged tenderstretched steak to be more tender, juicy, tasty, and acceptable compared to conventionally hung steaks (Ford 1981). Tenderstretch hanging has been assessed at the industry level (Troy 1995) and although it is a simple method that contributes significantly to tenderness, it has one main drawback: It does not affect all muscles equally. It tenderizes the strip loin and topside, it toughens the fillet, and it has little effect on forequarter muscles. Other drawbacks include its use of 25% in extra space when used with existing chill rails and its effects on the shape of certain cuts such as the topside and the sirloin.

Tendercut is another intervention technique that involves gravity to generate tension by cutting the skeleton of the prerigor carcass while maintaining the Achilles tendon suspension. It is based on the fact that muscles can be stretched more extensively if selected bones and ligaments are severed (Wang, Claus, and Marriot 1994). It involves the severing of the ischium of the pelvic bone, the junction between the 4th and 5th sacral vertebrae and the connective tissue at the round and loin region at about 45 minutes postslaughter. This helps to stretch many of the major loin and round muscles and has been shown to improve tenderness in the loin and round muscles by up to 32% (Wang, Claus, and Marriott 1995). In a study of four different sections along the longissimus muscle, the tenderness of all sections was improved by tendercut (Ludwig, Claus, Marriot, Johnson, and Wang 1997). However, as with tenderstretch, this method does not affect all muscles equally. As one of the main problems encountered with intervention techniques mentioned so far is the nonuniformity of muscles on a carcass, it would be beneficial to apply the intervention techniques directly to the muscle rather than the carcass as a whole. Therefore, achieving the optimum conditions for the production of tender beef for individual muscles is more effective for hot-boned muscles than muscles attached to a carcass.

4.4 HOT-BONING

Hot-boning can be defined as the removal of muscle or muscle systems from the carcass prior to chilling (within 90 minutes postslaughter; see figure 4.1). The hot-boning process was developed in response to commercial demands to lower energy usage and chiller space requirements. It allows for the carcass to be treated as a set of individual muscles and cuts by removing muscles from the carcass in the prerigor state shortly after slaughter. This technique has not yet been developed in the Irish beef

FIGURE 4.1 Different stages of hot-boning loin muscle from a carcass.

industry but it is practiced in many countries, including Australia, New Zealand, Norway, South Africa, and Sweden.

4.4.1 Benefits of Hot-Boning

Hot-boning offers several economic benefits. For example, it reduces weight loss during chilling. A beef carcass can lose between 1% and 2.2% of its weight by evaporation during cooling. There is also a reduction of drip from hot-boned meat during storage of vacuum-packed cuts by 0.1 % to 0.6%, depending on the muscle and chilling parameters (Pisula and Tyburcy 1996). Prerigor meats are well recognized for their superior functional characteristics. As prerigor beef has a higher water holding capacity and better fat emulsifying properties than postrigor beef, it is better suited to making comminuted meat products such as sausages (Hamm 1982). Therefore hot-boning is an advantage in this case as it allows for processing of prerigor meat. An excessively large refrigeration space is needed to accommodate hanging carcasses. However, hot-boned beef can be boxed, resulting in a reduction in cooler space of 50% to 55%. This in turn results in savings on refrigeration input (energy), and capital costs for buildings. There is also a quicker turnover of meat at the plant, a 20% savings on labor, and savings in transport costs (primal cuts vs. carcasses; Pisula and Tyburcy 1996). Hot meat is also easier to bone out when compared to postrigor meat.

4.4.2 Disadvantages of Hot-Boning

Despite the advantages of hot-boning, there has been a delay in the implementation of this process. Pisula and Tyburcy (1996) suggested that a more gradual introduction of a new system might be more attractive and safer for many meat plant managers. A major problem facing the industry regarding hot-boning is high initial investment for construction of purpose-built equipment or for retrofit of existing plants, new equipment, and training of staff. Also there is a need for a change in system from commercial trading of carcasses to separate primals, limiting possibilities for traditional quality and grading of carcasses. As muscles must be boned out within 90 minutes postslaughter, there needs to be careful synchronization of the slaughter, boning, and processing operations. Hot-boning also requires a higher standard of hygiene as the surface area and temperature of the meat is increased. However, Miller, Bawcom, Wu, Meade, and Ramsey (1995) showed that removed fat cover did not enhance the microbial contamination during storage when compared to cold-boned meat. Research carried out on the microbiology of hot-boned meat from a laboratory or from closely controlled factory experiments would be of limited value and would not reflect factory operations (Pisula and Tyburcy 1996). Another concern regarding hot-boning is changes in the quality of hot-boned meat, mostly associated with tenderness. During hot-boning, muscles are removed in the prerigor state and are more prone to contract because the muscle is not held in a stretched state in the framework of a carcass. When hot-boned muscle is chilled quickly before the onset of rigor, cold shortening, or severe contraction of the muscle fibers, will occur and significantly reduce tenderness.

4.5 INCREASING THE TENDERNESS OF HOT-BONED BEEF: A NEW PERSPECTIVE

To make hot-boned meat as tender as possible it is necessary to prevent it from contracting by using some intervention technique involving restriction of contraction in the individual muscles. Mechanical devices have been constructed for the fixation or stretching of prerigor muscle to avoid contraction. This was done by either gluing or clamping the muscle before stretching it to a certain length (Sørheim et al. 2001).

Devine, Wahlgren, and Thornburg (1999) tightly wrapped hot-boned muscles in cling polyethylene film to direct forces against the diametrical expansion of the muscles and prevent shortening of the muscle lengths. This method reduced sarcomere shortening and increased the tenderness of meat that entered rigor in the critical high temperature range of 20°C to 35°C, and also 4°C; however meat entering rigor at 15°C and 12°C was unaffected by wrapping. When *M. longissimus* and *M. semimembranosus* were compared, wrapping had no effect on *M. semimembranosus,* which was presumed to be due to differences in the physical dimensions of the muscles as *M. semimembranosus* would be considered more difficult to wrap effectively.

This wrapping technique was further developed by the Pi-Vac Elasto-Pack system (Meixner and Karinitzschky 2001; see figures 4.2 and 4.3). The Pi-Vac packaging system involves stretching tubes of elastic film to the inside walls of the packaging chamber. After the muscle is inserted into the chamber, pressure is released and the elastic film returns to its original dimensions. The elastic film then hinders the diametrical expansion of the muscle, restricting muscle contraction. It was found that Pi-Vac increased the tenderness of beef longissimus muscles incubated at 4°C and 14°C and that rapid chilling did not have a detrimental effect on the tenderness

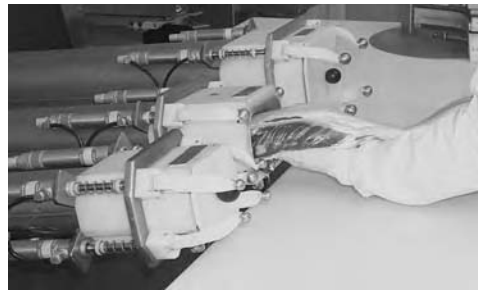


FIGURE 4.2 Packaging a hot-boned loin in a Pi-Vac machine (three different-sized tunnels accommodate different-sized muscles).

FIGURE 4.3 Sealing Pi-Vac film after insertion and loins that have been Pi-Vac packaged.

of Pi-Vaced meat (Wahlgren and Hildrum 2001). It was also stated that Pi-Vaced muscles had a more attractive shape than conventional vacuum-packaged muscles (Hildrum, Nilsen, and Wahlgren 2002).

4.5.1 Results

A series of trials were carried out at The National Food Centre, Dublin, linking hot-boning with this novel packaging machine. The effect of restraining techniques on hot-boned beef *Longissimus dorsi* muscle was investigated. Hot-boned muscles were randomly assigned to three different postmortem treatments: restraint using weights (4-kg weight suspended from a hanging muscle), tenderbound (hot-boned and Pi-Vac packaged), and control (no restraint applied to the muscle). Two chilling regimes were also examined, 2°C until 48 hours postmortem (fast chilling) or 10°C for 10 hours postmortem (slow chilling) followed by 2°C until 48 hours postmortem. Under the fast chilling regime muscles stretched by weights or packaged using the tenderbound technique had significantly ($p < .05$) lower Warner Bratzler shear force (WBSF) values than control muscles after 7 and 14 days of aging. Drip loss was also significantly ($p < .05$) lower for tenderbound muscles. In contrast to the unrestrained muscle, the shape of the tenderbound muscle was not distorted. Sensory analysis revealed that panelists ranked tenderbound muscle higher than the control for the attributes tenderness, flavor, and overall acceptability. Table 4.1 summarizes results from this trial. Similar trends were noted for both chilling regimes, but the difference between the treatments was greater for the fast chilled muscles than the slow chilled. It was concluded from this trial that when hot-boned meat is Pi-Vac packaged it can be chilled quickly without adversely affecting tenderness. This system produces a consistent quality product with improved shape and lower drip.

TABLE 4.1
Effect of No Restraint (Control) and Restraint (Weighted and Tenderbound) on Warner Bratzler Shear Force After 7 and 14 Days Postmortem, Sarcomere Length (SL), CIE a* Color (Redness), Drip Loss, and Sensory Attributes of Hot-Boned Beef Loins Aged at 2°C or 10°C

	2°C Chilling Temperature			10°C Chilling Temperature		
Attributes	**Control**	**Weighted**	**Tenderbound**	**Control**	**Weighted**	**Tenderbound**
WBSF 7 days (*N*)	89.50	51.40	54.88	45.00	34.70	39.37
WBSF 14 days (*N*)	71.52	46.45	39.94	37.94	29.00	30.75
SL (μm)	1.34	1.64	1.63	1.68	1.90	1.82
Color (CIE a*)	14.22	14.37	14.96	14.44	14.89	15.17
Drip loss (%)	1.67	1.57	0.93	1.13	0.88	0.94
Sensory tender	3.81	5.88	5.75	6.42	7.06	6.67
Sensory flavor	3.98	4.06	4.25	4.39	4.47	4.44
Sensory juiciness	5.06	5.16	5.05	5.64	5.47	5.30
Sensory overall acceptibility	3.03	3.98	4.09	4.55	4.80	4.64

A comparison of electrical stimulation to the tenderbound process on the tenderness of fast chilled hot-boned beef was investigated. Hot-boned muscles were randomly assigned to the following treatments: control (no treatment), high-voltage electrical stimulation (HVES, 700V), low-voltage electrical stimulation (LVES, 90V), and Pi-Vac packaging. All muscles were then subjected to a fast chilling regime as they were chilled in water baths at 2°C until 8 hours postmortem, followed by air chilling. Both LVES and HVES accelerated the pH decline of the hot-boned *M. Longissimus dorsi* (figure 4.1), but they did not prevent cold shortening during fast chilling. Sarcomere lengths were significantly ($p > .05$) longer for the Pi-Vac packaged muscles when compared to muscles subjected to LVES, HVES, and the control. WBSF measurements showed that only the Pi-Vac packaging had a significant positive effect on tenderness (see table 4.2). Also there was no significant difference in tenderness of Pi-Vac packaged beef between Day 7 and Day 14 postmortem. These results were confirmed by sensory analysis as Pi-Vac packaged beef scored highest in tenderness and overall acceptability among trained panelists. Pi-Vac packaged had the lowest drip loss when compared to electrically stimulated meat and the control product. The overall conclusion of this trial was that Pi-Vac packaging of hot-boned beef allowed for fast chilling of this beef without the risk of cold shortening, ensuring consistent quality and improved tenderness. HVES and LVES both accelerated pH decline but did not prevent cold shortening of fast chilled hot-boned beef loins, and had no positive effect on beef tenderness.

TABLE 4.2
A Comparison of Three Treatments: Aitch-Bone Hung (AB), Hot-Boned and Pi-Vac Wrapped (Tenderbound), and Immersed in 5°C Water Bath (TB5) or 15°C Water Bath (TB15)

Treatment Muscle	TB5 Loin	TB15 Loin	AB Loin	TB5 Topside	TB15 Topside	AB Topside	TB5 Rump	TB15 Rump	AB Rump
Shear force	36.52	35.48	30.70	45.52	45.56	40.33	36.73	31.91	33.71
Sarcomere length	1.85	1.83	2.36	2.28	2.32	2.77	2.26	2.13	2.49
Hunter L	36.43	36.85	37.51	35.52	35.81	37.08	36.34	35.14	37.25
Hunter a	15.17	14.74	15.18	15.67	15.21	15.92	14.36	14.84	16.33
Hunter b	8.66	8.76	8.94	8.64	8.50	8.81	8.06	8.26	9.31
Sensory tender	6.29	—	6.79	5.02	—	5.60	6.17	—	6.60
Sensory overall acceptability	4.31	—	4.38	3.85	—	4.04	4.31	—	4.44

A trial was completed involving the comparison of two postmortem processing systems for meat eating quality traits and microbiological shelf life. The processing systems compared were conventional cold-boned vacuum-packed meat chilled at 10°C for 10 hours followed by 2°C until 48 hours postmortem (typically conventional commercial processing) and hot-boning followed by Pi-Vac packaging (tenderbound) with chilling at 0°C. Bovine *M. Longissimus dorsi*, *M. Semimembranosus* were analyzed for quality traits and *M. Semitendinosus* was analysed for microbiological

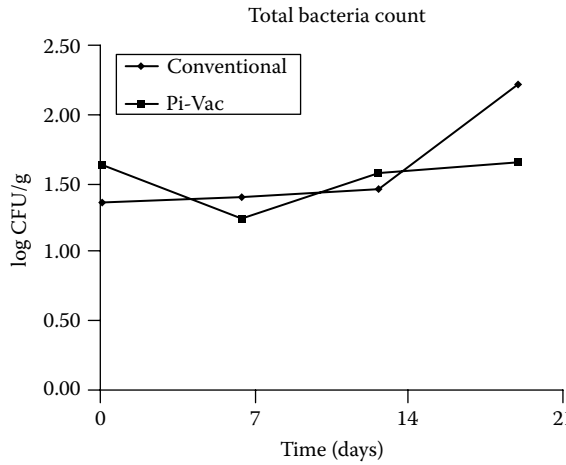


FIGURE 4.4 A comparison of the total bacterial counts of conventionally hung and cold-boning (control) versus hot-boning and Pi-Vac packaging (tenderbound) of the silverside muscles.

shelf life. Sarcomere lengths were longer ($p < .05$) in the tenderbound muscles. After 14 days aging the tenderbound topside muscle had lower ($p < .05$) WBSF values then the conventional topside. There was no significant difference for WBSF or sensory evaluation scores for loin muscle processed by both systems. Therefore it is evident from this trial that when tenderbound meat is fast chilled, tenderness and sensory attributes are not adversely affected. Bacterial counts were low in both tenderbound and conventional meat after 7 and 14 days, but after 21 days bacterial counts were lower for tenderbound meat than for conventionally packaged beef (figure 4.4) This trial highlighted the fact that Pi-Vac packaging of hot-boned meat managed to overcome the two main disadvantages of hot-boning: increased toughness and shape distortion caused by muscle contraction. In addition, it was also possible to extend the shelf life of hot-boned meat.

A comparison of the eating quality of aitch-bone hanging and the tenderbound systems was also studied. Muscles were excised after 48 hours from aitch-bone hung carcasses (tenderstretched; AB). Hot-boned muscles were Pi-Vac packaged (tenderbound) within 90 minutes postmortem and either cooled by immersion in a 5°C (TB5) or a 15°C (TB15) water bath. Color was analyzed and it was found that lightness did not differ between the treatments, although the yellowness and redness of the AB muscles were greater ($p < .001$) compared to TB5 and TB15. WBSF or sensory analysis rankings did not differ between the treatments. There was also no significant difference between TB5 and TB15 for any of the attributes measured. This trial emphasized the benefits of tenderbound muscle as it is as tender, juicy, and flavorsome as muscles from carcasses that were aitch-bone hung, but tenderbound muscles do not require a large amount of chill space as do aitch-bone hung carcasses. Also tenderbound muscles do not cold shorten at low temperatures. Results from this trial are highlighted in table 4.2.

From the research carried out at the National Food Centre, Dublin, it was concluded that of all the different manipulations carried out on the muscles, such as HVES and LVES, weights, and the tenderbound process, the latter had the most impact on producing a tender, flavorsome, uniform steak. The tenderbound process produced striploin and topside that was mechanically superior to conventionally processed muscle and of equal quality to aitch-bone hung muscles. In addition, a survey was completed by panels of industry experts, retailers, and consumers. Each group found tenderbound meat to be more consistent, tender, juicy, and flavorsome compared to conventionally managed beef.

REFERENCES

Carse, W. A. 1973. Meat quality and the acceleration of post-mortem glycolysis by electrical stimulation. *J. Food Technol.* 8:163–166.

Chrystall, B. B., C. E. Devine, and C. L. Davey. 1980. Studies in electrical stimulation: Post-mortem decline in nervous response in lambs. *Meat Sci.* 4:69–76.

Cross, H. R. 1979. Effects of electrical stimulation on meat tissue and muscle properties—A review. *J. Food Sci.* 44:509–523.

Devine, C. E., N. M. Wahlgren, and E. Thornburg. 1999. Effect of rigor temperature on muscle shortening and tenderisation of restrained and unrestrained beef M. *longissimus thoracicus et lumborum*. *Meat Sci.* 51:61–72.

Devine, C. E. R. Wells, C. J. Cook, and S. R. Payne. 2001. Does high voltage electrical stimulation of sheep affect the rate of tenderisation? *NZ. J. Agric. Res.* 44:53–58.

Dransfield, E. 1992. Meat tenderness: A unified model for meat tenderness. *Meat Focus Int.* September, 237–239.

Dransfield, E., D. J. Etherington, and M. A. J. Taylor. 1992. Modelling post-mortem tenderisation: II. Enzyme changes during storage of electrically stimulated and non-stimulated beef. *Meat Sci.* 31:75–84.

Dransfield, E, E., D. K. Wakefield, and I. D. Parkman. 1980. Modelling post-mortem tenderisation:I. Texture of electrically stimulated and non-stimulated beef. *Meat Sci.* 31:57–73.

Dutson, T. R., G. C. Smith, and Z. I. 1980. Lysosomal enzyme distribution in electrically stimulated ovine muscle. *J. Food. Sci.* 45:1097–1098.

Eikelenboom, G., F. J. M. Smulders, and H. Ruderus. 1985. The effect of high and low voltage electrical stimulation on beef quality. *Meat Sci.* 28:99–109.

Ferguson, D. M., S.-T. Jiang, H. Hearnshaw, and S. R. Rymill. 2000. Effect of electrical stimulation on protease activity and tenderness of *M. longissimus* from cattle with different proportions of *Bos indicus* content. *Meat Sci.* 55:265–272.

Ford, A. L. 1981. Consumer assessment of "tenderstretched" loin steak. *Food Res. Quart.* 41:1–4.

Hamm, R. 1982. Post-mortem changes in muscle with regard to processing of hot-boned beef. *Food Technol.* 36:105–115.

Harsham, A., and F. E. Deatherage. 1951. U.S. Patent No. 2,544,681.

Hendrick, H. B., E. D. Aberle, J. C. Forrest, M. D. Judge, and R. A. Merkel. 1994. *Principles of Meat Science* (3rd ed). Des Moines, IA: Kendall/Hunt.

Herring, H. K., R. G. Cassens, and E. J. Briskey. 1965. Further studies on bovine muscle tenderness as influenced by carcass position, sarcomere length and fibre diameter. *J. Food Sci.* 30:1049–1054.

Ho, C. Y., M. H. Stromer, and R. M. Robson. 1996. Effect of electrical stimulation on post-mortem titin, nebulin, desmin, and troponin-T degradation and ultrastructural changes in bovine longissimus muscle. *J. Anim. Sci.* 74:1563–1575.

Hoffmann, K. 1990. Definition and measurement of meat quality. In *Proceedings of 36th Ann. Int. Cong. of Meat Science and Technology, Cuba.* 941.

Hwang, H. I., and J. M. Thompson. 2001. The effect of time and type of electrical stimulation on the calpain system and meat tenderness in beef longissimus dorsi muscle. *Meat Sci.* 58:135–144

Jeremiah, L. E. 1982. A review of factors influencing consumption, selection and acceptability of meat purchases. *J. Consumer Studies and Home Economics.* 6:137–154.

Jeremiah, L. E., A. H. Martin, and A. C. Murray. 1985. The effect of various post-mortem treatments on certain physical and sensory properties of three different bovine muscles. *Meat Sci.* 12:155–176.

Jeremiah, L. E., A. K. W. Tong, and L. L. Gibson. 1991. The usefulness of muscle color and pH for segregating beef carcasses into tenderness groups. *Meat Sci.* 30:97–114.

Koohmaraie, M. 1998. The role of the endogenous proteases in meat tenderness. *Proc. Recip. Meat Conf.* 41:89–100.

Koohmaraie, M., M. Kent, S. Shakelford, E. Veisteth, and T. Wheeler. 2002. Meat tenderness and muscle growth: Is there any relationship? *Meat Sci.* 62:345–352.

Lopez, C. A., and E. W. Herbert. 1975. *The private Franklin: The man and his family.* New York: Norton.

Ludwig, C. J., J. R. Claus, N. G. Marriot, J. Johnson, and H. Wang. 1997. Skeletal alteration to improve beef longissimus muscle tenderness. *J. Anim. Sci.* 75:2404–2410.

Maher, S.C., A. M. Mullen, A. P. Moloney, D. J. Buckely, and J. P. Kerry. 2004. Quantifying the extent of variation in the eating quality traits of the *M. longissimus dorsi and M. semimembranosus* of conventionally processed Irish beef. *Meat Sci.* 66:351–360.

Meixner, H.-W., and I. Karinitzschky. 2001. Correctly packaging hot-boned meat. *Fleischwirtsch Int.* 3:24–25.

Miller, M. F., D. B. Bawcom, C. K. Wu, M. K. Meade, and C. B. Ramsey. 1995. Microbiology of hot-fat-trimmed beef. *J. Anim. Sci.* 73:1368–1371.

Miller, M. F., M. A. Carr, C. B. Ramsey, K. L. Crockett, and L. C. Hoover. 2001. Consumer thresholds for establishing the value of beef tenderness. *J. Anim. Sci.* 79:3062–3068.

Morgan, J. B., J. W. Savell, D. S. Hale, R. K. Miller, D. B. Griffin, H. R. Cross, and S. D. Shackelford. 1991. National beef tenderness survey. *J. Anim. Sci.* 69:3274–3283.

O'Halloran, G. R., D. J. Troy, and D. J. Buckley. 1997. The relationship between early post-mortem pH and the tenderisation of beef muscles. *Meat Sci.* 45:239–251.

Olsson, U., C. Hertzman, and E. Tornberg. 1994. The influence of low temperature, type of muscle and electrical stimulation on the course of rigor mortis, ageing and tenderness of beef muscles. *Meat Sci.* 37:115–131.

Ouali, A. 1990. Meat tenderisation: Possible causes and mechanisms. A review. *J. Muscle Foods.* 50:129–165.

Pisula, A., and A. Tyburcy. Hot processing of meat. *Meat Sci.* 43:S125–S134.

Rhee, M. S., and B. C. Kim. 2001. Effect of low voltage electrical stimulation and temperature conditioning on post-mortem changes in glycolysis and calpains activities of Korean native cattle (Hanwoo). *Meat Sci.* 58:231–237.

Savell, J. W. 1979. Update: Industry acceptance of electrical stimulation. *Proc. Recip. Meat Conf.* 32:113–118.

Savell, J. W., T. R. Dutson, G. C. Smith, and Z. L. Carpenter. 1978. Structural changes in electrically stimulated beef muscle. *J. Food Sci.* 43:1606–1609.

Savell, J. W., F. K. McKeith, and G. C. Smith. Reducing post-mortem aging time of beef with electrical stimulation. *J. Food Sci*. 46:1777–1781.

Savell, J. W., and G. C. Smith. 1979. Electrical stimulation—Effects on meat tenderness, muscle structure and the quality indicating characteristics of meat. *Proc. Annu. Meet. Res. Dev. Assoc. Mil. Food Packag. Syst., New York,* 1–14.

Smulders, F. J. M., G. Eikelenboom, and G. van Logtestijn. 1986. The effect of electrical stimulation on the quality of three bovine muscles. *Meat Sci*. 16:91–101.

Sørheim, O., J. Idland, E. C. Halvorsen, T. Froystein, P. Lea, and K. I. Hildrum. 2001. Influences of beef carcass stretching and chilling rate on tenderness of *M. longissimus dorsi*. *Meat Sci*. 57:79–85.

Sorinmade, S.O., H. R. Cross, K. Ono, and W. P. Wergin. 1982. Mechanisms of ultrastructural change in electrically stimulated beef longissimus muscle. *Meat Sci*. 6:71–77.

Takahashi, G. S.-M. Wang, J. V. Lochner, and B. B. Marsh. 1987. Effects of 2-Hz and 60-Hz electrical stimulation on the microstructure of beef. *Meat Sci*. 19:65–76.

Troy, D. J. 1995. Modern methods to improve and control meat quality. *Int. Dev. in Process Efficiency and Quality in the Meat Ind. Dublin Castle, Ireland,* 57–72.

Utterhaegan, L., E. Claeys, and D. Demeyer. 1992. The effect of electrical stimulation on beef tenderness, protease activity and myofibrillar protein fragmentation. *Biochimie*. 74:275–281.

Wahlgren, N. M., and K. I. Hildrum. Improving the tenderness of hot-boned strip loins using a novel packaging method. *Proc 47th Int. Congr. Meat Sci. Technol. Krakow,* 116–117.

Wang, H., J. R. Claus, and N. G. Marriott. 1994. Stretched skeletal alterations to improve tenderness of beef round muscles. *J. Muscle Foods*. 5:137–147.

Wang, H., J. R. Claus, and N. G. Marriott. 1995. A research note—Tenderness of pre-rigor stretched porcine longissimus muscle. *J. Muscle Foods*. 6:75–82.

Warkup, C., S. Marie, and G. Harrington. 1995. Consumer perceptions of texture; the most important quality attribute of meat? In *Expression of tissue proteinases and regulation of protein degradation as related to meat quality,* ed. A. Ouali, D. I. Demeyer, and F. J. M. Smulders, 225. Utrecht, The Netherlands: ECCEAMST.

White, A., A. O'Sullivan, D. J. Troy, and E. E. O'Neill. in press. Manipulation of the pre-rigor glycolytic behaviour of bovine M. *longissimus dorsi* in order to identify the causes of inconsistencies in tenderness. *Meat Sci*.

5 New Spectroscopic Techniques for Online Monitoring of Meat Quality

Kjell Ivar Hildrum, Jens Petter Wold, and Vegard H. Segtnan
Norwegian Food Research Institute

Jean-Pierre Renou
STIM INRA Theix

Eric Dufour
Dépt Qualité & Economie Alimentaires, ENITA Clermont Ferrand

CONTENTS

5.1 What Is Spectroscopy? ... 88
5.2 Meat Processing ... 89
5.2.1 What Are the Measurement Needs in Meat Processing? ... 89
5.3 NIR Spectroscopy: Theory And Applications ... 91
5.3.1 Principles of Measurement ... 91
5.3.2 NIR Instrumentation and Spectral Sampling ... 92
5.3.3 NIR Online Applications ... 93
5.3.3.1 NIR Analysis of Fresh Meat ... 93
5.3.3.2 NIR Analysis of Semifrozen Meat ... 97
5.3.3.3 NIR Transflectance Analysis of Whole (Unground) Muscle Meat ... 97
5.4 Fluorescence Spectroscopy ... 100
5.4.1 Background and Principle for Method ... 100
5.4.2 Fluorescence Techniques ... 101
5.4.3 Fluorescence Applications ... 102
5.4.3.1 Connective Tissue and Fat ... 102
5.4.4 Tenderness and Muscle Types ... 105

5.4.5 Lipid Oxidation 107
5.4.6 Fluorescence Potential for Online Analysis of Meat 109
5.5 Raman Spectroscopy 109
5.5.1 Background 109
5.5.2 Principle of Measurements 110
5.5.3 The Major Challenges in Raman 110
5.5.4 Potential Use of Raman in Meats 111
5.6 Microwave Spectrometry 112
5.6.1 Introduction 112
5.6.2 Principle of Measurements 112
5.6.3 Applications and Instrumentation 113
5.6.3.1 Noncontact Reflectance Mode Microwave 113
5.6.3.2 Guided Microwave Spectrometry 114
5.7 Nuclear Magnetic Resonance 115
5.7.1 Background 115
5.7.2 Principle of Operation 115
5.7.3 Constraints Regarding Use in Online Analysis 115
5.7.4 NMR Applications in Meat Research 116
5.7.4.1 Fat Content and Distribution 116
5.7.4.2 Connective Tissue 117
5.7.4.3 Meat Quality Parameters 117
5.7.4.4 Processing 118
5.7.5 Conclusions 119
5.8 X-Ray Spectroscopic Techniques 120
5.8.1 Principle of X-Ray Measurements in Meat 120
5.8.2 X-Ray Techniques Using One Energy Level 120
5.8.3 X-Ray Techniques Using Two Energy Levels (DEXA) 121
5.9 Methods for Grading of Carcasses 121
5.10 Comments on Sampling Problems in Online Spectroscopic Analysis 121
5.11 Concluding Remarks 122
References 123

5.1 WHAT IS SPECTROSCOPY?

Spectroscopy is the study of the interaction between electromagnetic radiation and atoms, molecules, or other chemical species. The use of spectroscopy in food science has increased tremendously in the last couple of decades as it has appeared that detection and estimation of a number of food constituents and properties may be achieved by measuring the amounts of this radiation that is either absorbed or emitted at different wavelengths. Absorption spectroscopy is now widely used in food analysis, including the estimation of proteins, carbohydrates, mineral elements, vitamins, and many additives. Emission spectroscopy has increased much in importance in the last decade, and is presently in wide use in estimation of fat oxidation, collagen, and certain elements.

Radiation is a form of energy that possesses both electrical and magnetic properties and is often described as electromagnetic radiation. Techniques such as ultraviolet, visible, infrared, and near-infrared spectroscopy derive their names from their use of a portion of this electromagnetic spectrum, and can be categorized according to the particular wavelength being utilized as shown in table 5.1, which also indicates the energy changes associated with each wavelength.

TABLE 5.1
The Electromagnetic Spectrum and Related Energy Changes

Typical Wavelength (nm)	Description	Associated Energy Changes
10^{-3}–10^{-2}	Gamma rays	Nuclear emissions from radioactive substances
10^{-2}–10	X-rays	Inner-shell electronic transitions
10–400	Ultraviolet	Valence electron transitions
400–700	Visible	Valence electron transitions
800–2,500	Near infrared (NIR)	Molecular vibrations
2,500–15,000	Infrared	Molecular vibrations
10^{6}–10^{7}	Microwaves	Molecular rotations
10^{7}–10^{8}	Radio	Spin orientation

5.2 MEAT PROCESSING

In slaughtering, live animal tissue is converted to meat. However, online analysis in the slaughter process itself is not addressed in this chapter. By meat processing here is meant unit operations from deboning of carcasses to packing and storing of processed meat products. The processes for making different products—bacon, salami sausages, canned hams, cooked sausages, and fried hamburgers—are rather different. Manufacturing procedures used by the small butcher and large meat processing plants differ in many respects.

The meat processes must handle large variations in raw material composition and other properties. Raw material costs account for a large share of the total production costs in meat processing. For example, in Norway, typical for raw material costs in percentage of total costs in the meat industry are around 70% (Tøgersen, Rødbotten, and Hildrum 2002). This stresses the need for optimal usage of valuable raw materials with stringent quality control procedures in meat processing. Meat is susceptible to deterioration, and low temperatures and rapid turnover are prerequisites for efficient processes. Handling of solid meat and viscous meat batters is often complicated, and present procedures often resemble a series of poorly coordinated unit operations more than continuously flowing, efficient processes.

5.2.1 What Are the Measurement Needs in Meat Processing?

As industrial manufacturing moves toward increasing levels of automation, faster turnover, lower cost margins, and integrated computer architecture in the plants,

there is an increasing pressure to provide real-time, accurate information for process control (Downey and Hildrum 2004). Delays in providing such analytical information rapidly add extra costs to the manufacturing of the final product. In food manufacturing processes, most conventional control systems today monitor physical conditions, such as temperature, humidity, and pressure. Generally the process is only controlled on the basis of these measurements. Measurements of chemical or physico-chemical properties, which are directly relevant to food quality, are found less frequently for process control in the meat industry. Measurements of hygienic conditions are often given high priority. However, analyses of the contents of fat and other major components of the meat are often performed, and the rapid introduction of online methods opens up the opportunity for efficient use of such data in process control. Among the physico-chemical properties of meat to be monitored in process control include the following:

- Contents of main constituents (fat, water, protein)
- Collagen proportion of protein (BEFFE)
- Salt concentration, pH, and acid concentration
- Color and appearance
- Rancidity, antioxidative capacity, fatty acids, and cholesterol
- Texture, tenderness, and binding properties
- Processing effects (i.e., heating, freezing, and packaging)

Thus focus should be on positions in the process where the possibilities for alterations and improvements are optimal. Generally the earlier in the process the measurements are being made, the better the possibilities to introduce the necessary corrections.

During the last couple of decades spectroscopic methods have found many applications in food and agriculture, in particular near infrared (NIR) and fluorescence spectroscopy. The main emphasis was earlier on laboratory applications, where the primary applications have been quantitative analysis of the major components, including protein, water, fat, salt, and sugars (Hildrum, Ellekjaer, and Isaksson 1995). Methods for direct analysis of important food quality parameters have also emerged, such as analysis of sensory properties, state of water, texture and tenderness, component interactions, and material properties.

The transition of the spectroscopic techniques from the laboratory to online use in industrial processes presents a number of problems. Online analysis does not permit sample pretreatment like grinding or homogenization, and the instruments must be able to handle large differences in homogeniety and particle size, for example. The analysis must be continuous and very rapid. The instruments must be robust and withstand the variations in temperature, humidity, external vibrations, and light conditions usually encountered under industry conditions.

Compared to other industry segments, process control is not well developed in the meat industry. However, in recent years a range of equipment for online analysis of meat has been developed, based on principles like fluorescence (Egelandsdal, Wold, Sponnich, Neegard, and Hildrum 2002), microwave (Borggaard and Bager Christensen 2003), X-rays (Hansen et al. 2003), NIR spectroscopy (Anderson and

Walker 2003; Hildrum, Nilsen, and Wahlgren 2003; Isaksson, Nilsen, Tøgersen, Hammond, and Hildrum 1996; Schwarze 1997; Tøgersen, Isaksson, Nilsen, Bakker, and Hildrum 1999), electrical conductivity or impedance, ultrasonics, and video image analysis. In this chapter, an up-to-date status for online analysis of meat quality by several different spectroscopic methods is given. The focus is on techniques that are within the overall experiences of the authors, so techniques like ultrasonics, video image analysis, and bioimpedance are only touched on briefly.

5.3 NIR SPECTROSCOPY: THEORY AND APPLICATIONS

5.3.1 Principles of Measurement

Molecular motions are caused by vibrational and rotational energy transitions. Several vibrational modes can occur that are either stretching or bending modes. The vibrational frequencies depend on the force constant of the molecular bond and the masses of the two molecules constituting the bond. NIR spectroscopy is one of the techniques that utilize the vibrational energy transitions of molecules. In the NIR region of the electromagnetic spectrum, which is defined from 780 to 2,500 nm, molecular vibrations that are overtones and combinations of the fundamental vibrations of the midinfrared (IR) spectral region are found.

Molecules absorb photons that have the same energy level as the molecules, resulting in molecular excitations. If the photons possess twice or three times the energy needed to excite a molecule, the molecule will be excited to the second or third energy level. Excitations to the first level produce the fundamental vibrations absorbing in the IR region, whereas excitations to higher levels produce overtones, absorbing in the NIR region. Because fewer molecules are excited to higher energy levels, the first overtone band will be weaker than the fundamental band, the second overtone band will be weaker than the first, and so on. Combination vibrations arise when the absorbed photon excites two or more vibrations simultaneously.

Harmonically oscillating atoms (oscillators) cannot form overtones. Thus, the molecular vibrations are required to have a certain deviation from harmonicity, forming so-called anharmonic oscillators. In practice, a very light atom like the hydrogen atom bound to a heavier atom favors the formation of anharmonic oscillators. The most important molecular vibrations that absorb NIR energy are therefore stretching and bending modes involving C-H, O-H and N-H bonds (Osborne, Fearn, and Hindle 1993; Williams and Norris 2001).

There are three basically different ways of obtaining NIR spectra from a sample: (a) by reflection measurements, where the energy reflected mainly from the surface of the sample is detected, (b) by transmission measurements, where the energy that has been transmitted through the sample is detected, and (c) by transflection measurements, which is a combination of the other two methods. The illumination and detection take place on the same side of the sample, like in reflection measurements, but the energy has been transported some distance into the sample and back to the surface prior to the detection. Normally, transflection measurements require contact between the sample and the instrument probe. However, a new instrument has

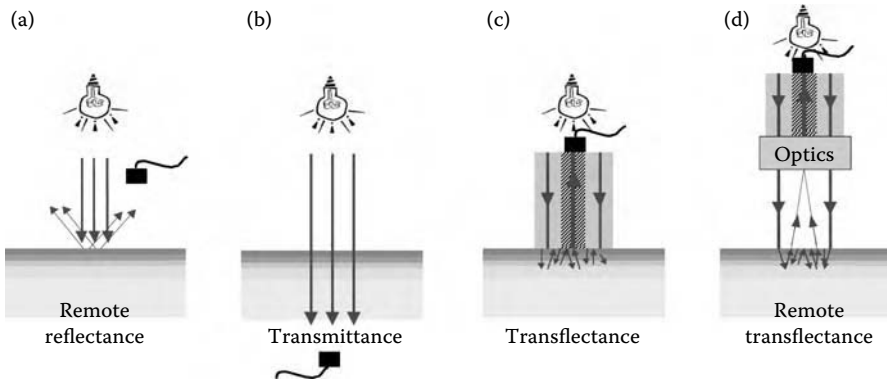


FIGURE 5.1 Measurement modes for NIR measurements on solid foods.

recently been patented that measures in remote transflection. This instrument is presented and discussed later. The different principles are illustrated in figure 5.1.

The reflection mode is the best option for optically dense samples with a surface that is representative for the response of interest. The detectors are normally mounted at an angle of 45° compared to the illumination source to avoid specular reflection, but the signals are still mainly representative of the sample surface and not the interior. Transmission measurements have traditionally been preferred for transparent liquids or very thin samples, but today it is also possible to use transmission for analysis of relatively thick and heterogeneous samples. Transflection measurements are ideal for heterogeneous samples with a surface that is not representative for the response of interest.

5.3.2 NIR Instrumentation and Spectral Sampling

The speed of acquisition and the level of detail and precision in NIR spectra have naturally increased substantially since the introduction of the technique in the early 1960s. The first instruments had discrete filters for separate wavelengths, and recorded at a relatively low number of different wavelengths. A high percentage of the online NIR instruments in use in industry today are still of this type, due to their speed and ruggedness. Tilting filters made it possible to measure sequences of wavelengths. The benefits of full spectral measurements are obvious, brought about grating monochromators and vibrating grating instruments, both based on physical wavelength switching. Electronic wavelength switching is utilized in diode array instruments and acousto-optical tunable filter instruments, offering higher speed and greater precision. The best spectral precision is probably offered by Fourier-transform instruments, which have entered the market during the last decade (Williams and Norris 2001).

For heterogeneous samples like meat, the spectral sampling is of great importance. Most standard spectrometers record spectra from discrete smaller or larger spots on the sample (see figure 5.2). If the sample moves, one will thus get more or less representative spectra from a line, for example, from the middle of the product stream. This spectral sampling is well suited for continuous streams of ground meat,

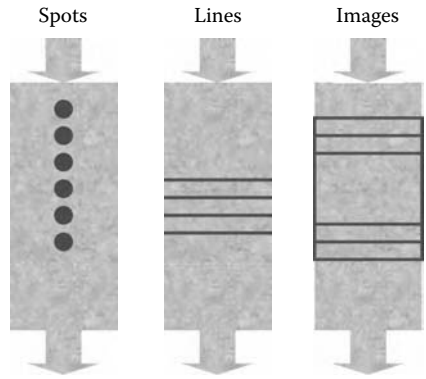


FIGURE 5.2 Acquisition patterns of NIR spectral samples from solid foods.

where this line is representative for the whole sample. If the samples are discrete, heterogeneous objects, the spot sampling needs to be triggered such that the spots are obtained from the same regions of the objects each time. However, for such samples, spot sampling is not the optimal sampling principle. The alternative to spot sampling has so far been multispectral imaging in the NIR range. This technique assures that the whole sample is represented, but the number of wavelengths and the speed of sampling may be limiting. An alternative that falls between these two sampling options is line scanning. Line scanning is a good option for continuous sample streams that have a gradient across the conveyor belt, and in particular for discrete, heterogeneous samples like meat cuts or fish fillets. The output from a line scanner is the same as that of an imaging instrument (i.e., multispectral images).

5.3.3 NIR Online Applications

Offline NIR applications regarding meat composition have been reported on many times, (see Downey and Hildrum 2004; Hildrum et al. 1995). The prediction errors for fat, water, and protein have typically been in the range of 0.3% to 0.7% for offline applications. During the last decade a number of dedicated NIR online instruments have appeared on the market. Among these are reflectance filter instruments (e.g., MM710 and MG710; NDC-Infrared Engineering; Isakssen et al. 1996; Tøgerson et al. 1999) and transmission instruments (CFA; Wolfking; Schwarze 1993), as well as reflectance instruments with diode array detectors (Anderson and Walker 2003; Hildrum et al. 2003; MSC 511 or Corona 45; Zeiss, DA-7000NIR/VIS analysis system; Perten Instruments; Springfield, IL).

5.3.3.1 NIR Analysis of Fresh Meat

5.3.3.1.1 Reflectance Filter Instruments

The first online meat application with an NIR instrument was reported by Isaksson et al. (1996), who used a noncontact reflectance, filter instrument mounted at the outlet of an industry meat grinder (MM55, NDC Infrared Engineering, Maldon, Essex, UK), as shown in figure 5.3.

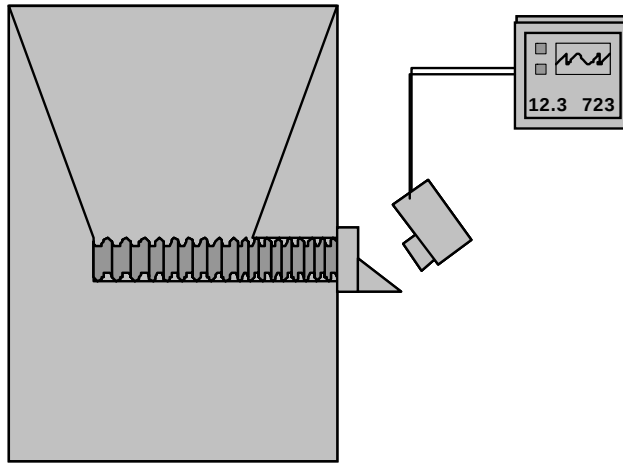


FIGURE 5.3 Instrumentation setup for online NIR reflectance analysis at a meat grinder (MM55 or MM710).

The instrument consisted of a sensing head with a quartz halogen lamp, five filters on a rotating wheel and two lead sulphide detectors. Calibrations were developed for fat, moisture, and protein on 48 small (20 kg) beef batches. The meat samples were produced by grinding meat through plates with 4-, 8-, 13-, and 19-mm diameter holes; and NIR spectral data were collected for 3–5sec on each batch. The root mean square error of cross-validation (RMSECV) was calculated to be in the range of 0.73% to 1.50% for fat, 0.75% to 1.33% for water, and 0.23% to 0.32% for protein. It was found that the prediction error increased with increasing hole size in the grinder plate, which was probably mainly due to a higher NIR sampling error in the relatively small batches.

Using the same instrument, online NIR prediction of fat, water, and protein in large industrial-scale batches of beef and pork was further studied by Tøgersen et al. (1999). By scaling industrial batches up to 400 kg to 800kg of ground beef (13 mm), the NIR samples increased in size by the longer measurement time. To improve sampling, reference samples were taken from batches that were reground through 4-mm hole plates. The average distance from the meat surface to the sensing head was approximately 25 cm. Three of the five filters were used for analysis of fat (1,728 nm) and water (1,441 nm and 1,510 nm), and the remaining two filters with low absorbance (1,655 nm and 1,810 nm) were used as reference filters. The prediction errors were 1.09% to 1.33% and 1.30% to 1.49% for moisture and fat, respectively, which were on the same level as reported earlier for the small batches.

An upgraded version of the MM55 instrument (MM710) was tested in two other meat processing plants. The MM710 had a rotating filter wheel with eight filters, which opened up for more robust and precise calibrations. With instruments installed at the outlet of a grinder (4- to 13-mm hole plates), the prediction errors for analysis of fat content in ground beef for the two plants were 0.51% and 0.48%. The same type of instrument was also tested in a third processing plant on 60 beef batches ground to sizes of 18 mm to –40 mm (the last size refers to grinding without a

grinder plate; Hildrum et al. 2003). The prediction results were found to be satisfactory for fat with a correlation coefficient of 0.48 and a standard error down to 0.72%. The lowest error was found for models with large batches (about 500 kg). The regression lines for the two ground meat sizes overlapped, indicating that the same calibration can be used for both grind sizes. Online estimation of water content was also possible with the MM710, but the standard error was larger. Due to the limited range of protein concentration in meat raw materials, the prediction of this component did not prove to be very useful. Nonlinearities were observed for batches with average fat content higher than 30%, but the reason for this is not clear. At present, the described applications regarding online analysis of proximal composition of ground meat have been implemented for regular use in four Norwegian meat processing plants.

In beef, tenderness is usually rated as being the most important quality variable for the consumer. This ranking reflects the wide variation in this property often observed in the market, and studies have shown that the consumer is willing to pay a higher price for cuts with superior tenderness (Shackelford, Wheeler, and Koohmaraie 2005). NIR spectroscopy has the ability to reveal changes in the state of water and hydrogen bond interactions in food. Such changes occur in beef during aging, which makes NIR an interesting option for beef tenderness assessment (Downey and Hildrum 2004).

One decade ago, Hildrum, Isaksson, Naes, Rødbotten, and Lea (1995) studied the prediction of beef tenderness using the InfraAlyzer 500. Slices of 120 aged beef loin samples were measured and regressed against sensory analysis and Warner-Brazier (WB) shear force. The multivariate correlation coefficients for sensory hardness and tenderness (and WB) were in the range from 0.70 to 0.74, which meant that the models explained 50% to 55% of the variation in the data. The corresponding correlation coefficient for WB shear force was of the same magnitude. Multiple scatter correction of the raw spectra removed most of the predictive information in the models, which indicated that the predictive ability relied on differences in light scatter between the samples.

Mahalanobis distances classification (MDC) in principal component subspaces was used in predicting sensory tenderness from NIR measurements of 90 beef samples (Downey and Hildrum 2004; Naes and Hildrum 1997). Average percentage correct classifications for three-way models were 49% to 63% (figure 5.4). A considerable overlap in the membership map was observed between neighbor subgroups. However, there were almost no overlaps between extreme groups. This means that NIR is capable of discriminating between the extreme tenderness groups in a three-way classification. If a meat packer wants to ensure that a shipment should not contain any tough steaks, he or she should select only the ones classified in the tender group and exclude both the intermediate and tough groups. For the two-way classification the average percentage correct classifications were 78% to 81%. These results were confirmed in later studies (Downey and Hildrum 2004; Shackelford et al. 2005). This technology could be useful for development of corresponding online applications, as with the use of the NIR scanner technique discussed later.

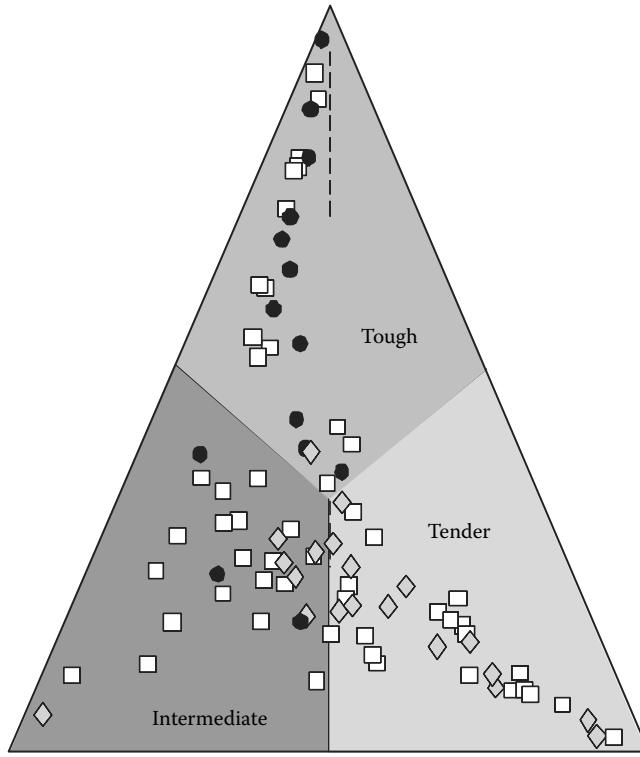


FIGURE 5.4 Membership map for 90 fresh beef samples in the MDC tenderness classification (◊ = tender, □ = intermediate, • = tough).

5.3.3.1.2 Reflectance Diode Array NIR Instruments

Whereas filter instruments record discrete bands in the NIR spectrum, diode array instruments monitor the spectrum with higher resolution than filter instruments. The instrument used in the following experiment was an industrial reflectance head for measurement in a wide spectral range (Corona 45, Carl Zeiss Jena GmbH, Jena, Germany). The light source was a Wolfram lamp, and the sensor array was an InGaAs array with 128 diodes. This instrument measured in the 950 nm to 1,700 nm range with a bandwidth of 6 nm per diode. The instrument was designed to measure at a distance of approximately 3 cm to 5cm above the sample, the surface of the ground meat stream on a conveyor belt. The measurements were performed under industrial conditions on 60 batches of 150 kg to 500 kg (Hildrum, Nilsen, Westad, and Wahlgren 2004) in 30-msec to 60-msec periods, and 1,500 spectra were recorded for each batch. As the meat flow on the conveyor belt was frequently discontinuous, interfering spectral readings from the belt itself had to be identified and removed by principal component analysis (PCA) and soft independent modeling of class analogy (SIMCA) classification (Westad and Martens 2000; Wold, Westad, and Heia 2001). Partial least squares (PLS) calibration models for all samples at two different grinding sizes (40 mm and 18 mm) yielded correlation coefficients in the range of .93 to .96,

and the full cross-validated errors for fat and water were between 1.6% and 2.4%. The corresponding errors for protein were 0.5% to 0.8%. A forward variable selection method based on jack-knifing yielded similar results. The predictions were generally best for the 18-mm grinding size. Before the implementation of these calibrations, they need to be reevaluated under industry conditions on new independent batches.

Anderson and Walker (2003), using a DA-7000NIR/VIS analysis system (Perten Instruments, Springfield, IL) estimated fat content in ground beef in a continuous stream on a conveyer belt. The instrument made use of a fixed grating and a diode array. The batches were relatively small (27 kg), which gave a measurement time of only 1.94 sec. The prediction errors for the validation set were in the range of 2.15% to 2.28% for fat.

The performance of an NIR online transmission instrument in a blender bypass grinder was reported by Schwarze (1997; Continuous Fat Analyzer; Wolfking). The sampling process in the blender was repeated several times to ensure that the concentration of the blend was accurate. It is reported that the instrument can achieve an accuracy on batch level corresponding to a prediction error of 0.4% to 0.8%, depending on the type of product and process specifications.

5.3.3.2 NIR Analysis of Semifrozen Meat

Due to periodical mismatch between demand and supply, meat raw materials are often frozen for storage. Semifrozen raw materials, which are partly thawed, are frequently used in manufacturing meat products, as they have the additional benefit of contributing to temperature control of batches (Tøgersen et al. 2002). Applications for online analysis of semifrozen ground meat would enable more complete control of meat raw materials.

The phase transition of liquid water to ice results in frequency shifts in the O-H sensitive wavelengths around 1,400 to 1,600 nm and 1,900 to 2,000 nm (Tøgersen, Arnesen, Nilsen, and Hildrum 2003). To avoid the spectral effects caused by these phase transitions, filters for the MM55 were selected outside O-H sensitive regions with central wavelengths of 1,630, 1,728, 1,810, 2,100 and 2,180 nm. In the calibration test, 38 samples were ground through 13-mm hole plates, and 17 samples were ground through 4-mm hole plates, adding up to a total of 55 batches of 400 kg to 800 kg of ground meat.

As earlier, the NIR instrument was mounted at the outlet of the grinder, and fat, moisture, and protein contents were estimated from readings throughout the grinding of the batch. A total of 55 beef batches of 400 kg to 800 kg in the range of 8% to 23% fat, 59% to 71% moisture, and 17% to 21% protein were ground through 4-mm or 13-mm hole plates. The prediction errors obtained (RMSECV) were on the same level as for fresh meat (i.e., 0.48%–1.11% for fat, 0.43%–0.97% for moisture, and 0.41%–0.47% for protein.

5.3.3.3 NIR Transflectance Analysis of Whole (Unground) Muscle Meat

As stressed earlier, careful consideration on how to obtain representative sampling is critical when developing applications based on NIR spectroscopy. Most foodstuffs

are highly heterogeneous with regard to distribution of major constituents, such as moisture, fat, and protein. This is a potential problem when one wants to obtain representative NIR measurements to get the best possible estimate of the chemical composition.

NIR remote reflectance measures mainly the surface (figure 5.1a) and is normally well suited for measuring homogeneous samples. However, as seen earlier, the method can work well even on highly heterogeneous materials as long as the samples monitored are large enough. Particularly difficult applications are single products (not batches) of whole muscles, such as beef cuts, chicken breasts, or salmon fillets. There is a limited surface to measure, and frequently the surface does not represent the average contents of the constituents of interest, as they are not evenly distributed within the muscles.

One example of a complicated product is fillet of whole Atlantic salmon, for which producers and buyers want to know the average fat content. The fat content increases from tail to head, decreases from the skin toward the backbone, and increases from the lateral line and down into the belly flaps (Rye 1991). So where on the fillet should one collect NIR spectra to get representative measurements of the whole fish? This problem has been studied by Isaksson, Tøgersen, Iversen, and Hildrum, (1995) and Wold, Jakobsen, and Krane (1996), who found that the prediction results vary dramatically with the site of measurement. For optimal results, NIR measurements should be performed on tissue from the upper part of the fish, just behind the dorsal fin.

One analytical solution for small heterogeneous samples is transmittance measurements (figure 5.1b), which can improve the accuracy significantly by increasing the NIR sample size. NIR transmission measures light that has been transmitted through the sample and gives a more representative average spectrum. Another possibility is transflectance performed in contact with the surface of the sample (figure 5.1c). Here the light probes deeper into the material as compared to reflectance, and a more representative sampling is obtained. For instance, transflectance is used for offline measurement of sugar content in single peaches (Kawano, Watanabe, and Iwamoto 1992) and melons (Greensill and Walsh 2000), where NIR spectra are collected from beneath the skin.

For online purposes, the transmittance mode can be cumbersome because sample thickness varies and gives rise to undesirable offset variations in the spectra, and some products are also too thick to transilluminate. Standard transflectance usually requires contact with the product (figure 5.1c), which can introduce difficulties with regard to mechanics and hygiene.

These problems have been addressed in a recent Norwegian collaboration project between Matforsk and Sintef, in which the goal was to develop an online system for moisture measurement in dried salted cod, also called split cod. Split cod is used to make the dish bacalao.

Split cod is an extremely heterogeneous product, just like most meat cuts. The moisture is unevenly distributed, with high contents in the thick loin parts and much lower levels in the thinner belly flaps and tail. The surface of the fish is usually much drier than the interior and covered by a layer of salt. The surface of the fish is rough and hard, which makes it difficult to obtain good contact between the sample

and the transflectance probe. The fishes vary in size and thickness (up to about 6 cm thick), which makes transmittance measurements difficult to use. In addition, the skin of the fish varies greatly in color and structure. Quality of split cod is traditionally judged manually by trained graders on criteria like size, shape, texture, color, odor, and moisture content. The water content is one of the most important criteria in the market; low content demands a higher price due to higher content of proteins and because drier fish has a longer shelf life.

Remote reflectance (figure 5.1a) measurements did not give good results on split cod, as the water content at the salty and dry surface does not correlate well with the average moisture content. With single point measurements, contact transflectance (figure 5.1c) gave fairly good calibrations with average water. However, as in most online situations, contact between the optical probe and the sample was not easy to achieve with this approach. By using a remote transflectance setup, shown in figure 5.1d, the results were much improved. The light is guided into the sample from a remote light source, scattered and absorbed in the sample, and then parts of it are back-scattered to the surface to be measured by a remote detector. Laboratory studies (not online) on well-defined samples of limited size showed that noncontact remote transflectance (figure 5.1d) gave results as good as contact transflectance (figure 5.1c) with regard to calibration results. From the split cod study we concluded that remote transflectance seemed to be a feasible technique for obtaining useful NIR spectra from the interior of heterogeneous food samples.

The remote reflectance principle was implemented in an existing rapid NIR reflectance scanner, a commercial system used for automatic plastic waste sorting (Titech Visionsort, Norway). There were two particularly attractive features with this system: It was very fast and could measure and analyze objects on a conveyor belt at a high speed (3 m/sec). Second, it was also a spectral imaging system, which could produce images with an NIR spectrum in each pixel of the image. A powerful illumination line was projected down on samples on the conveyor belt. A vertical black shield protected the detector from the main part of the direct reflected light. Well-adjusted optics enabled measurement of light emerging from the samples approximately 2 cm from the illumination line. This gave an online system, which both recorded spectral images of each sample, and at the same time yielded spectra of each pixel measured in transflectance mode. This means that both the surface and the interior of the sample were being measured simultaneously. Experiments so far indicate that the system measures as deep as 15 mm to 40 mm, depending on product (fish, meat, cheese). Examples of raw images of dried salted cod and whole beef entrecôte are shown in figure 5.5.

For a sample set of 70 dried cod a regression model for water with a correlation coefficient of .96 and an accuracy of ±0.70% was obtained. As a comparison, the manual graders generally have an accuracy of about ±2.0% to 2.5%. The split cod industry regards the new moisture determination by the NIR tranflectance as very promising, and plans for implementation of the online method are underway.

The NIR transflectance system has also been evaluated on other heterogeneous food products with promising results. Figure 5.6 shows a whole entrecôte being scanned by the system. The deep light penetration assures that a considerable portion

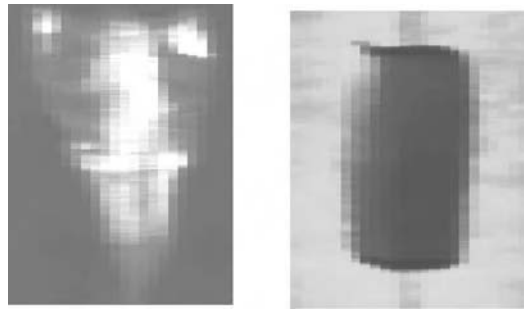


FIGURE 5.5 Images of dried salted cod (left) and entrecote produced by the transflectance scanner.

FIGURE 5.6 NIR scanning of whole entrecotes.

of the muscle is being monitored. Entrecôtes are highly heterogenous beef cuts, with a variation in fat content that makes it interesting for butchers to be able to sort them. In a preliminary experiment, 15 whole entrecôtes were scanned, and the average NIR spectrum from each image was used for calibration. A tentative correlation of .95 and a prediction error of ±1.4% were obtained, and work is underway to confirm this result.

The use of noncontact transflectance measurement in combination with smart sensors and data treatment will probably increase the usefulness of NIR spectroscopy for online meat applications. The described NIR scanner is presently a prototype (patent pending), but commercialization with custom-made versions for meat products are being considered.

5.4 FLUORESCENCE SPECTROSCOPY

5.4.1 Background and Principle for Method

Fluorescence spectroscopy is a promising method for online quality measurements of meat. However, as compared with NIR, online applications are still in their infancy. In the following sections we give an update on offline applications, in particular meat applications, and comment on the feasibility of these for online use.

Fluorescence offers several inherent advantages for the characterization of molecular interactions and reactions. First, it is 100 to 1,000 times more sensitive than other spectrophotometric techniques. Second, fluorescent compounds are extremely sensitive to their environment. For example, tryptophan residues that are buried in the hydrophobic interior of a protein have different fluorescent properties than residues that are on a hydrophilic surface. This environmental sensitivity enables characterization of conformational changes such as those attributable to the thermal, solvent, or surface denaturation of proteins (Lakowicz 1983), as well as the interactions of proteins with other food components. Third, most fluorescence methods are relatively rapid and a spectrum is recorded in less than 1 sec with a CCD detector.

Fluorophores can be broadly divided into two main classes: intrinsic and extrinsic. Intrinsic fluorophores are those that occur naturally in the product sample. In meat these include the aromatic amino-acids tryptophan, tyrosine, and phenylalanine; structural proteins such as elastin and collagen; the enzymes and coenzymes NADH, FAD, and NADPH; the vitamins A, K, and D; derivatives of pyridoxal; porphyrins; phospholipids; and the lipid pigments lipofuscin and ceroids (Ramanujam 2000). Riboflavin is another prominent fluorophore that is likely to occur in meat products.

5.4.2 Fluorescence Techniques

If sample absorbance is less than 0.1, the intensity of the emitted light is proportional to fluorophore concentration and excitation and emission spectra are accurately recorded by classical right-angle solution fluorescence device. When the absorbance of the sample exceeds 0.1, emission and excitation spectra are both decreased and excitation spectra are distorted. To avoid these problems, a dilution of samples can be performed so that their total absorbance would be less than 0.1. However, the results obtained on diluted solutions of food samples cannot be extrapolated to native concentrated samples because the organization of the food matrix is lost. Moreover, the approach is not suited for online analysis.

To avoid the problems already described, the method of front-face fluorescence spectroscopy can be used (Parker 1968). The surface of the samples is simply illuminated by excitation light, and the emitted fluorescence from the same surface is measured. Front-face fluorescence allows investigation of the fluorescence of powdered, turbid, and concentrated samples. The method has been used to quantitatively determine hemoglobin in undiluted blood (Blumberg, Doleiden, and Lamola 1980), to study hemoglobin R->T transition kinetics (Hirsch and Nagel 1989), or proteins in wheat gluten (Genot, Tonetti, Montenaygarestier, Marion, and Drapron 1992). But searching in the literature, few studies deal with the application of front-face fluorescence in the characterization of food products, and there are no reports on implementation of online applications. This is probably because foods are complex products containing numerous fluorescent compounds. In such cases the signals of the different chromophores may overlap, and it becomes more complicated to predict the concentration of particular compounds.

Recently, front-face fluorescence spectroscopy in combination with multivariate statistical methods has been more commonly used for studying quality parameters of "native" samples of milk, cheese, meat, and meat products. Although many of

these studies indicate interesting potentials for solid sample measurements, the implemented applications are, however, rather rare. Munck's (1989) broad and inspiring overview demonstrates the versatility of fluorescence techniques for quality assessment of cereals, meat, and fish. Within the field of autofluorescence in meat science, Swatland (1987, 1991, 1993, 1996) is one of the main contributors. Through numerous articles on fluorescence properties related to meat quality parameters, connective tissue in particular, he pinpointed the potential of direct measurements on meat. Another important source of both practical and fundamental knowledge within front-face fluorescence is the medical literature (Ramanujam 2000).

Instrumentation for front-face fluorescence is not complicated and should not be very expensive. The main components are a stable excitation light source with a specified narrow bandwidth output, a spectrograph, and a CCD detector. A cutoff filter in front of the spectrograph is recommended to suppress the excitation light. Systems like this can be put together in different ways depending on the desired applications. For instance, systems can be designed to measure large surfaces, or to do point measurements based on fiber optics.

5.4.3 Fluorescence Applications

Some fluorescence applications have already been mentioned. In the following subsections, selected meat applications that both are feasible for online use and that have a significant industry and public interest are dealt with.

5.4.3.1 Connective Tissue and Fat

Collagenous connective tissue (CT) is an important parameter (constituent) of meat quality, which is related to tenderness and texture. CT is beneficial due to binding properties, but high levels in ground meat products can have detrimental effects on the end quality, such as unwanted gelatin formation, graininess, and brittle texture (Bailey and Light 1989). Knowledge of the amount of CT in ground beef and different kinds of beef blends is important for monitoring raw materials and for optimizing beef product recipes. Today's common technique for CT quantification is to determine hydroxyproline, a tedious, chemically demanding, and not particularly precise method. Thus, a rapid and preferably online measurement is desired.

It has long been known that CT and adipose tissues are autofluorescent (Jensen, Reenberg, and Munck 1989; Newman 1984). The sources of this bluish fluorescence are not fully understood at the molecular level, but it is well known that different types of collagen crosslinks such as hydroxylycyl pyridoline, lysyl pyridinoline, and pentosidine are contributors (Bailey, Asims, Avery, and Halligan 1995; Bailey and Light 1989; Eyre, Paz, and Gallop 1984). Collagen exists in several different genetic forms, four of which have been found to be present in muscle, Types I, III, IV, and V. Types I, III, and IV have similar fluorescent properties for excitation in the region of 330 nm to 380 nm, whereas Type V differs from the others. Another powerful fluorophore in meat is elastin, which exhibits fluorescence quite similar to collagen Types I, III, and IV (Egelandsdal, Dingstad, Tøgersen, Lundby, and Langsrud 2005).

Adipose tissues contain a CT network, but they also include other fluorescent components more specific for fat, such as the age-related pigments lipofuscin and ceroid (Yin 1996). The fat-soluble vitamins A, D, and K exhibit fluorescence in the region 387nm to 480 nm when excited in the 308 nm to 340 nm region (Dufour and Riaublanc 1997; Ramanujam 2000; Skjervold et al. 2003).

Swatland (1987) suggested monitoring the gristle content in beef slurries by using front-face fluorescence spectroscopy. He also utilized the fluorescent properties of collagen and elastin to develop a prototype of a CT probe based on fluorescence. The system consists of ultraviolet illumination, a sensitive sensor, a fiber-optic cable, and an insertion probe with an optical window. When pushing the probe through the meat, fluorescence peaks are registered at intersections with CT. Swatland reported a correlation of .85 between the biochemically determined collagen and the fluorescence in beef meat. He obtained rather high correlations with a taste panel evaluating chewiness, ranging from .61 to .86 for *Semitendinosus* and .47 to .65 for *Longissimus dorsi* (LD; Swatland and Findlay 1997). To our knowledge, the system has not been commercialized or adapted for online application.

Jensen et al. (1989) proposed 340 nm as a feasible excitation wavelength for general quality control of products from fish, cattle, swine, and poultry. They reported that bovine fat and CT had local emission maxima at 455 nm and 475 nm, respectively, and suggested that the difference could be used specifically to quantify fat. The fluorescence spectra from fatty tissue and CT are, however, severely overlapped, as indicated in figure 5.7, so the use of multivariate analysis and regression is needed for curve resolution and useful quantitative measurements. Note from the figure that the spectrum from pork is generally more intense than that from beef, because the meat is brighter and less reabsorption of fluorescence occurs. The peak at 450 nm is mostly associated with CT, the one at 475 nm with fat, and the one at 385 nm is connected with both constituents. The valley at about 420 nm is probably due to reabsorption of myoglobin, and disappears more or less after heat treatment. The

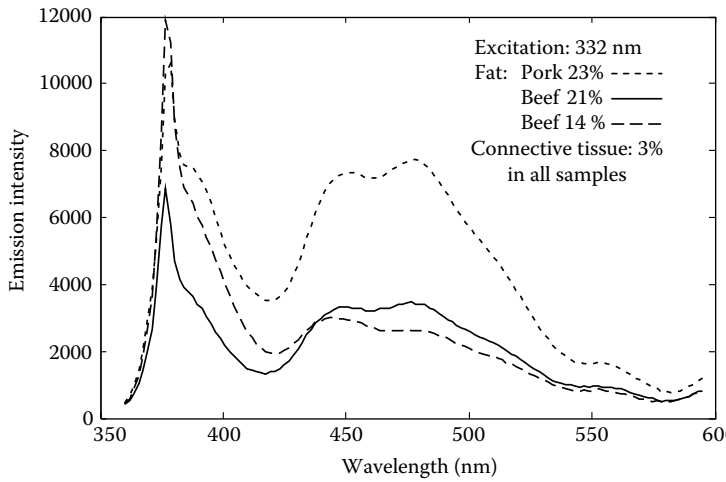


FIGURE 5.7 Fluorescence spectra from ground beef.

complexity of the spectra has motivated studies of how various biological variations will affect the predictive ability of the method with regard to collagen.

Wold, Lundby, and Egelandsdal (1999) designed a sample set ($n = 66$) of ground beef that spanned the range of both CT and fat to a high, but realistic amount with regard to meat products. Front-face fluorescence spectra were collected with an optical bench system. A circular sample area of 5 cm diameter was measured. Five excitation wavelengths were investigated (300, 332, 365, 380, and 400 nm). A smaller set of independent samples ($n = 24$) were introduced as a test set. Partial least squares regression resulted in the lowest root mean square error of prediction at 0.37% CT ($R = .97$) and 1.89% fat ($R = 0.84$) for excitation wavelengths 380 and 332 nm, respectively. This system was quite idealized, as it was based on materials only from bovine LD. Egelandsdal et al. (2005) showed that the greatest source of variation in fluorescence from sausage batters was not different levels of collagen and fat, but rather the type of muscle that was used in those batters. A dark muscle like beef *Masseter* contains much myoglobin, whereas a pork muscle like *Gluteus medius* contains significantly less. More realistic follow-up studies were performed. In one experiment 50 batch samples of ground meat of each of the quality grades beef 14% fat, beef 21% fat, and pork 23% fat, were collected randomly at eight different production plants in Norway. Again PLSR was used to calibrate between fluorescence spectra and CT (measured as hydroxyproline), and promising results were obtained. Overall, when a common calibration model was made including all 150 samples, a RMSEP of 0.55% CT was obtained, although a calibration on only beef of 14% fat yielded somewhat better results (RMSEP = 0.49%; Egelandsdal et al. 2002). Egelandsdal et al. (2005) also showed that similar prediction results could be obtained on complex sausage batters consisting of different kinds of muscles, a large span in myoglobin, and realistic ranges of CT and fat. One of the main conclusions from that study is that multivariate regression is necessary to obtain any kind of meaningful calibration with chemical composition. Another notable result from this study was that fluorescence performed better than NIR in predicting collagen content. The conclusion of these surveys is that autofluorescence spectroscopy might be well suited for rapid online determination of collagen in ground beef. Excitation wavelength around 380 nm is optimal for determination of CT, whereas excitation at 332 nm is feasible for simultaneous determination of fat and CT. It is important to include expected biological variation in the calibration model. The precision obtained in the studies is relevant to the industry, but as far as we know, the method has so far not been implemented.

The ability to classify whole meat cuts according to the amount of CT is of interest, particularly for cuts in which the amount of CT varies over a wide range and directly affects tenderness and other technical properties. To achieve representative measures from whole cuts, imaging and image analysis would probably be needed. It should be pointed out that the best excitation–emission pairs to use to highlight myofibers, fat, and CT for imaging are 290/332 nm, 322/440 nm, and 380/440nm, respectively (Skjervold et al. 2003). Using these filter combinations for imaging, it is easy to distinguish the three different tissues. In that way, it would be possible to perform a quantitative analysis of each cut as long as the surface is representative enough for the interior.

5.4.4 Tenderness and Muscle Types

When there is a substantial range in collagen levels within a muscle or between muscles, there is a significant relationship between collagen content and the variation in tenderness (Light, Champion, Voyle, and Bailey 1985). Consequently, fluorescence can probably be used to estimate tenderness in such muscles. Determination of tenderness in a muscle like LD based on fluorescence emission spectroscopy (excitation between 332 nm and 380 nm) has, however, proven to be difficult (Egelandsdal et al. 2002). The collagen content in LD is low and stable. In addition, variation in sarcomere length and other factors, which are not picked up by fluorescence, reduce the ability to obtain reliable calibration models for tenderness. A combination of fluorescence and measurement of light scattering by, for instance, NIR could improve feasibility, but preliminary work suggests that there is not much to gain.

The tryptophan fluorescence spectra from meat have also been evaluated for tenderness measurement and for discrimination between different muscle types. Dufour and Frencia (2001) measured emission spectra of protein tryptophan residues for the meat samples from *Longissimus thoracis* (LT) and *Infraspinatus* (IS) at 2 and 14 days postmortem. The maximum emission was observed at about 336 nm and shifted slightly as a function of meat sample and aging (figure 5.8). Based on the spectral profiles, it was possible to discriminate between the muscles (figure 5.9).

Frencia, Thomas, and Dufour (2003) continued this work by recording the tryptophan fluorescence spectra on five muscle types (*Tensor fasciae latae* [TFL], LT, *Semi-tendinosus* [ST], IS, and *Triceps brachii* [TB]) at two points (2 and 14 days) during aging. By discriminant analysis, 82% of the samples were correctly classified. It was concluded that tryptophan fluorescence spectra are characteristic fingerprints allowing a relatively good identification of muscle type at 2 and 14 days postmortem. These results indicate the possibility of classification of muscle type by fluorescence spectroscopy.

Preliminary studies also suggest that the tryptophan spectra might contain information related to rheology and sensory variables related to tenderness. The

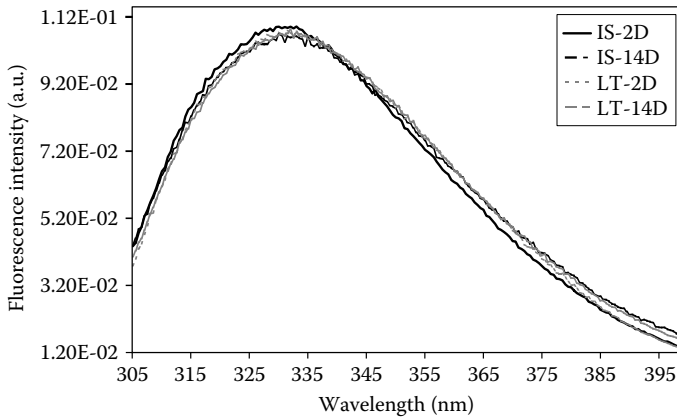


FIGURE 5.8 Normalized emission spectra of LT and IS at 2 and 14 days postmortem at excitation wavelengths of 290 nm.

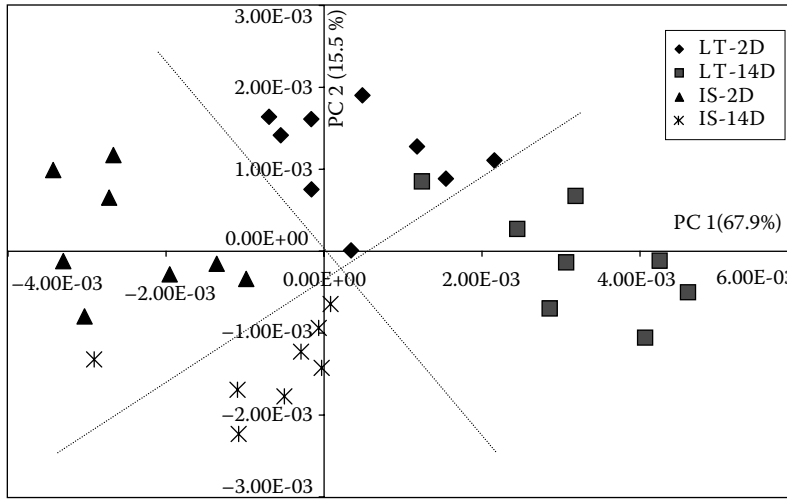


FIGURE 5.9 PCA similarity map defined by the principal components 1 and 2 for the tryptophan spectral data of LT and IS muscles at 2 and 14 days postmortem.

fluorescence spectra (spectrometer with a front-face device or coupled to a fiber optic), the mechanical properties, and the sensory characteristics were recorded at 2, 6, and 11 days postmortem on three muscles (LT, ST, and TB) sampled on two bovine carcasses (Frencia et al. 2003). As the three methods were able to discriminate between the samples, the correlation among the results obtained with sensory analysis, rheology, and fluorescence spectroscopy were investigated by canonical correlation analysis (CCA). Table 5.2 shows strong correlations between the different data tables. Considering sensory and spectral data, the canonical coefficient for the canonical variates was 0.95.

Lebecque, Laguet, Chanonat, Lardon, and Dufour (2003) designed a sample set of ST muscles of 25 Charolais carcasses of different sex and age (30 months–8 years), which spanned a wide range of meat tenderness. Sensory analysis (eight texture attributes) and fluorescence analysis (tryptophan residues) were performed

TABLE 5.2
Canonical Correlation Coefficients (*R*) for the First Canonical Variates of CCA Performed on the Sensory, Rheology, and Spectral Data

	R
Sensory analysis/spectroscopy with optic fiber	.93
Sensory analysis/spectroscopy with front-face device	.95
Texturometer/spectroscopy with front-face device	.95
Spectroscopy with front-face device/with optic fiber	.96

after 7 and 12 days of aging. CCA performed on the sensory and fluorescence data showed that the first two canonical variates were correlated with squared canonical correlation coefficient equal to .57 (level of confidence, $p < .001$). This result indicates that the texture attributes of meat may be derived from the fluorescence spectra of protein tryptophans. Allais, Viaud, Pierre, and Dufour (2004) also showed that similar results could be obtained on meat emulsions and frankfurters. A laptop-compatible spectrofluorimeter that may be used in the abattoir for texture measurements is currently under development.

5.4.5 Lipid Oxidation

Increasing efforts have been devoted to the development of methods to detect and quantify lipid oxidation in model systems as well as in food systems. Besides being important for product quality and shelf life, lipid oxidation in foods has attracted increased attention as a health issue in recent years. Most of these methods have been developed for use in pure oil systems, and some of the methods have been adapted to more complex matrices like muscle foods, but with questionable reliability (Gullién-Sans and Guzmán-Chozas 1998; Jo and Ahn 1997). Generally, for some food systems the most sensitive and reliable method for assessing lipid oxidation is therefore sensory analysis (Frankel 1998), which is time consuming, very expensive, and requires a trained expert panel.

In the food industry, quality control of fat is achieved by a small set of methods for unspecific determination of oxidation products like peroxides and aldehydes, conjugated dienes and other secondary oxidation compounds. One of the main needs in the area of product quality in relation to lipids is the development of reliable methods, preferably rapid and noninvasive, to evaluate lipid oxidation progress and for early prediction of oxidative stability. The industrial needs are in this respect a mirror image of consumer demands for improved processed foods with a minimum of oxidative changes. Although techniques such as gas sensors (Haugen and Kvaal 1998), ultraviolet-absorbance spectroscopy (Baron, Bro, Skibsted, and Andersen 1997), and fluorescence spectroscopy (Wold and Mielnik 2000) have potential for online applications, no good methods for this purpose are as yet established.

Oxidative processes involve many complex red-ox reactions, and a variety of lipid oxidation products are formed. Some of the oxidation products are unstable and will react with other compounds in the matrix. Some of these reaction products are autofluorescent; that is, they emit light in the visible region when illuminated by ultraviolet light. These compounds are formed from reactions of oxidizing fatty acids or lipid oxidation breakdown products (hydroperoxides and aldehydes) with compounds containing primary amino groups (proteins, amino acids, DNA; Kikugawa and Beppu 1987). Autofluorescence is by now recognized as a sensitive method for determining the level of lipid oxidation in complex foods such as fish and meat (Melton 1983). However, autofluorescence, as used until recently, has been regarded as a destructive technique, because it has been performed on extracts of lipids or proteins. More recently it has been reported that good estimates of the degree of rancidity in ground poultry meat as well as on more complex meat products, can be obtained by autofluorescence measurements directly on the product (Wold

and Mielnik 2000; Wold, Mielnik, Pettersen, Aaby, and Baardseth 2002). The method correlates well with both thiobarbituric acid reactive substances (TBARS), sensory measured oxidation, and with volatile compounds measured with gas chromatography-mass spectroscopy (GC-MS). The fluorescence spectra exhibited by the oxidation products are rather broad and featureless, but the resulting solid-sample spectra can be complex because signals from other fluorophores such as CT and porphyrins will also be present. In spite of the complexity, with chemometric techniques it is possible to extract quantitative information related to rancidity. Figure 5.10 shows the fluorescence response after adding small amounts of different aldehydes to pork meat (Veberg, Wold, and Vogt, in press). The samples were stored for seven days at 4°C before these measurements. The blank was lean pork meat.

Solid sample fluorescence spectroscopy has several interesting properties with regard to determination of rancidity:

1. It is rapid and nondestructive; that is, it has potential for online/at-line use.
2. State of oxidation can be determined without any kind of extraction step; that is, it might give a more "correct" measure than other traditional measures where extraction is required.
3. Several different lipid oxidation products might be measured simultaneously, probably giving the opportunity to instantly model different sensory properties of the foods.
4. It has potential for use on raw and cooked foods, as well as raw materials and end products (Wold et al. 2002).
5. It seems to be at least as sensitive as sensory analysis.
6. It is possible to create maps of lipid oxidation by spectral imaging of the fluorescence, enabling detailed studies of lipid oxidation distribution and progression in meat and meat products (Wold and Kvaal 2000).

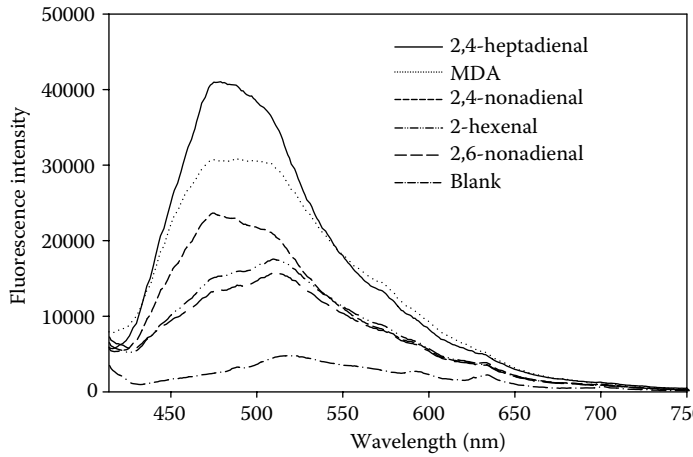


FIGURE 5.10 Fluorescence from pork meat after addition of five different aldehydes. MDA = malondialdehyde.

Central components in lipid oxidation processes are believed to occur in the following sequence: free radicals, hydroperoxides, thiobarbituric acid (TBA)-reactive substances, and finally fluorescent oxidation products. This sequence indicates that fluorescence is not sensitive to early formation of oxidation. However, these chemical processes can run in parallel, some fast and others slow. Some unsaturated aldehydes will react to form strong fluorochromes, and because they react quickly with the surroundings, within hours (Verberg et al., in press), and fluorescence is a very sensitive method, early detection should be possible. It is also suggested that fluorescent compounds can be formed already by lipid radicals reacting with amino acids (Yamaki, Kato, and Kikugawa 1992). How early fluorescence is capable of detecting lipid oxidation is uncertain and probably dependent on the food product. Olsen et al. (2005) reported that for pork backfat, front-face fluorescence was more sensitive to lipid oxidation than was a trained sensory panel.

The concept of determining oxidative status by using solid sample spectroscopy in combination with chemometrics is promising. It is one of the very few fluoroscence methods with an online potential. However, the approach is rather new and requires further investigation. It is obvious that specific calibration models have to be developed for each product category. The application of combining oxidation detection by fluorescence with image analysis offers new ways to study lipid oxidation and its progression in meat and meat products. It can be convenient and useful to actually see how fast and where the oxidation starts and develops.

5.4.6 Fluorescence Potential for Online Analysis of Meat

As seen earlier, there are several potential online applications for fluorescence spectroscopy. With reference to several studies, the method seems to be robust enough for online situations, as long as the relevant variability of the food system is taken into consideration. Interfering phenomena like quenching, reabsorption, and spectral overlap can be modeled and accounted for in multivariate calibrations. Because the instrumental requirements for fluorescence systems are rather modest, it is probably just a question of time before the first online systems for rancidity screening, for example, are up and running in the meat industry.

5.5 RAMAN SPECTROSCOPY

5.5.1 Background

Raman spectroscopy has great potential for biochemical analysis of tissue at both the macroscopic and microscopic levels. One major advantage of this technique is its ability to provide information about concentration, structure, and interaction of biochemical molecules within intact cells and tissues, nondestructively, without homogenization or extraction. Although discovered in 1928, this field has until recently received rather little attention, most likely due to expensive instrumentation, a cumbersome user interface, and some inherent problems associated with measurements of biomaterials. However, in recent years Raman systems have become much more affordable and easy to use due to the development of dedicated detectors,

lasers, and optics. Also within different areas of food science, Raman spectroscopy has been recognized as a promising analytical tool (LiChan 1996; Ozaki, Cho, Ikegaya, Muraishi, and Kawauchi 1992), and one such area is rapid and nondestructive quality assessment of foods for inline purposes.

5.5.2 Principle of Measurements

Raman is a relatively specific spectroscopic technique that measures rocking, wagging, scissoring, and stretching fundamental vibrations of molecules containing bonds such as C-C, C-O, C-H, -S-S-, -C-S-, and -C=C-. The specificity of the spectral bands is comparable with that of IR, but the methods are based on different selection rules. Whereas the IR signals depend on vibrations of polar functional groups, the ability of a bond to polarize forms the basis of Raman scattering. The two methods are therefore complementary regarding the molecular structural information. As a consequence, Raman is, as opposed to IR, almost insensitive to water, enabling efficient measurement of samples such as meat and liquids.

5.5.3 The Major Challenges in Raman

Raman scattering is a relatively weak optical effect that requires laser light for efficient excitation. This way of excitation introduces two major challenges with regard to biomaterials, including meat.

In most biomaterials, the incident laser light will produce autofluorescence, which is usually much more intense than the Raman scattered light. This fluorescence can make the Raman signals difficult or even impossible to measure. The problem can be avoided by using a Fourier-transform (FT) Raman system with laser excitation at 1064 nm, as negligible fluorescence occurs for this low-energy excitation wavelength (Keller, Lochte, Dippel, and Schrader 1993). However, 1064 nm excitation results in weak Raman signals and requires long exposure times to obtain spectra with sufficient signal-to-noise ratios. This approach is therefore impractical for online purposes. From biomedical research it is suggested that the optimal wavelength region for Raman excitation for rapid tissue analysis is between 780 and 850 nm (Brennan, Wang, Dasari, and Feld 1997). Excitation in this region minimizes the fluorescence emission to an acceptable level and allows the use of sensitive CCD cameras that can capture high signal-to-noise spectra in short time exposures (a few seconds). Several biomedical studies demonstrate that 785 nm and 850 nm Raman spectroscopy can be used for precise quantitative histochemical analysis of various types of human tissue (Buschman et al. 2001; Manoharan, Wang, and Feld 1996; Romer et al. 2000). Algorithms to remove the fluorescence background from Raman spectra have been developed (Brennan et al. 1997; Lieber and Mahadevan-Jansen 2003), and excellent quantitative calibrations have been obtained, for instance, for carotenoids and fat in ground salmon muscle (Wold, Marquardt, Dable, Robb, and Hatlen 2004). Figure 5.11 shows raw and background corrected Raman spectra from beef muscle and intramuscular fat.

The second challenge is that the area that is actually measured is very small, typically 250 μm^2, basically limited by the diameter of the laser beam. The small

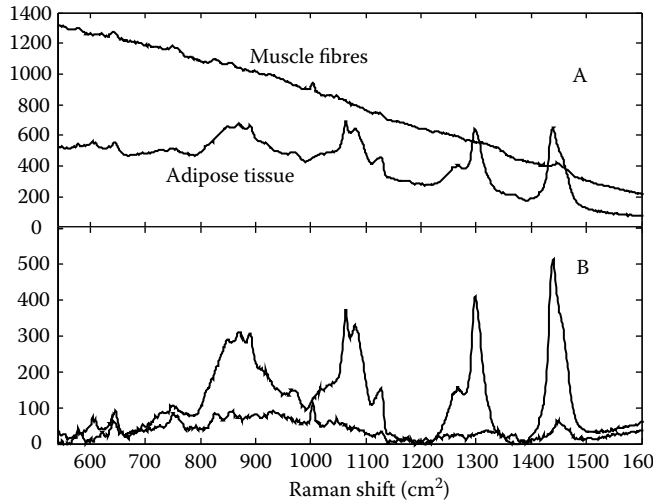


FIGURE 5.11 (a) Raw Raman spectra from muscle fibers and adipose tissue from beef. (b) Same spectra but background corrected.

sampling spot requires careful consideration of how to obtain representative measurements from heterogeneous samples. The samples can be homogenized, multiple measurements can be performed on each sample, or—if measurements are done batchwise—several measurements from the same batch can be averaged. Some new Raman developments use larger diameter lasers (around 3–4 mm) to improve representation and close the sampling gap between NIR and Raman. The small spot size certainly also has some benefits. It is possible to collect quantitative Raman spectra from very small and specific parts of tissue.

One previous difficulty with Raman spectroscopy of heterogeneous materials and solutions was the problem of optical focusing. Much of this problem has recently been overcome by the development of new probes. An example is a so-called ballprobe (see figure 5.12), a novel immersion probe designed and optimized for performing Raman measurements in both laboratory and industrial environments (Marquardt 2001). The spherical lens probe is an efficient sampling interface for the analysis of heterogeneous multiphase samples including solids, tissues, slurries, and liquids. High-quality Raman spectra can be collected simply by ensuring physical contact between the sample and the probe, as the focal point is fixed at the tip of the sapphire ball. New probes like this make it possible to transfer any successful Raman method to an online application.

5.5.4 Potential Use of Raman in Meats

The potential of Raman spectroscopy for determination of meat quality has so far been briefly investigated. Some of the components contributing to the Raman scattering in muscle tissues are certain amino acids, collagen, elastin, carotenoids, fatty acids, and cholesterols, all of which can be useful to describe meat quality. Raman

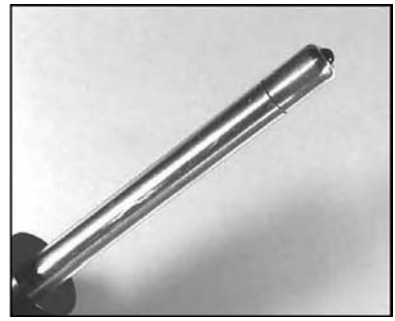


FIGURE 5.12 Immersion ball probe for robust Raman measurements.

is well known for the ability to determine the degree of saturation in fatty acids, and high correlations have been established with the iodine number in oils (Sadeghijorabchi, Wilson, Belton, Edwardswebb, and Coxon 1991). Because there is an increased consciousness of fat composition, especially with focus on the proportions of saturated, monounsaturated, and polyunsatured fatty acids, there might be an interest in transferring this application to intact meat.

Another interesting Raman feature is the ability to measure and describe changes in secondary protein structure. Certain Raman bands can be assigned to α-helix and β-sheet, and the ratio can be measured. Beattie, Bell, Farmer, Moss, and Desmond (2004) suggested that Raman can be useful for determination of textural properties like tenderness and shear force based on this ratio.

Raman is generally very well suited for online use. Fiber optics (up to hundreds of meters in length) enables remote analyses in difficult-to-access spots and harsh environments. Instruments are robust, stable over time, and designed for online purposes. The limited Raman sampling spot will in many cases require multiple measurements to obtain representative sampling. Measurements in continuous streams of ground meat, batters, and powders are feasible, but representative sampling of larger heterogeneous products is difficult.

5.6 MICROWAVE SPECTROMETRY

5.6.1 Introduction

Traditional microwave spectroscopy is defined as high-resolution absorption spectroscopy of molecular rotational transitions in the gas phase. During the last few years, however, instrumentation for solid sample analysis has been developed. Most applications using microwave spectroscopy on solid samples deal with water analysis, as water is a very strong absorber of microwave energy. Technical principles and examples of applications are described next.

5.6.2 Principle of Measurements

Microwave spectrometry is based on the orientation and relaxation of polar molecules in an electromagnetic field. Microwaves cover the frequency range from 200 MHz

to 80 GHz. When a sample is irradiated with microwave energy, two basic molecular processes take place. The first is described by the dielectric constant (ε'), which reflects the field reduction due to the dielectric molecule. As an electromagnetic wave passes through a sample, it causes an alternating polarization within the material. The material stores some of the energy, and releases it back to the wave slowly, thereby reducing the wave velocity. ε' has the value of one for a vacuum and greater than one for a dielectric substance. The small dipoles of water molecules can easily be oriented in a rapidly oscillating electromagnetic field, giving water a very high dielectric constant compared to almost all other molecules ($\varepsilon' = 80.2$ at 20°C). The second molecular process is described by the dielectric loss (ε''). This is a heat energy loss caused by friction between the orienting molecules, resulting in a wave amplitude reduction. Measuring ε' or ε'' as a function of frequency provides a microwave spectrum (Walmsley and Loades 2001).

5.6.3 Applications and Instrumentation

Unlike waves in many other regions of the electromagnetic spectrum, microwaves can penetrate through large volumes of meat. Microwave spectrometry should thus be well suited for bulk online measurements of ground meat for standardization purposes. It should be noted that microwave spectrometry has two main drawbacks that could concern analysis of ground meat samples: First, frozen meat or ice will not give microwave signals; second, the presence of salt will disturb the measurements substantially. In addition, microwave spectra are not as easily interpretable as those of most other comparable techniques. The great benefit of microwaves is their penetration depth, which is on the order of several centimeters. The penetration depth makes this technique well suited for very heterogeneous materials like ground meat.

Online microwave spectrometry can be performed using two basically different instrumental principles: noncontact measurements using antennas mounted over the sample stream, (i.e., reflectance measurements), and guided microwave spectrometry, which utilizes a waveguide chamber (i.e., transmission measurements). The latter technique is probably the one best suited for online analysis of ground meat.

5.6.3.1 Noncontact Reflectance Mode Microwave

Knöchel, Daschner, and Taute (2001) reported that open microwave resonators were well suited for online moisture monitoring of cereal products. The same principle was tested by Kent, Knochel, Daschner, and Berger (2001) for determination of water uptake and protein, fat, water, salt, and phosphate contents in pork and chicken. Also the use of reflectance microwave for analyzing fat content in ground meat on a conveyer belt has been studied (unpublished results). This implies noncontact measurements, which have advantages in several aspects. A vector network analyzer from Rohde and Schwarz was used, where the frequency range 4 GHz to 8 GHz was scanned, and the microwave signal was delivered to the meat sample by a horn antenna 40 cm over the conveyer belt. The measurements were performed on the same 60 batches (120–180 kg) of ground beef as described earlier in discussing NIR. As reference measurements, scans of the conveyer belt were used. After belt correction and FT of the data, regression

was performed against fat values. The explained variance of the model was 0.74, which was not impressive. However, by using similar physical waveguides as previously described to eliminate external interferences and freak reflections, the potential for improving the results could be considerable.

5.6.3.2 Guided Microwave Spectrometry

In online guided microwave spectrometry (GMS) analysis, the sample has to move through and fill a waveguide chamber. The main purposes of the waveguide chamber are to assure constant sample thickness and to guide the microwaves toward the receiver, as exemplified by the parallel horizontal metal plates shown in figure 5.13. The metal plates reflect the microwave energy and restrict the wavelength range reaching the receiver. Only waves that can fit into the chamber (i.e., wavelengths shorter that 2a) can reach the detector. The spectroscopic response is an attenuation spectrum for the different microwave frequencies involved. The GMS utilizes the lower frequency range of the microwave region (i.e., 200–3,200 MHz; Dane, Rea, Walmsley, and Haswell 2001; Wellock and Walmsley 2004).

The GMS is suited for samples that can be pumped through the waveguide chamber, like ground meat. The instrument can be mounted on transportation pipes or directly onto a meat grinder. Grinder mounting does not require any additional pumping of the meat. The main assets of GMS are that practically all the material is measured, and it has a low sensitivity toward particle size and color differences.

GMS is a relatively new technique, and not many food applications have been reported in scientific literature. However, there are industrial GMS applications running, and one such implementation test is presented shortly (unpublished results). A GMS instrument from Thermo Electron Corp. (Round Rock, TX) was tested on a meat production site in Norway (Gilde Hed-Opp) in the fall of 2004. The equipment was mounted directly onto the meat grinder, and the target was measuring total fat content in ground beef batches of up to 1,000 kg. It should be noted that fats and oils do not produce microwave spectra, and that this application relies on the relationships with water and protein responses. The calibration was performed on 47 samples in the range of 3.1% to 77.3% fat. The calibration samples were not in motion while the spectra were taken; that is, the calibration samples were obtained with the waveguide chamber filled with meat not in motion (~1 kg). The whole calibration sample was then removed from the chamber, homogenized, and analyzed using the fat reference method. The validation was based on spectra generated online from 19 full batches in the range of 3.0% to 32.4% fat. The validation reference samples were collected after 10 minutes of mixing. Approximately 40 kg of sample

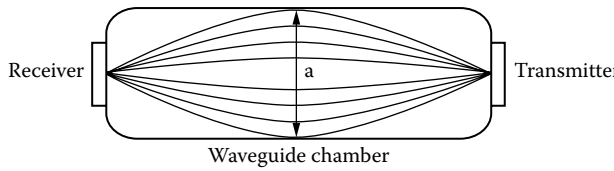


FIGURE 5.13 Technical principle guided microwave spectroscopy (GMS).

were taken from each batch, homogenized, and analyzed using the same reference analysis as in the calibration step. This experiment gave an average prediction error (RMSEP) of 1.5% fat after a bias adjustment.

5.7 NUCLEAR MAGNETIC RESONANCE

5.7.1 Background

When Purcel, Torrey, and Pound (1946), as well as Bloch (1946) demonstrated nuclear magnetic resonance (NMR) in condensed matter for the first time in 1946, they probably had no idea that this finding would lead to many important applications in all branches of science and medicine. NMR has seen spectacular development. One of the reasons for the success comes from its ability to provide nondestructive information on molecular structure, molecular dynamics, chemical analysis, and imaging.

5.7.2 Principle of Operation

Before reviewing applications of NMR to meat products, the choice of magnet is highlighted. It depends on the sample and the measurement. The higher the frequency of the magnetic field, the better the spectral resolution. In MRS (NMR spectroscopy), the frequency range of the magnetic field is typically between 1.4 and 18.8T (60–800 MHz for hydrogen nuclei), whereas in magnetic resonance imaging (MRI) it ranges between 0.4 and 2T. The low-field magnets are sufficient for most applications, because the relaxation time values are more favorable and the water concentration is large enough to have sufficient signal-to-noise ratio.

5.7.3 Constraints Regarding Use in Online Analysis

In the literature, many studies show potentially useful correlations between NMR parameters and the intrinsic quality of food materials. However, these results have not so far been used in online quality assurance in the meat industry. This fact can be explained by some constraints: the sampling, the difficulty of implanting magnets on a production line, the sophisticated methodology used, and the cost of NMR spectrometers.

The sample size to analyze can vary in a large range from a carcass to a small piece of meat. A single small sample is not enough to characterize the carcass or meat products. Analyses have to be performed on the whole product or many samples. Because NMR experiments are time consuming, the analysis time is not compatible with the speed of the production line.

An NMR experiment needs to have a high spatial homogeneity field. The movement of ferromagnetic mass outside and in the vicinity of a magnet induces great perturbation field lines. Each sample also has its own geometry and susceptibility and moves through the magnet. All those factors disturb the field homogeneity and hinder the online NMR spectrometer implant even if these perturbations can be corrected with expensive and time-consuming devices.

The last point is that there is no actual commercial NMR spectrometer currently built for the industrial environment. No NMR spectrometer is available at low price, easy to use for unqualified staff, or designed for online chains. The spectrometer

price and maintenance costs are high because the NMR technology is quite sophisticated and the market is small. The electronic devices used are not absolutely consistent with clean-up operations as required by hygienic safety. Moreover, production lines are not designed to receive an NMR spectrometer.

The use of NMR in on-line analysis of meat quality parameters must therefore be seen in a long time perspective. However, the potential of the technique in this area should be large. In the following subsections, the most interesting applications, as we see it today, are outlined.

5.7.4 NMR Applications in Meat Research

Quantity and distribution of fat and CT are of great importance for the evaluation of meat quality.

5.7.4.1 Fat Content and Distribution

NMR relaxometry (Renou, Kopp, and Valin 1985; Toussaint et al. 2002) or spectroscopy (Foucat, Donnat, Humbert, Martin, and Renou 1997; Renou, Briguet, Gatellier, and Kopp 1987) allows the determination of fat content with great accuracy. The NMR results are always closely correlated with the reference chemical methods. Acceptability of meat by the consumer is also related to the intramuscular fat distribution, which is usually estimated by visual inspection of carcasses or quantified from visible light digital images of meat cuts (Monin 1998). Compared to these methods, MRI is a potential alternative tool for examining fat distribution noninvasively and quantitatively because of the intrinsic contrast due to the different NMR properties of water and lipids (Bonny, Santé-Lhoutellier, and Renou, in press; Fuller, Fowler, McNeill, and Foster 1994; Laurent, Bonny, and Renou 2000). The extracellular intramuscular fat distribution in meat was determined in different cattle breeds (figure 5.14). MRI can be very useful in genetic selection and stock breeding.

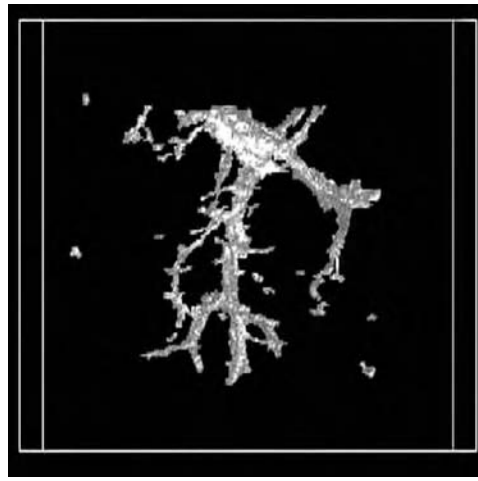


FIGURE 5.14 Extracellular intramuscular fat 3D distribution in meat of a cattle breed as measured by NMR.

5.7.4.2 Connective Tissue

The contribution of the amount of intramuscular CT to meat toughness has long been recognized (Purslow 1999). In contrast, the role of the spatial distribution of CT in meat quality is still unknown. Muscles with similar CT contents but with different arrangements of CT (orientation, thickness, length) may exhibit different textural properties, such as tenderness, because of the resulting differences in the resistance of the connective network to deformation during mastication and the temperature increase during cooking.

In heterogeneous samples, inherent magnetic field variations occur owing to the coexistence of two adjacent phases with different magnetic susceptibilities. Because of its low water content, the magnetic susceptibility of collagen-rich CT differs from that of soft tissue (Posse and Aue 1990; Schenck 1996; Yablonskiy 1998). Quantitative assessment of susceptibility effects was performed by specific pulse sequence (Posse and Aue 1990; Yablonskiy 1998). Comparison with histological pictures indicates that these maps exhibit the overall organization of the primary perimysium at the scale of the whole muscle. Figure 5.15 illustrates the potential of MRI for characterizing muscle CT structure.

5.7.4.3 Meat Quality Parameters

5.7.4.3.1 Energy Metabolism

The rate of postmortem catabolism in muscle determines meat quality. This trait is under partial genetic control. The muscle metabolic defect connected with porcine halothane sensitivity (also known as malignant hyperthermia syndrome) leads to pale, soft, exudative (PSE) meat of low technological and organoleptic quality. The pH value and levels of several metabolites, reflecting the rate of muscle glycogenolysis and glycolysis, were measured postmortem in muscle of normal and PSE pigs (Miri, Talmant, Renou, and Monin 1992). The difference observed after 30 minutes postmortem in ^{31}P spectrum of the normal and PSE pigs was large. For the normal pig, the spectrum contained seven resonances corresponding to sugar phosphates (SP), inorganic phosphates (Pi), glycerophosphoryl choline (GPC), phosphocreatine

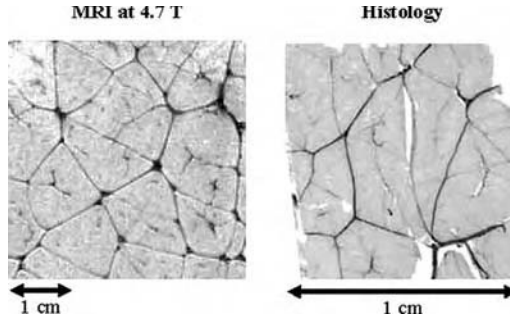


FIGURE 5.15 Potential of MRI for characterizing muscle CT structure. Images of transverse section of bovine *Gluteo biceps* muscle obtained by MRI and histology. Comparison with histological picture indicates that NMR map exhibit the overall organization of the primary perimysium at the scale of the whole muscle.

(PCr), and the three phosphate groups of adenosine triphosphate (ATP). For the PSE biopsy most of the resonances disappeared and only the SP and Pi resonances were observed. Moreover the chemical shift of Pi depends on pH ranging from 7.2 to 5. The study demonstrated a more than threefold accelerated PCr decay in heterozygote malignant-hyperthermia compared with normal pigs (Lahucky et al. 1993). Combining ^{31}P NMR with a rapid and efficient technique for taking biopsies allows the prognosis of these defects in live animals, which is particularly useful for genetic selection (Liu, Lirette, Fairfull, and McBride 1994).

5.7.4.3.2 Water Holding Capacity

The interactions between water and macromolecules determine the water holding capacity (WHC) of meat. Meat WHC depends primarily on the extent of postmortem myofibrillar shrinkage and the correlative changes in the extracellular water compartments (Offer and Knight 1998a, 1998b). WHC of fresh meat was assessed at low field by NMR relaxation measurement of water protons (Brondum et al. 2000; Renou, Kopp, Gatellier, Monin, and Kozakreiss 1989). Highly significant relationships were found between relaxation NMR parameters and some other characteristics such as pH measured 30 minutes postmortem (pH_{30}), reflectance, and cooking yield, whereas T_{2s} was correlated only with pH_{30} (Renou, Monin, and Sellier 1985). Many NMR studies have since been conducted to assess meat quality (Borowiak, Adamski, Olszewski, and Bucko 1986; Brown et al. 2000). From these NMR studies different water compartments have been shown according to their different interactions with macromolecules. Working on pig muscles, Fjelkner-Modig and Tornberg (1986) and Tornberg, Tornberg, Andersson, Göransson, and Von Seth (1993) identified three water compartments: extracellular water, water in myofibrils and reticulum, and water in interaction with macromolecules. This histological picture of water compartments between intra- and extracellular domains is attractive, yet there is little evidence to support this concept (Bertram, Andersen, and Karlsson 2001; Hills 1992; Laurent et al. 2000; 109. Traore, Foucat, and Renou 2000). MRI provides morphological images that can be associated with parametric images of relaxation times, or diffusion in the tissue. Diffusion MRI is a well-established tool (Le Bihan et al. 2001) for noninvasive investigation of muscle structure. This makes it possible to probe the influence of intracellular diffusional barriers (Kinsey, Locke, Penke, and Moerland 1999) during the postmortem structural changes (Foucat, Benderbous, Bielicki, Zanca, and Renou 1995). DTI have confirmed the free water accumulation that diffuses more freely and isotropically than in the rest of the muscle (figure 5.16).

These results show an interstitial space that appears postmortem between fascicles of muscle fibers (Offer and Cousins 1992) and underline the usefulness of diffusion tensor measurements to characterize muscle structure and help understand the mechanisms of postmortem water exudation (Bonny and Renou 2002).

5.7.4.4 Processing

5.7.4.4.1 Freezing-Thawing

Freezing is currently used for extending the shelf life of meat by inhibiting microbiological growth. However, the price of fresh or chilled meat is higher than that of

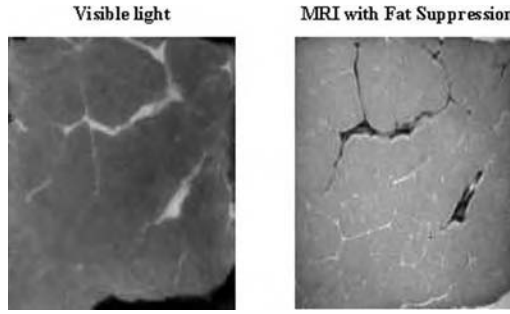


FIGURE 5.16 DTI confirmation of free water accumulation in muscle.

frozen-thawed meat. MRI has been used to measure the effects of freezing in beef, lamb, and pork meat (Evans, Nott, Kshirsagar, and Hall 1998) and trout muscle (Foucat, Taylor, Labas, and Renou 2001; Nott, Evans, and Hall 1999). The variations in the dynamic NMR parameters agree with histological observations (Foucat et al. 2001).

5.7.4.4.2 Brine Composition and Properties

Salt (sodium chloride) is added as a flavoring or flavor enhancer, as a preservative and as an ingredient contributing to desirable textural characteristics of meat products. Except for NMR, no method is able to measure in situ the bound–free ion ratio. The quantitative NMR data are closely correlated with the chemical method. In addition, for each ^{35}Cl and ^{23}Na ion, the bound–free ion ratio reveals significant differences according to technological processing method (Foucat, Donnat, and Renou 2003). NMR approaches allow correlation of the fluxes of water and ions in fish meat during salting (Erikson, Veliyulin, Singstad, and Aursand 2004), and also to determine the effect of raw fish (fat content, freshness, etc.) on the salt distribution.

5.7.4.4.3 Drying

Food characteristics are greatly influenced by moisture content. Methods for processing or stabilizing solid foods involve coupled water and heat transfer. Internal water migration is a function of chemical composition and structure, and drives the overall water transfer. The water diffusitivity coefficient (D) varies with water content. It can be derived from the time course of the moisture profile measured by NMR with 50 μm spatial resolution and 15 minutes temporal resolution (Ruiz-Cabrera, Gou, Foucat, Renou, and Daudin 2004). The lipid content has a negative effect on D while the temperature induces an increase in D values for low water content and a decrease for high water content (Ruiz-Cabrera et al. 2004).

5.7.5 Conclusions

NMR studies in spectroscopy and imaging afford quantitative determination of meat composition such as fat and CT, and the techniques have a useful potential as reference methods. A better understanding of water interactions with meat structure and the underlying meat quality can also be obtained from relaxation and diffusion NMR parameters. In view of the marked industrialization of meat processing, NMR may be

useful for optimizing technological processes such as brining, drying, and freezing. The NMR analytical methods are highly sensitive, accurate, and robust, but are at present expensive and complicated, which limits their applicability for implementation in the meat industry. However, the potential of the technique is high and could have an important role in quality assurance systems in future meat processing.

5.8 X-RAY SPECTROSCOPIC TECHNIQUES

5.8.1 Principle of X-Ray Measurements in Meat

The use of penetrating energy such as X-rays has proved useful in many areas of medicine and technology. The principle of operation of X-ray measurement systems is that various components of muscle—lean meat, fat, and bone—have different properties when exposed to physical energy from X-rays. The relative density is the critical property, as lean tissue has a consistent density of 1.07 to 1.08, whereas fat varies depending on the temperature. Skin has a similar density to lean meat, which means that collagen measurement is hardly possible. As materials attenuate X-rays depending on their energy, use can be made of the selective attenuation of one, two, or more energy levels; that is, mono, dual (DEXA), or multiple energy X-ray absorptiometry (MEXA).

5.8.2 X-Ray Techniques Using One Energy Level

The X-ray technique first appeared in the meat industry in the 1960s with the offline AnylRay device (Gordon 1973), which is still in practical use. When the meat was exposed to X-rays at one low energy level, fat and lean meat absorbed different amounts of X-rays. Although AnylRay was an improvement for the industry over most chemical standard methods, the method was still hampered with errors due to the presence of air and other materials than fat and lean meat, as well as limited sample size.

To reduce the last source of errors, online systems that made use of the same principle were developed (AVS Raytech, Safeline, Ashwell, Hertfordshire, UK). Such systems have been reported and implemented in the meat industry (Groves and Donovan 1979; Hildrum et al. 2003). With known X-ray output, known meat temperature, constant flow, and constant cross-sectional area, the fat content can be continuously monitored with the X-ray system using one energy level. By pumping ground meat through the X-ray cell of fixed dimensions one energy level has been found satisfactory. The system is also in industry use for analyzing fat content in whole pork.

The Raytech instrument was calibrated with selected ceramic standards. The same 60 batches as in the third processing plant discussed earlier were pumped through the measurement cell, and the data collected as a series of samples over 1-sec periods. Once the whole batch had been scanned, the values were averaged. The instrument readings yielded an explained variance of 99% against standard fat methods (Hildrum et al. 2003). The coarseness of the ground beef did not seem to affect the performance of the instrument.

5.8.3 X-Ray Techniques Using Two Energy Levels (DEXA)

DEXA allows the determination of material properties independently of their thickness by calculating the ratio of the attenuated X-ray beams (Bartle, Kroger, and West 2004). DEXA has been used in medical applications for many years, for bone density and fat quantity estimations, for example. Several research teams are currently working on this technology (Bartle et al. 2004; Brienne, Denoyelle, Baussart, and Daudin 2001; Hansen et al. 2003; Tan 2004). A method for determining the fat content (or lean) in boneless meat that is packed in standard size meat boxes has been developed (Bartle et al. 2004), with explained variance of 97% to 98%, against the Babcock fat standard fat method. The method has been successfully launched in industry in New Zealand. Brienne et al. (2001) studied DEXA absorption on three types of pork and beef, and obtained correlations with fat content that were good to very good (R^2 = 0.70–0.97). The DEXA (DXR) was also investigated for the same purpose in Denmark (Hansen et al. 2003). The prediction error (RMSEP) for fat was reported to be in the range of 0.34% to 0.57%, depending on the batch size. The equipment was released for sale in 2002 as the MeatMaster in-line fat content analyzer (Foss A/S, Hillerød, Denmark).

5.9 METHODS FOR GRADING OF CARCASSES

This large area of research deals with measurement technology to aid in the grading and value assessment of both live animals and carcasses. Although they belong to the class of spectroscopic methods, they are only briefly mentioned in this review. Applying computer vision in meat quality evaluation has been an active area of recent research (Tan 2004), and has been recognized as a promising approach. It is claimed that quality attributes such as muscle color, marbling, maturity, and muscle texture can be predicted to a satisfactory accuracy. The existing research has formed a foundation so that a grading assistant can be implemented. Electromagnetic scanning uses the conductivity differential between fat and lean tissue to measure the total body electrical conductivity (TOBEC; Forrest 1995). A carcass or parts of it can be passed through a magnetic field on a conveyer belt to predict lean mass in ham, loin, and shoulder. Ultrasound techniques have also been used for grading purposes. Ultrasound utilizes sound waves that are far beyond the frequency that can be detected by the human ear. Finally, the large number of optical fat or lean probes, mostly handheld, that are in use for classification purposes in the meat industry should be mentioned. According to a review (Forrest 1995), they account for 68% to 86% of the variation in dissected lean percentages of meat. The potential in replacing these with efficient online spectroscopic methods is evident.

5.10 COMMENTS ON SAMPLING PROBLEMS IN ONLINE SPECTROSCOPIC ANALYSIS

A careful consideration of how to obtain representative sampling is most important when developing applications based on spectroscopy. Some of the problems regarding this have already been touched on earlier in this chapter. The calibration and

prediction errors given in the preceding sections are the accumulated errors from different sources. This includes, for example, the analytical error in the reference analysis, sampling error for reference analysis, spectroscopic sampling error, and modeling errors.

The heterogeneity of meat raw materials often results in a large amount of uncertainty in sampling for online analysis. Sampling and the subsequent preparation steps for reference analysis are frequently found to be the greatest sources of errors in the analysis, as with many other food materials. An ideal sample should be identical in all of its intrinsic properties with the bulk of the material from which it is taken. A sample is satisfactory if the properties under investigation correspond to those of the bulk material within the limits set by the nature of the test. Very often the focus is on the analytical uncertainty of the instrument, and sampling problems are overlooked. As a rule of thumb, if the analytical uncertainty is less than one-third of the sampling uncertainty, additional reduction of the analytical uncertainty is deemed to be of little value (Pomeranz and Meloan 1994).

One common way to reduce the sampling error is to increase the sample size. For online calibration work at least 10% of the batch size needs to be sampled, finely ground, and mixed before further preparation for reference analysis. With batches of 1,000 kg and more this is a laborious task.

The spectroscopic sampling error is particularly critical for surface measuring techniques, such as fluorescence and reflectance NIR. With a penetration depth of only a few millimeters, only a small fraction of the overall meat flow is being monitored. The scanning time on the meat material (conveyer belt, tube, stream) needs to be of a certain length to obtain a sufficient sample. However, the longer the measurements are recorded, the more representative the measurements will be for the whole batch. Therefore both a slow flow rate of meat and a large batch will increase the size of the spectral sample, given a fixed scanning rate of the instrument for surface measuring instruments.

For the diode array NIR Corona 45 instrument with 80-sec scanning time, the spectral sample was estimated to be about 0.5 kg from a batch of 180 kg, which is more than 100 times the amount of an average offline scan. Increasing the illuminated area by using more multiple sensors (i.e., the Titech scanner) will also improve the size of the sample, and consequently decrease the instrument sampling size error. For X-ray, GMS, and NIR transmission techniques in which the energy penetrates through the meat material, the spectroscopic sampling is less critical. However, even for an X-ray technique the prediction error was halved by increasing the sample from 27 kg to 241 kg (Hansen et al. 2003).

5.11 CONCLUDING REMARKS

As stressed in the introduction, the material presented in this chapter is predominantly based on our experiences with selected spectroscopic techniques. Some of these, like NIR, GMS, and X-ray, are already implemented for online use in meat processing. Others have similar or even higher potential, but have to be developed further before they are mature for such purposes.

It has not been our intention to provide comprehensive coverage of the large and bewildering specter of techniques and instruments on the market. This would be an impossible task in itself, and unfortunately many interesting devices do not have satisfactory documentation regarding their performance in practical use.

The focus has been to investigate and demonstrate the potential of spectroscopic techniques for online applications in the meat sector, rather than presenting robust models for prediction purposes that are ready for the marketplace. A large portion of the studies reported in this chapter are based on models with a limited number of independent samples. However, the success of the presented research strongly supports the contention that these applications can be successfully developed should the industry want them. Even as screening techniques without the complete level of accuracy, the methods merit further study as the food industry moves toward an increased level of surveillance to protect market share and satisfy consumer concerns. It is our belief that in a couple of decades, meat processing lines will be surveyed at the critical points by a number of spectroscopic measuring devices to secure those goals.

REFERENCES

Allais, I., C. Viaud, A. Pierre, and E. Dufour. 2004. A rapid method based on front-face fluorescence spectroscopy for the monitoring of the texture of meat emulsions and frankfurters. *Meat Sci.* 67:219–229.

Anderson, N. M., and P. N. Walker. 2003. Blending ground beef to control fat content using simulated on-line spectroscopic measurements. *Trans. of the ASAE.* 46:1135–1141.

Bailey, A. J., T. J. Asims, N. C. Avery, and E. P. Halligan. 1995. Non-enzymatic glycation of fibrous collagen: Reaction products of glucose and ribose. *Biochem. J.* 305:385–390.

Bailey, A. J., and N. D. Light. *Connective tissue in meat and meat products*. London: Elsevier Applied Science.

Baron, C. P., R. Bro, L. H. Skibsted, and H. J. Andersen. 1997. Direct measurement of lipid peroxidation in oil-in-water emulsions using multiwavelength derivative UV-spectroscopy. *J. Ag. and Food Chem.* 45:1741–1745.

Bartle, C. M., C. Kroger, and J. G. 2004. New uses of X-ray transmission techniques in the animal-based industries. *Rad. Phys. and Chem.* 71:843–851.

Beattie, R. J., S. J. Bell, L. J. Farmer, B. W. Moss, and P. D. Desmond. 2004. Preliminary investigation of the application of Raman spectroscopy to the prediction of the sensory quality of beef silverside. *Meat Sci.* 66:903–913.

Bertram, H. C., H. J. Andersen, and A. H. Karlsson. 2001. Comparative study of low-field NMR relaxation measurements and two traditional methods in the determination of water holding capacity of pork. *Meat Sci.* 57:125–132.

Bloch, F. 1946. Nuclear induction. *Phys. Rev.* 70:460–474.

Blumberg, W. E., F. H. Doleiden, and A. A. Lamola. 1980. Hemoglobin determined in 15 mu-L of whole-blood by front-face fluorometry. *Clin. Chem.* 26:409–413.

Bonny, J. M., and J. P. Renou. 2002. Water diffusion features as indicators of muscle structure ex vivo. *Mag. Res. Imaging.* 20:395–400.

Bonny, J. M., V. Santé-Lhoutellier, and J. P. Renou. in press. Multiexponential diffusion in meat at high b values by MRI. In *Magnetic resonance in food science: The multivariate challenge,* eds. P. S. Belton, A. M. Gil, G. A. Webb, and S. B. Engelsen,. Cambridge, UK: Royal Society of Chemistry.

Borggaard, C., L. Bager Christensen, and B. L. Jespersen. 2003. *Reflection mode microwave spectroscopy for on-line measurement of fat in trimmings*. Presented at the 49th ICOMST, September, Campinas, Brazil.

Borowiak, P., J. Adamski, K. Olszewski, and J. Bucko. 1986. *The identification of normal and watery pork by pulsed nuclear magnetic resonance measurements*. Presented at the 32nd European Meeting of Meat Research Workers, Ghent.

Brennan, J. F., Y. Wang, R. R. Dasari, and M. S. Feld. 1997. Near-infrared Raman spectrometer systems for human tissue studies. *Appl. Spectroscopy.* 51:201–208.

Brienne, J.P., C. Denoyelle, H. Baussart, and J. D. Daudin. 2001. Assessment of meat fat content using dual energy X-ray absorption. *Meat Sci.* 57:235–244.

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

Brown, R. J. S., F. Capozzi, C. Cavani, M. A. Cremonini, M. Petracci, and G. Placucci. 2000. Relationships between H-1 NMR relaxation data and some technological parameters of meat: A chemometric approach. *J. of Mag. Res.* 147:89–94.

Buschman, H. P., G. Deinum, J. T. Motz, M. Fitzmaurice, J. R. Kramer, A. van der Laarse, A. V. Bruschke, and M. S. Feld. 2001. Raman microspectroscopy of human coronary atherosclerosis: Biochemical assessment of cellular and extracellular morphologic structures in situ. *Cardiovascular Pathology.* 10:69–82.

Dane, A. D., G. J. Rea, A. D. Walmsley, and S. J. Haswell. 2001. The determination of moisture in tobacco by guided microwave spectroscopy and multivariate calibration. *Anal. Chim. Acta.* 429:185–194.

Downey, G., and K .I. Hildrum. Analysis of meats. In Near infrared spectroscopy in agriculture (Agron. Monogr. 44), ed. C. A. Roberts, 599–632. Madison, WI:ASA, CSSA, SSSA.

Dufour, E., and J. P. Frencia. 2001. Les spectres de fluorescence frontale: Une empreinte digitale de la viande. *Viandes & Produits Carnés.* 22:9–14.

Dufour, E., and A. Riaublanc. 1997. Potentiality of spectroscopic methods for the characterisation of dairy products: I. Front face fluorescence study of raw, heated and homogenised milks. *Le Lait.* 77:657–670.

Egelandsdal, B., G. Dingstad, G. Tøgersen, F. Lundby, and Ø. Langsrud. 2005. Autofluorescence quantifies collagen in sausage batters with a large variation in myoglobin content. *Meat Sci.* 69:35–46.

Egelandsdal, B., J. P. Wold, A. Sponnich, S. Neegard, and K. I. Hildrum. 2002. On attempts to measure the tenderness of Longissimus dorsi muscles using fluorescence emission spectra. *Meat Sci.* 60:187–202.

Erikson, U., E. Veliyulin, T. E. Singstad, and M. Aursand. 2004. Salting and desalting of fresh and frozen-thawed cod (Gadus morhua) fillets: A comparative study using Na-23 NMR, Na-23 MRI, low-field H-1 NMR, and physicochemical analytical methods. *J. Food Sci.* 69:E107–E114.

Evans, S. D., K. P. Nott, A. A. Kshirsagar, and L. D. Hall. 1998. The effect of freezing and thawing on the magnetic resonance imaging parameters of water in beef, lamb and pork meat. *Int. J. Food Sci. Tech.* 33:317–328.

Eyre, D. R., M. A. Paz, and P. M. Gallop. 1984. Cross-linking in collagen and elastin. *Ann. Rev. Biochem.* 53:717–748.

Fjelkner-Modig, S., and E. Tornberg. 1986. Water distribution in porcine M-Longissimus dorsi in relation to sensory properties. *Meat Sci.* 17:213–231.

Forrest, J. 1995. *Value-based marketing systems: Technology implementation*. Presented at the Annual International Congress of Meat Science and Technology, San Antonio, TX, August 20–25.

Foucat, L., S. Benderbous, G. Bielicki, M. Zanca, and J. P. Renou. 1995. Effect of brine injection on water dynamics on postmortem muscle: Study of T-2 and diffusion-coefficients by MR microscopy. *Mag. Res. Imaging*. 13:259–267.

Foucat, L., J. P. Donnat, F. Humbert, G. Martin, and J. P. Renou. 1997. On-line determination of fat content in ground beef. *J. Magn. Reson. Anal*. 108–112.

Foucat, L., J. P. Donnat, and J. P. Renou. 2003. 23Na and 35Cl NMR studies of the interactions of sodium and chloride ions with meat products. In *Magnetic resonance in food science: Latest developments,* eds. P. S. Belton, A. M. Gil, G. A. Webb, and D. Rutledge, 180–185. Cambridge, UK: Royal Society of Chemistry.

Foucat, L., R. G. Taylor, R. Labas, and J. P. Renou. 2001. Characterization of frozen fish by NMR imaging and histology. *Am Lab,* 33:38ff.

Frankel, E. N. 1998. *Lipid oxidation* (Vol. 10). Dundee, Scotland: The Oily Press.

Frencia, J. P., E. Thomas, and E. Dufour. 2003. Measure of meat tenderness using front-face fluorescence spectroscopy. *Sciences Des Aliments*. 23:142–145.

Fuller, M. F., P. A. Fowler, G. McNeill, and M. A. Foster. 1994. Imaging techniques for the assessment of body-composition. *J. of Nutrition*. 124:S1546–S1550.

Genot, C., F. Tonetti, T. Montenaygarestier, D. Marion, and R. Drapron. 1992. Front face fluorescence applied to structural studies of proteins and lipid-protein interactions of viscoelastic food-products:2. Application to wheat gluten. *Sciences Des Aliments*. 12:687–704.

Gordon, A. 1973. AnylRay determines lean/fat ratio. *Food Pros. Ind*. 42:495.

Greensill, C. V., and K. B. Walsh. 2000. A remote acceptance probe and illumination configuration for spectral assessment of internal attributes of intact fruit. *Measurement Sci. & Tech*. 11:1674–1684.

Groves, W. H., and A. D. Donovan. 1979. *Continuous X-ray analysis for meat blending system*. U.S. Patent 4,171,164.

Gullién-Sans, R., and M. Guzmán-Chozas. 1998. The thiobarbituric acid (TBA) reaction in foods: A review. *Crit. Rev. in Food Sci. and Nutrition*. 38:315–330.

Hansen, P. W., I. Tholl, C. Christensen, H. C. Jehg, J. Borg, O. Nielsen, B. Ostergaard, J. Nygaard, and O. Andersen. 2003. Batch accuracy of on-line fat determination. *Meat Sci*. 64:141–147.

Haugen, J. E., and K. Kvaal. 1998. Electronic nose and artificial neural network. *Meat Sci*. 49:S273–S286.

Hildrum, K. I., M. R. Ellekjaer, and T. Isaksson. 1995. *Near infrared spectroscopy in meat analysis*. Presented at Meat Focus International, April.

Hildrum, K. I., T. Isaksson, T. Naes, R. Rødbotten, and P. Lea. 1995. Near infrared spectroscopy in the prediction of sensory properties of beef. *J Near Infrared Spec*. 3:81–87.

Hildrum, K. I., B. N. Nilsen, and M. Wahlgren. 2003. *On-line assessment of the proximal composition of ground meat by NIR and other instrumental techniques*. Presented at the International Conference of Near Infrared Spectroscopy, Cordoba, Spain.

Hildrum, K. I., B. N. Nilsen, F. Westad, and M. Wahlgren. 2004. In-line analysis of ground beef by a diode array NIR instrument on a conveyor belt. *J Near Infrared Spec*. 12:367–376.

Hills, B. P. 1992. The proton-exchange cross-relaxation model of water relaxation in biopolymer systems. *Molec. Phys*. 76:489–508.

Hirsch, R.E., and R. L. Nagel. 1989. Stopped-flow front-face fluorometer: A prototype design to measure hemoglobin R-]T transition kinetics. *Analyt. Biochem*. 176:19–21.

Isaksson, T., B. N. Nilsen, G. Tøgersen, R. P. Hammond, and K. I. Hildrum. 1996. On-line, proximate analysis of ground beef directly at a meat grinder outlet. *Meat Sci*. 43:245–253.

Isaksson, T., G. Tøgersen, A. Iversen, and K. I. Hildrum. 1995. Nondestructive determination of fat, moisture and protein in salmon fillets by use of near-infrared diffuse spectroscopy. *J. Sci. Food and Agri.* 69:95–100.

Jensen, S. A., S. Reenberg, and L. Munck. 1989. Fluorescence analysis in fish and meat technology. In *Fluorescence analysis in foods,* ed. A. D. Francisco, 181–192. Essex, UK: Longman Group.

Jo, C., and D. U. Ahn. 1997. Fluorometric analysis og 2-thiobarbituric acid reactive substances in turkey. *Poultry Sci.* 77:475–480.

Kawano, S., H. Watanabe, and M. Iwamoto. 1992. Determination of sugar content in intact peaches by near-infrared spectroscopy with fiber optics in interactance mode. *J. Japanese Soc. for Hort. Sci.* 61:445–451.

Keller, S., T. Lochte, B. Dippel, and B. Schrader. 1993. Quality-control of food with near-infrared-excited Raman-spectroscopy. *Fresenius J. Analyt. Chem.* 346:863–867.

Kent, M., R. Knochel, F. Daschner, and U. K. Berger. 2001. Composition of foods including added water using microwave dielectric spectra. *Food Control.* 12:467–482.

Kikugawa, K., and M. Beppu. 1987. Involvement of lipid oxidation-products in the formation of fluorescent and cross-linked proteins. *Chem. and Phys. of Lipids.* 44:277–296.

Kinsey, S.T., B. R. Locke, B. Penke, and T. S. Moerland. 1999. Diffusional anisotropy is induced by subcellular barriers in skeletal muscle. *Nmr. in Biomed.* 12:1–7.

Knochel, R., F. Daschner, and W.Taute. 2001. Resonant microwave sensors for instantaneous determination of moisture in foodstuffs. *Food Control.* 12:447–458.

Lahucky, R., J. Mojto, J. Poltarsky, A. Miri, J. P. Renou, A. Talmant, and G. Monin. 1993. Evaluation of halothane sensitivity and prediction of postmortemmuscle metabolism in pigs from a muscle biopsy using P-31 Nmr-spectroscopy. *Meat Sci.* 33:373–384.

Lakowicz, J. R. 1983. *Principles of fluorescence spectroscopy.* New York: Plenum.

Laurent, W. M., J. M. Bonny, and J. P. Renou. 2000. Imaging of water and fat fractions in high-field MRI with multiple slice chemical shift-selective inversion recovery. *J. Mag. Res. Imaging.* 12:488–496.

Le Bihan, D., J. F. Mangin, C. Poupon, C. A. Clark, S. Pappata, N. Molko, and H. Chabriat. 2001. Diffusion tensor imaging: Concepts and applications. *J. Mag. Res. Imaging.* 13:534–546.

Lebecque, A., A. Laguet, M. Chanonat, S. Lardon, and E. Dufour. 2003. Joint analysis of sensory and instrumental data applied to the investigation of the texture of Charolais meat. *Sciences Des Aliments.* 23:172–175.

LiChan, E. C. Y. 1996. The applications of Raman spectroscopy in food science. *Trends Food Sci. Tech.* 7:361–370.

Lieber, C. A., and A. Mahadevan-Jansen. 2003. Automated method for subtraction of fluorescence from biological Raman spectra. *App. Spectroscopy.* 57:1363–1367.

Light, N., A. E. Champion, C. Voyle, and A. J. Bailey. 1985. The role of epimysial, perimysial and endomysial collagen in determining texture in 6 bovine muscles. *Meat Sci.* 13:137–149.

Liu, Z., A. Lirette, R. W. Fairfull, and B.W. McBride. 1994. Embryonic adenosine triphosphate/phosphodiesters ratios obtained with in-vivo nuclear-magnetic-resonance spectroscopy (P-31): A new technique for selecting leaner broiler-chickens. *Poultry Sci.* 73:1633–1641.

Manoharan, R., Y. Wang, and M. S. Feld. 1996. Histochemical analysis of biological tissues using Raman spectroscopy. Spectrochimica Acta Part A: Mol. and Biomol. *Spectroscopy.* 52:215–249.

Marquardt, B. J. 2001. Novel applications of Raman spectroscopy to process monitoring and materials characterization. *Abstracts of Papers of the American Chemical Society.* 222:U111.

Melton, S. L. 1983. Methodology for following lipid oxidation in muscle foods. *Food Tech.* 37:105ff.

Miri, A., A. Talmant, J. P. Renou, and G. Monin. 1992. P-31 Nmr-study of post-mortem changes in pig muscle. *Meat Sci.* 31:165–173.

Monin, G. 1998. Recent methods for predicting quality of whole meat. *Meat Sci.* 49:S231–S243.

Munck, L. 1989. *Fluorescence in food analysis*. Essex, UK: Longman Group.

Naes, T., and K. I. Hildrum. 1997. Comparison of multivariate calibration and discriminant analysis in evaluating NIR spectroscopy for determination of meat tenderness. *Appl. Spectrosc.* 51:350–357.

Newman, P. B. 1984. The use of video image-analysis for quantitative measurement of fatness in meat: 2. Comparison of via, visual assessment and total chemical fat estimation in a commercial environment. *Meat Sci.* 10:161–166.

Nott, K. P., S. D. Evans, and L. D. Hall. 1999. Quantitative magnetic resonance imaging of fresh and frozen-thawed trout. *Mag. Res. Imaging,* 17:445–455.

Offer, G., and T. Cousins. 1992. The mechanism of drip production: Formation of 2 compartments of extracellular-space in muscle postmortem. *J. Sci. Food Agr.* 58:107–116.

Offer, G., and P. Knight. 1998a.The structural basis of water-holding in meat. Part 1: General principles and water uptake in meat processing. In *Development in meat science,* ed. R. A. Lawrie, 63–171. London: Elsevier Science.

Offer, G., and P. Knight. 1998b. The structural basis of water-holding in meat. Part 2: Drip losses. In *Development in meat science,* ed. R. A. Lawrie, 172–243. London: Elsevier Science.

Olsen, E., G. Vogt, D. Ekeberg, M. Sandbakk, J. Pettersen, and A. Nilsson. 2005. Analysis of the early stages of lipid oxidation in freeze-stored pork back fat and mechanically recovered poultry meat. *J. Ag. Food Chem.* 53(2):338–348.

Osborne, B. G., T. Fearn, and P. H. Hindle. 1993. *Practical NIR spectroscopy with applications in food and beverage analysis* (2nd ed.). Essex, UK: Longman Scientific & Technical.

Ozaki, Y., R. Cho, K. Ikegaya, S. Muraishi, and K. Kawauchi. 1992. Potential of near-infrared Fourier-transform Raman-spectroscopy in food analysis. *App. Spectroscopy.* 46:1503–1507.

Parker, C. A. 1968. Apparatus and experimental methods. In *Photoluminescence of solutions with applications to photochemistry and analytical chemistry,* ed. C. A. Parker, 128–302. Amsterdam: Elsevier.

Pomeranz, Y., and C. E. Meloan. 1994. *Food analysis: Theory and practice*. New York: Chapman & Hall.

Posse, S., and W. P. Aue. 1990. Susceptibility artifacts in spin-echo and gradient-echo imaging. *J. Mag. Res.* 88:473–492.

Purcell, E. M., H. C. Torrey, and R. V. Pound. 1946. Resonance absorption by nuclear magnetic moments in a solid. *Phys. Rev.* 69:37–38.

Purslow, P. P. 1999. *The intramuscular connective tissue matrix and cell/matrix interactions in relation to meat toughness*. Presented at ICoMST, Yokohama, Japan, August 1–6.

Ramanujam, N. 2000. Fluorescence spectroscopy in vivo. In *Encyclopedia of analytical chemistry,* ed. R. A. Meyers, 20–56. Chichester, UK: Wiley.

Renou, J. P., A. Briguet, P. Gatellier, and J. Kopp. 1987. Determination of fat and water ratios in meat-products by high-resolution Nmr at 19.6 Mhz. *Int. J. Food Sci. Tech.* 22:169–172.

Renou, J. P., J. Kopp, P. Gatellier, G. Monin, and G. Kozakreiss. 1989. Nmr relaxation of water protons in normal and malignant hyperthermia-susceptible pig muscle. *Meat Sci*. 26:101–114.

Renou, J. P., J. Kopp, and C. Valin. 1985. Use of low resolution Nmr for determining fat-content in meat products. *J. Food Tech*. 20:23–29.

Renou, J. P., G. Monin, and P. Sellier. 1985. Nuclear-magnetic-resonance measurements on pork of various qualities. *Meat Sci*. 15:225–233.

Romer, T. J., J. F. Brennan, G. J. Puppels, A. H. Zwinderman, S. G. van Duinen, A. van der Laarse, A. F. W. van der Steen, N. A. Bom, and A. V. G. Bruschke. 2000. Intravascular ultrasound combined with Raman spectroscopy to localize and quantify cholesterol and calcium salts in atherosclerotic coronary arteries. *Arteriosclerosis Thrombosis and Vasc. Bio*. 20:478–483.

Ruiz-Cabrera, M. A., P. Gou, L. Foucat, J. P. Renou, and J. D. Daudin. 2004. Water transfer analysis in pork meat supported by NMR imaging. *Meat Sci*. 67:169–178.

Rye, M. 1991. Prediction of carcass composition in Atlantic salmon by computerized-tomography. *Aquaculture*. 99:35–48.

Sadeghijorabchi, H., R. H. Wilson, P. S. Belton, J. D. Edwardswebb, and D. T. Coxon. 1991. Quantitative analysis of oils and fats by Fourier-transform Raman-spectroscopy. *Spectrochimica Acta Part A: Mol. and Biomol. Spectroscopy.* 47:1449–1458.

Schenck, J. F. 1996. The role of magnetic susceptibility in magnetic resonance imaging: MRI magnetic compatibility of the first and second kinds. *Med. Phys*. 23:815–850.

Schwarze, H. 1997. Continuous fat analysis in the meat industry. *Process Control and Qual*. 9:133–138.

Shackelford, S. D., T. L. Wheeler, and M. Koohmaraie. 2005. On-line classification of US Select beef carcasses for longissimus tenderness using visible and near-infrared reflectance spectroscopy. *Meat Sci*. 69:409–415.

Skjervold, P. O., R. G. Taylor, J. P. Wold, P. Berge, S. Abouelkaram, J. Culioli, and E. Dufour. 2003. Development of intrinsic fluorescent multispectral imagery specific for fat, connective tissue, and myofibers in meat. *J. Food Sci*. 68:1161–1168.

Swatland, H. J. 1987. Measurement of the gristle content in beef by macroscopic ultraviolet fluorometry. *J. Anim. Sci*. 65:158–164.

Swatland, H. J. 1991. Evaluation of probe designs to measure connective-tissue fluorescence in carcasses. *J. Anim. Sci*. 69:1983–1988.

Swatland, H. J. 1993. An anomaly in the effect of temperature on collagen fluorescence in beef. *Food Res. Int*. 26:271–276.

Swatland, H. J. 1996. Connective tissue distribution patterns in beef detected by ultraviolet fibre optics. *Food Sci. Technol.-Leb.* 29:272–277.

Swatland, H. J., and C. J. Findlay. 1997. On-line probe prediction of beef toughness, correlating sensory evaluation with fluorescence detection of connective tissue and dynamic analysis of overall toughness. *Food Qual. and Pref.* 8:233–239.

Tan, J. L. 2004. Meat quality evaluation by computer vision. *J. Food Eng*. 61:27–35.

Tøgersen, G., J. F. Arnesen, B. N. Nilsen, and K. I. Hildrum. 2003. On-line prediction of chemical composition of semi-frozen ground beef by non-invasive NIR spectroscopy. *Meat Sci*. 63:515–523.

Tøgersen, G., T. Isaksson, B. N. Nilsen, E. A. Bakker, and K. I. Hildrum. On-line NIR analysis of fat, water and protein in industrial scale ground meat batches. *Meat Sci*. 51:97–102.

Tøgersen, G., Rødbotten, R., and K. I. Hildrum. 2002. Recent advances in prediction of meat quality by NIR spectroscopy. In *Research advances in the quality of meat and meat products,* ed. F. Toldrá, 1–15. Trivandrum, India: Research Signpost, Physical Sciences/Technology.

Tornberg, E., A. Andersson, A. Göransson, and G. Von Seth. 1993. Water and fat distribution in pork in relation to sensory properties. In *Pork quality, genetic and metabolic factors,* eds. E. Puolanne and D. Demeyer, 239–263. Townbridge, UK: CAB International.

Toussaint, C. A., F. Medale, A. Davenel, B. Fauconneau, P. Haffray, and S. Akoka. 2002. Determination of the lipid content in fish muscle by a self-calibrated NMR relaxometry method: Comparison with classical chemical extraction methods. *J. Sci. Food. Agr.* 82:173–178.

Traore, A., L. Foucat, and J. P. Renou. 2000. H-1-NMR study of water dynamics in hydrated collagen: Transverse relaxation-time and diffusion analysis. *Biopolymers* 53:476–483.

Veberg, A., J. P. Wold, and G. Vogt. In press. Fluorescence in aldehyde model systems related to lipid oxidation. *J. Food Sci. Tech.*

Walmsley, A. D., and V. C. Loades. 2001. Determination of acetonitrile and ethanol in water by guided microwave spectroscopy with multivariate calibration. *Analyst.* 126:417–420.

Wellock, R., and A. D. Walmsley. 2004. Applications of microwave spectroscopy in process analysis. *Spectroscopy Europe* 16(4):23–26.

Westad, F., and H. Martens. 2000. Variable selection in near infrared spectroscopy based on significance testing in partial least squares regression. *J. Near Infrared Spec.* 8:117–124.

Williams, P., and K. Norris. 2001. *Near-infrared technology in the agricultural and food industries* (2nd ed.). St. Paul, MN: AACC.

Wold, J. P., T. Jakobsen, and L. Krane. 1996. Atlantic salmon average fat content estimated by near-infrared transmittance spectroscopy. *J. Food Sci.* 61:74–77.

Wold, J. P., and K. Kvaal. 2000. Mapping lipid oxidation in chicken meat by multispectral imaging of autofluorescence. *App. Spectroscopy* 54:900–909.

Wold, J. P., F. Lundby, and B. Egelandsdal. 1999. Quantification of connective tissue (hydroxyproline) in ground beef by autofluorescence spectroscopy. *J. Food Sci.* 64:377–383.

Wold, J. P., B. J. Marquardt, B. K. Dable, D. Robb, anc B. Hatlen. 2004. Rapid quantification of carotenoids and fat in Atlantic salmon (Salmo salar L.) by Raman spectroscopy and chemometrics. *App. Spectroscopy* 58:395–403.

Wold, J. P., and M. Mielnik. 2000. Nondestructive assessment of lipid oxidation in minced poultry meat by autofluorescence spectroscopy. *J. Food Sci.* 65:87–95.

Wold, J. P., M. Mielnik, M. K. Pettersen, K. Aaby, and P. Baardseth. 2002. Rapid assessment of rancidity in complex meat products by front face fluorescence spectroscopy. *J. Food Sci.* 67:2397–2404.

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

Yablonskiy, D. A. 1998. Quantitation of intrinsic magnetic susceptibility-related effects in a tissue matrix: Phantom study. *Mag. Res. in Med.* 39:417–428.

Yamaki, S., T. Kato, and K. Kikugawa. 1992. Characteristics of fluorescence formed by the reaction of proteins with unsaturated aldehydes, possible degradation products of lipid radicals. *Chem. & Pharm. Bull.* 40:2138–2142.

Yin, D. Z. 1996. Biochemical basis of lipofuscin, ceroid, and age pigment-like fluorophores. *Free Radical Bio. and Med.* 21:871–888.

6 Real-Time PCR for the Detection of Pathogens in Meat

Petra Wolffs and Peter Rådström
Lund Institute of Technology

CONTENTS

6.1 Real-Time PCR Principle 132
6.1.1 Fluorescent Compounds 135
6.2 Pre-PCR Processing 136
6.2.1 Elimination of PCR Inhibition 138
6.2.2 Concentration of Target Nucleic Acids or Cells 141
6.2.3 Conversion of Heterogeneous Samples into Homogeneous PCR Samples 141
6.2.4 Avoiding False Positive and False Negative Results 142
6.2.5 Enabling Quantification 143
6.3 Real-Time Detection in Meat: Examples 143
6.3.1 Enrichment and Qualitative Real-Time PCR Detection 144
6.3.2 DNA Purification Prior to Real-Time PCR 145
6.3.3 Floatation Prior to Quantitative Real-Time PCR 146
6.4 Concluding Remarks and Future Outlook 147
Acknowledgments 147
References 147

Food-borne disease is recognized as one of the most serious public health concerns today (Wallace et al. 2000). Bacterial enteric pathogens contribute significantly to these concerns and are estimated to lead to approximately 5 million illnesses per year in the United States (Mead et al. 1999). Food-producing animals (cattle, pigs, chickens, and turkeys) are seen as the major sources for many of these pathogens. Aside from disease-causing bacteria, spoilage micro-organisms also lead to significant economic losses. Traditional microbial analysis of meat relies on selective enrichment and isolation of micro-organisms on solid media, usually followed by biochemical or serological confirmation (Fleet 1999). However, although many of the traditional methods are

standardized, modern food processing and delivery systems coupled with increasingly strict regulations impose requirements for speed and specificity that traditional methods cannot meet. Other serious disadvantages of traditional methods are that because they are growth based, viable but nonculturable (VNC) cells (e.g., stressed *Campylobacter* spp. or *Vibrio* spp. (Barer and Harwood 1999) will lead to false negative results. Furthermore, due to the preenrichment or enrichment in liquid medium, only qualitative data can be generated. Because growth-based methods cannot fulfil the demands for modern food-borne microbial diagnostics, new methods need to be developed and validated. Ideally, such methods would be able to quantify pathogens down to levels of 1 cell per 25 grams of food (Sharpe 1994), detect both viable and VNC cells (no false negatives), not detect any dead cells (no false positives), and deliver results within a few hours.

Because of speed, specificity, and sensitivity, molecular methods are increasingly being developed to detect, identify, and quantify micro-organisms in food. The introduction of one of these methods, the polymerase chain reaction (PCR) (Mullis et al. 1986) has revolutionized molecular diagnostics with its speed (down to five hours or less), specificity, selectivity, and sensitivity (single nucleic acid target; for review see Lübeck and Hoorfar 2003). Further development of the method in the 1990s has led to real-time PCR, which combines the DNA amplification of conventional PCR with nucleic acid detection by fluorescent substances during amplification, rather than after the completion of amplification (Higuchi, Dollinger, Walsh, and Griffith 1992; Higuchi, Fockler, Dollinger, and Watson 1993). This further reduces the analysis time and sample handling during the process and also offers the opportunity to acquire quantitative data (Orlando, Pinzani, and Pazzagli 1998). Nonetheless, there are still limitations to the use of PCR for food-borne quantification that need to be overcome (Klein 2002).

This chapter gives a description of real-time PCR as a methodology for detection of micro-organisms in meat. Focus then shifts to pre-PCR processing (i.e., the steps prior to the actual PCR analysis that have to convert meat into an actual PCR amplifiable sample). Finally, several examples of integrated real-time PCR systems for microbial analysis of meat are discussed.

6.1 REAL-TIME PCR PRINCIPLE

Real-time PCR is based on the three-temperature-step cycling procedure in which nucleic acids are enzymatically synthesized by a thermostable DNA polymerase with specific DNA oligonucleotides called primers, as first described by Saiki and Mullis (Mullis et al. 1986; Saiki et al. 1985) (see figure 6.1). Each cycle starts with a denaturation step, usually at a temperature around 95°C, in which the double-stranded DNA is separated into single strands. The next step, performed at temperatures between 45°C and 65°C depending on the primer composition, is the annealing step in which the primers bind to the single stranded DNA. The temperature is then elevated to around 72°C, the optimal temperature for the thermostable DNA polymerase, for the elongation step in which the DNA polymerase synthesizes strands complementary to

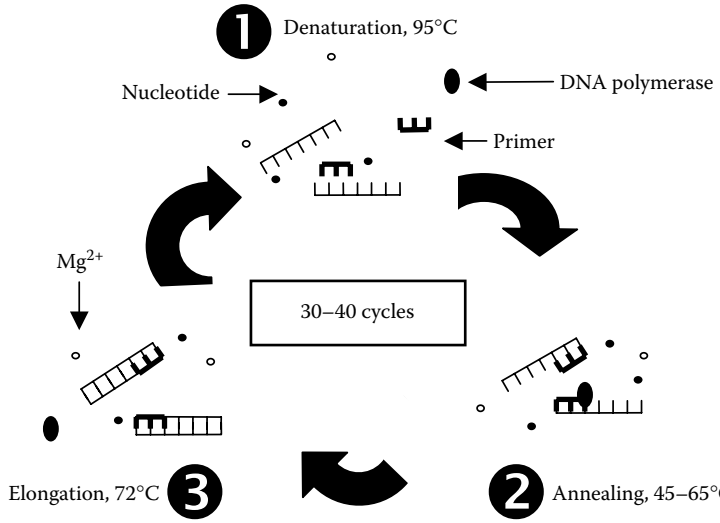


FIGURE 6.1 Schematic overview of the three-step cycling procedure on which PCR is based.

both single strands starting at the primers. The process described so far is known as conventional PCR, the difference being that in real-time PCR product detection is possible in the same reaction vessel during the amplification procedure with help of fluorescent compounds (Higuchi et al. 1992; Higuchi et al. 1993). Ideally a doubling of the target sequence can be obtained during each cycle, leading to a theoretical amount of $x(n) = x(0) \times 2^n$ targets, where x is the amount of target and n is the number of cycles. As the number of targets increases during amplification, the fluorescence also increases.

The possibility of following the increase in the number of targets during the amplification by monitoring the increase in the fluorescent signal is the key to performing real-time PCR. By observing the point where the fluorescence crosses a threshold level, or crossing point value or Cp value (depending on the equipment, also known as a Ct value), a cycle number can be acquired for samples with different initial DNA concentrations. If the initial concentration is high, the threshold level will be crossed earlier than when the initial concentration is low (figure 6.2). By measuring the Cp value for samples with known concentrations, standard curves can be made that can then be used for absolute quantification.

The standard curve that is created prior to quantification of unknown samples gives important information about two parameters. First, it shows the detection window, or the range over which data points can be acquired. It is, however, important to notice that a linear relationship is used for quantification (Livak, Flood, Marmaro, Giusti, and Deetz 1995), and that sometimes not all points (especially at the window borders) fit a linear relationship (figure 6.2). That is why a distinction can be made between the detection window (i.e., the window over which detection is obtained) and the linear range of amplification (i.e., the window over which a linear relationship of the standard curve can be obtained).

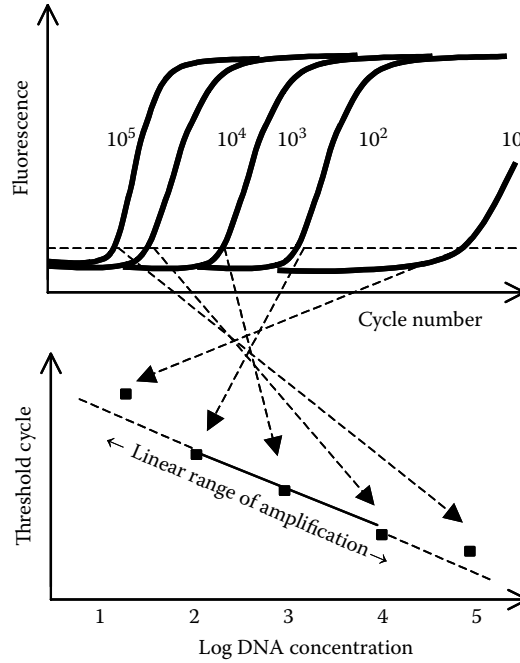


FIGURE 6.2 Schematic overview of the generation of a standard curve used for real-time quantitative PCR.

The second parameter that can be derived from the standard curve is the amplification efficiency (AE) through the following equation: $AE = (10^{(-1/slope)}) - 1$ (Kyger, Krevolin, and Powell 1998). When the theoretical optimum of a target doubling in each cycle is reached, the slope of the standard curve will be –3.32 and the value of AE will be 1.00. The AE can be used in several ways. First of all, deviations from the optimal value of 1.00 indicate that the PCR is not performing optimally, either because of inhibition or because of a suboptimal PCR setup. Therefore, the AE is an excellent tool with which to perform PCR optimization.

Unfortunately, there seems to be no consensus yet in the scientific community about the correct way to analyze quantitative data and to create standard curves for real-time PCR. Most published data show standard curves constructed of one data set (Hein, Schellenberg, Bein, and Hackstein 2001; Malinen, Kassinen, Rinttila, and Palva 2003; Nogva, Bergh, Holck, and Rudi 2000) whereas others analyze and use multiple data sets to calculate the AE (Brinkman et al. 2003; Ibekwe and Grieve 2003). In recent work, the effect of using single or multiple data sets for standard curve generation on the quantitative data that are obtained with that standard curve has been studied (Wolffs, Grage, Hagberg, and Rådström 2004). Results showed that depending on the PCR mixture composition, the choice for analysis of individual or multiple data sets significantly influenced the outcome of the results. Furthermore, there is also no general consensus regarding the inclusion or exclusion of data points that fall within the detection window but seem to be outside the linear range of

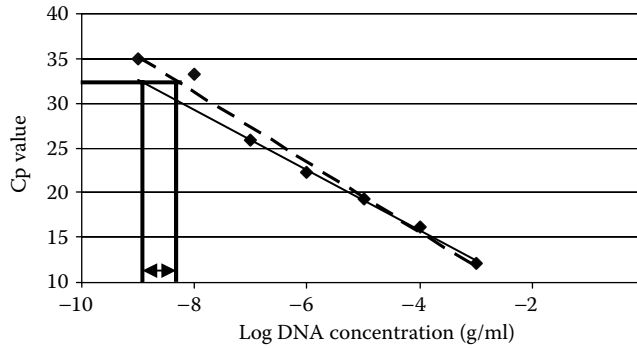


FIGURE 6.3 Effect of inclusion and exclusion of data points in the standard curve on final quantification of unknown samples. Series 1 (– –) includes all data points in the detection window for calculation of the standard curve. Series 2 (—) includes data points in the range from 1 mg/ml to 0.1 μg/ml. When an unknown sample with a Cp value of 32 is quantified with both standard curves, a clear difference between the estimated DNA concentration is observed (↔).

amplification, and whether extrapolation of the linear range of amplification is acceptable. Some groups seem to include the whole detection window in the linear range of amplification (Hein et al. 2001; Nogva, Rudi, Naterstad, Holck, and Lillehaug 2000), whereas others divide in both linear and nonlinear areas within the detection window (Knutsson, Löfström, Grage, Hoorfar, and Rådström 2002), and others extrapolate the linear range of amplification beyond the observed detection window (Brinkman et al. 2003). However, it is clear that inclusion or exclusion of data points as well as extrapolation of the linear range of amplification will influence the equation describing the nature of the linear relationship and thus also influence the quantification. Figure 6.3 demonstrates the effect of inclusion and exclusion of data points on quantification.

6.1.1 Fluorescent Compounds

As mentioned, fluorescent compounds are used during real-time PCR to follow the synthesis of the target sequence. The interaction between target and fluorescent compounds occurs in different ways, based on the type of compound used. They can be divided into two groups: (a) unspecific double-stranded DNA binding dyes such as SYBR Green I (Wittwer et al. 1997), and (b) sequence-specific fluorescent probes. As the name double-stranded DNA binding dyes describes, these dyes bind to double-stranded DNA and can then be excited and emit light. The advantage to using such dyes is that the cost is lower and the assays are easier to develop than a DNA probe assay. However, because the dyes bind to all double-stranded DNA, a signal generated by binding of the dyes to primer-dimer products is often seen at the end of the cycling, and this can negatively affect the detection window.

Probes consist of oligonucleotides bound to one or more fluorescent dyes. Due to the need for hybridization between the probe and the target, probes can be designed

to specifically bind to a single target. There are currently a number of fluorescent probe systems available. The most commonly used are hydrolysis or TaqMan probes (Heid, Stevens, Livak, and Williams 1996; Livak et al. 1995), hybridization probes (Cardullo, Agrawal, Flores, Zamecnik, and Wolf 1988; Wittwer, Herrmann, Moss, and Rasmussen 1997), or molecular beacons (Tyagi and Kramer 1996). All these systems have been developed for a number of targets and systems. Apart from these, new probe systems are constantly being developed. Examples are systems in which the probe is combined with the PCR primer, such as in Amplisensor primers (Chen et al. 1997), Sunrise primers (Nazarenko, Bhatnagar, and Hohman 1997; Winn-Deen 1998), and Scorpion primers (White, Arnheim, and Ehrlich 1989). All these systems are based on the interaction of two fluorescent molecules. In all cases except hybridization probes, one dye acts as a fluorochrome and the other works as a matching quencher. During amplification or on binding with the target the fluorochrome and the quencher are separated, removing the quenching effect, after which fluorescence measurements can take place. In the case of hybridization probes, one is the donor dye and the other the acceptor. On binding to the target, the excited donor dye uses its energy to excite the acceptor dye, the emission of which then can be measured. Finally, new groups of probes have been designed based on DNA analogs. These groups have a peptide nucleic acid (PNA) backbone, and just one fluorochrome instead of two, or consist of locked nucleic acids (LNA), in which the 2' and 4' positions of the furanose ring are joined by a methylene containing moiety (Singh, Kumar, and Wengel 1998). PNA is a synthetic achiral nucleic acid in which the sugar-phosphate backbone of DNA is replaced by peptide-like N-(2-aminoethyl) glycine units (Frank-Kamenetskii 1991; Nielsen, Egholm, Berg, and Buchardt 1991). Due to the change in backbone, PNA monomers are uncharged, which leads to the absence of electrostatic repulsion between a PNA-DNA double strand. This results in such double strands having a higher thermal stability, and a mismatch in such a duplex leads to a relatively greater drop in thermal stability. LNA is also characterized by a higher affinity toward complementary nucleic acids. Also, the hybridization process is independent of the salt concentration used. All these features have led to the expectation that PNA and LNA will be highly suitable for the production of fluorescent probes. Recently published studies confirm these indications (Ørum, Jakobsen, Koch, Vuust, and Borre 1999; Svanvik, Westman, Wang, and Kubista 2001; Wolffs, Knutsson, Sjöback, and Rådström 2001).

6.2 PRE-PCR PROCESSING

The process from meat to PCR signal comprises several steps: sampling, sample treatment, nucleic acid amplification, and detection and quantification (figure 6.4). Pre-PCR processing consists of all steps prior to the detection and quantification of the PCR signal. Thus it includes sampling, sample treatment, and the composition of the PCR mixture, in particular the choice of thermo-stable DNA polymerase and the use of amplification facilitators (Rådström, Knutsson, Wolffs, Dahlenborg, and Löfström 2003; Rådström, Knutsson, Wolffs, Lovenklev, and Löfström 2004). It is important to understand that the steps in pre-PCR processing affect the final PCR results as much as the actual design of the PCR assay with its primer composition does.

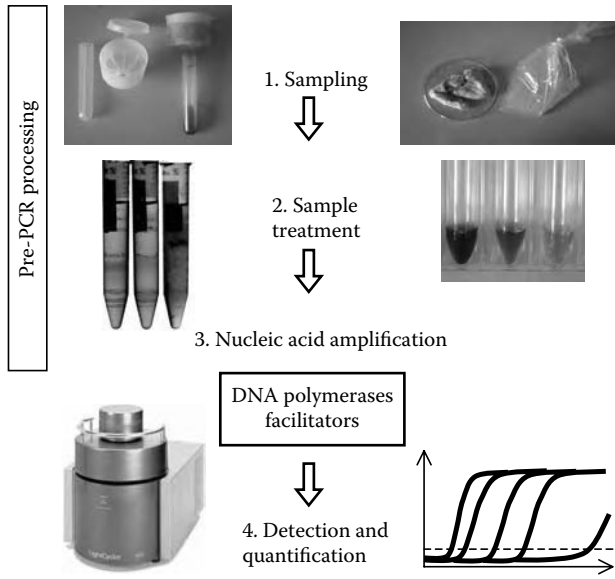


FIGURE 6.4 Schematic overview of pre-PCR processing. (Adapted from Rådström, Knutsson, Wolffs, Dahlenborg, and Löfström 2003).The figure shows the different steps in diagnostic PCR. Pre-PCR processing refers to sampling, sample treatment, and nucleic acid amplification with the use of an appropriate DNA polymerase and amplification facilitators.

The first step in the pre-PCR processing procedure is sampling. To perform microbial analysis on a biological matrix, a representative sample of the whole matrix has to be taken. There are different ways in which sampling can be performed, such as rinsing, swabbing, direct sampling from liquids, and maceration (Knutsson 2001). The choice of a specific sampling method depends on different aspects. It is important, first of all, to have a good understanding of the distribution of the target organism in the matrix. Second, the surroundings of the target organism, such as the amount of background flora and the matrix particles, can influence the choice of sampling method, as they can both inhibit the PCR reaction. Furthermore, the sampling method can influence the recovery and thus the concentration of the target.

The second step in pre-PCR processing, sample treatment, can be divided into four main types (for review see Lantz, Abu Al-Soud, Knutsson, Hahn-Hägerdal, and Rådström 2000): immunological methods, physical methods, biochemical methods, and enrichment methods. The basic principle behind immunological methods is the binding of antibody-coated magnetic beads to target cells. The target cells can in this way be separated from the background matrix and be concentrated if needed (Rudi et al. 2002). Physical methods separate target cells from the original sample based on physical properties such as cell size and buoyant density of the cell. Biochemical methods focus mainly on DNA extraction from the target cells, whereas enrichment methods involve cultivation of the target. Hundreds of sample preparation methods in all categories have been developed over time, for all kinds of different targets and sample types and focusing on different aims of pre-PCR processing (Lantz et al. 2000; Lantz, Hahn-Hägerdal, and Rådström 1994).

The final pre-PCR processing step is the adjustment of the PCR mixture. Many different factors are of influence, such as concentrations of the basic PCR mixture components, DNA polymerase, buffer, dNTP, and primers (Hosta and Flick 1991–1992). Many studies have shown that modification of the basic PCR mixture by, for example, addition of beneficial components called amplification facilitators, or replacing the commonly used *Taq* DNA polymerase with an alternative DNA polymerase, can improve the PCR performance. These two PCR mixture modifications can have different effects on PCR performance, such as improving sensitivity and specificity (Dahlenborg, Borch, and Rådström 2001; Laigret, Deaville, Bove, and Bradbury 1996), reducing variability (Mullan, Kenny-Walsh, Collins, Shanahan, and Fanning 2001), or amplification errors (Cline, Braman, and Hogrefe 1996). One study also showed that choice of a DNA polymerase with a different 5'-exonuclease activity drastically affected real-time detection with the so-called TaqMan or hydrolysis probes (Kreuzer, Bohn, Lass, Peters, and Schmidt 2000). Finally, studies have shown that the choice of DNA polymerase and amplification facilitators may reduce or eliminate the effect of PCR inhibitors (Abu Al-Soud, Jönsson, and Rådström 2000; Abu Al-Soud and Rådström 1998, 2000). Amplification facilitators can be divided into different groups: proteins, organic solvents, nonionic detergents, biologically compatible solutes, and polymers. Each group has its own mechanism for improving PCR performance, although many of them remain unclear. To use the technological advancements of real-time PCR to their full potential, several requirements must be met before amplification and detection can take place. Those requirements can be classified as follows: (a) elimination of PCR inhibition, (b) concentration of target nucleic acids or cells, (c) conversion of heterogeneous samples into homogeneous PCR samples, (d) avoiding false positive and false negative results, and (e) enabling quantification.

There are currently few pre-PCR processing strategies that can fulfil all five requirements simultaneously. Furthermore, obvious needs for speed, low cost, and simplicity of the procedure are naturally of importance as well. Therefore, when a choice is to be made concerning sampling, sample treatment, and composition of the PCR mixture, the different requirements must be prioritized. The following sections discuss these five different requirements individually and give examples of different pre-PCR processing strategies that have been applied to fulfil them.

6.2.1 Elimination of PCR Inhibition

The use of conventional and real-time PCR may be restricted by the presence of PCR inhibitors (Lantz 1998). PCR inhibitors originate either from the original complex biological sample or from sample preparation prior to PCR, or both (Rossen, Nøskov, Holmstrøm, and Rasmussen 1992). Although many biological samples have been reported to inhibit PCR amplification, the identities and biochemical mechanisms of many inhibitors remain unclear. In a review by Wilson (1997), a systematic list of inhibitors of conventional PCR was presented, and the mechanisms by which the inhibitors may act were divided into the following three categories: (a) inactivation of the thermostable DNA polymerase, (b) degradation or capture of the nucleic acids, and (c) interference with the cell lysis step. Examples of PCR inhibitors found

in blood and meat are polysaccharides and glycogen in oyster meat (Atmar, Metcalf, Neill, and Estes 1993); sucrose, ovalbumin, and phenolic compounds in cold-smoked salmon (Simon, Gray, and Cook 1996); and immunoglobulin G (Al-Soud and Rådström 2001) and heparin (Satsangi, Jewell, Welsh, Bunce, and Bell 1994) from blood.

Apart from the three mentioned categories of PCR inhibition that are encountered during conventional PCR, the addition of fluorogenic substances such as fluorescent probes or fluorochromes to the PCR to allow real-time PCR has made the system more complex. Therefore, three additional possible mechanisms of PCR inhibition can be identified (Rådström, Knutsson, Wolffs, Lovenklev, and Löfström 2004). The first additional factor (d) involves fluorescent substances, which are either quenched by sample components or cause autofluorescence (Stults, Snoeyenbos-West, Methe, Lovley, and Chandler 2001). Autofluorescence is the onset of a fluorescent signal by something other than the target DNA, such as high amounts of nontarget DNA. When DNA binding dyes such as SYBR Green I are used, the presence of high amounts of nontarget DNA may result in such a high unspecific signal that it dominates the signal of the specific product. The second additional mechanism (e) concerns substances other than the fluorescent probes or dyes used for real-time PCR that have a background fluorescence of their own. Substances or complexes with a high absorbance can also cause PCR interference by scattering the excitation light (e.g., blood or charcoal-based enrichment media). Finally, (f) some PCR vessels have been adapted to increase the speed of heat transfer during cycling (e.g., capillaries used for the LightCycler™ [Roche Molecular Biochemicals] or Smart Cycler® reaction tubes used for the Smart Cycler® [Cepheid]). The higher surface-to-volume ratio of these adapted tubes might lead to increased binding or adherence of inhibitors to the walls and thus reduce their effect. However, large particles from complex biological samples (e.g., denatured blood proteins or meat particles from homogenized samples) might also negatively affect the flow of PCR components through the more narrow PCR tubes.

It is of great importance that PCR inhibitors from all six categories be removed from the PCR sample or neutralized in the PCR mixture to allow real-time PCR. Because many inhibitors have not yet been identified, real-time PCR can be used to determine the "cleanliness" of the sample as a whole, rather than to determine the degree of removal or neutralization of separate inhibitors. By studying the AE and the linear range of amplification after real-time PCR in a sample, possible remaining PCR inhibition can be detected by comparing these values with AE and linear range of amplification for purified DNA samples. This approach has, for example, been applied to follow the decrease of PCR inhibition during the optimization of PCR conditions (Wolffs, Grage, Hagberg, and Rådström 2004; Wolffs, Norling, and Rådström 2004). Mathematical models can also be used to study PCR inhibition because they enable comparison of the effects of different inhibitors, kinetic analysis of the DNA polymerase in the presence and absence of inhibitors, and evaluation of the effects of amplification facilitators. Such a model was developed and used to study the effect of buffered peptone water (BPW) on real-time PCR performance (Knutsson et al. 2002; Knutsson, Blixt, Grage, Borch, and Rådström 2002). Results showed that BPW partially inhibited Ampli*Taq* Gold, and r*Tth* maintained the same PCR performance as the positive control.

Examples of methods or strategies for removing or inactivating PCR inhibitors can be found during all three steps of pre-PCR processing (sampling, sample treatment, and adjustment of the PCR mixture). In the work reported here, examples of removal or inactivation of PCR inhibitors during all three steps are described. First, the effect of the composition of different rinse sampling solutions for carcass rinses on PCR inhibition was studied (table 6.1). The results indicate that physiological saline is preferable as a rinse solution due to its low PCR inhibition.

TABLE 6.1
Effect of Chicken Rinse Solution on PCR Detection of *Y. enterocolitica*

	PCR Results at Different Concentrations of the Chicken Rinse in PCR Mixture[a]		
Rinse Solution	**20 (Vol/Vol)%**	**2.0 (Vol/Vol)%**	**0.2 (Vol/Vol)%**
Peptone broth, whole skin rinse	– –	+ +	+ +
Peptone broth, neck skin rinse	– –	+ +	+ +
Physiological saline, whole skin rinse	+ +	+ +	+ +
Physiological saline, neck skin rinse	– +	+ +	+ +

[a] Results of independent duplicate analysis are shown as +: specific band visible after gel electrophoresis, or as -: no band visible after gel electrophoresis.

As mentioned previously, due to the complexity of the real-time PCR mixture and the use of fluorescent compounds, the removal of PCR and fluorescence inhibitors has become very important. This has led to the use of a very limited number of sample preparation methods being applied prior to real-time PCR, and these seem to almost exclusively involve DNA extraction. Recent work has described a new physical sample preparation method called floatation to be used prior to real-time PCR (Wolffs, Knutsson, Norling, and Rådström 2004). Floatation is based on traditional buoyant density centrifugation, and because the sample floats upward instead of being concentrated at the bottom of the tube, contamination during recovery of the sampling is reduced. Results showed that application of this method could remove PCR inhibitors to levels comparable to commercial DNA purification.

Finally, in the last step in PCR processing, adjustment of the PCR mixture, changes can be made to deal with PCR inhibition. Amplification facilitators can be added to the PCR mixture to relieve PCR inhibition (Abu Al-Soud and Rådström 2000). Especially the use of the proteins bovine serum albumin (BSA) and the single-stranded DNA-binding protein coded by gene 32 of bacteriophage T4 (gp32) are well documented (Abu Al-Soud and Rådström 2000; Kreader 1996). Their mechanisms for the relief of PCR inhibition seem to be based on their binding capacities to inhibitory compounds such as haeme or phenolics (Kreader 1996) or by being a target for proteases, thereby preventing degradation of the DNA polymerase (Powell, Gooding, Garrett, Lund, and McKee 1994). With the introduction of new commercial DNA polymerases, several suppliers have added different amplification facilitators to their buffers. An example is the addition of BSA and Tween 20 to the buffer of *Tth* polymerase (Roche Diagnostics). Previous research indicated that different DNA

polymerases have different susceptibilities to PCR inhibitors from, for example, meat, feces, or blood samples (Abu Al-Soud and Rådström 1998). For this reason, recently developed PCR assays incorporated more resistant DNA polymerases, such as the use of r*Tth* for amplification of *Clostridium botulinum* from fecal samples (Dahlenborg et al. 2001) and the use of *Tth* for amplification of *Salmonella* in feed samples (Löfström, Knutsson, Axelsson, and Rådström 2004). In recent work, the choice of a DNA polymerase resistant to the food samples, in this case chicken rinse, was taken into account in the design of a new PCR assay for *Campylobacter* spp. (Lübeck, Cook, Wagner, Fach, and Hoorfar 2003). The study showed that *Tth* DNA polymerase was most resistant to chicken rinse particles, and this DNA polymerase was therefore used in a newly developed PCR assay, which aimed to detect *Campylobacter* spp. in chicken rinse samples.

6.2.2 Concentration of Target Nucleic Acids or Cells

Real-time PCR is an extremely sensitive method, principally able to detect as little as only one copy of the target in the reaction tube. However, traditional methods for the detection of food-borne pathogens have set an ideal standard of detecting one single viable cell in 25 grams of sample (Sharpe 1994). Because the volume of sample added to real-time PCR analysis is so small (i.e., between 1 and 10 µl), it is impossible to obtain a sample that can adequately represent the chemical and microbial composition of the original sample of 25 grams without some kind of sample treatment. It is therefore necessary that the single target cell is either concentrated into a volume the size of a PCR sample or multiplied to such a level that the PCR sample will surely contain copies of the target. This requirement has to be fulfilled during the first two pre-PCR processing steps (i.e., sampling and sample treatment). An example of how sampling influences the target concentration is given by a study that showed the difference in *Salmonella* recovery between different sampling techniques such as rinsing and swabbing (Sarlin et al. 1998), whereas another study showed that different cotton-tipped swabs affected the recovery of *Y. enterocolitica* (Knutsson 2001). Regarding sample treatment methods, many of them have been developed or have been shown to concentrate the target. One of the most commonly used sample treatment methods to increase the concentration of the target is culture enrichment (Candrian 1995; Hoorfar, Ahrens, and Rådström 2000; Knutsson, Fontanesi, Grage, and Rådström 2002; Scheu, Berghof, and Stahl 1998). Examples of other sample treatment methods that may concentrate the original amount of target are filtration (Lantz, Stalhandske, Lundahl, and Rådström 1999; Starbuck, Hill, and Stewart 1992), buoyant density centrifugation (Lindqvist, Norling, and Lambertz 1997), and floatation (Wolffs, Knutsson, Norling, and Rådström 2004).

6.2.3 Conversion of Heterogeneous Samples Into Homogeneous PCR Samples

For real-time PCR and in particular for quantitative analysis to be reliable, it is of great importance that the sample generated after pre-PCR processing has a reproducible composition concerning the chemical composition of the sample, the presence of

inhibitors, and the amount of target per unit volume sample. This is especially important when there is a great sample-to-sample variation, for example, in meat juice samples in which the contents of PCR inhibiting groups such as hemoglobin or myoglobin (Al-Soud and Rådström 2001) may vary, even after sample treatment. In recent work AEs were used to check sample homogeneity, by comparing AEs before and after sampling and between different samples.

When samples are routinely analyzed, it is important to maintain constant control over the performance in each individual sample. Internal controls that are co-amplified in each sample can be developed and used for this purpose (Stocher, Leb, Holzl, and Berg 2002). Not only do the internal controls allow a qualitative way of checking that amplification is possible in the sample (Bai, Hosler, Rogers, Dawson, and Scheuermann 1997), but they can also be used to check the amplification efficiency in the sample, providing information necessary to allow quantification (Stocher, Leb, and Berg 2003).

6.2.4 Avoiding False Positive and False Negative Results

Within food microbiology and the detection of food-borne pathogens and spoilage micro-organisms, one of the major concerns about the use of a nucleic acid-based detection method such as PCR has been the risk of false positive results caused by the detection of DNA from dead cells (Scheu et al. 1998). This can theoretically be expected because such methods detect nucleic acids in the sample, rather than the presence of viable cells, as standardized growth-based methods do. Numerous studies have been performed to determine the risk of false positive results due to detection of residual dead cell material with different outcomes. It has been found that dead cells of several food-borne pathogens such as *Salmonella*, *E. coli,* and *Campylobacter* spp were detected by PCR (Allmann et al. 1995; Dupray, Caprais, Derrien, and Fach 1997; Josephson, Gerba, and Pepper 1993). However, other studies indicated that only viable cells of, for example, *Vibrio vulnificus,* were detected by PCR (Brauns, Hudson, and Oliver 1991), or that the detectability of the dead cells depended on the way they were killed (Herman 1997). Recent studies have used real-time PCR to study degradation of *Campylobacter* DNA (Nogva et al. 2000) and degradation rates of free *Y. enterocolitica* DNA in selected food samples (Wolffs, Knutsson, Norling, and Rådström 2004). Results showed that the degradation rate of the DNA depended on temperature and sample type, with the fastest degradation of 0.5 h per log unit DNA in chicken homogenate at 20°C and the slowest degradation of 120.5 h per log unit DNA in pork rinse at 20°C. Furthermore, by following dying cell cultures over time it was found that cell death occurred slowly (i.e., 7.6 days per log unit in chicken rinse and 10.2 days per log unit in pork rinse). Also, in both cases, performing qPCR directly on tenfold diluted samples showed false positive results (Wolffs, Knutsson, Norling, and Rådström 2004).

Due to the rapid degradation of mRNA (Alifano, Bruni, and Carlomagno 1994), it has been used as an indicator of cell viability. By applying reverse transcriptase-PCR (RT-PCR), only signals of viable cells of many micro-organisms such as *Mycobacterium tuberculosis* (Patel, Banerjee, and Butcher 1993), *Listeria monocytogenes* (Klein and Juneja 1997; Norton and Batt 1999), and *E.coli* (McIngvale, Selhanafi, and Drake 2002) have been detected. However even for mRNA, some

studies show that rapid degradation after cell death is not always guaranteed and can depend on the way in which the cells died, and storage conditions after killing (Sheridan, Szabo, and Mackey 1999). This implies that detectable mRNA may not in all cases be a good indicator of cell viability.

It must be remembered when one wants to detect viable cells that these cells can be part of two different groups. The first group consists of viable culturable cells, whereas the second group are VNC cells. Differences can also be seen in the group of VNC cells. A distinction can be made between bacteria that have, as yet, been uncultured (AYU), and those that can be cultured, but are now temporarily or permanently unculturable (Barer and Harwood 1999). The significance of these cells in the diagnostics of food-borne pathogens is still controversial. For example, for *C. jejuni*, some studies have shown that these organisms in the VNC state cannot colonize baby chicks, and consider the VNC state for that reason to be a degenerative form (Hald, Knudsen, Lind, and Madsen 2001; Medema, Schets, van de Giessen, and Havelaar 1992), whereas others showed that VNC cells of this organism can in fact colonize baby chicks and suggested that the VNC state is a dormant form of the cell (Stern 1994). Because there is no conclusive evidence, one should assume that there is a possibility that VNC cells may become infective again and they should thus be detected by diagnostic methods for food-borne pathogens.

6.2.5 Enabling Quantification

In cases where real-time PCR is used to obtain quantitative measurements, one additional requirement is made on pre-PCR processing. To be able to correctly quantify the initial amount of target in the sample, pre-PCR processing methods should ideally not influence the amount. However, in cases where this cannot be avoided, the influence on the target amount should be predictable and regulated. With the development of qPCR, this last aim has placed major restrictions especially on the use of sample preparation methods. Thus, culture enrichment-based methods are not to be used anymore, as these methods influence the initial amount of cells in an uncontrolled way. The requirement of quantification also affects the PCR mixture. Recently, for the first time, the effect of different DNA polymerases and the buffer components on qPCR in a "clean" system without PCR inhibitors, has been studied in a systematic way (Wolffs, Grage, Hagberg, and Rådström 2004). Results showed that the choice of DNA polymerase affected the amplification efficiency, the intralab reproducibility, and the detection window. The effect of the buffer composition was also studied because some buffers contain amplification facilitators such as BSA and Tween 20. Results suggested that both the choice of DNA polymerase and the addition of amplification facilitators can affect the detection window and the qPCR performance.

6.3 REAL-TIME DETECTION IN MEAT: EXAMPLES

When real-time PCR is used for detection of pathogens in meat in a "real-life" case, it has to be noted that, as mentioned before, no method is perfect yet. It is therefore essential to evaluate the case concerning expected bacterial load, PCR inhibition levels of the sample, need for quantitative data, possibility of having VNC cells

present, time and cost limitations, and so on. When priorities have been determined from all those points, it becomes possible to select the most suitable method. For example, in cases with a very low bacterial load, sensitivity of the method is a priority and therefore a likely choice is real-time PCR combined with an enrichment step. In cases with a higher bacterial load, as for a spoilage organism, either the speed or the ability to quantify the amount of organisms present might be the most important factor. In that case rapid DNA purification or density centrifugation combined with real-time PCR might be a fast, suitable option. New PCR assays and matching sample treatments for all kinds of pathogens found in meat are developed every day and it goes beyond the scope of this chapter to list all currently published methods. To our knowledge no examples of such methods concerning rapid detection of spoilage organisms found in meat have been published. This final part focuses on describing three types of integrated sample preparation and real-time PCR used for detection of pathogens in meat.

6.3.1 Enrichment and Qualitative Real-Time PCR Detection

As mentioned before, due to the possible low contamination levels of pathogens in meat, most frequently real-time PCR detection is proceeded by an enrichment step. After enrichment no quantitative data are acquired, but due to the combination of enrichment and the increased sensitivity and speed of real-time PCR, conventional PCR setups can be improved. Currently there are methods available for most foodborne pathogens in a wide variety of meat samples (table 6.2). Most of the assays mentioned in table 6.2 reported sensitivity levels of 10 to 100 CFU per gram of meat sample. It has to be pointed out that most enrichment strategies discussed are not developed for specific use in combination with (real-time) PCR; often DNA purification is performed to remove PCR inhibitors present in the enrichment medium. One example is that BPW from some suppliers is shown to inhibit PCR (Knutsson et al. 2002). A recent study has followed a novel approach to develop an enrichment medium for growth of *Yersinia entercolitica* that does not contain any PCR inhibitors or compounds interfering with fluorescence measurements, and is therefore excellently suited to be combined with real-time PCR(Knutsson, Blixt, Grage, Borch, and Rådström 2002; Knutsson, Fontanesi, Grage, and Rådström 2002). An additional development, not only in this area, is the combined detection of several pathogens in one food sample by using multiplex PCR. Examples of this are simultaneous detection of *Listeria* and *Salmonella* in chicken samples (Soultos, Koidis, and Madden 2003) and *Listeria monocytogenes* and *Salmonella* in raw sausage meat after six to eight hours of enrichment (Wang, Jothikumar, and Griffiths 2004). Finally, all methods discussed so far deal with enrichment in liquid enrichment media; however, PCR can also be used for rapid identification to the species level when rough isolates on plates are available. This setup has been used to develop an automated qualitative real-time PCR assay for identification of presumptive *Salmonella* colonies (Hoorfar, Ahrens, and Rådström 2000).

TABLE 6.2
Examples of Real-Time PCR Assays Combined with Enrichment for Detection of Food-Borne Pathogens in Meat Samples

Micro-Organism	Sample	Enrichment Time (Hours)	Reference
Campylobacter jejuni	Chicken rinse	14	Cheng and Griffiths (2003)
Campylobacter jejuni	Poultry, pork, beef, lamb, shellfish	48–72	Sails, Fox, Bolton, Wareing, and Greenway (2003)
Enterohemorrhagic *Escherichia coli*	Beef	16	Sharma (2002)
Verotoxogenic *Escherichia coli*	Minced beef	6	O'Hanlon, Catarame, Duffy, Blair, and McDowell (2004)
Salmonella	Raw and ready-to-eat beef	6	Ellingson, Anderson, Carlson, and Sharma (2004)
Salmonella	Poultry carcass	Overnight	Eyigor and Carli (2003)
Listeria monocytogenes	Sausage	6–8	Wang, Jothikumar, and Griffiths (2004)
Vibrio parahaemolyticus	Crab and mussels	24	Davis et al. (2004)
Vibrio vulnificus	Oysters	5	Panicker, Myers, and Bej (2004)
Yersinia enterocolitica	Pork swab	9–18	Knutsson, Blixt, Grage, Borch, and Rådström (2002)

6.3.2 DNA Purification Prior to Real-Time PCR

It is important to mention that due to the high sensitivity of real-time PCR to inhibitors present in meat samples (as in all biological samples), DNA purification is to our knowledge exclusively used prior to real-time PCR except for the examples described later. Often it is combined with another treatment such as after the enrichment steps described in the previous paragraph or after use of immuno-magnetic separation (Rudi et al. 2002). Here we focus on the methods that use DNA purification directly on meat.

There are currently only a few published examples of direct DNA purification of pathogen DNA from food. The obvious obstacle is again the low pathogen load in the sample. Nonetheless, there are examples of situations in which direct detection by DNA purification followed by real-time PCR can work very elegantly. A first example was a trace-back of a *Salmonella* contamination from a gastroenteritis outbreak in Texas (Daum et al. 2002). After a buffet-style picnic an outbreak of acute gastroenteritis was caused by a *Salmonella* infection. All foods at the buffet were tested for presence of *Salmonella* by direct purification of DNA, followed by real-time PCR. The data showed that the barbecued chicken was contaminated with

Salmonella. Another example is the quantification of *Campylobacter jejuni* from highly contaminated chicken samples, surface and ground water samples, and milk (Yang, Jiang, Huang, Zhu, and Yin 2003). Results showed that levels up to 2.3×10^5/ml sample were accurately quantified in the samples. The whole process takes less than 60 minutes and in cases of high contamination levels, this method is very promising. The same idea can be seen in other cases in which DNA is directly purified from the samples. The pathogen load is expected to be high, as, for example, in fecal or cecal samples of chickens (Rudi et al. 2004).

6.3.3 Floatation Prior to Quantitative Real-Time PCR

Floatation is based on traditional buoyant density centrifugation (Pertoft, Rubin, Kjellen, Laurent, and Klingeborn 1977). The principle of such a method is the separation of particles with different buoyant densities. Buoyant density centrifugation has frequently been applied as a sample preparation method for, for example, separation of bacteria from food particles (Basel, Richter, and Banwart 1983), separation of subpopulations of bacteria (Håkansson, Granlund-Edstedt, Sellin, and Holm 1990), and separation of cells in different growth stages (Makinoshima, Nishimura, and Ishihama 2002; Whiteley, Barer, and O'Donnell 2000). The method has the advantages that it is nondisruptive to bacterial cells and is easy to perform (Pertoft 2000). In contrast to traditional applications where the target is usually layered on top of the density centrifugation medium and after centrifugation is concentrated at the bottom, with floatation the target is loaded below the centrifugation medium and floats during centrifugation upward. The benefit of applying floatation compared to sedimentation is that the target can be retrieved with less risk of contamination. To direct the location of the target after floatation, layers of density centrifugation media with different densities can be used (Wolffs, Knutsson, Norling, and Rådström 2004).

When the buoyant densities of the target cell, background flora (BGF), and food particles are known, the different layers in the floatation setup can be chosen. The target cells are concentrated at the interface between two layers by choosing a top layer with a lower density than the target and a lower layer with a higher density than the target. The opposite strategy can be adopted for the BGF and the sample matrix. This ensures that BGF and sample matrix particles will concentrate at locations other than the target. By choosing this density window to be as narrow as practically possible, the target is separated from most of the BGF and the food matrix.

Recently, a floatation-PCR strategy has been developed, bearing in mind the requirements for pre-PCR processing strategies mentioned earlier. This particular setup was developed for separation of *Y. enterocolitica* from meat juice from pork (Wolffs, Knutsson, Norling, and Rådström 2004). A floatation system was developed consisting of three layers of density centrifugation medium aimed at separating *Y. enterocolitica* from meat juice and its accompanying BGF. Results showed that applying this floatation-PCR to undiluted meat juice, human blood, or pig feces led to significant reduction in PCR inhibition. This indicates that very heterogeneous samples can be turned into homogeneous samples for PCR. Furthermore, false positive results caused by DNA originating from dead cells is avoided due to the low floatation speed of free DNA. This ensures that the free DNA remains in the

bottom layer, where the sample is added prior to floatation (Wolffs, Knutsson, Norling, and Rådström 2004).

6.4 CONCLUDING REMARKS AND FUTURE OUTLOOK

The use of real-time PCR for detection and quantification of micro-organisms in meat has increased over the past decade. To perform real-time PCR in such a way, pre-PCR processing (i.e., sampling, sample treatment, and PCR mixture composition) should be optimized to fulfill five requirements as well as possible: (1) elimination of PCR inhibition, (2) concentration of target nucleic acids or cells, (3) conversion of heterogeneous samples into homogeneous PCR samples, (4) avoiding false positive and false negative results, and (5) enabling quantification (if quantification is desired). For detection of pathogens in meat, an increasing number of integrated sample treatment and real-time PCR assays have been published and several have been discussed in the final part of this chapter. Even though most assays do not fulfill all requirements, depending on the expected bacterial load, sample type, and whether quantitative or qualitative data are needed, these assays can be used in defined circumstances. As of yet, no methods for detection of spoilage organisms have been published.

ACKNOWLEDGMENTS

This work was financially supported by the Commission of the European Communities within the program FOOD-PCR, QLK1-1999-00226; the Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning, 2001-4068; and the Nordic Innovation Centre (Campyfood).

REFERENCES

Abu Al-Soud, W., L. Jönsson, and P. Rådström. 2000. Identification and characterization of immunoglobulin G in blood as a major inhibitor of diagnostic PCR. *J. Clin. Microbiol.* 38:345–350.

Abu Al-Soud, W., and P. Rådström. 1998. Capacity of nine thermostable DNA polymerases to mediate DNA amplification in the presence of PCR-inhibiting samples. *Appl. Environ. Microbiol.* 64:3748–3753.

Abu Al-Soud, W., and P. Rådström. 2000. Effect of amplification facilitators on diagnostic PCR in the presence of blood, feces and meat. *J. Clin. Microbiol.* 38:4463–4470.

Alifano, P., C. B. Bruni, and M. S. Carlomagno. 1994. Control of mRNA processing and decay in prokaryotes. *Genetica* 94:157–172.

Allmann, M., C. Höfelein, E. Köppel, J. Lüthy, R. Meyer, C. Niederhauser, B. Wegmüller, and U. Candrian. 1995. Polymerase chain reaction (PCR) for detection of pathogenic microorganisms in bacteriological monitoring of dairy products. *Res. Microbiol.* 146:85–97.

Al-Soud, W. A., and P. Rådström. 2001. Purification and characterization of PCR-inhibitory components in blood cells. *J. Clin. Microbiol.* 39:485–493.

Atmar, R. L., T. G. Metcalf, F. H. Neill, and M. K. Estes. 1993. Detection of enteric viruses in oysters by using the polymerase chain reaction. *Appl. Environ. Microbiol.* 59:631–635.

Bai, X., G. Hosler, B. B. Rogers, D. B. Dawson, and R. H. Scheuermann. 1997. Quantitative polymerase chain reaction for human herpes virus diagnosis and measurement of Epstein-Barr virus burden in posttransplant lymphoproliferative disorder. *Clin. Chem.* 43:1843–1849.

Barer, M. R., and C. R. Harwood. 1999. Bacterial viability and culturability. *Adv. Microb. Physiol.* 41:93–137.

Basel, R. M., E. R. Richter, and G. J. Banwart. 1983. Monitoring microbial numbers in food by density centrifugation. *Appl. Environ. Microbiol.* 45:1156–1159.

Brauns, L. A., M. C. Hudson, and J. D. Oliver. 1991. Use of the polymerase chain reaction in detection of culturable and nonculturable *Vibrio vulnificus* cells. *Appl. Environ. Microbiol.* 57:2651–2655.

Brinkman, N. E., R. A. Haugland, L. J. Wymer, M. Byappanahalli, R. L. Whitman, and S. J. Vesper. 2003. Evaluation of a rapid, quantitative real-time PCR method for enumeration of pathogenic Candida cells in water. *Appl. Environ. Microbiol.* 69:1775–1782.

Candrian, U. 1995. Polymerase chain reaction in food microbiology. *J. Microbiol. Meth.* 23:89–103.

Cardullo, R. A., S. Agrawal, C. Flores, P. C. Zamecnik, and D. E. Wolf. 1988. Detection of nucleic acid hybridization by nonradiative fluorescence resonance energy transfer. *Proc. Natl. Acad. Sci. USA.* 85:8790–8794.

Chen, S., A. Yee, M. Griffiths, C. Larkin, C. T. Yamashiro, R. Behari, C. Paszko-Kolva, K. Rahn, and S. A. D. Grandis. 1997. The evaluation of a fluoregenic polymerase chain reaction assay for the detection of *Salmonella* species in food commodities. *Int. J. Food Microbiol.* 35:239–250.

Cheng, Z., and M. W. Griffiths. 2003. Rapid detection of Campylobacter jejuni in chicken rinse water by melting-peak analysis of amplicons in real-time polymerase chain reaction. *J. Food Prot.* 66:1343–1352.

Cline, J., J. C. Braman, and H. H. Hogrefe. 1996. PCR fidelity of *Pfu* DNA polymerase and other thermostable DNA polymerases. *Nucleic Acids Res.* 24:3546–3551.

Dahlenborg, M., E. Borch, and P. Rådström. 2001. Development of a combined selection and enrichment PCR procedure for *Clostridium botulinum* Types B, E, and F and its use to determine prevalence in fecal samples from slaughtered pigs. *Appl. Environ. Microbiol.* 67:4781–4788.

Daum, L. T., W. J. Barnes, J. C. McAvin, M. S. Neidert, L. A. Cooper, W. B. Huff, L. Gaul, W. S. Riggins, S. Morris, A. Salmen, and K. L. Lohman. 2002. Real-time PCR detection of salmonella in suspect foods from a gastroenteritis outbreak in Kerr County, Texas. *J. Clin. Microbiol.* 40:3050–3052.

Davis, C. R., L. C. Heller, K. K. Peak, D. L. Wingfield, C. L. Goldstein-Hart, D. W. Bodager, A. C. Cannons, P. T. Amuso, and J. Cattanii. 2004. Real-time PCR detection of the thermostable direct hemolysin and thermolabile hemolysin genes in a *Vibrio parahaemolyticus* cultured from mussels and mussel homogenate associated with a foodborne outbreak. *J. Food Prot.* 67:1005–1008.

Dupray, E., M. P. Caprais, A. Derrien, and P. Fach. 1997. Salmonella DNA persistence in natural seawaters using PCR analysis. *J. Appl. Microbiol.* 82:507–510.

Ellingson, J. L., J. L. Anderson, S. A. Carlson, and V. K. Sharma. 2004. Twelve hour real-time PCR technique for the sensitive and specific detection of Salmonella in raw and ready-to-eat meat products. *Mol. Cell. Probes* 18:51–57.

Eyigor, A., and K. T. Carli. 2003. Rapid detection of *Salmonella* from poultry by real-time polymerase chain reaction with fluorescent hybridization probes. *Avian Dis.* 47:380–386.

Fleet, G. H. 1999. Microorganisms in food ecosystems. *Int. J. Food Microbiol.* 50:101–117.

Frank-Kamenetskii, M. 1991. Oligonucleotide drugs: A change of backbone. *Nature*. 354:505.

Håkansson, S., M. Granlund-Edstedt, M. Sellin, and S. E. Holm. 1990. Demonstration and characterization of buoyant-density subpopulations of group B S*treptococcus* type III. *J. Infect. Dis.* 161:741–746.

Hald, B., K. Knudsen, P. Lind, and M. Madsen. 2001. Study of the infectivity of saline-stored *Campylobacter jejuni* for day-old chicks. *Appl. Environ. Microbiol.* 67:2388–2392.

Heid, C. A., J. Stevens, K. J. Livak, and P. M. Williams. 1996. Real time quantitative PCR. *Genome Res.* 6:986–994.

Hein, J., U. Schellenberg, G. Bein, and H. Hackstein. 2001. Quantification of murine IFN-gamma mRNA and protein expression: Impact of real-time kinetic RT-PCR using SYBR green I dye. *Scand. J. Immunol.* 54:285–291.

Herman, L. 1997. Detection of viable and dead *Listeria monocytogenes* by PCR. *Food Microbiol.* 14:103–110.

Higuchi, R., G. Dollinger, P. S. Walsh, and R. Griffith. 1992. Simultaneous amplification and detection of specific DNA sequences. *Biotech.* 10:413–416.

Higuchi, R., C. Fockler, G. Dollinger, and R. Watson. 1993. Kinetic PCR analysis: Real-time monitoring of DNA amplification. *Biotech.* 11:1026–1030.

Hoorfar, J., P. Ahrens, and P. Rådström. 2000. Automated 5' nuclease PCR assay for identification of *Salmonella enterica*. *J. Clin. Microbiol.* 38:3429–3435.

Hosta, L., and P. Flick. 1991–1992. Enhancement of specificity and yield in PCR. *Ed. Comments*. 18:1–5.

Ibekwe, A. M., and C. M. Grieve. 2003. Detection and quantification of *Escherichia coli* O157:H7 in environmental samples by real-time PCR. *J. Appl. Microbiol.* 94:421–431.

Josephson, K. L., C. P. Gerba, and I. L. Pepper. 1993. Polymerase chain reaction detection of nonviable bacterial pathogens. *Appl. Environ. Microbiol.* 59:3513–3515.

Klein, D. 2002. Quantification using real-time PCR technology: Applications and limitations. *Trends Mol. Med.* 8:257–260.

Klein, P. G., and V. K. Juneja. 1997. Sensitive detection of viable *Listeria monocytogenes* by reverse transcription-PCR. *Appl. Environ. Microbiol.* 63:4441–4448.

Knutsson, R. 2001. *Diagnostic PCR for the detection of* Yersinia enterocolitica *and* Salmonella *in the food-chain: Reliability of PCR performance*. Lund, Sweden: Institute of Technology.

Knutsson, R., Y. Blixt, H. Grage, E. Borch, and P. Rådström. 2002. Evaluation of selective enrichment PCR procedures for *Yersinia enterocolitica*. *Int. J. Food Microbiol.* 73:35–46.

Knutsson, R., M. Fontanesi, H. Grage, and P. Rådström. 2002. Development of a PCR-compatible enrichment medium for *Yersinia enterocolitica*: Amplification precision and dynamic detection range during cultivation. *Int. J. Food Microbiol.* 72:185–201.

Knutsson, R., C. Löfström, H. Grage, J. Hoorfar, and P. Rådström. 2002. Modeling of 5' nuclease real-time responses for optimization of a high-throughput enrichment PCR procedure for *Salmonella enterica*. *J. Clin. Microbiol.* 40:52–60.

Kreader, C. A. 1996. Relief of amplification inhibition in PCR with bovine serum albumin or T4 gene 32 protein. *Appl. Environ. Microbiol.* 62:1102–1106.

Kreuzer, K. A., A. Bohn, U. Lass, U. R. Peters, and C. A. Schmidt.2000. Influence of DNA polymerases on quantitative PCR results using TaqMan probe format in the LightCycler instrument. *Mol. Cell. Probes.* 14:57–60.

Kyger, E. M., M. D. Krevolin, and M. J. Powell. 1998. Detection of the hereditary hemochromatosis gene mutation by real-time fluorescence polymerase chain reaction and peptide nucleic acid clamping. *Anal. Biochem.* 260:142–148.

Laigret, F., J. Deaville, J. M. Bove, and J. M. Bradbury. 1996. Specific detection of *Mycoplasma iowae* using polymerase chain reaction. *Mol. Cell. Probes.* 10:23–29.

Lantz, P. 1998. PCR based detection of microorganisms in complex biological samples (doctoral thesis). Lund, Sweden: Department of Applied Microbiology, Lund University.

Lantz, P.-G., W. Abu Al-Soud, R. Knutsson, B. Hahn-Hägerdal, and P. Rådström. 2000. Biotechnical use of the polymerase chain reaction for microbial analysis of biological samples. In *Biotechnology annual review,* ed. M. R. El-Gewely, 87–130. Amsterdam: Elsevier Science.

Lantz, P.-G., B. Hahn-Hägerdal, and P. Rådström. 1994. Sample preparation methods in PCR-based detection of food pathogens. *Trends Food Sci. Tech.* 5:384–389.

Lantz, P.-G., F. I. Stalhandske, K. Lundahl, and P. Rådström. 1999. Detection of yeast by PCR in sucrose solutions using a sample preparation method based on filtration. *World J. Microbiol. Biotech.* 15:345–348.

Lindqvist, R., B. Norling, and S. T. Lambertz. 1997. A rapid sample preparation method for PCR detection of food pathogens based on buoyant density centrifugation. *Lett. Appl. Microbiol.* 24:306–310.

Livak, K. J., S. J. Flood, J. Marmaro, W. Giusti, and K. Deetz. 1995. Oligonucleotides with fluorescent dyes at opposite ends provide a quenched probe system useful for detecting PCR product and nucleic acid hybridization. *PCR Meth. Appl.* 4:357–362.

Löfström, C., R. Knutsson, C. E. Axelsson, and P. Rådström. 2004. Rapid and specific detection of *Salmonella* spp. in animal feed samples by PCR after culture enrichment. *Appl. Environ. Microbiol.* 70:69–75.

Lübeck, P. S., N. Cook, M. Wagner, P. Fach, and J. Hoorfar. 2003. Toward an international standard for PCR-based detection of food-borne thermotolerant Campylobacters: Validation in a multicenter collaborative trial. *Appl. Environ. Microbiol.* 69:5670–5672.

Lübeck, P. S., and J. Hoorfar. 2003. PCR technology and applications to zoonotic food-borne bacterial pathogens. *Meth. Mol. Biol.* 216:65–84.

Makinoshima, H., A. Nishimura, and A. Ishihama. 2002. Fractionation of Escherichia coli cell populations at different stages during growth transition to stationary phase. *Mol. Microbiol.* 43:269–279.

Malinen, E., A. Kassinen, T. Rinttila, and A. Palva. 2003. Comparison of real-time PCR with SYBR Green I or 5'-nuclease assays and dot-blot hybridization with rDNA-targeted oligonucleotide probes in quantification of selected faecal bacteria. *Microbiol.* 149:269–277.

McIngvale, S. C., D. Elhanafi, and M. A. Drake. 2002. Optimization of reverse transcriptase PCR to detect viable Shiga-toxin-producing *Escherichia coli*. *Appl. Environ. Microbiol.* 68:799–806.

Mead, P. S., L. Slutsker, V. Dietz, L. F. McCaig, J. S. Bresee, C. Shapiro, P. M. Griffin, and R. V. Tauxe. 1999. Food-related illness and death in the United States. *Em. Infect. Dis.* 5:607–625.

Medema, G. J., F. M. Schets, A. W. van de Giessen, and A. H. Havelaar. 1992. Lack of colonization of 1 day old chicks by viable, non-culturable *Campylobacter jejuni*. *J. Appl. Bacteriol.* 72:512–516.

Mullan, B., E. Kenny-Walsh, J. K. Collins, F. Shanahan, and L. J. Fanning. 2001. Inferred Hepatitis C Virus quasispecies diversity is influenced by choice of DNA polymerase in reverse transcriptase-polymerase chain reactions. *Anal. Biochem.* 289:281–288.

Mullis, K., F. Faloona, S. Scharf, R. Saiki, G. Horn, and H. Erlich. 1986. Specific enzymatic amplification of DNA *in vitro*: The polymerase chain reaction. *Cold Spring Harb. Symp. Quant. Biol.* 51:Pt. 1, 263–273.

Nazarenko, I. A., S. K. Bhatnagar, and R. J. Hohman. 1997. A closed tube format for amplification and detection of DNA based on energy transfer. *Nucleic Acids Res.* 25:2516–2521.

Nielsen, P. E., M. Egholm, R H. Berg, and O. Buchardt. 1991. Sequence-selective recognition of DNA by strand displacement with a thymine-substituted polyamide. *Science.* 254:1497–1500.

Nogva, H. K., A. Bergh, A. Holck, and K. Rudi. 2000. Application of the 5'-nuclease PCR assay in evaluation and development of methods for quantitative detection of *Campylobacter jejuni*. *Appl. Environ. Microbiol.* 66:4029–4036.

Nogva, H. K., K. Rudi, K. Naterstad, A. Holck, and D. Lillehaug. 2000. Application of 5'-nuclease PCR for quantitative detection of *Listeria monocytogenes* in pure cultures, water, skim milk, and unpasteurized whole milk. *Appl. Environ. Microbiol.* 66:4266–4271.

Norton, D., and C. Batt. 1999. Detection of viable *Listeria monocytogenes* with a 5'nuclease PCR assay. *Appl. Environ. Microbiol.* 65:2122–2127.

O'Hanlon, K. A., T. M. Catarame, G. Duffy, I. S. Blair, and D. A. McDowell. 2004. RAPID detection and quantification of *E. coli* O157/O26/O111 in minced beef by real-time PCR. *J. Appl. Microbiol.* 96:1013–1023.

Orlando, C., P. Pinzani, and M. Pazzagli. 1998. Developments in quantitative PCR. *Clin. Chem. Lab. Med.* 36:255–269.

Ørum, H., M. H. Jakobsen, T. Koch, J. Vuust, and M. B. Borre. 1999. Detection of the factor V Leiden mutation by direct allele-specific hybridization of PCR amplicons to photoimmobilized locked nucleic acids. *Clin. Chem.* 45:1898–1905.

Panicker, G., M. L. Myers, and A. K. Bej. 2004. Rapid detection of *Vibrio vulnificus* in shellfish and Gulf of Mexico water by real-time PCR. *Appl. Environ. Microbiol.* 70:498–507.

Patel, B. K., D. K. Banerjee, and P. D. Butcher. 1993. Determination of Mycobacterium leprae viability by polymerase chain reaction amplification of 71-kDa heat-shock protein mRNA. *J. Infect. Dis.* 168:799–800.

Pertoft, H. 2000. Fractionation of cells and subcellular particles with Percoll. *J. Biochem. Biophys. Meth.* 44:1–30.

Pertoft, H., K. Rubin, L. Kjellen, T. C. Laurent, and B. Klingeborn. 1977. The viability of cells grown or centrifuged in a new density gradient medium, Percoll(TM). *Exp. Cell. Res.* 110:449–457.

Powell, H. A., C. M. Gooding, S. D. Garrett, B. M. Lund, and R. A. McKee. 1994. Proteinase inhibition of the detection of *Listeria monocytogenes* in milk using the polymerase chain reaction. *Letters in App. Microbiol.* 18:59–61.

Rådström, P., R. Knutsson, P. Wolffs, M. Dahlenborg, and C. Löfström. 2003. Pre-PCR processing of samples. *Meth. Mol. Biol.* 216:31–50.

Rådström, P., R. Knutsson, P. Wolffs, M. Lovenklev, and C. Löfström. 2004. Pre-PCR processing: Strategies to generate PCR-compatible samples. *Mol. Biotechnol.* 26:133–146.

Rossen, L., P. Nøskov, K. Holmstrøm, and O. F. Rasmussen. 1992. Inhibition of PCR by components of food samples, microbial diagnostic assays and DNA-extraction solution. *Int. J. Food Microbiol.* 17:37–45.

Rudi, K., H. K. Hoidal, T. Katla, B. K. Johansen, J. Nordal, and K. S. Jakobsen. 2004. Direct real-time PCR quantification of *Campylobacter jejuni* in chicken fecal and cecal samples by integrated cell concentration and DNA purification. *Appl. Environ. Microbiol.* 70:790–797.

Rudi, K., H. K. Nogva, B. Moen, H. Nissen, S. Bredholt, T. Moretro, K. Naterstad, and A. Holck. 2002. Development and application of new nucleic acid-based technologies for microbial community analyses in foods. *Int. J. Food Microbiol.* 78:171–180.

Saiki, R. K., S. Scharf, F. Faloona, K. B. Mullis, G. T. Horn, H. A. Erlich, and N. Arnheim. 1985. Enzymatic amplification of β-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. *Science.* 230:1350–1354.

Sails, A. D., A. J. Fox, F. J. Bolton, D. R. Wareing, and D. L. Greenway. 2003. A real-time PCR assay for the detection of *Campylobacter jejuni* in foods after enrichment culture. *Appl. Environ. Microbiol.* 69:1383–1390.

Sarlin, L. L., E. T. Barnhart, D. J. Caldwell, R. W. Moore, J. A. Byrd, D. Y. Caldwell, D. E. Corrier, J. R. Deloach, and B. M. Hargis. 1998. Evaluation of alternative sampling methods for Salmonella critical control point determination at broiler processing. *Poult. Sci.* 77:1253–1257.

Satsangi, J., D. P. Jewell, K. Welsh, M. Bunce, and J. I. Bell. 1994. Effect of heparin on polymerase chain reaction. *Lancet.* 343:1509–1510.

Scheu, P., K. Berghof, and U. Stahl. 1998. Detection of pathogenic and spoilage microorganisms in food with the polymerase chain reaction. *Food Microb.* 15:13–31.

Sharma, V. K. 2002. Detection and quantitation of enterohemorrhagic *Escherichia coli* O157, O111, and O26 in beef and bovine feces by real-time polymerase chain reaction. *J. Food Prot.* 65:1371–1380.

Sharpe, A. N. 1994. Preparing samples for rapid detection of microbes. In *Rapid methods and automation in microbiology and immunology,* eds. R. C. Spencer, E. P. Wright, and S. W. B. Newsom, 97–105. Andover, UK: Intercept.

Sheridan, G. E., E. A. Szabo, and B. M. Mackey. 1999. Effect of post-treatment holding conditions on detection of tufA mRNA in ethanol-treated *Escherichia coli*: Implications for RT-PCR-based indirect viability tests. *Letters Appl. Microbiol.* 29:375–379.

Simon, M. C., D. I. Gray, and N. Cook. 1996. DNA extraction and PCR methods for the detection of *Listeria monocytogenes* in cold-smoked salmon. *Appl. Environ. Microbiol.* 62:822–824.

Singh, S. K., R. Kumar, and J. Wengel. 1998. Synthesis of novel bicyclo[2.2.1] ribonucleosides: 2'-Amino- and 2'-Thio-LNA monomeric nucleosides. *J. Org. Chem.* 63:6078–6079.

Soultos, N., P. Koidis, and R. H. Madden. 2003. Presence of *Listeria* and *Salmonella* spp. in retail chicken in Northern Ireland. *Letters Appl. Microbiol.* 37:421–423.

Starbuck, M. A. B., P. J. Hill, and G. S. A. B. Stewart. 1992. Ultra sensitive detection of *Listeria monocytogenes* in milk by polymerase chain reaction (PCR). *Letters App. Microbiol.* 15:248–252.

Stern, N. J. 1994. Mucosal competitive exclusion to diminish colonization of chickens by *Campylobacter jejuni*. *Poult. Sci.* 73:402–407.

Stocher, M., V. Leb, and J. Berg. 2003. A convenient approach to the generation of multiple internal control DNA for a panel of real-time PCR assays. *J. Virol. Meth.* 108:1–8.

Stocher, M., V. Leb, G. Holzl, and J. Berg. 2002. A simple approach to the generation of heterologous competitive internal controls for real-time PCR assays on the LightCycler. *J. Clin. Virol.* 25:S47–S53.

Stults, J. R., O. Snoeyenbos-West, B. Methe, D. R. Lovley, and D. P. Chandler. 2001. Application of the 5' fluorogenic exonuclease assay (TaqMan) for quantitative ribosomal DNA and rRNA analysis in sediments. *Appl. Environ. Microbiol.* 67:2781–2789.

Svanvik, N., G. Westman, D. Wang, and M. Kubista. 2000. Light-up probes; thiazole orange conjugated PNA for detection of target nucleic acid in homogeneous solution. *Anal. Biochem.* 281:26–35.

Tyagi, S., and F. R. Kramer. 1996. Molecular beacons: Probes that fluoresce upon hybridization. *Nature Biotech.* 14:303–308.

Wallace, D., T. V. Gilder, S. Shallow, T. Fiorentino, S. Segler, K. Smith, B. Shiferaw, R. Etzel, W. Garthright, and F. Angulo. 2000. Incidence of food-borne illnesses reported by the food-borne diseases active surveillance network. (FOODNET)-1997. *J. Food Prot.* 63:807–809.

Wang, X., N. Jothikumar, and M. W. Griffiths. 2004. Enrichment and DNA extraction protocols for the simultaneous detection of *Salmonella* and *Listeria monocytogenes* in raw sausage meat with multiplex real-time PCR. *J. Food Prot.* 67:189–192.

White, T. J., N. Arnheim, and H. A. Ehrlich. 1989. The polymerase chain reaction. *Tech. Focus.* 5:185–188.

Whiteley, A. S., M. R. Barer, and A. G. O'Donnell. 2000. Density gradient separation of active and non-active cells from natural environments: *Antonie Van Leeuwenhoek.* 77:173–177.

Wilson, I. G. 1997. Inhibition and facilitation of nucleic acid amplification. *App. Env. Microbiol.* 63:3741–3751.

Winn-Deen, E. S. 1998. Direct fluorescence detection of allele-specific PCR products using novel energy-transfer labeled primers. *Mol. Diagn.* 3:217–221.

Wittwer, C. T., M. G. Herrmann, A. A. Moss, and R. P. Rasmussen. 1997. Continuous fluorescence monitoring of rapid cycle DNA amplification. *BioTechniques.* 22:130–131, 134–138.

Wittwer, C. T., K. M. Ririe, R. V. Andrew, D. A. David, R. A. Gundry, and U. J. Balis. 1997. The LightCycler: A microvolume multisample fluorimeter with rapid temperature control. *Biotechniques.* 22:176–181.

Wolffs, P., H. Grage, O. Hagberg, and P. Rådström. 2004. Impact of DNA polymerases and accompanying buffer systems on quantitative real-time PCR. *J. Clin. Microbiol.* 42:408–411.

Wolffs, P., R. Knutsson, R. Sjöback, and P. Rådström. 2001. PNA-based light-up probes for real-time detection of sequence-specific PCR products. *Biotechniques.* 31:766, 769–771.

Wolffs, P., R. Knutsson, B. Norling, and P. Rådström. 2004. Rapid quantification of *Yersinia enterocolitica* in pork samples by a novel sample preparation method, flotation, prior to real-time PCR. *J. Clin. Microbiol.* 42:1042–1047.

Yang, C., Y. Jiang, K. Huang, C. Zhu, and Y. Yin. 2003. Application of real-time PCR for quantitative detection of *Campylobacter jejuni* in poultry, milk and environmental water. *FEMS Immunol. Med. Microbiol.* 38:265–271.

7 Meat Decontamination by Irradiation

D. U. Ahn, E. J. Lee, and A. Mendonca
Iowa State University

CONTENTS

7.1 Food Irradiation 156
7.1.1 History of Food Irradiation 156
7.1.2 Irradiation Process 159
7.2 Microbial Decontamination of Meat by Irradiation 162
7.2.1 Factors Affecting Radiation Destruction of Micro-Organisms in Meat 163
7.2.1.1 Irradiation Dose 163
7.2.1.2 Meat Composition 163
7.2.1.3 Temperature 164
7.2.1.4 Gaseous Composition 164
7.2.1.5 Microbial Factors 165
7.2.2 Combinations of Irradiation and Other Antimicrobial Interventions 166
7.3 Quality Changes in Meat by Irradiation 167
7.3.1 Lipid Oxidation 167
7.3.2 Sources and Mechanisms of Off-Odor Production 169
7.3.2.1 Sources of Off-Odor Production in Irradiated Meat 169
7.3.2.2 Mechanism of Off-Odor Production in Irradiated Meat 170
7.3.3 Color Changes in Meat by Irradiation 171
7.3.3.1 Color Changes in Irradiated Raw and Cooked Meat 171
7.3.3.2 Mechanism of Color Changes in Irradiated Meat 173
7.3.4 Water Holding Capacity and Texture 175
7.3.5 Consumer Attitude and Acceptance of Irradiated Meat 175
7.3.6 Control of Quality Changes 176
7.3.6.1 Additives 176
7.3.6.2 Packaging 178
7.3.6.3 Packaging and Additive Combinations 180
7.4 Future Research Needed 180
References 181

Meat is one of the major sources for pathogens that cause food-borne illness in humans. The intervention strategies for pathogens in meat can be divided into preharvest reduction of micro-organisms in livestock and postharvest decontamination of carcass and meat. The reduction of bacteria in the 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, chemical, and physical and chemical method combinations during slaughtering or processing steps.

Irradiation is among the most effective physical decontamination technologies for inactivating food-borne pathogens and improving the safety of meats. Irradiation of meat for the purpose of killing indigenous microflora, and thereby extending shelf life, has been recognized as a preservation technique for several decades. The major advantages of irradiating meat are that 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 largely linked to the damage of bacterial DNA from the production of free radicals during the irradiation process. The effect of irradiation in inhibiting food-borne pathogens and spoilage bacteria in meat products is dose dependent. The survival of microbial cells after 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 micro-organisms are suspended also have significant effects on the microcidal efficiency of irradiation.

Although very effective in controlling pathogens, irradiation can deplete antioxidants in muscle, which could reduce storage stability, induce color change, increase production of off-odor volatiles, and negatively alter the sensory characteristics of meat products. Formation of 2-alkylcyclobutanones, benzene, and methylbenzene (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 chemical treatments that can improve the efficacy of irradiation and the quality and consumer acceptance of irradiated meat products are also discussed.

7.1 FOOD IRRADIATION

7.1.1 History of Food Irradiation

Food irradiation has a 60-year history of scientific research and testing, with more than 40 years preceding approval of the process for any foods in the United States. To date, no other food technology has as long a history of scientific research and testing before gaining approval (American Medical Association 1993). Research has

been comprehensive and has included toxicological and microbiological evaluation, as well as testing for wholesomeness (World Health Organization 1994).

Since Willhelm von Roentgen discovered X-rays in 1895 and Pierre and Marie Curie discovered the breakdown of uranium into polonium and radium with the accompanying production of radiation in 1899, the use of ionizing radiation to preserve foods by destroying spoilage micro-organisms was proposed (Brynjolfsson 1989; Minsch 1896). Although two patents were filed in 1905 (Appleby and Barks 1905; Lieber 1905) and X-ray technology was applied to kill *Trichina* in pork in 1921 (Schwartz 1921), food irradiation was economically unfeasible in the United States until World War II because of the high cost of ionizing radiation sources (Urbain 1989). In the 1940s, machines that could produce high-energy electron beams of up to 24 million electron volts became available. This energy was sufficient to penetrate and sterilize a 6-inch No. 10 can of food when electron beams were "fired" from both sides of the can. 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 (Urbain 1989). The availability of these sources stimulated research in food irradiation aimed at the development of commercial processes. In the mid-1940s, interest in food irradiation was renewed when it was suggested that electron accelerators could be used to preserve foods. However, the accelerators in those days were rather costly and unreliable for industrial application.

From 1940 through 1953, exploratory research in food irradiation in the United States was sponsored by the Department of the Army, the Atomic Energy Commission, and private industry (Thayer, Lachica, Huhtanen, and Wierbicki 1986). In 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 cathode ray radiation (electrons) had the necessary characteristics of efficiency, safety, and practicality. They considered X-rays impractical because of very low conversion efficiency from electron to X-ray at that time (Hayashi 1991; Urbain 1986). Ultraviolet light and alpha particles were also considered impractical because of their limited ability to penetrate matters. Neutrons exhibited a great penetration capability and were very effective in the destruction or 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 medium in which micro-organisms were irradiated was a factor 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 in milk and orange juice. Most in-depth studies in food irradiation since 1952 were government sponsored because of military interest in this type of food processing. Much of the early research was done to sterilize foods by the Quartermaster Corps of the U.S. Army at the Food and Container Institute in Chicago because of the Army's 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. Because the U.S. Army Medical Department began to assess the safety of irradiated foods in 1955 (CAST 1986), petitions to the Food and Drug Administration (FDA) for the approval of irradiation of specific foods were followed

and commercial radiation equipment and sources were developed. In the meantime, the International Atomic Energy Agency (IAEA), which promotes 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, and development and practical application of atomic energy for peaceful uses throughout the world." As a result, the 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 its research laboratories in Natick, Massachusetts, and conducted research in sterilizing meat products using high-dose irradiation. The Army sponsored studies for the development of shelf-stable bacon, ham, pork, beef, hamburger, corned beef, pork sausage, codfish cakes, and shrimp. The first products approved by the FDA were wheat and wheat powder in 1963 (Federal Register 1999; Mason 1992). In the early 1970s, the National Aeronautics and Space Administration (NASA) adopted irradiation processes to sterilize meats for astronauts to consume in space, and this practice has continued (Karel 1989). NASA had been investigating irradiation as a method for sterilizing spacecraft to ensure that microbes from Earth did not contaminate Mars. NASA, a pioneer in the use of irradiated food, first used irradiated meats in 1972 when irradiated ham processed by the U.S. Army Natick Research and then Development and Engineering Center was included on the flight menu of *Apollo 17*. In 1975, irradiated ham, turkey, beef steak, and corned beef were used on the *Apollo-Soyuz* Test Project (ASTP), and irradiated foods were shared with the Russian cosmonauts.

In 1980, the Food and Agriculture Organization 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 problems; hence toxicological testing of foods so treated is no longer required" (World Health Organization 1981). The residual Army food irradiation program (chicken) was transferred to the U.S. Department of Agriculture (USDA). This agency assigned the responsibility to the Eastern Regional Research Center in Philadelphia, Pennsylvania (Skala, McGown, and Waring 1987). In 1981, the FDA proposed that certain foods irradiated at dosages not exceeding 100 krad (1 kGy) would be considered unconditionally safe (Henkel 1998). During the 1980s, the FDA approved petitions for irradiation of spices and seasonings, pork, fresh fruits, and dry or dehydrated substances. The USDA approved irradiation of pork to destroy *Trichinella spirallis* (USDA 1986), and pathogen control for poultry (USDA 1992), and red meats (USDA 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 recommended removing dose limit so that irradiation could be used to commercially sterilize foods 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 atomic bombs and nuclear radiation. However, irradiation has several applications in the food

industry to increase safety, preserve foods, improve quality compared to heat-processed foods, maintain nutrient content during storage, and preserve nutrients compared to other preservative processes such as cooking and sterilizing. Currently, 40 countries have permitted irradiation of food, and more than half a million tons of food are irradiated annually in the world (IAEA 1999; Loaharanu 1992; North American Plant Protection Organization 1995). The United States also has approximately 40 licensed irradiation facilities, most of which are used to sterilize medical and pharmaceutical supplies. The use of irradiation in meat is restricted to raw, packaged poultry at 1.5 kGy to 3.0 kGy, and fresh and frozen red meat at a maximum dose up to 4.5 kGy and 7.0 kGy, respectively (Sommers 2004; see table 7.1).

TABLE 7.1
Food Irradiation Rules from the U.S. Food and Drug Administration

Product	Purpose of Irradiation	Dose Permitted (kGy)	Date of Rule
Wheat and wheat powder	Disinfest insects	0.2–0.5	August 21, 1963
White potatoes	Extend shelf life	0.05–0.15	November 1, 1965
Spices and dry vegetable seasoning	Decontamination/ disinfest insects	30 (maximum)	July 15, 1983
Dry or dehydrated enzyme preparations	Control insects and micro-organisms	10 (maximum)	June 10, 1985
Pork carcasses or fresh noncut processed cuts	Control *Trichinella spiralis*	0.3 (minimum)–1.0 (maximum)	July 22, 1985
Fresh fruits	Delay maturation	1	April 18, 1986
Dry or dehydrated enzyme preparations	Decontamination	10	April 18, 1986
Dry or dehydrated aromatic vegetable substances	Decontamination	30	April 18, 1986
Poultry	Control illness-causing micro-organisms	3	May 2, 1990
Red meat	Control illness-causing micro-organisms	4.5 (minimum for refrigerated)–7 (maximum for frozen)	December 3, 1997

Adopted from O. B. Wood and C. M. Bruhn (2000). Position of the American Dietetic Association: Food irradiation. *J. Amer. Diet. Assoc.* 100:246–253.

7.1.2 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 a 1:1 to 1:1.5 ratio (Thakur and Singh 1994). If any change of number and arrangement in the forces of the nuclear particles occur, they lose balance and consequently become an unstable or radioactive atom. This unstable atom can restabilize 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 1999).

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-rays and gamma rays; and very high energy radiations occur in 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 nonionizing radiation, and if it also has sufficient energy to break chemical bonds it is called ionizing radiation (Josephson and Peterson 2000). Because high-energy sources such as accelerated electrons, gamma rays, and X-rays have short wavelength (< 300 nm) and higher energy that can create ions or free radicals from atoms, these are ionizing radiations. The amount of energy needed to break a carbon-carbon bond is about 33 electron volts (eV) and a dose of 1 kGy has been estimated to break less than one chemical bond in 1 million bonds present (CAST 1989).

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* (Lagunas-Solar 1995). Alpha particles are emitted in very large atoms such as radium, uranium, and plutonium when the neutron:proton ration is too low. Alpha particles travel slowly and lose energy rapidly due to their charge and mass, expending 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 the nucleus and X-rays are from electron fields. Gamma rays have such high energy and they are so small that they pass through living tissues without interacting with them (Jarrett 1982). 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 materials, the energy can be transferred or absorbed by an electron of an atom in materials. The electron of an atom in materials increases in energy level and leaves its orbit. The ejected electron, called the 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 there is no longer enough energy to cause electrons to leave their orbits (Venugopal, Doke, and Thomas 1999).

By this basic principle of the radiation process, irradiation energy applied to biological materials ejects electrons from the atoms or 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 materials. The dispersion of ions and free radicals is higher when free water is present in liquid form than in limited free-water form (dried products) or the crystalline form (frozen products; Thakur and Singh 1994). The hydroxyl radical (HO•), the primary radiolytical product of water, is a powerful oxidizing agent and thus free radicals tend to recombine to form stable products (Taub, Karielian, and Halliday 1978; Taub et al. 1979). Because dispersion and capture of electrons are purely random, large molecules and compounds have a greater probability of being affected than smaller molecules. Cellular components such as DNA, pigments, fatty acids, and membrane lipids can be damaged by ionizing radiation (Olson 1998b). When the DNA of living cells is exposed to hydroxyl radicals by radiation, both single and double strands in the molecule are broken. Therefore, humans suffer greater damage than micro-organisms when they are exposed to radiation energy, and higher doses of radiation energy are required to kill micro-organisms compared with bigger animals (Thayer 1995).

Radionuclides such as ^{137}Cs and ^{60}Co are used as the major sources for gamma rays, and the linear accelerator, the electron beam (e-beam) machine, is used to generate high-energy electrons and X-rays. ^{137}Cs is produced when uranium and plutonium absorb neutrons and 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 is the most common energy source for irradiation that produces 1.33 MeV gamma radiations as it decays to nonradioactive nickel by emitting beta particles and strong 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. ^{60}Co produces highly penetrable γ-rays, which 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 electron beam is a stream of high-energy electrons that are generated electrically and then propelled out of 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 used with single-sided treatment and 10 MeV electrons can give satisfactory 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 to 10 cm thickness with a density of 1 g/cm^3.

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; readily availability; and low environmental risk. The disadvantages include the fact that 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 the linear accelerator compared with the gamma irradiators are that it can simply be turned off when not in use, does not need to be replenished, has an established history of use, and has 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, they are not used in food irradiation due to poor conversion of accelerated electrons to X-rays (Hayashi 1991). The quantity of energy absorbed by something (food) as it passes through a radiation field is called the *radiation absorbed dose*. The unit (SI) for irradiation dose is the Gray (Gy), which is equal to the absorption of energy equivalent to one Joule per kilogram of absorbing material (1 Gy = 1 $J.kG^{-1}$ = 6,200 billion MeV absorbed/kg of food = 0.01 calorie/lb. of food = 100 rad, 1 rad = 100 erg/g; Dragnic and Dragnic 1964).

7.2 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 and is well described in reviews (Farkas 1987; Lee, Sebranek, Olson, and Dickson 1996; Murano 1995; Thayer 1993). Use of this technology with the aim of destroying meat-borne pathogenic micro-organisms will also result in a reduction in numbers of spoilage micro-organisms to increase the shelf life of meats (Olson 1998a). The destruction of micro-organisms in meats using irradiation is impacted by several factors including irradiation dose, meat composition, temperature, gaseous atmosphere, and microbial factors. Large reductions in microbial populations in meats can be achieved by using high radiation doses; however, such an approach can have negative 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, high hydrostatic pressure, etc.) 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.

7.2.1 Factors Affecting Radiation Destruction of Micro-Organisms in Meat

7.2.1.1 Irradiation Dose

Generally, larger populations of food-borne micro-organisms are destroyed when high doses of radiation are applied to meats. Relatively high doses of irradiation negatively impact the organoleptic qualities of the meat; therefore, there is a need for application of optimal doses to achieve microbial safety in raw or ready-to-eat (RTE) meats while preserving the sensory quality of these products. The extent of destruction of meat-borne micro-organisms at a given irradiation dose may be reduced under anaerobic conditions or very low water activity (a_W) because of the lower rate of oxidizing reactions that generate free radicals and toxic oxygen products.

7.2.1.2 Meat Composition

Meat composition affects the destruction of micro-organisms by irradiation. Meats are known to be high in protein and increasing amounts of protein may protect micro-organisms 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 micro-organisms in meats and dairy products compared to nonprotein foods of similar moisture content. Proteins and other meat constituents, including natural antioxidants such as carnosine and vitamin E, compete for free radicals formed by the radiolysis of water. This competition for free radicals decreases the antimicrobial efficacy of ionizing radiation. Carnosine has been reported to increase the radiation resistance of *Aeromonas hydrophilia* in minced turkey meat (Steccheni et al. 1998). Sweeteners, including dextrose, are commonly incorporated in the formulation of fine emulsion sausages such as bologna and frankfurters. The irradiation of dextrose has been shown to result in the production of peroxides (Kawakishi, Okumura, and Namki 1971), which theoretically should further contribute to microbial inactivation during irradiation of dextrose-containing RTE meats. Sommers and Fan (2002) reported that the radiation resistance of *L. monocytogenes* was unaffected in beef bologna with dextrose concentration of 0 (control), 2, 4, 6, or 8%. Interestingly, fat content of meat does not seem to influence the extent of microbial destruction by irradiation (Clavero, Monk, Beuchat, Doyle, and Brackett 1994; Monk, Clavero, Beuchat, Doyle, and Brackett 1994; Thayer and Boyd 1994; Thayer, Boyd, Fox, Lakritz, and Hampson 1995). During irradiation of meat, free fatty acids, carbonyl compounds, hydrogen peroxide, and hydroperoxides are produced from fats. Radiation-induced production of hydrogen peroxide and other toxic oxygen products should increase the killing effect of irradiation in foods that contain fat. However, the reported ineffectiveness of fat levels in meat to influence the radiation resistance of certain bacterial pathogens may be attributed to other meat constituents. Such constituents, mainly proteins, may protect bacteria and other micro-organisms from the antimicrobial products of radiation-induced chemical changes in fats (Diehl 1995).

7.2.1.3 Temperature

The temperature of meat during irradiation is an important factor that affects the extent of irradiation destruction of micro-organisms. Microbial resistance to irradiation increases with decreases in temperature below the freezing point of water. The impact of meat temperature on survival of pathogenic bacteria following irradiation has been reported for *Campylobacter jejuni* (Clavero et al. 1994), *Salmonell*a (Thayer and Boyd 1991a, 1991b), *Escherichia coli* O157:H7 (Clavero et al. 1994; Lopez-Gonzalez, Murano, Brennan, and Murano 1999; Thayer and Boyd 1992), *Staphylococcus aureus* (Thayer and Boyd 1992), *Listeria monocytogenes* (Thayer and Boyd 1995), and *C. botulinum* spores (El-Bisi, Snyder, and Levin 1966). Generally, micro-organisms exhibit a greater sensitivity to irradiation at ambient temperatures than at subfreezing temperatures. Matsuyama, Thornley, and Ingram (1964) reported the need for an 8.5-fold higher radiation dose to destroy 90% of *Pseudomonas* spp. at subfreezing temperature than at ambient temperature. 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 kGy 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 (Lopez-Gonzalez et al. 1999). Freezing meat reduces its water activity by converting the water to ice. Reduced water activity increases the irradiation resistance of micro-organisms by drastically reducing the generation of free radicals from the radiolysis of water (Diehl 1995). Additionally, the frozen state of meat 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).

7.2.1.4 Gaseous Composition

The gaseous composition of packaged meats can influence microbial destruction by irradiation. Most published research indicates that meat-borne micro-organisms were more susceptible to destruction by irradiation in the presence of oxygen. Gamma irradiation treatments were significantly more lethal to *L. monocytogenes* in aerobically packaged turkey meat than in either vacuum packaging or modified atmosphere packaging (MAP; Thayer and Boyd 1999). In contrast, Patterson (1988) observed greater radiation sensitivities of *S. typhimurium* and *E. coli* in vacuum-packaged poultry meat or meat packaged under CO_2 compared to aerobic packaging. *Lactobacillus sake*, *Lactobacillus alimentarius*, and *Lactobacillus curvatus* were more sensitive to gamma radiation in ground meat packaged under 100% carbon dioxide (CO_2) than under nitrogen (N_2; Hastings, Holzapfel, and Niemand 1986). Other studies have reported no significant differences in total bacterial numbers or numbers of *E. coli* O157:H7 surviving electron beam irradiation of ground beef packaged under air versus vacuum (Fu, Sebranek, and Murano 1995; Lopez-Gonzalez et al. 1999). A small but significant increase in the radiation sensitivity of *L. monocytogenes* in turkey meat packaged in 100% CO_2 compared to 100% N_2 has been reported (Thayer and Boyd 1999).

Inconsistencies in published research regarding the influence of gaseous composition on the radiation sensitivity of meat-borne bacteria may be attributed to differences in factors such as microbial recovery methods and irradiation temperature. Based on the results of two independent studies (El-Shenawy, Yousef, and Marth 1989; Patterson 1989) the type of agar media used for recovery of *L. monocytogenes* after irradiation significantly affected D_{10} values for this organism. Low temperatures have been shown to increase microbial sensitivity to irradiation under anoxic conditions (Hollaender, Stapleton, and Martin 1951). Lee et al. (1996) suggested that more complete information is needed to optimize the use of vacuum packaging or MAP combined with irradiation for ensuring the microbial safety of fresh meat and poultry.

7.2.1.5 Microbial Factors

Microbial factors including numbers and types of micro-organisms in meats as well as the physiological state of the micro-organisms can affect the extent of microbial destruction by irradiation. As observed with other food preservation processes, the presence of large populations of micro-organisms reduces the effectiveness of a given irradiation dose. Therefore, decontamination of meat using irradiation would be more effective if the meat to be treated is of good microbial quality. With regard to types of micro-organisms, 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 bacterial vegetative cells. Bacterial vegetative cells are more radiation resistant than fungi (yeast and molds). Generally, more complex life forms have a higher sensitivity to irradiation than simpler life forms. For example, meat-borne parasites (e.g., roundworms, tapeworms) are more sensitive to irradiation than bacteria or fungi, which are more sensitive than viruses. 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 generally 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 (Ehioba et al. 1988; Lambert, Smith, and Dodds 1992; Thayer, Boyd, and Jenkins 1993). Non-spore-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 important, meat-borne bacteria that have adapted to certain environmental stress demonstrate even greater radiation resistance than stationary-phase bacteria. For example, Buchanan, Edelson, and Boyd (1999) observed increased resistance of *Escherichia coli* O157:H7 strains to gamma radiation following induced acid adaptation. Those authors stated that growth of this meat-borne pathogen under conditions that would induce a stress response would also cause a higher radiation resistance, and recommended that this factor be considered when determining the radiation resistance (D_{10} values) for *E. coli* in meats. More recently, Mendonca, Romero,

Lihono, Nannapaneni, and Johnson (2004) demonstrated significant increases in the radiation resistance (D_{10} value) of starved *Listeria monocytogenes* cells in ground pork at 4°C. 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.

7.2.2 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. To avoid compromising the desirable sensory characteristics of meats the applied irradiation dose may be reduced when used in combination with other food preservation methods. 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 addition of chemical food preservatives can improve the decontamination efficacy of irradiation by increasing the radiation sensitivity of meat-borne micro-organisms 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% [w/w] ascorbic acid or 0.5% [w/w] glucono-delta-lactone) in vacuum-packaged minced meat prepared with pork and beef with spices and cereal fillings. Experimental batches of meat were stored at 0° to 2°C for 4 weeks or temperature abused at 10°C for 1 week. Acidulants delayed growth of *Enterobacteriaceae* in nonirradiated meat for two weeks at 0° to 2°C; however, a combination of acidulant with irradiation completely prevented growth of this microbial group during refrigerated storage and during temperature abuse (10°C). Bhide, Paturkar, Sherikar, and Waskar (2001) sprayed sheep carcasses with 1% propionic acid, 2% lactic acid, or 2% acetic acid at a pressure of 3 kg/cm^2 for 2 to 4 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 most effective in reducing total viable count and *B. cereus* count during refrigerated storage (5–7°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 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, Fan, Handel, Sokorai 2003). More recently, Kim, Jang, Lee, Min, and Lee (2004) investigated the combined effects of organic acids (lactic, citric, and acetic) at 2% and electron beam irradiation (1, 2, and 3 kGy) on the shelf life of pork loins. Combinations of organic acid and irradiation were more effective than each inter-

vention used alone for controlling growth of total microbial counts and coliforms in pork during storage at 4°C for 14 days.

Various other antimicrobial treatments have shown good potential for enhancing destruction of micro-organisms by irradiation. Kim and Thayer (1996) exposed a suspension of *Salmonella Typhimurium* ATCC 14028 cells to heating (65°C for 2.0 minutes) before or after treatment with gamma irradiation at a dose rate of 0.114 kGy/min. Both irradiation and heating significantly decreased survival of *S. Typhimurium* irrespective of the order of application of each treatment. Interestingly, application of irradiation before rather than following heating was consistently more lethal to the pathogen. Two cycles of vacuum–steam–vacuum (VSV) technology combined with 2.0 kGy of gamma radiation reduced initial populations of *Listeria innocua* on ham meat and skin by 4.40 and 4.85 log, respectively. This treatment combination resulted in an additive antimicrobial effect and did not produce significant changes in desirable quality characteristics of the ham (Sommers, Kozempel, Fan, and Radewonuk 2002). By using a combination of hurdles (low a_W, vacuum packaging, and irradiation) Kanatt, Chawla, Chander, and Bongirwar (2002) developed a number of 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 6 months (spiced chicken cubes) or 9 months (mutton kababs). No viable micro-organisms were detected in meat products treated with 10 kGy and those products retained acceptable sensory quality for up to 9 months. More recently, Chen, Sebranek, Dickson, and Mendonca (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. Generally, the combined treatments did not negatively alter the sensory characteristics of the frankfurters.

7.3 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 adoption of irradiation technology by the meat industry is limited because of quality and health concerns about irradiated meat products. Irradiation produces a characteristic aroma and alters meat flavor and color, which significantly impact consumer acceptance. Consumers associate the brown or gray color in 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 important for implementing irradiation technology in the meat industry.

7.3.1 Lipid Oxidation

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 75% or more of water, and irradiation is expected to accelerate oxidative changes in meat, irradiation-induced oxidative chemical changes in meat are dose-dependent (Ahn et al. 1997). The presence of oxygen also has a significant effect on the development of oxidation and odor production (Merritt, Angelini, Wierbicki, and Shuts 1975). 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 correlation with the amount 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 very important to stop oxidative chain reactions (Ahn, Jo, and Olson 2000; Ahn, Kawamoto, Wolfe, and Sim 1995; Ahn, Wolfe, Sim, and Kim 1992). Ahn et al. (1998) reported that preventing oxygen exposure after cooking was more important for cooked meat quality than packaging, irradiation, or storage conditions of raw meat (Ahn, Jo, Olson, and Nam 2000; Ahn, Olson, Jo, Love, and Jin 1999). Aerobically packaged sausages irradiated at higher irradiation dose produced greater amounts of TBARS than those irradiated at lower doses. The TBARS of aerobic- or vacuum-packaged sausages with higher polyunsaturated fatty acids was higher than those with lower polyunsaturated fatty acids.

Diehl (1995) indicated 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. Nawar (1986) reported that a series of dienes, trienes, and tetraenes were formed from unsaturated triacylglycerols by irradiation at 60 kGy under vacuum conditions. Shahidi and Pegg (1994) reported that aldehydes contributed the most to oxidation flavor and rancidity in cooked meat and hexanal was the major volatile aldehyde.

Lee and Ahn (2003) reported that TBARS values of oil emulsion samples immediately after irradiation were lower than those of nonirradiated samples. After 10 days of storage, however, irradiated samples developed higher TBARS values than nonirradiated emulsions. Especially arachidonic acid, linolenic acid, and fish oil, which had a high proportion of multi-double-bonded fatty acids, had accelerated lipid oxidation after irradiation. Longer storage time increased the amount of aldehydes and TBARS values in these oil emulsions, but irradiation had minimal effect on the increase of aldehydes and TBARS.

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 (Ahn, Jo, Olson, and Nam 2000; Nam and Ahn 2002a). Luchsinger et al. (1997) also showed that TBARS values of both chilled and frozen boneless pork chops were stable, regardless of display day, dose, and irradiation source. These results indicated that the radiation chemistry of refrigerated and frozen meat could be different. 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. Tarte (1996) reported that temperature has significant effects on the formation of radiolytic products, and that the reactive intermediates of water radiolysis were trapped in deep-

frozen materials and were kept from reacting with each other or with the substrates. 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 (Diehl 1995).

7.3.2 Sources and Mechanisms of Off-Odor Production

7.3.2.1 Sources of Off-Odor Production in Irradiated Meat

All irradiated meat produced characteristic, readily detectable, irradiation odor regardless of degree of lipid oxidation (Ahn et al. 1997; Ahn et al. 1998a, 1998b; Ahn et al. 1999). Huber, Brasch, and Waly (1953) reported that meat sterilized through irradiation developed 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 a "bloody and sweet" (Hashim, Resurreccion, and MaWatters 1995), "hot fat," "burned oil," or "burned feathers" (Heath, Owens, Tesch, and Hannah 1990), and "barbecued corn-like" (Ahn, Jo, and Olson 2000).

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. Patterson and Stevenson (1995) reported that dimethyl trisulfide, bis(methylthio-)methane, *cis*-3- and *trans*-6-nonenals, and oct-1-en-3-one are important for irradiation off-odor in chicken meat. 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, hydrogen sulfide, sulfur dioxide, mercaptomethane, dimethyl sulfide, methyl thioacetate, dimethyl disulfide, and trimethyl sulfide from meat (Ahn, Jo, and Olson 2000; Fan, Sommers, Thayer, and Lehotay 2002; Jo and Ahn 2000; Jo, Lee, and Ahn 1995; Nam, Du, Jo and Ahn 2002). Irradiating various amino acid homopolymers produced different odor characteristics, but irradiation of sulfur-containing amino acids produced an odor characteristic similar to irradiation odor of meat (Ahn 2002; Ahn and Lee 2002).

Champaign and Nawar (1969) found that hydrocarbons are the major radiolytic products in fat and are related to the fatty acid composition of the fat. Merritt, Angelini, and Graham (1978) postulated that carbonyls are formed in irradiated meats, due to the reactions of hydrocarbon radicals with molecular oxygen, which follows the same pathway as normal lipid oxidation. Sensory results, however, clearly indicated that the main source of irradiation off-odor was caused by sulfur compounds. The odor intensity of sulfur compounds was much stronger and stringent than that of other compounds. Volatiles from lipids accounted for only a small part of the off-odor in irradiated meat (Lee and Ahn 2003). Most sulfur compounds have low odor thresholds and were considered important for irradiation odor (Angelini, Merritt, Mendelshon, and King 1975). 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 distinctly different from those of warmed-over flavor in oxidized meat.

7.3.2.2 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 newly generated and increased volatiles by irradiation were sulfur compounds indicating that sulfur-containing amino acids are among the most susceptible amino acid groups to irradiation (Ahn and Lee 2002).

The perception of odor from samples containing sulfur volatiles changed greatly depending on their composition and amounts present in the sample. Sulfur compounds were not only produced by the radiolytic cleavage of side chains (primary reaction), but also by the secondary reactions of primary sulfur compounds with other compounds around them. The amounts and kinds of sulfur compounds produced from irradiated methionine and cysteine indicated that methionine is the major amino acid responsible for irradiation off-odor. The total amount of sulfur compounds produced from cysteine is only about 0.25% to 0.35% of methionine even after the proportion of cysteine or methionine in each of the dimmer, trimer, or tetramer was considered. Therefore, the contribution of methionine to the irradiation odor is far greater than that of cysteine (Ahn 2002). 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 as suggested by 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 et al. 1997; Ahn, Jo, Olson, and Nam 2000; Ahn et al. 1999; Ahn et al. 1998b). Jo and Ahn (1999) reported that the amount of volatiles released from oil emulsion correlated negatively with fat content.

Mechanisms related to the radiolysis of amino acids are not fully understood, but deamidation during irradiation is one of the main steps involved in amino acid radiolysis (Dogbevi, Vachon, and Lacroix 1999). The degradation of amino acids by oxidative deamination-decarboxylation via Strecker degradation produces branched chain aldehydes (Mottram, Wedzicha, and Dodson 2002), which may be the mechanism for the formation of 3-methyl butanal and 2-methyl butanal during irradiation from leucine and isoleucine, respectively (Jo and Ahn 2000). 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 alpha-carbon positions) and the side chain. Besides amino acids, fatty acids are also radiolyzed by irradiations. When triglycerides or fatty acids are irradiated, hydrocarbons are formed by cutting CO_2 and CH_3COOH off from fatty acids in various free-radical reactions. The yield of these radiolytically generated hydrocarbons was linear with absorbed dose (Morehouse, Kiesel, and Ku 1993). Radiolytic

degradation of fatty acid methyl ethers was affected by irradiation dose, irradiation temperature, oxygen pressure, and fatty acid components (Miyahara et al. 2002). Polyunsaturated fatty acids (PUFA) are more susceptible to radiolysis than monounsaturated or saturated fatty acids and irradiation caused a significant reduction in PUFA (Formanek, Lynch, Galvin, Farkas, and Kerry 2003). Jo, Lee, and Ahn (1999) showed that the content of 1-heptene and 1-nonene in volatiles was positively correlated to irradiation dose, and the production of alkenes and alkanes, the degradation products of fatty acids, also increased proportionally to irradiation dose (Du, Nam, and Ahn 2001).

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, Guadagni, and Ling 1973). The interactions among food components such as carbohydrates, lipids, and proteins (Godsall 1997), and the physicochemical conditions of foods, which influence conformation of proteins, also affect the release of volatile compounds in foods (Lubbers, Landy, and Voilley 1998). 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).

7.3.3 Color Changes in Meat by Irradiation

7.3.3.1 Color Changes in Irradiated Raw and Cooked Meat

The color of meat depends on the concentration and chemical status of heme pigments. Heme pigments are composed of heme ring and globin protein. The amino acid residues of globin are oriented so that their hydrophobic portion points inward and the only polar amino acids inside myoglobin are two histidines, which have a critical function at the heme-binding sites (Bandman 1987). The oxidation status of iron in the heme ring is very important because the ability of heme iron to coordinate with a sixth ligand, which is very important for color expression, is determined by the chemical states of heme iron. 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 (ferrous state; Judge, Aberle, Forrest, Hendrick, and Merkel 1989).

Although three common forms of myoglobin exist in different proportions, fresh meat color is imparted by mainly bright red oxymyoglobin and purple deoxymyoglobin (Ghorpade and Cornforth 1993). The color of fresh meat is determined by oxygen partial pressure, oxygen diffusion rate, and oxygen consumption rate at meat surface (Giddings 1977). Under normal conditions, enzymes use up all oxygen available and generate reducing conditions inside meat block. Thus, the pigments in the middle of meat block are usually in the reduced form and weakly bind with water molecules or are stabilized by distal histidine of globin (Lehninger 1982). The color of such pigment is purple and is called deoxymyoglobin or reduced myoglobin. 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 brown oxidized color can be turned into bright red color under air (blooming

or oxygenation) if the meat has strong enough reducing power or purple red under absolute 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; Nanke, Sebranek, and Olson 1999; Shahidi, Pegg, and Shamsuzzaman 1991). Millar, Moss, MacDougall, and Stevenson (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 oxygen-permeable film. Nam and Ahn (2002a) also reported that irradiation increased redness of both aerobically and vacuum-packaged raw turkey breast. The color changes were not localized in any specific area but evenly distributed over the whole meat sample. The increased redness was irradiation dose-dependent and was stable during the two-week storage periods in raw turkey meat. Jo, Jin, and Ahn (2000) found a significant increase in redness of cooked pork sausages after irradiation (Nam, Ahn, Du, and Jo 2001). Irradiation and subsequent storage of pork improved the red color even in PSE pork, indicating that irradiation can be used to increase the acceptability of low-quality pork (Nam, Du, Jo, and Ahn 2002).

Packaging environment is an important factor that influences the color of irradiated meat during storage. Irradiation increased the a-value of both aerobically and vacuum-packaged turkey breast and pork steaks, but vacuum-packaged meat was redder than aerobically packaged meat and was stable during storage (Grant and Patterson 1991; Luchsinger et al. 1986; Luchsinger et al. 1987; Nam and Ahn 2002b; Nanke, Sebranek, and Olson 1998; Nanke, Sebranek, and Olson 1999). During frozen storage, irradiation increased pink color in both aerobically and vacuum-packaged turkey breast, and the pink color was stable (Nam, Hur, Ismail, and Ahn 2002). 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, Thibault, Charbonneau, and Piette 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 2003d). The surface color of cooked meat was grayish brown regardless of irradiation, and the pink color inside of aerobically packaged cooked meat also changed to brown or yellow regardless of irradiation after storage because of pigment oxidation. Tappel (1957) noted that when precooked meat was irradiated, the normal gray-brown hematin pigments were converted to uncharacteristic red pigments. An objectionable red color in radiation-sterilized cooked chicken meat was found in the absence of oxygen (Hanson, Brushway, Pool, and Lineweaver 1963).

Irradiation of red meat changes the red color to brown or gray under aerobic conditions. In beef, color values were significantly influenced by the aging time. Immediately after irradiation, the color of ground beef changed from a bright red to a greenish brown, which would be unattractive beef color for consumers. Color L* value increased as the aging time of beef increased. During storage after irradiation, L* values of ground beef increase as the storage time increases, and the increase in

L* value was more apparent in meat from "long-term-aged" beef than other ones (Nam and Ahn 2003b).

7.3.3.2 Mechanism of Color Changes in Irradiated Meat

Irradiation produces ligand-forming compounds that can act as a sixth ligand of myoglobin. Ferrylmyoglobin can be formed from metmyoglobin due to the production of hydrogen peroxide and other radiolytic products of water by irradiation (Giddings and Markakis 1972). Thiols are particularly susceptible to attack by free radicals and hydrogen sulfide was produced when cysteine was irradiated (Swallow 1984). Green pigment was formed during gamma irradiation of meat because of hydrosulfide produced from glutathione or thiol-containing compounds (Fox and Ackerman 1968). When sulfhydryl group and peptide bonds were attacked by hydrated electrons, gas compounds such as hydrogen sulfide and ammonia were produced (Swallow 1984). Brown and Akoyunoglou (1964) proposed that gamma irradiation split small peptides from globin protein and induced deamination from myoglobin molecule. The brown color of cooked meat is partially converted to red by ionizing radiation. Satterlee, Wilhelm, and Barnhart (1971) suggested that the red pigment after irradiation could be formed by the loss of amide nitrogen from heme protein and the addition of the compound to heme iron. Irradiation might produce nitric oxide or other precursors to the cured meat pigment, nitrosyl hemochrome, particularly if nitrite or nitrate ions are present (Cornforth, Vahabzadeh, Carpenter, and Bartholomew 1986), and nitric oxide radical could be generated from nitrogen-containing amino acids side chain (e.g., arginine, glutamine) by an oxidative stress such as irradiation (Thomas 1999).

Tappel (1956) postulated a bright red color after gamma irradiating fresh meat in an inert atmosphere was oxymyoglobin formed by the reaction between metmyoglobin and hydroxyl radicals. Nanke, Sebranek, and Olson (1988, 1999) also proposed that the pigment in vacuum-packaged irradiated raw meat is an oxymyoglobin-like pigment. Giddings and Markakis (1972) proposed that oxymyoglobin-like pigment was formed by the reduction of heme iron by a radiolytic water product, hydrated electrons, and the oxygenation from either residual oxygen or generated oxygen during irradiation. However, it is very difficult to accept the pigment as an oxymyoglobin because the red color formed by irradiation has been produced mainly in anoxic conditions. Millar et al. (1995) postulated that the red or pink color in irradiated meat was due to a ferrous myoglobin derivative such as carboxy-myoglobin or nitric oxide-myoglobin other than oxymyoglobin. Nam and Ahn (2002a, 2002c) characterized the pink pigment formed in irradiated raw and cooked turkey breast as carbon monoxide-myoglobin (CO-Mb). They identified the pigment by comparing the absorption spectra of meat juice and myoglobin derivatives, and the reflectance spectra of meat surfaces. The absorption spectra of meat drip from irradiated turkey breast were similar absorption maxima to that of CO-Mb (absorption maxima at 541 and 577 nm) and concluded that CO-Mb was the major heme pigment responsible for the red or pink color in irradiated turkey breast. The reflectance of meat and the absorption spectra of myoglobin solution supported the conclusion that the CO-Mb was the pigment in irradiated precooked turkey breast

(Nam and Ahn 2002c). In cooked meat, both undenatured and denatured heme pigments in cooked meat may have been involved in heme-complex formations (with ligands available under the conditions), which is important for the pink color formation. In both aerobically and vacuum-packaged turkey breast, the a* values of turkey breast were positively correlated with the irradiation dose and the amount of CO gas produced (Nam and Ahn 2003d). 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. The formation of CO-Mb intensified the red color greatly.

Considerable amounts of carbon monoxide were produced by radiolysis of organic components such as alcohols, aldehydes, ketones, carboxylic acids, amides, and esters, and frozen meat and poultry (Furuta, Dohmaru, Katayama, Toratoni, and Takeda 1992; Nam and Ahn 2002a, 2002c; Woods and Pikaev 1994). Lee and Ahn (2004) reported that glycine, asparagine, glutamine, pyruvate, glyceraldehydes, α-ketoglutarate, and phospholipids were the major 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.

Hydrated electrons (aqueous e^-), a radiolytic radical, can act as a powerful reducing agent and react with ferricytochrome to produce ferrocytochrome (Swallow 1984). Irradiated meats need reducing conditions to maintain heme iron in ferrous state. Watts, Wolfe, and Brown (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 very important role in CO-Mb formation because the CO-Mb complex can only be formed when heme pigment is in reduced form (Cornforth et al. 1986). Nam and Ahn (2002a, 2002c) showed that irradiation lowered ORP of both aerobically and vacuum-packaged raw and cooked turkey breast meat. However, the ORP in irradiated meat increased rapidly during storage under aerobic conditions while it was maintained under vacuum-packaging conditions. The red pigments generated by irradiation were fairly stable against the increased oxidative environment stress during the storage time. 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, the storage of irradiated meat under aerobic conditions means CO-Mb receives a continuous challenge from oxygen to form Mb-O_2: Although the affinity of CO to Mb is 200-fold higher than that of oxygen (Stryer 1981), the concentration of oxygen in the atmosphere is much higher than that of CO. Continuous challenge of oxygen under aerobic conditions thus eventually replaces or removes all CO from heme pigments and reduces the intensity of pink color.

The mechanisms of color change in irradiated beef are different from those of light meats, and the proposed color-changing mechanism in irradiated beef is this: Irradiation produces aqueous electrons (e_{aq}-) and hydrogen radicals that have reducing power from water molecules (Thakur and Singh 1999). 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 ·H, respectively (Giddings 1977). Therefore, irradiation under aerobic conditions favors ferric Mb (brown color) but produces ferrous Mb (red color) under vacuum conditions. The content of heme pigments in beef is about 10 times greater than that of light meats and the proportion of CO-Mb, the compound responsible for color changes in irradiated light meats, to total heme pigments in irradiated beef is small because the amounts of CO produced in those meats are about the same. Thus, overall beef color is mainly determined by the status of heme pigments, which is determined by the reducing potential of meat. Irradiation of meat under vacuum conditions or addition of ascorbic acid to aerobically packaged meat creates reducing environments (Wheeler, Koohmaraie, and Shackelford 1996) and can prevent brown color development in ground beef.

7.3.4 Water Holding Capacity and Texture

Zhu, Mendonca, and Ahn (2004) found that irradiation significantly increased centrifugation loss of water from pork loins that 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 meat. The mechanism for irradiation-induced water loss could be caused by (a) the damage in the integrity of membrane structure of muscle fibers (Lakritz, Carroll, Jenkins, and Maerker 1987) and (b) denaturation of muscle proteins, which reduced water holding capacity (Lynch, Macfie, and Mead 1991) by irradiation. Transmission electron microscopy showed significant differences in size of myofibril units (sarcomeres) between irradiated and nonirradiated breasts. Shrinkage in sarcomere width (myofiber diameter) and disruption of myofibrils in irradiated breast meat were also noticed when compared with nonirradiated breast meat (Yoon 2003). Lewis, Velasquez, Cuppett, and McKee (2002) found that the texture attributes were lower in irradiated (1.0 kGy and 1.8 kGy) chicken breasts 14 and 28 days after irradiation. However, others reported that irradiation had minimal effects on texture of frozen, raw, and precooked ground beef patties; frozen boneless beef steaks; vacuum-packaged, chilled, boneless beef steaks (Luchsinger et al. 1997), and vacuum-packaged RTE turkey breast rolls (Zhu et al. 2004).

7.3.5 Consumer Attitude and Acceptance of Irradiated Meat

Consumers easily distinguished odor differences between nonirradiated and irradiated meat. Lynch et al. (1991) reported that a set of unpleasant odors was produced from irradiated turkey breast fillet and was different from nonirradiated samples. Consumers preferred the odor of aerobically packaged irradiated meats to vacuum-packaged meats. 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 (Lee, Love, and Ahn 2003).

Surveys (American Meat Institution Foundation 1993) showed that most supermarket shoppers believed that irradiated foods pose a health risk. Risk perception studies indicated that the public viewed food irradiation as moderately or highly risky. Frenzen et al. (2001) found that consumers' willingness to buy irradiated foods was associated with other factors, such as gender, education level, income, exposure to irradiated food products, and geographic location, whereas there was no difference in consumer acceptance by any risk factors of food-borne illness. The acceptance of irradiated food was also affected by consumers' knowledge about food irradiation (Bord and O'Connor 1989; Lusk, Fox, and McIlvain 1999; Nayga 1996). Market simulation studies showed that the proportion of consumers buying irradiated meat and poultry increased after the participants of study received additional information 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. Johnson, Reynolds, Chen, and Resurreccion (2004) surveyed and compared consumer attitudes toward irradiated food between 1993 and 2003, and found that more consumers were willing to buy irradiated products in 2003 than in 1993 (69% vs. 29%). Several reports indicated that positive attitudes toward irradiation are increasing (Bruhn 1995b; Resurreccion and Galvez 1999) and consumer education was very important for the acceptance of food irradiation (American Meat Institution Foundation 1993; Bruhn 1995a). However, the effects of positive and negative information about irradiation on consumer response were different: A favorable description of irradiation increased willingness to pay, and an unfavorable description decreased willingness to pay. When participants were given both positive and negative descriptions about irradiation, 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, Hayes, and Shogren 2002).

7.3.6 Control of Quality Changes

7.3.6.1 Additives

Schwarz et al. (1997) reported that pink color in cooked uncured ground turkey was successfully inhibited by the addition of 3% nonfat dry milk or metal chelators in the presence of pink generating ingredients (150 ppm nitrite and 1% nicotinamide). Others also reported that dairy proteins reduced a*-values in nicotinamide-treated samples (Slesinski et al. 2000a, 2000b). These authors found that whey protein concentrates at the 1.5% level was effective in reducing a*-value regardless of ligand treatment. Chelators added to meat have the potential to bind heme iron, particularly on unfolding or denaturation of the globin during heat processing.

Antioxidants added to nonirradiated fresh and further processed meat prevented oxidative rancidity, retarded development of off-flavors, and improved color stability (Morrissey, Brandon, Buckley, Sheehy, and Frigg 1997; Xiong, Decker, Robe, and Moody 1993). Huber et al. (1953) found that the use of antioxidants such as ascor-

bate, citrate, tocopherol, gallic esters, and polyphenols was effective in reducing the off-odor of irradiated meat. Antioxidants may be effective in controlling and reducing the discoloration of irradiated meat because they either produce reducing conditions or scavenge free radicals. Vitamin E functions as a lipid-soluble antioxidant and is capable of quenching free radicals in meat during storage (Gray, Gomaa, and Buckley 1996). Some phenolic compounds are believed to interrupt autoxidation of lipids either by donating hydrogen atoms or quenching free radicals. Therefore, addition of phenolic antioxidants may be effective in reducing the oxidative reactions in irradiated meat by scavenging free radicals produced by irradiation (Chen and Ahn 1998; Hsieh and Kinsella 1989; Nam and Ahn 2003c). Ascorbic acid and sesamol + tocopherol lowered the amounts of dimethyl disulfide in irradiated ground beef (Nam, Min, Park, Lee, and Ahn 2003).

The commercial use of natural antioxidants such as rosemary oleoresin by the meat industry is growing because of consumer demands for natural products. When rice hull extract treated by far-infrared was added to irradiated turkey breast, it was as effective in reducing volatile aldehydes and dimethyl disulfide as sesamol or rosemary oleoresin (Lee, Nam, Kim, and Ahn 2003). Dietary antioxidant treatments also have shown to stabilize lipids in membranes and reduce the extent of lipid oxidation in meat during storage (Morrissey et al. 1997; Wen, Morrissey, Buckley, and Sheehy 1996; Winne and Dirinck 1996). However, the antioxidant effects of dietary tocopherol in chicken meat differ among muscle types (Ahn et al. 1995).

Acid is commonly used as a preservative in further processed meat (Stivarius, Pohlman, McElyea, and Waldroup 2002). Incorporation of 0.3% citric acid to ground turkey reduced the pinkness of nicotinamide (1%)-treated and sodium nitrite (10 ppm)-treated cooked meat (Kieffer, Claus, and Wang 2000). Polyphosphates like sodium tripolyphosphate are excellent metal chelators and inhibitors against lipid oxidation. However, when added to raw meat, they are ineffective due to rapid hydrolysis to monophosphate by endogenous phosphatase enzymes (Lee, Hendricks, and Cornforth 1998). Food-grade oxidants were compared for prevention of undesirable raw appearance of cooked dark-cutting beef patties (Trout 1989). Lactic acid showed acceptable cooked appearance and increased myoglobin denaturation during cooking, but produced a tangy off-flavor.

The decrease of ORP in turkey breast by irradiation (Nam and Ahn 2002a, 2002c) suggested that irradiation was the source of solvated electrons. The solvated electrons attack the distal histidine of methemoglobin, which drives out the ligand at the sixth site to allow hemochrome formation via a covalent bond of the distal histidine to the iron atom. This process is accelerated when a substantial amount of hydroxide anion is present. Lowering pH, thus, was expected to decrease the amount of hydroxide anion present and decrease redness. However, acid (citric or ascorbic acid) did not affect the redness of irradiated turkey breast (Nam and Ahn 2002b).

Ascorbic acid incorporated to ground beef at the level of 0.1% (w/w) was very effective in maintaining redness of irradiated ground beef and the color stabilizing effect of ascorbic acid was more distinct in long-term-aged than in pre-aged irradiated ground beef (Nam et al. 2003). Satterlee et al. (1971) reported that the formation of red, MbO_2-like pigment formed from $MbFe^{3+}$ was greatest in a nitrogen atmosphere, slightly inhibited in air, and greatly inhibited in an oxygen atmosphere. Oxygen is

an effective scavenger of aqueous electrons (e_{aq}^-). Therefore, in the absence of oxygen, a reducing environment is established in the irradiated meat, which converts ferric myoglobin to ferrous form (Giddings and Markakis 1972). The addition of ascorbic acid with or without sesamol + tocopherol significantly lowered the ORP values of irradiated ground beef regardless of the age of meat. The lowered ORP values by ascorbic acid maintained heme pigments in ferrous status and stabilized the color of irradiated ground beef. On the other hand, sesamol + tocopherol had no effect in preventing color changes, and did not show any synergistic effect between ascorbic acid and sesamol + tocopherol in ground beef by irradiation (Nam and Ahn 2003b).

Addition of antimicrobial agents had synergistic effects with irradiation in killing micro-organisms in meat, and generally had positive effects on the quality of meat products: Injection of sodium lactate (SL) into vacuum-packaged beef top rounds resulted in higher cooking yields and darker, redder color with less gray surface area. Flavor notes associated with fresh beef were also enhanced by the addition of SL, and flavor deterioration during storage was minimized (Papadopoulos, Ringer, and Cross 1991). SL increased hardness, springiness, cohesiveness, chewiness, and resilience of turkey breast rolls (Zhu et al. 2004; Zhu et al. 2005), but resulted in more rapid surface discoloration in fresh pork sausage (Lamkey, Leak, Tuley, Johnson, and West 1991). Lactate/diacetate-enhanced chops maintained higher a* and b* values during display and had less visual discoloration, and more tender, juicier, and stronger pork flavor than controls (Jensen et al. 2003). Others reported that addition of antimicrobial agents such as lactate, acetate, sorbate, and benzoate salts had no effect on the texture, color, and sensory properties of meat products when used within regulatory limits (Bradford, Huffman, Egbert, and Jones 1993; Choi and Chan 2003; Sommers and Fan 2003). This suggests that combined use of antimicrobial agents with irradiation can improve the safety of meat products without significant impact on meat quality. The addition of potassium benzoate, however, greatly increased the content of benzene in the volatiles of irradiated RTE turkey ham and breast rolls. Therefore, caution is needed in using benzoate salt in products for irradiation (Zhu et al. 2004; Zhu et al. 2005).

7.3.6.2 Packaging

Packaging turned out to be the major factor influencing color and the amounts and types of volatiles detected in irradiated meat. Vacuum packaging prevented oxidative changes and color fading but retained S-volatiles such as methanethiol, dimethyl sulfide, dimethyl disulfide, and dimethyl trisulfide inside the packaging bag during storage, which reduced the odor acceptance of irradiated meat (Nam, Du, Jo, and Ahn 2002). Packaging with high-oxygen partial pressure can extend the shelf life of fresh meat color (Taylor and MacDougall 1973). 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, Nam, Du, and Jo 2001). At high-oxygen tension, oxymyoglobin can persist for several days before discoloration occurs. Vacuum-packaged meats have mainly purple deoxymyoglobin if the partial oxygen pressure reaches zero (Lawrie 1983). Failure to remove oxygen (to less than 1%)

completely, however, can result in oxidizing conditions associated with low partial oxygen pressure. The use of modified atmosphere packaging can discolor fresh meat because the inner gases such as carbon dioxide or nitrogen lower the pH or oxygen partial pressure and results in brown color (Seideman, Cross, Smith, and Durland 1984). Low pH also facilitates oxidation of myoglobin to metmyoglobin.

The impacts of irradiation on meat color are related to oxygen availability and the amount of free radicals formed at the time of irradiation. Nanke et al. (1999) reported that irradiated meat in aerobic packaging discolor more rapidly than non-irradiated samples during display. In general, vacuum packaging or controlled atmosphere packaging is a satisfactory measure in preventing color and rancidity problems in nonirradiated raw meat during storage. In irradiated meat, vacuum packaging was better than aerobic packaging in preventing lipid oxidation and oxidation-dependent volatile production, but increased pink color intensity during frozen storage (Nam, Hur, Ismail, and Ahn 2002; Nam, Kim, Du, and Ahn 2002). Aerobic packaging was more desirable for the irradiated meat color than vacuum packaging if lipid oxidation could be controlled (Ahn, Jo, Olson, and Nam 2000; Ahn et al. 2001). Exposing meat samples to aerobic conditions for a certain period of time was helpful in reducing irradiation off-color because of competition between atmospheric oxygen and carbon monoxide produced by irradiation (Nam and Ahn 2002b, 2003a). Exposing irradiated meats to aerobic conditions increased ORP and increased the competition of CO with O_2, which decreased the chances for CO-Mb ligand formation, and thus, pink color intensity (Nam and Ahn 2003a).

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 the storage, and it also was effective in reducing the generation of pink color in irradiated meat compared to vacuum packaging alone (Nam and Ahn 2003a, 2003d). 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 (2003a, 2003d) found that irradiation and aerobic packaging promoted the production of aldehydes (propanal and hexanal) related to lipid oxidation in turkey breast and thigh meats. The term *double packaging* is to describe 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. The a*-value of double-packaged meats was lower than that of the vacuum-packaged meats, but was not enough to reduce the pink color of irradiated raw turkey meat (Nam and Ahn 2002b, 2003a). Packaging condition was more critical in irradiated ground beef. The greenish-brown color was problematic when ground beef was irradiated under aerobic conditions, but anaerobic conditions protected the beef from discoloration. When vacuum-packaged irradiated beef was exposed to aerobic conditions in the middle of storage, the color bloomed to vivid fresh red color and was maintained during the remaining aerobic storage (Nam et al. 2005).

7.3.6.3 Packaging and Additive Combinations

Addition of antioxidant to irradiated meat was very effective in complementing problems of double packaging. Addition of sesamol + tocopherol (S + E) or gallate + tocopherol (G + E) combinations lowered the amount of propanal and total volatiles in double-packaged and irradiated raw turkey meat (Nam and Ahn 2003d). After 10 days of refrigerated storage, however, volatile profiles of irradiated turkey breast were highly dependent on antioxidant and packaging conditions. Sulfur volatiles were not detected in irradiated aerobically or double-packaged meat. However, aerobically packaged irradiated meat without antioxidants produced large amounts of aldehydes (propanal, hexanal) and 2-butanone at 10 days, which coincided with the degree of lipid oxidation (TBARS). Double-packaged meat had lower lipid oxidation products compared with aerobically packaged meat, but antioxidant combinations significantly reduced the amounts. Therefore, the combination of double-packaging (vacuum for 7 days, aerobic for 3 days) with antioxidants in irradiated raw turkey breast was very effective in reducing total and sulfur volatiles responsible for the irradiation off-odor without any problem in lipid oxidation.

The beneficial effects of double packaging and antioxidant combinations on volatiles were more clearly shown in irradiated cooked turkey breast (Nam and Ahn 2003d). Irradiated cooked turkey breast meat from double packaging and antioxidant combinations produced significantly lower a* values than the vacuum-packaged irradiated cooked meat. 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. However, the combination of antioxidant with double packaging was more effective in reducing both sulfur and lipid oxidation volatiles in irradiated cooked meat. The total amounts of sulfur volatiles in double-packaged irradiated turkey meat with antioxidants were only about 5% to 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 (vacuum then aerobic packaging) and ascorbic acid was also very effective in reducing off-odor volatiles and maintaining bright red color of irradiated ground beef (Nam et al. 2005). Both irradiating under vacuum conditions and adding reducing agent was helpful in maintaining low ORP of irradiated beef and caused myoglobin to remain in a reduced form.

7.4 FUTURE RESEARCH NEEDED

Most of the irradiation studies are done with raw meat because irradiation is not permitted for meats with additives, further processed, or precooked RTE meat products. Therefore, future studies should be focused on flavor, color, and taste changes in further processed and precooked RTE meat products by irradiation. Methods to prevent quality changes in irradiated further processed or precooked RTE meat products should also be developed. 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 or flavor changes in irradiated cooked meat is available. Therefore, research is needed to elucidate the causes and mechanisms of taste changes in irradiated cooked meat, determine the roles of spices and additives on taste or flavor of irradiated processed meat, and develop methods that can control taste or flavor changes in irradiated further processed meat. The effect of those additives on the microcidal efficiency of irradiation also should be determined.

REFERENCES

Ahn, D. U. 2002. Production of volatiles from amino acid homopolymers by irradiation. *J. Food Sci.* 67:2565–2570.

Ahn, D. U., C. Jo, and D. G. Olson. 2000. Analysis of volatile components and the sensory characteristics of irradiated raw pork. *Meat Sci.* 54:209–215.

Ahn, D. U., C. Jo, D. G. Olson, and K. C. Nam. 2000. Quality characteristics of pork patties irradiated and stored in different packaging and storage conditions. *Meat Sci.* 56:203–209.

Ahn, D. U., C. Kawamoto, F. H. Wolfe, and J. S. Sim. 1995. Dietary alpha-linolenic acid and mixed tocopherols, and packaging influences lipid stability in broiler chicken breast and leg muscle tissue. *J. Food Sci.* 60:1013–1018.

Ahn, D. U., and E. J. Lee. 2002. Production of off-odor volatiles from liposome-containing amino acid homopolymers by irradiation. *J. Food Sci.* 67:2659–2665.

Ahn, D. U., K. C. Nam, M. Du, and C. Jo. 2001. Effect of irradiation and packaging conditions after cooking on the formation of cholesterol and lipid oxidation products in meats during storage. *Meat Sci.* 57:413–418.

Ahn, D. U., D. G. Olson, C. Jo, X. Chen, C. Wu, J. I. Lee. 1998a. Effect of muscle type, packaging, and irradiation on lipid oxidation, volatile production and color in raw pork patties. *Meat Sci.* 49:27–39.

Ahn, D. U., D. G. Olson, J. I. Lee, C. Jo, C. Wu, X. Chen. 1998b. Packaging and irradiation effects on lipid oxidation and volatiles in pork patties. *J. Food Sci.* 63:15–19.

Ahn, D. U., D. G. Olson, C. Jo, J. Love, and S. K. Jin. 1999. Volatiles production and lipid oxidation of irradiated cooked sausage with different packaging during storage. *J. Food Sci.* 64:226–229.

Ahn, D.U., J. L. Sell, M. Jeffery, C. Jo, X. Chen, C. Wu, and J. I. Lee. 1997. Dietary vitamin E affects lipid oxidation and total volatiles of irradiated raw turkey meat. *J. Food Sci.* 62:954–959.

Ahn, D. U., F. H. Wolfe, J. S. Sim, and D. H. Kim. 1992. Packaging cooked turkey meat patties while hot reduces lipid oxidation. *J. Food Sci.* 57:1075–1077, 1115.

American Meat Institute Foundation. 1993. *Consumer awareness, knowledge, and acceptance of food irradiation*. Washington, DC: Author.

American Medical Association. 1993. *Irradiation of food* (Council on Scientific Affairs Report 4). Chicago: Author.

Angelini, P., C. Merritt, Jr., J. M. Mendelshon, and F. J. King. 1975. Effect of irradiation on volatile constituents of stored haddock flesh. *J. Food Sci.* 40:197–199.

Appleby, J., and A. J. Barks. 1905. British Patent No. 1609.

Bandman, E. 1987. Chemistry of animal tissues. In *The science of meat and meat products,* eds. J. F. Price and B. S. Schweigert, 61–101. Westport, CT: Food Nutrition Press.

Batzer, O. F., and D. M. Doty. 1955. Nature of undesirable odors formed by gamma irradiation of beef. *J. Agric. Food Chem.* 3:64.

Bhide, M. R., A. M. Paturkar, A. T. Sherikar, and V. S. Waskar. 2001. Presensitization of microorganisms by acid treatments to low dose gamma irradiation with special reference to *Bacillus cereus*. *Meat Sci.* 58:253–258.

Bord, R. J., and R. E. O'Connor. 1989. Who wants irradiated food? Untangling complex public opinion. *Food Technol.* 43:87–90.

Bradford, D. D., D. L. Huffman, W. R. Egbert, and W. R. Jones. 1993. Low-fat fresh pork sausage patty stability in refrigerated storage with potassium lactate. *J. Food Sci.* 58:488–491.

Brown, W. D., and J. A. Akoyunoglou. 1964. Gamma irradiation of purified myoglobin. *Arch. Biochem. Biophys.* 107:239–244.

Bruhn, C. M. 1995a. Consumer attitudes and market response to irradiated food. *J. Food Prot.* 58:175–181.

Bruhn, C. M. 1995b. Strategies for communicating the fact on food irradiation to consumers. *J. Food Prot.* 58:213–216.

Brynjolfsson, A. 1989. Future radiation sources and identification of irradiated foods. *Food Technol.* 43(7):84–89, 97.

Buchanan, R. L., S. G. Edelson, and G. Boyd. 1999. Effects of pH and acid resistance on the radiation resistance of enterohemorrhagic *Escherichia coli*. *J. Food Prot.* 62:219–228.

Buttery, R. G., D. G. Guadagni, and L. C. Ling. 1973. Flavor compounds: Volatilities in vegetable oil and oil-water mixtures: Estimation of odor thresholds. *J. Agric. Food Chem.* 21:198–201.

CAST. 1986. Ionizing energy in food processing and pest control: I. Wholesomeness of food treated with ionizing energy (Task Force Report No. 109). Ames, IA: Author.

CAST. 1989. Ionizing energy in food processing and pest control: II. Applications (Task Force Report No. 115). Ames, IA: Author.

Champaign, J. R., and W. W. Nawar. 1969. The volatile components of irradiated beef and pork fats. *J. Food Sci.* 34:335–340.

Chen, C. M., J. G. Sebranek, J. S. Dickson, and A. F. Mendonca. 2004. Combining Pediocin with postpackaging irradiation for control of *Listeria monocytogenes* on frankfurters. *J. Food Prot.* 67:1866–1875.

Chen, X., and D. U. Ahn. 1998. Antioxidant activities of six phenolics from natural sources against lipid oxidation induced by Fe^{2+} or ultraviolet light (UV). *J. Am. Oil Chem. Soc.* 75:1717–1721.

Choi, S. H., and K. B. Chin. 2003. Evaluation of sodium lactate as a replacement for conventional chemical preservatives in comminuted sausages inoculated with *Listeria monocytogenes*. *Meat Sci.* 65:531–537.

Clavero, M. R., J. D. Monk, L. R. Beuchat, M. P. Doyle, R. E. Brackett. 1994. Inactivation of *Escherichia coli* O157:H7, salmonellae, and *Campylobacter jejuni* in raw ground beef by gamma irradiation. *Appl. Environ. Microbiol.* 60:2069–2075.

Cornforth, D. P., F. Vahabzadeh, C. E. Carpenter, and D. T. Bartholomew. 1986. Role of reduced hemochromes in pink color defect of cooked turkey rolls. *J. Food Sci.* 51:1132–1135.

Davies, M. J. 1996. Protein and peptide alkoxyl radicals can give rise to C-terminal decarboxylation and backbone cleavage. *Arch. Biochem. Biophys.* 336:163–172.

Diehl, J. F. 1995. *Safety of irradiated foods* (2nd ed.). New York: Marcel Dekker.

Dogbevi, M. K., C. Vachon, and M. Lacroix. 1999. Physicochemical and microbiological changes in irradiated fresh pork loins. *Meat Sci.* 51:349–354.

Dragnic, I. G., and Z. O. Dragnic. 1963. The radiation chemistry of water. New York: Academic Press.

Du, M., K. C. Nam, and D. U. Ahn. 2001. Cholesterol and lipid oxidation products in cooked meat as affected by raw-meat packaging and irradiation and by cooked-meat packaging and storage time. J. Food Sci. 66:1396–1401.

Efiok, B. S. 1996. Basic calculations for biomedical and biological analyses. Gaithersburg, MD: AOAC International.

Ehioba, R. M., A. A. Kraft, R. A. Molins, H. W. Walker, D. G. Olson, G. Subbaraman, and R. P. Skowronski. 1988. Identification of microbial isolates from vacuum-packaged ground pork irradiated at 1 kGy. J. Food Sci. 53:278–279, 281.

El-Bisi, H. M., O. P. Snyder, and R. E. Levin. 1966. Radiation death kinetics of *C. botulinum* spores at cryogenic temperatures. In *Botulism: Proceedings of the 5th International Symposium on Food Microbiology,* eds. M. Ingram and T. A. Roberts, 89–107. London: Chapman & Hall.

El-Shenawy, M. A., A. E. Yousef, and E. H. Marth. 1989. Inactivation and injury of *Listeria monocytogenes* in tryptic soy broth or ground beef treated with gamma irradiation. *Lebensm. Wiss. Technol.* 22:387–390.

Fan, X., C. H. Sommers, D. W. Thayer, and S. J. Lehotay. 2002. Volatile sulfur compounds in irradiated precooked turkey breast analyzed with pulsed flame photometric detection. *J. Agric. Food Chem.* 50:4257–4261.

Farkas, J. 1987. Decontamination, including parasite control, of dried, chilled and frozen foods by irradiation. *Acta Aliment.* 16:351–358.

Farkas, J., and E. Andrassy. 1993. Interaction of ionizing radiation and acidulants on the growth of the microflora of a vacuum-packaged chilled meat. *Int. J. Food Microbiol.* 19:145–152.

Federal Register. 1999. Irradiation of meat and meat products. *Federal Register.* 64:9089–9105.

Formanek, Z., A. Lynch, K. Galvin, J. Farkas, and J. P. Kerry. 2003. Combined effects of irradiation and the use of natural antioxidants on the shelf-life stability of overwrapped minced beef. *Meat Sci.* 63:433–440.

Fox, J. A., D. J. Hayes, and J. F. Shogren. 2002. Consumer preferences for food irradiation: How favorable and unfavorable descriptions affect preferences for irradiated pork in experimental auctions. *J. Risk Uncertainty,* 24:75–95.

Fox, J. B., and S. A. Ackerman. 1968. Formation of nitric oxide myoglobin: Mechanisms of the reaction with various reductants. *J. Food Sci.* 33:364–370.

Frenzen, P. D., E. E. DeBess, K. E. Hechemy, H. Kassenborg, M. Kennedy, K. McCombs, and A. McNees. 2001. Consumer acceptance of irradiated meat and poultry in the United States. *J. Food Prot.* 64:2020–2026.

Fu, A. H., J. G. Sebranek, and E. A. Murano. 1995. Survival of *Listeria monocytogenes*, *Yersinia enterocolitica*, and *Escherichia coli* O157:H7 and quality changes after irradiation of beef steak and ground beef. *J. Food Sci.* 60:972–977.

Furuta, M., T. Dohmaru, T. Katayama, H. Toratoni, and A. Takeda. 1992. Detection of irradiated frozen meat and poultry using carbon monoxide gas as a probe. *J. Agric. Food Chem.* 40:1099–1100.

Ghorpade, V. M., and D. P. Cornforth. 1993. Spectra of pigments responsible pink color in pork roasts cooked to 65 or 82°C. *J. Food Sci.* 58:51–52, 89.

Giddings, G. G. 1977. Symposium: The basis of quality in muscle foods, the basis of color in muscle foods. *J. Food Sci.* 42:288–294.

Giddings, G.G., and P. Markakis. 1972. Characterization of the red pigments produced from ferrimyoglobin by ionizing radiation. *J. Food Sci.* 37:361–364.

Godshall, M. A. 1997. How carbohydrate influence flavor. *Food Technol.* 51:63–67.

Grant, I. R., and M. F. Patterson. 1991. Effect of irradiation and modified atmosphere packaging on the microbiolical safety of minced pork stored under temperature abuse conditions. *Int. J. Food Sci. Technol.* 26:507–519.

Gray, J. I., E. A. Gomaa, and D. J. Buckley. 1996. Oxidative quality and shelf life of meats. *Meat Sci.* 43:S111–S123.

Halliwell, B. J. M., and C. Gutteridge. A consideration of atomic structure and bonding. In *Free radicals in biology and medicine* (2nd ed.), eds. B. Halliwelland J. M. C. Gutteridge, 508–524. London: Clarendon.

Hanson, H. L. M. J. Brushway, M. P. Pool, and H. Lineweaver. 1963. Factors causing color and texture differences in radiation-sterilized chicken. *Food Technol.* 17:1188–1194.

Hashim, I. B., A. V. A. Resurreccion, and K. H. MaWatters. 1995. Disruptive sensory analysis of irradiated frozen or refrigerated chicken. *J. Food Sci.* 60:664–666.

Hastings, J. W., W. H. Holzapfel, and J. G. Niemand. 1986. Radiation resistance of lactobacilli isolated from radurized meat relative to growth and environment. *Appl. Environ. Microbiol.* 52:898–901.

Hayashi, T. 1991. Comparative effectiveness of gamma rays and electron beams in food irradiation. In *Food irradiation,* ed. S. Thorne, 168–206. London: Elsevier Applied Science.

Heath, J. L., S. L. Owens, S. Tesch, and K. W. Hannah. 1990. Effect of high-energy electron irradiation of chicken on thiobarbituric acid values, shear values, odor, and cook yield. *Poult. Sci.* 69:313–319.

Henkel, J. 1998. Irradiation: A safe measure for safer food. *FDA Consumer,* May/June.

Hollaender, H., G. E. Stapleton, and F. L. Martin. 1951. X-ray sensitivity of *E. coli* as modified by oxygen tension. *Nature.* 167:103–104.

Hsieh, R. J., and J. E. Kinsella. 1989. Oxidation of polyunsaturated fatty acids: Mechanisms, products, and inhibition with emphasis on fish. *Adv. Food Nutr. Res.* 33:233–237.

Huber, W., A. Brasch, and A. Waly. 1953. Effect of processing conditions on organoleptic changes in foodstuffs sterilized with high intensity electrons. *Food Technol.* 7:109–115.

IAEA. 1991. *Facts about food irradiation.* Vienna, Austria: International Consultative Group on Food Irradiation, IAEA.

IAEA. 1999. *Facts about food irradiation.* Accessed July 8, 1999 at http://www.iaea.or.at/worldatom/inforesource/other/food. Vienna, Austria: Author.

Jarrett, R. D. 1982. Isotope (gamma) radiation sources. In *Preservation of food by ionizing radiation* (Vol. 1), eds. E. S. Josephson and M. S. Peterson, 137–163. Boca Raton, FL, CRC Press.

Jensen, J. M., K. L. Robbins, K. J. Ryan, C. Homco-Ryan, F. K. McKeith, and M. S. Brewer. 2003. Effects of lactic and acetic acid salts on quality characteristics of enhanced pork during retail display. *Meat Sci.* 63:501–508.

Jo, C., and D. U. Ahn. 1999. Fat reduces volatiles production in oil emulsion system analyzed by purge-and-trap dynamic headspace/gas chromatography. *J. Food Sci.* 64:641–643.

Jo, C., and D. U. Ahn. 2000. Production of volatiles from irradiated oil emulsion systems prepared with amino acids and lipids. *J. Food Sci.* 65:612–616.

Jo, C., S. K. Jin, and D. U. Ahn. 2000. Color changes in irradiated cooked pork sausage with different fat sources and packaging during storage. *Meat Sci.* 55:107–113.

Jo, C., J. I. Lee, and D. U. Ahn. 1999. Lipid oxidation, color changes and volatiles production in irradiated pork sausage with different fat content and packaging during storage. *Meat Sci.* 51:355–361.

Johnson, A. M., A. E. Reynolds, J. Chen, and A. V. A. Resurreccion. 2004. Consumer attitudes towards irradiated food: 2003 vs. 1993. *Food Prot. Trends.* 24:408–418

Josephson, E. S., and M. S. Peterson. 2000. *Preservation of food by ionizing radiation* (II). Boca Raton, FL; CRC Press.

Judge, M. D., E. D. Aberle, J. C. Forrest, H. B. Hendrick, and R. A. Merkel. 1989. Properties of fresh meat. In *Principles of meat science.* eds. E. D. Aberle, J. C. Forrest, D. E. Gerrard and E. W. Mills, 109–116. Dubuque, IA: Kendall/Hunt.

Kanatt, S.R., S. P. Chawla, R. Chander, and D. R. Bongirwar. 2002. Shelf-stable and safe intermediate-moisture meat products using hurdle technology. *J. Food Prot.* 65:1628–1631.

Karel, M. 1989. The future of irradiation applications on earth and in space. *Food Technol.* 41(7):95–97.

Kawakishi, S., J. Okumura, and M. Namki. 1971. Gamma radiolysis of carbohydrate in aqueous solution. *Food Irrad.* 6:80–86.

Kieffer, K. J., J. R. Claus, and H. A. Wang. 2000. Research note: Inhibition of pink color development in cooked, uncured ground turkey by the addition of citric acid. *J. Muscle Food,* 11:235–243.

Kim, A. Y., and D. W. Thayer. 1996. Mechanism by which irradiation increases the sensitivity of *Salmonella Typhimurium* ATCC 14028 to heat. *Appl. Environ. Microbiol.* 62:1759–1763.

Kim, B. H., A. Jang, S. O. Lee, J. S. Min, and M. Lee. 2004. Combined effect of electron-beam (beta) irradiation and organic acids on shelf-life of pork loins during cold storage. *J. Food Prot.* 67:168–171.

Lagunas-Solar, M. C. 1995. Radiation processing of food: An overview of scientific principles and current status. *J. Food Prot.* 58:186–192.

Lakritz, L., R. J. Carroll, R. K. Jenkins, and G. Maerker. 1987. Immediate effects of ionizing-radiation on the structure of unfrozen bovine muscle-tissue. *Meat Sci.* 20:107–117.

Lambert, A. D., J. P. Smith, and K. L. Dodds. 1992. Physical, chemical, and sensory changes in irradiated fresh pork packaged in modified atmosphere. *J. Food Sci.* 57:1294–1299.

Lamkey, J. W., F. W. Leak, W. B. Tuley, D. D. Johnson, and R. L. West. 1991. Assessment of sodium lactate addition to fresh pork sausage. *J. Food Sci.* 56:220–223.

Lawrie, R. A. 1983. Review: Aspects of the biochemistry of meat. *Int. J. Biochem.* 15:233–242.

Lee, B. J., D. G. Hendricks, and D. P. Cornforth. 1998. Effect of sodium phytate, sodium pyrophosphate, and sodium tripolyphosphate on physico-chemical characteristics of restructured beef. *Meat Sci.* 50:273–283.

Lee, E. J., and D.U. Ahn. 2003. Production of off-odor volatiles from fatty acids and oils by irradiation. *J. Food Sci.* 68:70–75.

Lee, E. J., and D. U. Ahn. 2004. Sources and mechanisms of carbon monoxide production by irradiation. *J. Food Sci.* 69:485–490.

Lee, E. J., J. Love, and D. U. Ahn. 2003. Effect of antioxidants on the consumer acceptance of irradiated turkey meat. *J. Food Sci.* 68:1659–1663.

Lee, M., J. G. Sebranek, J. G. Olson, and J. S. Dickson. 1996. Irradiation and packaging of fresh meat and poultry. *J. Food Prot.* 59:62–72.

Lee, S. C., K. C. Nam, J. H. Kim, and D. U. Ahn. (2003). Antioxident properties of far infrared-treated rice hull extracts in irradiated raw and cooked turkey breast. *J. Food Sci.* 68(6):1904–1909.

Lefebvre, N., C. Thibault, R. Charbonneau, and J. P. G. Piette. 1994. Improvement of shelf-life and wholesomeness of ground beef by irradiation 2: Chemical analysis and sensory evaluation. *Meat Sci.* 36:371–380.

Lehninger, A. L. 1982. Globular proteins: The structure and function of hemoglobin. In Principles of biochemistry, eds. D. L. Nelson and M. M. Cox, 169–206. New York: Worth Publishers.

Lewis, S. J., A. Velasquez, S. L. Cuppett, and S. R. McKee. 2002. Effect of electron beam irradiation on poultry meat safety and quality. *Poult. Sci.* 81:896–903.

Lieber, H. 1905. U.S. Patent 788480.

Loaharanu, P. 1994. Status and prospects of food irradiation. *Food Technol.* 48(5):124–130.

Lopez-Gonzalez, V., P. S. Murano, R. E. Brennan, and E. A. Murano. 1999. Influence of various commercial packaging conditions on survival of *Escherichia coli* O157:H7 to irradiation by electron beam versus gamma rays. *J. Food Prot.* 62:10–15.

Lubbers, S., P. Landy, and A. Voilley. 1998. Retention and release of aroma compounds in foods containing proteins. *Food Technol.* 52:68–74, 208–214.

Luchsinger, S. E., D. H. Kropf, C. M. Garcia-Zepeda, M. C. Hunt, J. L. Marsden, E. J. Rubio-Canas, C. L. Kastner, W. G. Kuecker, and T. Mata. 1996. Color and oxidative rancidity of gamma and electron beam irradiated boneless pork chops. *J. Food Sci.* 61:1000–1005, 1093.

Luchsinger, S. E., D. H. Kropf, C. Garcia-Zepeda, M. C. Hunt, S. L. Stroda, J. L. Marsden, and C. L. Kastner. 1997. Color and oxidative properties of irradiated ground beef patties. *J. Muscle Foods.* 8:445–464.

Lusk, J. L., J. A. Fox, and C. L. McIlvain. 1999. Consumer acceptance of irradiated meat. *Food Technol.* 53:56–59.

Lynch, J. A., H. J. H. Macfie, and G. C. Mead. 1991. Effect of irradiation and packaging type on sensory quality of chill-stored turkey breast fillets. *Int. J. Food Sci. Technol.* 26:653–668.

Mason, J. 1992. Food irradiation: Promising technology for public health. Public Health Rep. 107:489–490.

Matsuyama, A. T., M. J. Thornley, and M. Ingram. 1964. The effect of freezing on the radiation sensitivity of vegetative bacteria. *J. Appl. Bacteriol.* 27:110–124.

Mendonca, A. F., M. G. Romero, M. A. Lihono, R. Nannapaneni, and M. G. Johnson. 2004. Radiation resistance and virulence of *Listeria monocytogenes* following starvation in physiological saline. *J. Food Prot.* 67:470–474.

Merritt, C., Jr., P. Angelini, and R. A. Graham. 1978. Effect of radiation parameters on the formation of radiolysis products in meat and meat substances. *J. Agric. Food Chem.* 26:29–36.

Merritt, C., Jr., P. Angelini, E. Wierbicki, and G. W. Shuts. 1975. Chemical changes associated with flavor in irradiated meat. *J. Agric. Food Chem.* 23:1037–1043.

Millar, S. J., B. W. Moss, D. B. MacDougall, and M. H. Stevenson. 1995. The effect of ionizing radiation on the CIELAB color co-ordinates of chicken breast meat as measured by different instruments. *Inter. J. Food Sci. Technol.* 30:663–674.

Minsch, F. 1896. *Münch Med. Wochensch,* 5:101, and 9, 202.

Miyahara, M., A. Saito, T. Kamimura, T. Nagasawa, H. Ito, and M. Toyoda. 2002. Hydrocarbon productions in hexane solutions of fatty acid methyl esters irradiated with gamma rays. *J. Health Sci.* 48:418–426.

Monk, J. D., L. R. Beuchat, and M. P. Doyle. 1995. Irradiation inactivation of food-borne microorganisms. *J. Food Prot.* 58:197–208.

Monk, J. D., R. S. Clavero, L. R. Beuchat, M. P. Doyle, and R. E. Brackett. 1994. Irradiation inactivation of *Listeria monocytogenes* and *Staphylococcus aureus* in low and high fat frozen and refrigerated ground beef. *J. Food Prot.* 51:969–974.

Morehouse, K. M., M. Kiesel, and Y. Ku. 1993. Identification of meat treated with ionizing radiation by capillary gas-chromatographic determination of radiolytically produced hydrocarbons. *J. Agric. Food Chem.* 41:758–763.

Morrissey, P. A., S. Brandon, D. J. Buckley, P. J. A. Sheehy, and J. Frigg. 1997. Tissue content of a-tocopherol and oxidative stability of broilers receiving dietary -tocopheryl acetate supplementation for various periods pre-slaughter. *Brit. Poult. Sci.* 38:84–88.

Mottram, D. S., B. L. Wedzicha, and A. T. Dodson. 2002. Acrylamide is formed in the Maillard reaction. *Nature.* 419:448–449.

Murano, E. A. 1995. Irradiation of fresh meats. *Food Technol.* 49(12):52–54.

Nam, K. C., and D. U. Ahn. 2002a. Carbon monoxide-heme pigment complexes are responsible for the pink color in irradiated raw turkey breast meat. *Meat Sci.* 60:25–33.

Nam, K. C., and D. U. Ahn. 2002b. Effect of double-packaging and acid combination on the quality of irradiated raw turkey patties. *J. Food Sci.* 67:3252–3257.

Nam, K. C., and D. U. Ahn. 2002c. Mechanisms of pink color formation in irradiated precooked turkey breast. *J. Food Sci.* 67:600–607.

Nam, K. C., and D. U. Ahn. 2003a. Combination of aerobic and vacuum packaging to control color, lipid oxidation and off-odor volatiles of irradiated raw turkey breast. *Meat Sci.* 63:389–395.

Nam, K. C., and D. U. Ahn. 2003b. Effects of ascorbic acid and antioxidants on the color of irradiated beef patties. *J. Food Sci.* 68:1686–1690.

Nam, K. C., and D. U. Ahn. 2003c. Use of antioxidants to reduce lipid oxidation and off-odor volatiles of irradiated pork homogenates and patties. *Meat Sci.* 63:1–8.

Nam, K. C., and D. U. Ahn. 2003d. Use of double-packaging and antioxidant combinations to improve color, lipid oxidation, and volatiles of irradiated raw and cooked turkey breast patties. *Poult. Sci.* 82:850–857.

Nam, K. C., D. U. Ahn, M. Du, and C. Jo. 2001. Lipid oxidation, color, volatiles, and sensory characteristics of aerobically packaged and irradiated pork with different ultimate pH. *J. Food Sci.* 66:1225–1229.

Nam, K. C., M. Du, C. Jo, and D. U. Ahn. 2002. Quality characteristics of vacuum-packaged, irradiated normal, PSE, and DFD Pork. *Innov. Food Sci. Emerg. Technol.* 3:73–79.

Nam, K. C., S. J. Hur, H. Ismail, and D. U. Ahn. 2002. Lipid oxidation, volatiles, and color changes in irradiated raw turkey breast during frozen storage. *J. Food Sci.* 67:2061–2066.

Nam, K. C., Y. H. Kim, M. Du, and D. U. Ahn. 2002. Off-odor and pink color development in precooked, ionizing-radiated turkey breast during frozen storage. *Poult. Sci.* 81:269–275.

Nam, K. C., B. R. Min, K. S. Park, S. C. Lee, and D. U. Ahn. 2003. Effects of ascorbic acid and antioxidants on the lipid oxidation and volatiles of irradiated beef patties. *J. Food Sci.* 68:1680–1685.

Nanke, K. E., J. G. Sebranek, and D. G. Olson. 1998. Color characteristics of irradiated vacuum-packaged pork, beef, and turkey. *J. Food Sci.* 63:1001–1006.

Nanke K. E., J. G. Sebranek, and D. G. Olson. 1999. Color characteristics of irradiated aerobically packaged pork, beef, and turkey. *J. Food Sci.* 64:272–276.

Nawar, W. W. 1986. Volatiles from food irradiation. *Food Rev. Int.* 2:45–78.

Nayga, R. M. 1996. Sociodemographic influences on consumer concern for food safety: The case of irradiation, antibiotics, hormones, and pesticides. *Rev. Agric. Econ.* 3:467–475.

North American Plant Protection Organization (NAPPO). Proceedings of the North American Plant Protection Organization Annual Meeting Colloquium on the Application of Irradiation Technology as a Quarantine Treatment. Nepean, Ontario, Canada.

Olson, D. G. 1998a. Irradiation of food. *Food Technol*. 52:56–62.

Olson, D. G. 1998b. Irradiation processing. In *Food irradiation: A sourcebook. Meat and poultry irradiation short course,* ed. E. Murano, 3–27. Ames, IA: Iowa State University Press.

Papadopoulos, L. S., L. J. Ringer, and H. R. Cross. 1991. Sodium lactate effect on sensory characteristics, cooked meat color and chemical composition. *J. Food Sci*. 56:621–626, 635.

Patterson, M. 1988. Sensitivity of bacteria to irradiation on poultry meat under various atmospheres. *Lett. Appl. Microbiol*. 7:55–58.

Patterson, M. F. 1989. Sensitivity of *Listeria monocytogenes* to irradiation on poultry meat and in phosphate-buffered saline. *Lett. Appl. Microbiol*. 8:181–184.

Patterson, R. L., and M. H. Stevenson. 1995. Irradiation-induced off-odor in chicken and its possible control. *Brit. Poult. Sci*. 36:425–441.

Proctor, B. E., and S. A. Goldblith. Food processing with ionizing radiations. *Food Technol*. 5:376.

Resurreccion, A.V. A., and F. C. F. Galvez. 1999. Will consumers buy irradiated beef? *Food Technol*. 53(3):52–55.

Satin, M. 1993. *Food irradiation: A guidebook*. Lancaster, PA: Technomic Publishing.

Satin, M. 2002. Use of irradiation for microbial decontamination of meat: Situation and perspectives. *Meat Sci*. 62:277–283.

Satterlee, L. D., M. S. Wilhelm, and H. M. Barnhart. 1971. Low dose gamma irradiation of bovine metmyoglobin. *J. Food Sci*. 36:549–551.

Schwartz, B. 1921. Effect of X-rays on *Trichinae*. *J. Agric. Res*. 20:845–849.

Schwarz, S. J., J. R. Claus, H. Wang, N. G. Marriott, P. P. Graham, and C. F. Fernandes. 1997. Inhibition of pink color development in cooked, uncured ground turkey through the binding of non-pink generating ligands to muscle pigments. *Poult. Sci*. 76:1450–1456.

Seideman, S. C., H. R. Cross, G. C. Smith, and P. R. Durland. 1984. Factors associated with fresh meat color: A review. *J. Food Qual*. 6:211–237.

Shahidi, F., and R. B. Pegg. Hexanal as an indicator of the flavor deterioration of meat and meat products. In *Lipids in food flavors*, eds. C.-T. Ho and T. G. Hartman (ACS Sym. Ser. 558), 256–279. Washington, DC: American Chemical Society.

Shahidi, F., R. B. Pegg, and K. Shamsuzzaman. 1991. Color and oxidative stability of nitrite-free cured meat after gamma irradiation. *J. Food Sci*. 56:1450–1452.

Skala, J. H., E. L. McGown, and P. P. Waring. 1987. Wholesomeness of irradiated foods. *J. Food Prot. 50*(2):150–160.

Slesinski, A. J., J. R. Claus, C. M. Anderson-Cook, W. E. Eigel, P. P. Graham, G. E. Lenz, and R. B. Noble. 2000a. Ability of various dairy proteins to reduce pink color development in cooked ground turkey breast. *J. Food Sci*. 65:417–420.

Slesinski, A. J., J. R. Claus, C. M. Anderson-Cook, W. E. Eigel, P. P. Graham, G. E. Lenz, and R. B. Noble. 2000b. Response surface methodology for reduction of pinking in cooked turkey breast mince by various dairy protein combinations. *J. Food Sci*. 65:421–427.

Sommers C. H. 2004. Food irradiation is already here. *Food Technol. 58*(11):22.

Sommers, C. H., and X. Fan. 2002. Antioxidant power, lipid oxidation, color, and viability of *Listeria monocytogenes* in beef bologna treated with gamma radiation and containing various levels of glucose. *J. Food Prot*. 65:1750–1755.

Sommers, C., and X. Fan. 2003. Gamma irradiation of fine-emulsion sausage containing sodium diacetate. *J. Food Prot.* 66:819–824.

Sommers, C. H., X. Fan, P. A. Handel, and K. B. Sokorai. 2003. Effect of citric acid on the radiation resistance of *Listeria monocytogenes* and frankfurter quality factors. *Meat Sci.* 63:407–415.

Sommers, C. H., M. Kozempel, X. Fan, and R. E. Radewonuk. 2002. Use of vacuum-steam-vacuum and ionizing radiation to eliminate *Listeria* innocua from ham. *J. Food Prot.* 65:1981–1983.

Steccheni, M. L., M. Del Torre, P. G. Sarais, F. Fuochi, F. Tubaro, and F. Ursini. 1998. Carnosine increases the radiation resistance of *Aeromonas hydrophila* in minced turkey meat. *J. Food Sci.* 61:979–987.

Stivarius, M.R., F. W. Pohlman, K. S. McElyea, and A. L. Waldroup. 2002. Effects of hot water and lactic acid treatment of beef trimmings prior to grinding on microbial, instrumental color and sensory properties of ground beef during display. *Meat Sci.* 60:327–334.

Stryer, L. 1981. *Biochemistry.* New York: Freeman.

Swallow, A. J. 1984. Fundamental radiation chemistry of food components. In *Recent advances in the chemistry of meat,* ed. A. J. Bailey, 165–177. London: The Royal Society of Chemists..

Tappel, A. L. 1956. Regeneration and stability of oxymyoglobin in some gamma irradiated meats. *Food Res.* 21:650–654.

Tappel, A. L. 1957. The red pigment of precooked irradiated meats. *Food Res.* 22:408–412.

Tarte, R. 1996. Sensitivity of Listeria to irradiation in raw ground meat, as affected by type of radiation, product temperature, packaging atmosphere, and recovery medium. Doctoral dissertation Ames, IO: Iowa State University.

Taub, I. A., R. A. Karielian, and J. W. Halliday. 1978. Radiation chemistry of high protein food irradiated at low temperature. In *Food preservation by irradiation,* 371–384. Vienna, Austria: International Atomic Energy Agency.

Taub, I. A., R. A. Karielian, J. W. Halliday, J. E. Walker, P. Angeline, and C. Merritt. 1979. Factors affecting radiolytic effects of food. *Rad. Phys. Chem.* 14:639–653.

Taylor, A. A., and D. B. MacDougall. 1973. Fresh beef packed in mixtures of oxygen and carbon dioxide. *J. Food Technol.* 8:453–461.

Thakur, B. R., and R. K. Singh. 1994. Food irradiation: Chemistry and applications. *Food Rev. Int.* 10:437–473.

Thayer, D. W. 1993. Extending the shelf-life of poultry and red meat by irradiation processing. *J. Food Prot.* 56:831–833, 846.

Thayer, D. W. 1995. Use of irradiation to kill enteric pathogens on meat and poultry. *J Food Safety.* 15:181–192.

Thayer, D. W., and G. Boyd. 1991a. Effect of ionizing radiation dose, temperature, and atmosphere on the survival of *Salmonella typhimurium* in sterile mechanically deboned chicken meat. *Poult. Sci.* 70:381–388.

Thayer, D. W., and G. Boyd. 1991b. Survival of *Salmonella typhimurium* ATCC 14028 on the surface of chicken legs or in mechanically deboned chicken meat gamma irradiated in air or vacuum at temperatures of –20 to +20 C. *Poult. Sci.* 70:1026–1033.

Thayer, D. W., and G. Boyd. 1992. Gamma ray processing to destroy *Staphylococcus aureus* in mechanically deboned chicken meat. *J. Food Sci.* 57:848–851.

Thayer, D. W., and G. Boyd. 1993. Elimination of *Escherichia coli* O157 in meats by gamma irradiation. *Appl. Environ. Microbiol.* 59:1030–1034.

Thayer, D. W., and G. Boyd. 1994. Control of enterotoxic *Bacillus cereus* on poultry or red meats and in beef gravy by gamma irradiation. *J. Food Prot.* 57:758–764.

Thayer, D. W., and G. Boyd. 1995. Radiation sensitivity of *Listeria monocytogenes* on beef as affected by temperature. *J. Food Sci.* 60:237–240.

Thayer, D. W., and G. Boyd. 1999. Irradiation and modified atmosphere packaging for the control of *Listeria monocytogenes* on turkey meat. *J. Food Prot.* 62:1136–1142.

Thayer, D. W., G. Boyd, and R. K. Jenkins. 1993. Low-dose gamma irradiation and refrigerated storage in vacuo affect microbial flora of fresh pork. *J. Food Sci.* 58:717–719, 733.

Thayer, D. W., G. Boyd, J. B. Fox, Jr., L. Lakritz, and J. W. Hampson. Variations in radiation sensitivity of foodborne pathogens associated with the suspending meat. *J. Food Sci.* 60:63–67.

Thayer, D. W., R. V. Lachica, C. N. Huhtanen, and E. Wierbicki. 1986. Use of irradiation to ensure the microbiological safety of processed meats. *Food Technol. 40*(4):159–162.

Thomas, J. A. 1999. Oxidative stress, oxidant defense, and dietary constituents. In *Modern nutrition in health and disease,* eds. M. E. Shils, J. A. Olson, M. Shike, and A. C. Ross, 751–760. Philadelphia: Lea & Febiger.

Trout, G. R. 1989. Variation in myoglobin denaturation and color of cooked beef, pork, and turkey meat as influenced by pH, sodium chloride, sodium tripolyphosphate, and cooking temperature. *J. Food Sci.* 54:536–540, 544.

Urbain, W. M. 1986. *Food irradiation.* Orlando, FL: Academic Press.

Urbain, W. M. 1989. Food irradiation: The past fifty years as prologue to tomorrow. *Food Technol. 43*(7):76, 92.

USDA Issues. 1999. *Meat and poultry irradiation proposal.* USDA Food Safety and Inspection Service. Accessed July 11, 2002 at www.fsis.usda.gov/oa/background/irradprop.htm.

USDA. 1986. FSIS. Irradiation of pork for control of *Trichinella spiralis. Federal Register.* 51:1769–1771.

USDA. 1992. FSIS. Irradiation of poultry products. *Federal Register.* 57:43588–43600.

Venugopal, V., S. N. Doke, and P. Thomas. 1999. Radiation processing to improve the quality of fishery products. *Crit. Rev. Food Sci. Nutr.* 39:391–440.

Watts, D. A., S. K. Wolfe, and W. D. Brown. 1978. Fate of [14C]carbon monoxide in cooked or stored ground beef samples. *J. Agric. Food Chem.* 26:210–214.

Wen, J., P. A. Morrissey, D. J. Buckley, and P. J. A. Sheehy 1996. Oxidative stability and a-tocopherol retention in turkey burgers during refrigerated and frozen storage as influenced by dietary tocopheryl acetate. *Brit. Poult. Sci.* 37:787–792.

Wheeler, T. L., M. Koohmaraie, and S. D. Shackelford. 1996. Effect of vitamin C concentration and co-injection with calcium chloride on beef retail display color. *J. Anim Sci.* 74:1846–1853.

Winne, A. D., and P. Dirinck. 1996. Studies on vitamin E and meat quality: 2. Effect of feeding high vitamin E levels on chicken meat quality. *J. Sci. Food Agric.* 44:1691–1696.

Woods, R. J., and A. K. Pikaev, eds. 1994. Interaction of radiation with matter: Selected topics in radiation chemistry. In *Applied radiation chemistry: Radiation processing,* 59–89, 165–210. New York: Wiley.

World Health Organization. 1981. *Wholesomeness of irradiated foods* (Technical report series 659). Geneva, Switzerland; Author.

World Health Organization. 1994. Food irradiation. In *Safety and nutritional adequacy of irradiated food,* 5–13. Geneva, Switzerland: Author.

World Health Organization. 1999. *High-dose irradiation? Wholesomeness of food irradiated with doses above 10 kGy* (Report of a Joint FAO/IAEA/WHO Study Group). Geneva, Switzerland: Author.

Xiong, Y. L., E. A. Decker, G. H. Robe, and W. G. Moody. 1993. Gelation of crude myofibrillar protein isolated from beef heart under antioxidant conditions. *J. Food Sci.* 58:1241–1244.

Yoon, K. S. 2003. Effect of gamma irradiation on the texture and microstructure of chicken breast meat. *Meat Sci.* 63:273–277.

Zhu, M. J., A. Mendonca, and D. U. Ahn. 2004. Effect of temperature abuse on the quality of irradiated pork loins. *Meat Sci.* 67:643–649.

Zhu, M. J., A. Mendonca, H. A. Ismail, M. Du, E. J. Lee, and D. U. Ahn. 2005. Impact of antimicrobial ingredients and irradiation on the survival of *Listeria monocytogenes* and quality of ready-to-eat turkey ham. *Poult. Sci.* 84(4):613–620.

Zhu, M. J., A. Mendonca, B. Min, E. J. Lee, K. C. Nam, K. Park, M. Du, H. A. Ismail, and D. U. Ahn. 2004. Effects of electron beam irradiation and antimicrobials on the volatiles, color and texture of ready-to-eat turkey breast roll. *J. Food Sci.* 69:C382–C387.

8 Application of High Hydrostatic Pressure to Meat and Meat Processing

Atsushi Suzuki, Ken Kim, Hiroyuki Tanji, and Tadayuki Nishiumi
Niigata University

Yoshihide Ikeuchi
Kyushu University

CONTENTS

8.1 Pressure-induced Meat Tenderization 194
8.1.1 High-Pressure Effects on Prerigor Muscle 195
8.1.2 High Pressure and Heat Treatments on Postrigor Muscle 195
8.1.3 Tenderization of Postrigor Muscle by High Pressure 195
8.1.4 Mechanism of Meat Tenderization and Acceleration of Meat Conditioning Induced by High Pressure 196
8.1.4.1 Effect on Modification of Actin–Myosin Interaction 196
8.1.4.2 Effect on Fragmentation of Myofibrils 197
8.1.4.3 Effect on Conversion of α-Connectin to β-Connectin 199
8.1.4.4 Effect on Connective Tissue 201
8.2 Effect of High-Pressure Treatment on Flavor-Related Components 203
8.3 Effect of High-Pressure Treatment on Color and Fat Stability 204
8.4 Effect of High Pressure on Micro-Organisms 205
8.5 Pressure-Processing of Meat Products 206
8.5.1 Effect of High-Pressure Treatment on Thermal Gel Formability of Muscle Proteins 206
8.5.2 Pressure-Processed Pork Ham 208
8.5.3 Pressure-Processed Cooked Ham 209
8.5.4 Pressure-Processed Dry-Cured Ham 209
8.5.5 High-Pressure Assisted Freezing and Thawing of Meat and Meat Products 211
8.6 Conclusion 211
Acknowledgments 212
References 212

Professor P. W. Bridgman (1914), a pioneer of high-pressure physics, reported that raw egg albumen and yolk in a shell were coagulated under high hydrostatic pressure of 500 to 600 MPa without the disruption of the shell. This observation suggested that the high hydrostatic pressure (high pressure) was a useful tool for food processing instead of heat treatment. However application of high pressure for food processing was almost ignored until the onset of the project "Development of High-Pressure Technologies and Fermentation Using Dense-Mass Cultivation," which was supported by the Ministry of Agriculture, Forestry and Fisheries (1989) in Japan. Exceptionally, Australian meat scientists have carried out the application of high pressure to meat since the early 1970s (Macfarlane 1973; Bouton, Ford, Harris, Macfarlane, O'Shea 1977). Since the onset of the projects in Japan, the application of high pressure for food processing has attracted much attention in Japan and Europe because changes in the properties of food materials induced by pressurization proceed in a different manner from the properties of heat processing (Cheftel 1992; Hayashi 1992; Johnston 1995; Knorr 1996). Several pressure-processed foods have already been placed on the market. (Suzuki 2002).

Among all foods and food constituents, muscle and muscle proteins are probably most susceptible to high pressure. There are many reviews describing the regulation of meat quality or processing of meat by high pressure (Cheftel and Culioli 1997; de Lambellerie-Anton, Taylor, and Culioli 2002; Macfarlane 1985; Suzuki, Kim, Tanji, and Ikeuchi 1992).

This chapter reviews the pressure effects on the postmortem muscle in view of understanding the mechanism of pressure-induced tenderization of meat or acceleration of meat conditioning, as well as recent progress in pressure processing of meat products.

8.1 PRESSURE-INDUCED MEAT TENDERIZATION

When an animal is slaughtered, rigor mortis develops within a few hours with the contraction of muscle fibers and an increasing toughness of meat. The meat immediately after death is soft but lacking in good flavor and taste, and the meat in rigor state is no good for cooking and processing because of the toughness and low water-holding capacity. If the meat is held at low temperature for a few days, the meat becomes soft again and there is a progressive tenderization of meat over the next several weeks. Thus the most widely used process for meat with improved of flavor and taste is called *conditioning* or *aging* of the carcass.

If the tenderization of tough meat, especially from old dairy cows, or the shortening of the aging time could be achieved by high-pressure treatment, application of high pressure to meat should be valuable from the standpoint of saving resources and energy for refrigeration.

A trial to tenderize meat by high pressure was first carried out by Macfarlane (1973) in Australia. It is very important to choose the appropriate postmortem time for the application of high pressure.

8.1.1 High-Pressure Effects on Prerigor Muscle

Macfarlane (1973) reported data for various measurements on prerigor ox *Biceps femoris* muscle pressurized at 100 MPa for 2 to 4 minutes, along with those for muscle not pressurized. As a result of pressure treatment, the muscle shortened by about 35% and this degree of shortening in non-pressure-treated muscle would be expected to result in considerable toughening of the cooked muscle. However the shear force measurements indicated that pressure treatment improved the tenderness of meat.

Since Macfarlane's observation that a brief exposure of prerigor muscle to high pressure for a few minutes at ambient temperature produced marked drop in shear value, a new tenderization method for meat by high pressure has been reported in a series of papers by Macfarlane (Macfarlane, Mckenzie, Turner, and Jones 1981; Macfarlane and Morton 1978) and others (Elgasim and Kennick 1982; Kennick, Elgasim, Holmes, and Meyer 1980; Riffero and Holmes 1983).

8.1.2 High Pressure and Heat Treatments on Postrigor Muscle

Although pressure treatment of warm prerigor meat is effective for avoiding myofibrillar toughness in meat, a treatment that is effective when applied to postrigor muscle obviously would be potentially useful. Bouton et al. (1977) suggested that postrigor bovine muscle proved less suitable to such improvement of shear value unless long exposure to high pressure at high temperature was used. They said that 150 MPa at 60°C for 30 minutes was required for improvement of shear value. Locker and Wild (1984) also reported that pressure-heat (P-H) treatment tenderized meat effectively after a considerable period at an elevated temperature.

Macfarlane (1985) presented a scheme involving a pressure-induced dissociation of proteins to account for the tenderization of meat by combined pressure and heat treatments. In his scheme, myofibrillar proteins dissociated by high pressure are denatured and unable to associate by heat treatment, resulting in meat tenderization. P-H treatment is effective for overcoming toughness associated with cold-shortened muscle. However this treatment is not good for meat due to the brownish color caused by pressure and heat.

8.1.3 Tenderization of Postrigor Muscle by High Pressure

From the standpoint of the commercial application of high pressure, tenderization of postrigor muscle is more important than that of prerigor muscle. Suzuki, Kim, Honma, Ikeuchi, and Saito (1992) measured the hardness and elasticity of postrigor shoulder muscle obtained from an old dairy cow exposed to high pressure of 100 to 300 MPa for 5 minutes by Rheo Meter (Fudoh Co., Japan) with a conical plunger. The hardness of the muscle measured immediately after pressurization decreased to 60%, 20%, and 10% of the control (untreated) at 100 MPa, 150MPa, and 300 MPa, respectively, whereas a significant difference in elasticity was not observed. This result indicates that brief exposure of postrigor muscle to high pressure induces the meat tenderization without heat treatment. The long P-H treatment proposed by

Macfarlane (1985) and others (Bouton et al. 1977; Riffero and Holmes 1983) might not be required for tenderizing postrigor muscle, if higher pressures than those used in their experiments are applied.

8.1.4 Mechanism of Meat Tenderization and Acceleration of Meat Conditioning Induced by High Pressure

It is well known that the postmortem tenderization of meat is due to the following changes in the muscle during conditioning, mainly as a result of the activity of endogenous proteases: (a) weakening of actin–myosin interaction, (b) fragmentation of myofibrils into short segments due to Z-line disintegration, (c) degradation of the elastic filaments consisting of connectin (also called titin), and (d) weakening of connective tissue.

To clarify the mechanism for pressure-induced tenderization of meat or acceleration of meat conditioning, the following subjects were reviewed : (a) pressure effect on modification of actin–myosin interaction , (b) pressure effect on fragmentation of myofibrils, (c) pressure effect on conversion of α-connectin to β-connectin, and (d) pressure effect on connective tissue.

8.1.4.1 Effect on Modification of Actin–Myosin Interaction

It is well established that actin–myosin interaction and myofibrillar structure are modified during postmortem aging as evidenced by changes in the ATPase activity of myofibrils. Ouali (1984) reported that Mg^{2+}-Ca^{2+}-enhanced ATPase activity increased at low ionic strength (below about 0.2 M KCl), whereas it decreased at higher ones (0.3 M or more) as storage time increased. He concluded that the slope value that quantifies the sensitivity to ionic strength could be an accurate indicator of the degree of aging of the myofibrillar structure and has been denominated the Biochemical Index of Miofibrillar Aging (BIMA).

Nishiwaki, Ikeuchi, and Suzuki (1996) measured Mg^{2+}-enhanced ATPase activities (ionic strength between 0.06–0.32 M KCl) of the myofibrils prepared from the conditioned (7days, 4°C) and pressurized (30–300 MPa, 5 minute) rabbit muscles. The changes in the BIMA value calculated from the ATPase activities are shown in figure 8.1. In the conditioned muscle, BIMA value gradually increased with the increase of the storage time and reached about 2.5 times that of the muscle at death (inset in figure 8.1). The BIMA value of the myofibrils prepared from the pressurized muscles increased with increasing pressure up to 200 MPa and reached the same level as that of the myofibrils conditioned for 7 days. However, an application of higher pressure (300 MPa) caused a remarkable decrease of BIMA value.

The pressure-induced structural changes of the thin filament must be the main factor affecting the BIMA value observed in the myofibrils prepared from the muscles exposed to high pressure for a short period (5 minutes). The drastic structural changes observed in the pressurized muscle are not observed in the myofibrils prepared from the conditioned muscle as reported elsewhere. This result suggested that the application of high pressure to postmortem muscle caused the changes in ATPase activity and BIMA values of myofibrils in a different manner from that of conditioning (aging).

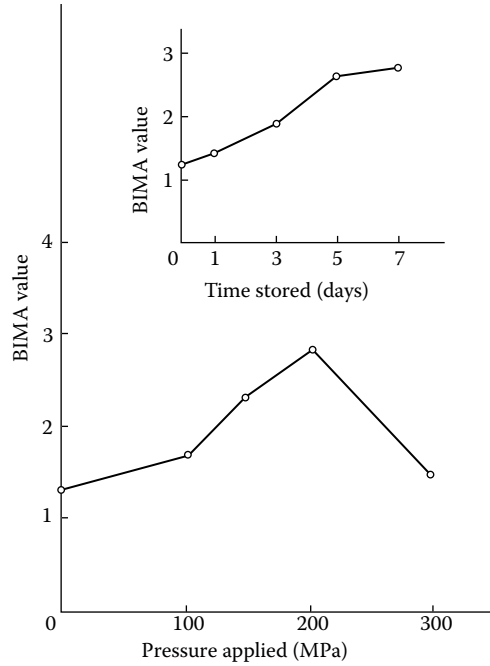


FIGURE 8.1 Effects of high pressure on the BIMA value. Inserted figure shows the changes in the BIMA value of the myofibrils prepared from the conditioned muscle. Adapted from Nishiwaki, Ikeuchi, and Suzuki (1996).

8.1.4.2 Effect on Fragmentation of Myofibrils

It is well known that the myofibrils prepared by homogenizing conditioned muscle were shorter and composed of fewer sarcomeres than those from at-death muscle (Takahashi, Fukazawa, and Yasui 1967) and that breaks in myofibrils at Z-line were correlated with the increase in meat tenderness (Davey and Gilbert 1967; Fukazawa and Yasui 1967; Takahashi et al. 1967). Therefore myofibrillar fragmentation is considered to be useful for predicting meat tenderness (Calkins and Davis 1980; Olson, Parrish, and Stromer 1977).

Suzuki, Watanabe, Iwamura, Ikeuchi, and Saito (1990) showed the degree of fragmentation in myofibrils prepared from the pressurized bovine muscles (100–300 MPa, 5 minutes) in figure 8.2. The degree of fragmentation is expressed as percentage of the number of myofibrillar fragments composed of one to four sarcomeres to the total number of myofibrils under a phase-contrast microscope. The degree of fragmentation, which was less than 10% in the untreated muscle, was accelerated by pressurization and reached over 30%, 70%, 80%, and 90% at 100, 150, 200, and 300 MPa, respectively. The degree of fragmentation, 80% to 90%, is over the maximal level of the fragmentation of myofibrils naturally occurring in the conditioned muscle. From the results of this fragmentation, a brief exposure of postrigor muscle to the high pressure seems to be useful for meat tenderization.

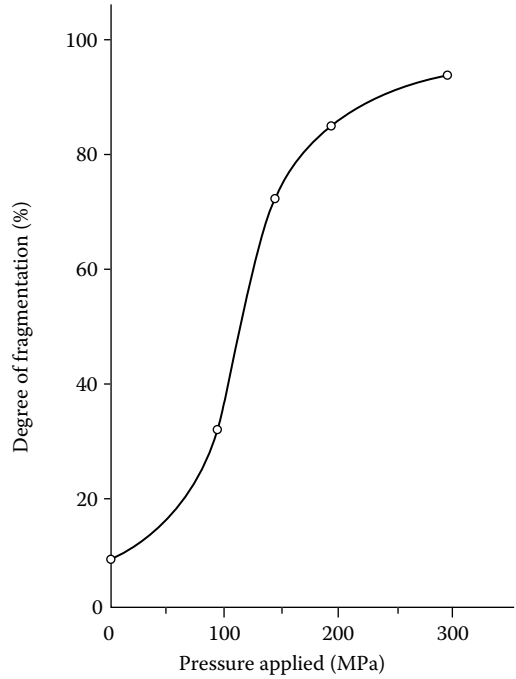


FIGURE 8.2 Effects of high pressure treatment on the degree of fragmentation in myofibrils. The degree of fragmentation of the myofibrils is expressed as a percentage of the number of myofibrillar fragments composed of one to four sarcomeres to the total number of myofibrils (about 100) observed. Adapted from Suzuki, Watanabe, Iwamura, Ikeuchi, and Saito (1990).

The effects of high pressure on the myofibrillar structure of the postrigor bovine muscle were characterized in by Suzuki et al. (1990). In the myofibrils prepared from the muscle pressurized at 100 MPa, a contraction of the sarcomere was observed, and the difference in density between the A-band and I-band became indistinguishable as compared with the control (untreated). Marked rupture of the filamentous structure of the I-band and a loss of the M-line materials were observed in the myofibrils from the muscle pressurized at 150 MPa. In the myofibrils from the muscle pressurized at 200 MPa, the structural continuity of the sarcomere was almost completely lost, with broken A- and I-filaments spread over the sarcomere. Complete loss of the M-line and thickening of the Z-line, probably due to collapse of the I-filament, were observed. Cleavage of the A-band in addition to the many changes already mentioned was observed in the myofibrils from the muscle pressurized at 300 MPa. The length of the sarcomere, initially contracted by pressurization at 100 MPa, seemed to have gradually recovered with the increase of pressure, because of the increasing loss of structural continuity. As already mentioned, fragmentation of the myofibrils during conditioning is derived from breakage of the myofibrils at Z-line, whereas the Z-line in the fragmented myofibrils from the pressurized muscle apparently remained intact.

In spite of the short time (5 minutes) and low temperature (about 10°C) of the pressure treatment applied to the postrigor muscle, changes in the ultrastructure of the myofibrils were principally in accordance with those reported by Macfarlane and Morton (1978) and Locker and Wild (1984). The application of high pressure is known to influence the state of aggregation of both actin and myosin, which are the major constituents of myofibrils. A number of reports describing the depolymerization of F-actin (Ikkai and Ooi 1966; Ivanov, Bert, and Lebedeva 1960; O'Shea, Horgan, and Macfarlane 1976), myosin polymer (Josephs and Harrington 1966, 1967, 1968), and actomyosin (Ikkai and Ooi 1969) under high pressure have been published. Although it is not clear whether depolymerization of F-actin occurred in the pressurized muscle in situ, degradation of the I-filament. (i.e., depolymerization of F-actin by the high pressure treatment) may be one of the causes of fragmentation. An acceleration of the fragmentation of myofibrils and coagulation of each myofibrillar protein in a dissociate state suggested by Macfarlane (1985) may cause tenderization of meat exposed to high pressure.

From the ultrastructural observation and SDS-PAGE analysis of myofibrils (data not shown; see Suzuki et al. 1990), the mechanism for the disruption of the structural continuity of myofibrils induced by pressurization may be different from that of conditioned muscle.

8.1.4.3 Effect on Conversion of α-Connectin to β-Connectin

Recent studies clearly indicate that a string-like protein that has been designated as connectin (also called titin) maintains the elasticity and mechanical stability of skeletal muscle. At death, the connectin exists as α-connectin (about 3,000 kDa) together with a small amount of its subfragment, β-connectin (about 2,000 kDa; Maruyama, Kimura, Yoshidomi, Sawada, and Kikuchi 1984; Wang, McClure and Tu 1979). α-Connectin has been shown to undergo degradation into β-connectin and a 1,200 kDa fragment during postmortem storage of muscle (Lusby, Ridpath, Parrish, and Robson 1983; Seki and Watanabe 1984; Suzuki, Hoshino, Sasaki, Sano, Nakane, and Ikenchi 1987). An entire molecule of α-connectin spans one half the width of a sarcomere and forms elastic connections between the end of the thick filament and the Z-line (Furst, Osborn, Nave, and Weber 1988; Maruyama, Yoshioka, Higuchi, Ohashi, Kimura, and Natori 1985). These elastic connectins keep the thick filaments centered within the sarcomere during the force developments (Horowits, Kempner, Bisher, and Podolsky 1986). The cleavage site converting α- to β-connectin is located in a region in the I-band (Kimura, Matsumura, Ohtsuki, Nakauchi, Matsuno, and Maruyama 1992), which indicates that the elastic connections linking the thick filament to the Z-line are cut off with increasing time postmortem. Many researchers have investigated the influence of connectin on meat tenderization during postmortem conditioning. It is obvious that the splitting of connectin from α to β is closely associated with the postmortem tenderization of meat (Anderson and Parrish 1989; Patterson and Parrish 1986; Takahashi and Saito 1979). Is it possible to induce these changes by high-pressure treatment?

Kim, Homma, Ikeuchi, and Suzuki (1993) obtained the results of SDS-PAGE of the whole muscle proteins prepared from the control (untreated) and pressurized rabbit muscle samples (figure 8.3). When muscles were exposed to high pressure of 100 MPa to 400 MPa for 10 minutes, the conversion of α-connectin into β-connectin was

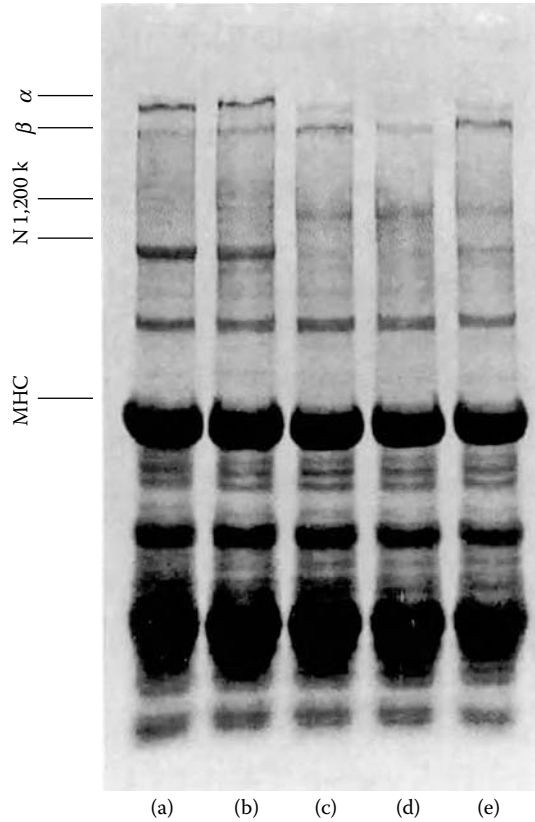


FIGURE 8.3 SDS-PAGE patterns of whole muscle proteins prepared from pressurized muscles. Whole muscle proteins were analyzed by electrophoresis on 2% polyacrylamide slab gel containing 0.1% SDS and 0.5% agarose. α = α-connectin; β = β-connectin; 1,200 k = 1,200 kDa peptide; N = nebulin; M = myosin heavy chain. Adapted from Kim, Homma, Ikeuchi, and Suzuki (1993).

markedly accelerated by pressurization at 200 MPa, and an approximately 1,200 kDa peptide was observed, accompanied by conversion of α-connectin into β-connectin. The conversion of connectin from α to β was most pronounced at pressure of 300 MPa however, connectin was relatively resistant to degradation at a pressure of 400 MPa. Nebulin disappeared on pressurization at 300 MPa, whereas it remained partly intact at 400 MPa. This result revealed that a brief exposure of muscle to a pressure as high as 300 MPa for 10 minutes could convert almost all of the α-connectin into β-connectin, which took about 1 week during conditioning at 2°C. As shown in the work of Suzuki et al. (1987), the extractability of β-connectin increased about 1.6 or 1.2 times as compared with that from untreated rabbit muscle by brief exposure (5 minutes) to 200 MPa or 300 MPa, respectively. The appearance of β-connectin isolated from the pressurized muscle was somewhat fragmented as compared with that from the untreated muscle. Because isolated β-connectin tends to aggregate due to intermolecular lateral association *in vitro* (Maruyama et al. 1984),

the fragmentation of β-connectin from the pressurized muscle possibly implies the weakening in intermolecular force of β-connectin. That is to say, the increase in extractability of β-connectin by pressurization might be due to the decrease in the intermolecular forces holding the connectin molecules and the weakening in the interaction between the connectin and myosin filaments.

Because the disruption of peptide bonds should not be induced by high pressure, it is of interest to establish the reason why the conversion of α- to β-connectin was caused by pressurization. There have been two theories about the mechanism of connectin splitting; one is the proteolytic cleavage by calpain (Ca^{2+}-activated protease) and cathepsin D (Kim et al. 1993, 1995; Suzuki, Kim, and Ikeuchi 1996), and the other one is direct action of the Ca^{2+} ion (Tatsumi, Hattori, and Takahashi 1996). Kim et al. (1993) reported that the effect of high pressure on connectin in the isolated myofibrils was similar to that of connectin in muscle, as seen using analysis by SDS-PAGE. They found that two kinds of protease inhibitors, 1 mM leupeptin and 1 mM E64, completely prevented the degradation of connectin at each stage of pressurization (100–400 MPa for 5 minutes), whereas connectin in the pressurized isolated myofibrils was almost the same as that in the control myofibrils (untreated), even though the myofibrils were pressurized in the presence of 3 mM $CaCl_2$. The degradation of connectin by the direct action of the calcium ion under high pressure is thus improbable. This result demonstrated the participation of some endogenous proteases, especially calpain, in the pressure-induced conversion of α-connectin to β-connectin.

This can be interpreted by assuming that the susceptibility of connectin to calpain was markedly increased by the application of pressure, but the ability of calpain to hydrolyze connectin was gradually reduced with increase of the pressure. It has been recognized that high pressure of 100 MPa or more denatures protein and increases its susceptibility to proteolysis. Because the calcium ion concentration in the sarcoplasmic fluid is near optimum for activation of calpain due to the release from sarcoplasmic reticulum during high-pressure treatment (Suzuki, Okamoto, Ikeuchi, and Saito 1993, 1994), the degree of conversion of α-connectin to β-connectin is thought to be mainly related to the pressure dependence of the structural changes of α-connectin and the inactivation of calpain.

The mechanism for the splitting of connectin under high pressure is probably the same as that in the muscle during conditioning (see Kim et al. 1995). The increase of the extractability of connectin may reflect the quality changes of connectin structure in the muscle induced by pressurization (Suzuki et al 1987).

8.1.4.4 Effect on Connective Tissue

Meat tenderness has been resolved at least into two different components: actomyosin toughness and background toughness. The *actomyosin toughness* is the toughness attributed to the myofibrillar proteins, whereas the *background toughness* is the toughness due to the presence of the connective tissue. Generally, it is accepted that changes in the connective tissue during conditioning of meat are only slight in comparison with those in the myofibrillar proteins.

There are few papers describing the effects of pressurization on connective tissue as compared with those on myofibrillar proteins. Ratcliff, Bouton, Ford, Harris, and

Macfarlane (1977) showed that although P-H treatment effectively eliminated the myofibrillar toughness (actomyosin toughness), the tenderness of the treated sample was limited by the connective tissue toughness (background toughness). Macfarlane et al. (1981) also revealed that a transition attributed to F-actin was absent; but that attributed to the connective tissue was not changed in the thermograms of the pressurized muscle. Beilken, Macfarlane, and Jones (1990) suggested that pressure treatment at temperatures ranging from 40°C to 80°C has little or no effect on the background toughness other than to raise the temperature at which heat treatment alone produced a decrease in this toughness. Suzuki, Watanabe, Ikeuchi, Saito, and Takahashi (1993) reported that no significant differences in the ultrastructure, electrophoretic pattern, thermal solubility, and thermogram of differential scanning calorimetry (DSC) analysis of the isolated intramuscular collagen from an old dairy cow were observed among the control (untreated) and pressurized muscles (100–400 MPa, 5 minutes). Effects of pressurization on denaturation temperature and enthalpy calculated from the thermogram are shown in table 8.1.

Recently Nishimura, Hattori, and Takahashi (1995) suggested that the weakening of the intramuscular connective tissue, endomysium and perimysium, caused during extended conditioning correlated with meat tenderization using scanning electron microscopy. Ueno, Ikeuchi, and Suzuki (1999) examined the intramuscular connective tissues in the conditioned and pressurized muscles by scanning electron microscopy. During conditioning the structural weakening of the endomysium and perimysium proceeded, and the disruption of the honeycomb structure was observed. In the pressurized muscle, deformation of the honeycomb structure of endomysium was accelerated with the increase of the pressure applied to the muscle, and expansion of the mesh of endomysium was observed in the muscle pressurized at 400 MPa.

TABLE 8.1
Effect of Pressurization on Denaturation Behavior of Intramuscular Collagen

	Denaturation Temperature (°C)			
	T_o	T_p	T_c	ΔH (mJ/mg)
Control	62.40 ± 1.03	65.65 ± 0.67	68.70 ± 0.65	15.00 ± 1.40
100 MPa	62.13 ± 0.66	65.35 ± 0.25	68.95 ± 0.64	15.53 ± 1.65
150 MPa	62.58 ± 1.03	66.05 ± 0.88	68.80 ± 0.64	15.78 ± 1.80
200 MPa	62.80 ± 1.65	66.03 ± 0.99	68.73 ± 0.72	16.93 ± 1.10
300 MPa	62.68 ± 0.63	65.90 ± 1.23	68.95 ± 0.83	15.50 ± 1.65

All values are the mean ± standard deviation of four different samples. Significant differences in denaturation temperature and enthalpy were not observed among the samples ($p < .05$).
T_o = temperature, onset of denaturation; T_p = temperature, peak of denaturation; T_c = temperature, conclusion of denaturation, ΔH = enthalpy of denaturation, per mg of dry weight.

Note: Adapted from Suzuki, Wantanabe, Ikenchi, Saito, and Takahashi (1993).

At present, it is not certain that the pressure-induced structural changes in the intramuscular connective tissue cause some significant effects on meat tenderness. Further studies are required to clarify this problem.

8.2 EFFECT OF HIGH-PRESSURE TREATMENT ON FLAVOR-RELATED COMPONENTS

It is well known that raw meat has a serum-like or blood-like flavor, which on heating is altered to produce compounds that impart a full, rich flavor. The conditioning of postmortem muscle causes this cooked flavor to be richer and stronger as the postrigor muscle becomes tenderized. Most of the compounds responsible for taste and "meaty" flavor consist of a reducing sugar (usually glucose), a source of amino acids and peptides, and a taste enhancer (e.g., inosinic acid; Baines and Mlotkiewicz 1984). It is generally accepted that most of these muscle tissue components increase with extended periods of conditioning, due to chemical breakdown of certain constituents of the muscle. Very little is known about the high-pressure effects on the components responsible for taste and meaty flavor.

Suzuki, Homma, Fukuda, Hirao, Uryu, and Ikeuchi (1994) reported that the amounts of peptides and amino acids as estimated by phenol reagent positive materials (PPM) apparently increased with increasing pressure applied to the rabbit *Longissimus dorsi* muscle up to 300 MPa for 5 minutes, but the differences between each treatment were not statistically significant. When the muscles were stored at 2°C for 7 days, increases in the amount of PPM (about 140–150%) were observed both in untreated and pressurized muscles. The amounts of PPM from the pressurized muscles were higher than those from untreated muscles at each stage of pressurization. It thus seems that the breakdown of the muscle protein, estimated as PPM, was accelerated by pressurization. The apparent decrease in PPM at 400 MPa, as compared with that at 300 MPa, may be due to a slight decrease of proteolytic activity of endogenous enzyme in the muscle induced by high intensity of pressure as suggested by Ohmori, Shigehisa, Taji, and Hayashi (1992), Homma, Ikeuchi, and Suzuki (1994), and Jung, de Lamballerie-Anton, Taylor, and Ghouk (2000). Homma et al. (1994) described the effects of high-pressure treatment on muscle proteolytic enzymes, especially catheptic enzymes that influence meat tenderization, and on acid phosphatase, used as an index of disruption of lysosomal membrane. Their report concluded that the pressure-induced increase in the amount of muscle protease activity was due to the release of the enzymes from lysosomes.

The content of inosinic acid (IMP), which is considered to contribute to the "umami" taste of meat (Suzuki et al. 1994), was not reduced by pressurization. The changes in amino acids contents and high-performance liquid chromatography (HPLC) pattern of soluble peptides in the extract from the pressurized rabbit muscles were also analyzed (data not shown; see Homma et al. 1994). From these results, it is suggested that high-pressure treatment on the postmortem muscle causes almost the same changes in the components responsible for the flavor of meat as those observed in conditioned muscle.

8.3 EFFECT OF HIGH-PRESSURE TREATMENT ON COLOR AND FAT STABILITY

There are many studies describing high-pressure treatments on meat color and fat stability. As suggested by MacDougall (1983), the color of meat depends on the amount and type of heme pigment, and the scattering properties of meat. Defaye, Ledward, MacDougall, and Tester (1995) showed that high-pressure treatment of myoglobin caused partial denaturation with later renaturation. It is also known that the effect of high-pressure treatment on myoglobin solutions depends on the temperature at which pressure treatment occurs. Zip and Kauzmann (1973) did not observe denaturation of myoglobin below 235 MPa at 20°C, and Ooi (1994) did not observe the denaturation until 500 MPa at 10°C. Carlets, Veciana-Nugues, and Cheftel (1995) studied color and myoglobin changes in minced bovine meat packaged under vacuum, air, or oxygen processed by high pressure for 10 minutes. They concluded that meat discoloration through pressure processing appeared to result from a whitening effect in the range of 200 MPa to 350 MPa, possibly due to globin denaturation or heme displacement or release, and oxidation of ferrous myoglobin to ferric metmyoglobin, at or above 400 MPa. Cheah and Ledward (1997) reported that application of pressure at 80 MPa to 100 MPa for 20 minutes improved the color stability, as measured by rate of metmyoglobin formation of *Longissimus dorsi* and *Psoas major* beef muscles exposed to air 2 days postslaughter (postrigor). However, pressure treatment of these muscles at 7 to 20 days postslaughter did not improve their color stability. These results suggest that pressure inhibits, at least partially, the mechanism responsible for the low color stability of very fresh beef. Although high-pressure treatment induced visible modification of the color of raw meat, the color difference was greatly reduced after cooking. As suggested by Cheftel and Culioli (1997), pressure processing of fresh red meat cannot be envisaged unless subsequent (or simultaneous) cooking is done before the final product is presented for sale and consumption. In contrast, pressure processing of cured meat or white meat is unlikely to cause any serious color problems.

Cheah and Ledward (1996) also studied the pressure effects on fat oxidation in minced muscle. On the basis of the measurement of thiobarbituric acid (TBA) value, they indicated that the TBA value did not increase in the minced muscle exposed to high pressure up to 200 MPa, but slightly increased in the muscle exposed to 300 MPa, and markedly increased in the muscle exposed to 800 MPa. High-pressure treatment above 300 MPa to 400 MPa caused conversion of reduced myoglobin and oxymyoglobin to the denatured ferric form, resulting in the acceleration of lipid oxidation. To the contrary, Orlien and Hansen (2000) reported that lipid oxidation at higher pressure was not related to the release of nonheme iron or catalytic activity of metmyoglobin, but could be linked to membrane damage.

As mentioned by Cheftel and Culioli (1997), this pressure-induced oxidation may limit the usefulness of this technology for meat-based products unless suitable packaging or antioxidants are used. Removing oxygen or adding carbon dioxide prior to pressurization may be useful to prevent the pressure-induced lipid oxidation.

8.4 EFFECT OF HIGH PRESSURE ON MICRO-ORGANISMS

High pressure is one of the physiological factors affecting cellular physiology of the micro-organisms. High pressure of a few hundreds MPa can decrease the viability of bacterial cells, and a pressure of a few tens MPa can decrease the growth rate. New high-pressure technology for food sterilization is being developed based on these facts. According to Yuste, Cappellas, Pla, Fung, and Mor-Mur (2001), the inactivation is due to widespread damages of micro-organisms through modification of morphology and of several vulnerable components such as cell membranes, ribosomes, and enzymes, including those involved in the replication and transcriptions of DNA. Microbial inactivation through high pressure application has been well reviewed by Cheftel (1995). The extent of inactivation depends on several parameters such as the type of micro-organisms, the pressure level, the process temperature and time, and the pH and composition of the food or the dispersion medium. High-pressure inactivation of micro-organisms is summarized in table 8.2 by Masoliver and Grebol (personal communication 2001).

In general, Gram-negative bacteria such as *Yersinia enterocollitica and Salmonella spp*. were found to be more sensitive than Gram-positive bacteria such as *Listeria monocytogenesis* and *Staphylococcus aureus*. Some strains of *Escherichia coli* O157:H7 were found to be relatively resistant to pressure. Patterson, Quinn, Simpson, and Gilmour (1996) reported the effect of substrate on pressure resistance of *S. aureus*, *S. enteridis* and one of the resistant *E. coli* O157:H7 strains. There was greater survival of *E. coli* and *S. enteridis* in ultra high-temperature treated (UHT) milk compared to poultry meat, whereas there was greater recovery of *S. aureus* in poultry meat than in the milk. The simultaneous applications of pressure with mild heating (up to 60°C) significantly increased the death of *E. coli* O157:H7 in poultry meat and UHT milk compared to either treatment alone. The variation in results

TABLE 8.2
High-Pressure Inactivation of Micro-Organisms

Less Sensitive	More Sensitive
Spores	Vegetative cells
Gram positive	Gram negative
Cells in stationary phase	Cells in exponential phase
Bacillus, Clostridium, Staphylococcus, Listeria, Escherichia coli O157:H7	*Yersinia, Vibrio, Salmonella,* parasites
Low a_W	High a_W
	Acidic pH
	Higher temperature
	Presence of bacteriocins

Prepared on the basis of a personal communication from Masoliver and Grebol.

obtained with different organisms, between strains of the same organisms and in different substrates should be recognized when recommendations for the pressure processing of foods are being considered.

In practical meaning, high-pressure processing is preferable as an additional final processing step to produce safety products. High-pressure processing can eliminate manufacturing contamination of *Salmonella*, *Listeria monocytogenesis,* and other food-borne pathogens in finished, packaged products without any adverse effects on color, flavor, texture, and moisture, and increase refrigerated shelf life.

8.5 PRESSURE-PROCESSING OF MEAT PRODUCTS

8.5.1 Effect of High-Pressure Treatment on Thermal Gel Formability of Muscle Proteins

Heat-induced gelation of the salt-soluble myofibrillar proteins leads to the formation of a three-dimensional network, which exhibits both viscous and elastic properties (Asghar, Samejima, and Yasui 1985). Myosin plays a very important role in this gelation. Actin is also important as a cofactor reinforcing this gel structure of myosin (Yasui, Ishioroshi, and Samejima 1980). Needless to say, pressure affects the properties of these proteins, depending on the extent of applied pressure, pH, salt concentration, and so on (Ikkai and Ooi 1969; Yamamoto, Miura, and Yasui 1990). For example, pressurization of rabbit myosin promotes formation of aggregates in high-salt solution at pH 6.5 and results in the formation of a gel consisting of a fine network in low-salt solution at pH 6.0 (O'Shea et al. 1976; Yamamoto et al. 1990). F-actin undergoes irreversible denaturation in the absence of ATP at a pressure of above 150 MPa, whereas ATP shows a significant protective effect against pressure-induced denaturation of actin (Ikkai and Ooi 1966). In actomyosin, a gel to solid transition is promoted as a result of pressure treatment. In the absence of ATP, an association remains between myosin and actin of actomyosin under pressure, whereas in the presence of ATP, actomyosin dissociates into the individual component (Ikkai and Ooi 1969).

The changes in the properties of myofibrillar proteins under the influence of pressure as described earlier may be used in meat processing. From this viewpoint, the effect of high-pressure treatment on the thermal gelation of the different types of skeletal muscle protein has recently been investigated, especially in Japan (Ikeuchi, Tanji, Kim, and Suzuki 1992a, 1992b; Ko, Tanaka, Nagashima, Taguchi, and Amano 1990; Sano, Noguchi, Matsumoto, and Tsuchiya 1988; Shoji, Saeki, Wakameda, and Nonaka 1990; Suzuki 1991). Shoji et al. (1990) reported that excellent gels could be produced from Alaska pollack by pressure treatment at 200 MPa to 400 MPa. Pressurized pork actomyosin was also reported to show higher work done values as an index of hardness than unpressurized actomyosin (Suzuki 1991). The heat-induced gelation of rabbit actomyosin (or natural actomyosin) treated with high pressure was investigated by Ikeuchi et al. (1992a). Figure 8.4 shows dynamic rheological behavior of actomyosin and myosin at 0.6 M KCl and pH 6.0 before and after pressure application. When actomyosin was subjected to a pressure of 150 MPa for 5 minutes (figure 8.4c), the dynamic rheological behavior during heat gelation

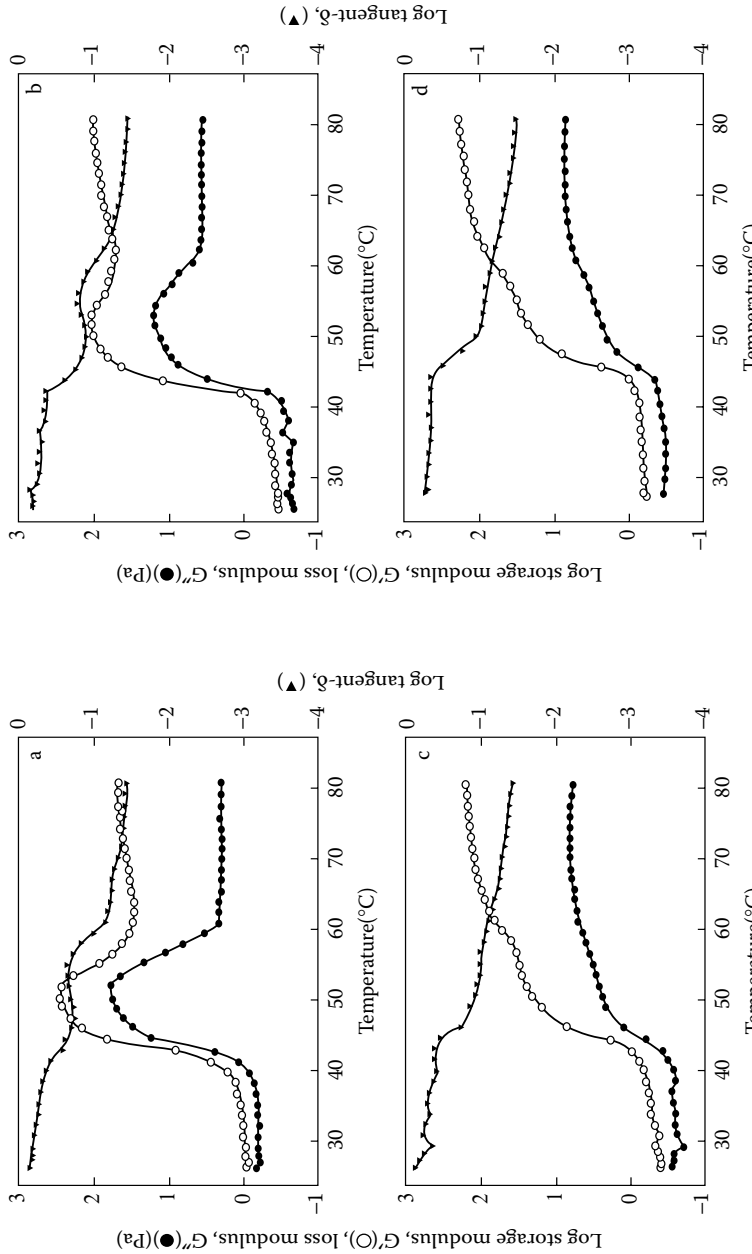


FIGURE 8.4 Dynamic rheological behavior of actomyosin and myosin at 0.6 M KCl and pH 6.0 before and after pressure application. (a) unpressurized actomyosin; (b) actomyosin pressurized at 100 MPa for 5 minutes; (c) actomyosin pressurized at 150 MPa for 5 minutes; (d) myosin pressurized at 150 MPa for 5 minutes. (Note: Unpressurized myosin gave almost the same pattern as pressurized one.) ○ = storage modulus, G'; ● = loss modulus, G"; ▼ = tangent-δ. The protein concentrations of actomyosin and myosin were 15 mg/ml and 10 mg/ml, respectively. Adapted from Ikeuchi, Tanji, Kim, and Suzuki (1992a).

showed a pattern similar to that of myosin (figure 8.4d). That is, the rheological transition in the 46°C to 53°C range induced by the presence of F-actin disappeared. This suggests that the greater part of actin in actomyosin was denatured or depolymerized into G-actin, which did not contribute to the heat-induced gel formation of myosin. The storage modulus (G') of pressurized actomyosin at 80°C was almost double that observed in unpressurized actomyosin. The dynamic rheological behavior of actomyosin and myosin at low ionic strength was also investigated by Ikeuchi et al. (1992a). In 0.2 M KCl at pH 6.0, where unpressurized actomyosin forms a very weak heat-induced gel, pressurized actomyosin formed a firm heat-induced gel having higher G' value than either pressurized or unpressurized actomyosin at 0.6 M KCl. The gel of pressurized actomyosin at 0.2 M KCl also resembled that of pressurized myosin at 0.2 M KCl in the dynamic rheological behavior. The remarkable increase in the storage modulus of pressurized actomyosin at low and high KCl concentration seemed to arise from pressure-induced denaturation in actomyosin. However, gel of pressurized actomyosin at 0.2 M KCl was a sponge-like gel showing apparently less elastic and less translucent nature than that formed at 0.6 M KCl as reported by Suzuki (1991). The pressurized actomyosin at low salt concentration probably forms a gel similar to myosin filamentous gel, because actin in actomyosin was mostly denatured under such a pressure (Ikeuchi et al. 1992b; Ishioroshi, Samejima, and Yasui 1983).

Mechanism of heat-induced gelation of pressurized actomyosin was investigated by Ikeuchi et al. (1992b). Judging from the data of dynamic rheological measurements, biochemical measurements (DNAase I inhibition capacity and ATPase activity of actomyosin), and electron microscopic observation, the acquisition of satisfactory gel-forming ability at low salt concentration, such as 0.2 M KCl, and the increased gel strength at high salt concentration of pressurized actomyosin are probably attributable to pressure-induced denaturation of actin in actomyosin. This is because a large amount of F-actin exhibits negative effect on the heat-induced gelation of myosin at low and high KCl concentrations according to Yasui et al.'s theory (1980). There is no doubt that increases of hydrophobicity and SH content in actomyosin by pressure is partly responsible for the increased gel strength of pressurized actomyosin (Ikeuchi et al. 1992b; Kinsella and Whitehead 1989). These results suggest that high hydrostatic pressure technology is potentially useful for improvement in the functional property (e.g., gel-forming ability) of muscle proteins. The authors also emphasized that a fact of the desired gelation of pressurized actomyosin at low salt concentration (0.2 M KCl) opens up the possibility for exploitation of new meat products.

Concerning the water retention of gels after pressurization, Cheftel et al. (personal communication 2005) observed that very finely minced beef muscle without any salt can give very smooth gels by pressurization at 5°C to 10°C, and the resulting gels have a high water retention capacity.

8.5.2 Pressure-Processed Pork Ham

Nose, Yamagishi, and Hattori (1992) introduced high pressure to processing pork meat products. Cured pork meat was exposed to high pressure at 250 MPa for 3 hours after smoking at 65°C for 90 minutes in a smoke house. Changes in some microbes and properties of pressure-processed ham during storage at 4°C are shown

TABLE 8.3
Changes in Microbes and Some Properties of Pressure-Processed Ham During Cold Storage at 4°C

	Days of Cold Storage at 4°C				
	2	**8**	**16**	**23**	**29**
Escherichia coli (SPC/g)	<10	<10	<10	<10	<10
Staphylococcus aureus	<100	<100	<100	<100	<100
Clostridium	0	0	0	0	0
Salmonella	Negative	Negative	Negative	Negative	Negative
Water activity	0.97	0.97	0.96	0.96	0.96
pH	6.0	6.0	5.95	6.0	6.0
Salt (%)	2.2	2.4	2.3	2.4	2.4
Moisture (%)	69.0	70.0	69.0	69.0	69.0

Note: Adapted from Nose, Yamagishi, and Hattori (1992).

in table 8.3. During the storage, the growth of microbes and changes in water activity, pH, salt concentration, and moisture content were not observed. Data not shown indicated that the color did not change during the storage and the texture was like raw meat. The organoleptic test indicated that the pressure-processed new product like a raw ham was acceptable to consumers (data not shown).

From this observation, it is clear that the pressure processing of pork ham has the advantages of reducing the level of microbes and extending the refrigeration life with a high quality. The company that developed this product obtained official approval in April 2000 from the Ministry of Health and Welfare in Japan, but is questioning expansion of production because of the huge sum of money invested in machines and facilities.

8.5.3 Pressure-Processed Cooked Ham

Pressure-processed sliced cooked ham has been commercialized in Spain since October 1998 (Grebol 2002). A cooked ham produced by conventional method is sliced and vacuum-packed with microfilm between the slices. After packaging, it is decontaminated with high pressure of 400 MPa for 10 minutes at around 10°C in a 320-liter industrial machine made by ACB (see figure 8.5). The pressurization at 400 MPa for 10 minutes allows a significant reduction of population, activity, and growth capacity of microorganisms that would otherwise produce unpleasant flavor in sliced cooked ham (lactic acid bacteria, Enterobacteriacea, yeasts, etc.). Therefore high-pressure processed ham is evaluated to have superior organoleptic qualities and a long shelf life of 8 weeks at 4°C.

8.5.4 Pressure-Processed Dry-Cured Ham

Minerich and Krug (2003) reported high-pressure effects on the microbiological quality of prosciutto (Italian dry-cured ham). *Listeria monocytogenes* can be one of the most challenging pathogens to control with its tendency to hide in the food-processing environment and threaten postprocessing contamination. High-pressure

FIGURE 8.5 Horizontal pressure vessel (courtesy of Esteban Espuña, Spain, and ACB Pressure System, France).

pasteurization has emerged as a method capable of delivering significant pathogen lethality with minimal to no adverse effects on the product. Being a very dense, dry, and relatively large product, the prosciutto ham did not lend itself to other postpackaging control methods without creating problems, such as off-flavors or soft textures.

High-pressure pasteurization of hams is conducted in ordinary water using heavy-duty processing vessels and pressure intensifiers capable of generating and holding the pressure at over 850 MPa. At this pressure, considerable compression of the product and water is achieved, but because the pressure is perfectly equalized throughout the vessel, the contents emerge with no detectable traces of the compression. The studies conducted on hams using known amounts of *Listeria monocytogenes* inoculated before pressurization show that a 5-log reduction is achievable. However, this level of processing does not render the product sterile. Unlike most pathogens, certain spoilage organisms tend to be pressure resistant. Although high-pressure pasteurization is effective as a control method, it does require a significant capital investment. Throughput volume (25–30 hams/basket) is somewhat limited compared to other methods as well. However, it does provide an alternative way to control pathogens in a postpackaging environment for products that are not amenable to other methods. High-pressure pasteurization can also be employed without the need for any special labeling provisions in the United States.

Saccani, Parolari, Tanzi, and Rabbuti (2004) also investigated sensory and microbiological properties of dried hams treated with high pressure. High-pressure treatment (9 minutes at 600 MPa) allowed reduction of *Listeria monocytogenes* to negligible levels in dry-cured hams. Treatments affect color (slight discoloration) and saltiness (enhanced perception) in such a way that changes are inversely related to the age of the ham. Typical maturated taste of dry-cured hams did not change after high-pressure treatment, whereas color properties, salty taste, and firmness were significantly ($p < .05$) affected by high pressure. However, it is noteworthy that maturing time and cold storage have an effect on sensory properties of packaged dry-cured ham. For maturing time, sensory properties and above all, color properties, were less affected in more aged hams (lower impact of pressure on more dehydrated

meat). For cold storage, sensory color intensity increased even after 7 days of storage at 4°C. These high-pressure processed dry-cured hams are now on the market.

8.5.5 High-Pressure Assisted Freezing and Thawing of Meat and Meat Products

The new pressure-assisted freezing or thawing processes based on the phase diagram of water have been well reviewed by Cheftel, Levy, and Dumay (2000), and Cheftel, Thiebaud, and Dumay (2002, 2003). Water can remain in the liquid state down to about –22°C at pressure up to 210 MPa. This property allows rapid freezing and thawing of foods through pressure applications. During freezing, use of high pressure facilitates supercooling, and promotes uniform and rapid ice nucleation throughout the sample on pressure release, producing smaller ice crystals. Inversely, pressurization of frozen foods allows rapid thawing of a part of the ice. This rapid thawing could limit mass transfers from cells, responsible for drip loss during the thawing by running water. It is generally accepted that slow freezing rate results in large ice crystals, which generally damage the texture of food, whereas a rapid freezing rate produces small ice crystals, preventing the the cellular damage to food materials.

Martino, Otero, Sanz, and Zaritzky (1998) reported that high-pressure-assisted freezing was particularly useful for freezing large pieces of food when uniform ice crystals are required. In high-pressure-assisted freezing, samples are cooled under 200 MPa to –20°C without ice formation, then the pressure is released and the high supercooling reached (approx. 20°C) promotes uniform and rapid nucleation. The size and location of ice crystals in large meat pieces (*Longissimus dorsi* pork muscle) as a result of high-pressure-assisted freezing were compared to those obtained by air-blast and liquid N_2. Samples from the surface and center of the frozen muscle were histologically analyzed using an indirect technique. Air-blast freezing, having thermal gradients, showed nonuniform ice crystal distributions. High-pressure-assisted frozen samples, both at the surface and at central zones, showed similar small-sized ice crystals.

The following two points are important to prevent the deterioration of meat quality during thawing of the frozen meat: (a) shortening of the time for thawing, and (b) thawing at the lowest temperature possible (Massaux, Bera, Steyer, Cindic, and Deroanne 1999a, 1999b). Okamoto and Suzuki (2002) compared physicochemical and histological parameters (thawing loss, tenderness, drip, color, and ultrastructure) of the thawed pork meat under high pressure (100–500 MPa) with those of the meat thawed by running water. They concluded that the most desirable results were obtained when the frozen pork was pressurized at 200 MPa. Zhao, Flores, and Olson (1998) reported that high-pressure thawing also maintained the organoleptic properties of the bovine meat.

8.6 CONCLUSION

In this chapter, we reviewed high-pressure effects on the postmortem muscle in view of understanding the mechanism of the pressure-induced tenderization of meat or

acceleration of meat conditioning, and recent progress of pressure processing of meat products.

We can say that the tenderization of meat or acceleration of meat conditioning could be induced by high-pressure treatment, and the improvement of thermal gel formability of pressurized actomyosin, especially at low salt concentration, opens up the possibility for exploitation of new types of meat products. We also have to pay more attention to extending the shelf life of cooked meat products prepared by conventional methods by exposing them to high pressure.

We are sure that pressure-processed meat or meat products will be launched in the market in the near future because of the development of true commercial-scale high-pressure machines and reduced running costs.

ACKNOWLEDGMENTS

We wish to thank Professor J. C. Cheftel, Universite des Sciences et Techniques, Montpellier, France, for his critical reading of this chapter. This chapter was supported in part by a Grant-in-Aid for Scientific Research from the Japan Society for the Promotion of Science (JSPS: No. 15208025).

REFERENCES

Anderson, T. J., and F. C. Parrish, Jr. 1989. Postmortem degradation of titin and nebulin of beef steaks varying in tenderness. *J. Food Sci.* 54:748–749.

Asghar, A., K. Samejima, and T. Yasui. 1985. Functionality of muscle proteins in gelation mechanism of structured meat products. *CRC Critical Reviews in Food Science and Nutrition.* 22:27–106.

Baines, D., and J. Mlotkiewicz. 1984. The chemistry of meat flavor. In *Recent advances in the chemistry of meat,* ed. A. J. Bailey, 119–164. London: Royal Society of Chemistry.

Beilken, S. L., J. J. Macfarlane, and P. N. Jones. 1990. Effects of high pressure during heat treatment on theWarner-Blatzler shear force values of selected beef muscles. *J. Food Sci.* 55:15–18, 42.

Bouton, P. E., A. L. Ford, P. V. Harris, J. J. Macfarlane, and J. M. O'Shea. 1977. Pressure-heat treatment of post-rigor muscle: Effect on tenderness. *J. Food Sci.* 42:132–135.

Bridgman, P. W. 1914. The coagulation of albumen by pressure. *J. Biol. Chem.* 19:511–512.

Calkins, C. R., and G. W. Davis. 1980. Fragmentation index of raw muscle as a tenderness predictor of steaks from good and standard steer and bullock carcasses. *J. Anim. Sci.* 55:1067–1072.

Carlez, A., T. Veciana-Nugues, and J. C. Cheftel. 1995. Changes in colour and myoglobin of minced beef meat due to high pressure processing. *Lebensm-Wiss Technol.* 28:528–538.

Cheah, P. B., and D. A. Ledward. 1996. High pressure effects on lipid oxidation in minced pork. *Meat Sci.* 43:123–134.

Cheah, P. B., and D. A. Ledward. 1997. Inhibition of metmyoglobin formation in fresh beef by pressure treatment. *Meat Sci.* 45:411–418.

Cheftel, J. 1992. Effects of high hydrostatic pressure on food constituents: An overview. In *High pressure and biotechnology,* eds. C. Balny, R. Hayashi, K. Heremans, and P. Masson, 195–209. Montrouge: John Libbey Eurotext.

Cheftel, J. C. 1995. Review: High-pressure, microbial inactivation and food preservation. *Food Sci. Technol. Intern.* 1:75–90.

Cheftel, J. C., and J. Culioli. 1997. Effects of high pressure on meat: A review. *Meat Sci.* 46:211–236.

Cheftel, J. C., J. Levy, and D. Dumay. 2000. Pressure-assisted freezing and thawing: Principles and potential applications. *Food Rev. Int.* 16:453–483.

Cheftel, J. C., M. Thiebaud, and E. Dumay. 2002. Pressure-assisted thawing and freezing of foods: A review of recent studies. *High Pressure Res.* 22:601–611.

Cheftel, J. C., M. Thiebaud, and E. Dumay. 2003. High pressure-low temperature processing of foods: A review. In *Advances in high pressure and biotechnology*, ed. R. Winter, 327–340.Berlin: Springer.

Davey, C. L., and K. V. Gilbert. 1967. Structural changes in meat during ageing. *J. Food Technol.* 2:57–59.

de Lambellerie-Anton, E. M., R. G. Taylor, and J. Culioli. 2002. High pressure processing of meat. In *Meat processing: Improving quality,* eds. J. Kerry, J. Kerry, and D. A. Ledward, 313–331. Boca Raton, FL:CRC/Wood Head.

Defaye, A., D. A. Ledward, D. A. MacDougall, and R. F. Tester. 1995. Renaturation of metmyoglobin subjected to high isostaticpressure. *Food Chem.* 52:19–22.

Elgasim, E. A., and W. H. Kennick. 1982. Effect of high pressure on meat microstructure. *Food Microstruct.* 1:75–82.

Fukazawa, T., and T. Yasui. 1967. The changes in the zigzag configuration of the Z-line of myofibrils. *Biochim. Biophys. Acta.* 140:534–537.

Furst, D. O., M. Osborn, R. Nave, and K. Weber. 1988. The organization of titin filaments in the half-sarcomere revealed by monoclonal antibodies in immunoelectron microscopy: A map of ten repetitive epitopes starting at the Z line extends close to the M line. *J Cell. Biol.* 106:1563–1572.

Grebol, N. 2002. Commercial use of high hydrostatic pressure in sliced cooked ham in Spain. In *Trends in high pressure bioscience and technology,* ed. R. Hayashi, 385–388. Amsterdam: Elsevier Science.

Hayashi, R. 1992. Utilization of pressure in addition to temperature in food science and technology. In *High pressure and biotechnology,* eds. C. Balny, R. Hayashi, K. Heremans, and P. Masson, 185–193. Montrouge: John Libbey Eurotext.

Homma, N., Y. Ikeuchi, and A. Suzuki. 1994. Effects of high-pressure treatment on the proteolytic enzymes in meat. *Meat Sci.* 38:219–228.

Horowits, R., E. S. Kempner, M. E. Bisher, and R. J. Podolsky. 1986. A physiological role for titin and nebulin in skeletal muscle. *Nature.* 323:160–164.

Ikeuchi, Y., H. Tanji, K. Kim, and A. Suzuki. 1992a. Dynamic rheological measurements on heat-induced pressurized actomyosin gels. *J. Agric. Food Chem.* 40:1751–1755.

Ikeuchi, Y., H. Tanji, K. Kim, and A. Suzuki. 1992b. Mechanism of heat-induced gelation of pressurized actomyosin: Pressure-induced changes in actin and myosin in actomyosin. *J. Agric. Food Chem.* 40:1756–1761.

Ikkai, T., and T. Ooi. 1966. The effects of pressure on G-F transformation of actin. *Biochem.* 5:1551–1560.

Ikkai, T., and T. Ooi. 1969. The effects of pressure on actomyosin systems. *Biochem.* 8:2615–2622.

Ishioroshi, M., K. Samejima, and T. Yasui. 1983. Heat-induced gelation of myosin filaments at a low concentration. *Agric. Biol. Chem.* 47:2809–2816.

Ivanov, I., Y. N. Bert, and N. A. Lebedeva. 1960. Changes in some properties of myosin, actomyosin and actin under the influence of high pressure. *Biokhimiya.* 25:505–510.

Johnston, D. J. 1995. High pressure effects on milk and meat. In *High pressure processing of foods,* eds. D. A. Edward, D. E. Johnston, R. G. Earnshaw, and A. P. M. Hasting, 99–121. Nottingham, UK: Nottingham University Press.

Josephs, R., and W. F. Harrington. 1966. Studies on the formation and physical chemical properties of synthetic myosin filaments. *Biochem.* 5:3474–3487.

Josephs, R., and W. F. Harrington. 1967. An unusual pressure dependence for a reversibly associating protein system: Sedimentation studies on myosin. *Proc. Natl. Acad. Sci. USA.* 58:1587–1594.

Josephs, R., and W. F. Harrington. 1968. On the stability of myosin filaments. *Biochem.* 7:2834–2845.

Jung, S., E. M. de Lamballerie-Anton, R. G. Taylor, and M. Ghouk. 2000. High pressure effects on lysosome integrity and lysosomal enzyme activity in bovine muscle. *J. Agric. Food Chem.* 48:2467–2471.

Kennick, W. H., E. A. Elgasim, Z. A. Holmes, and P. F. Meyer. 1980. The effect of pressurization of pre-rigor muscle on post-mortem meat characteristics. *Meat Sci.* 4:33–40.

Kim, K., Y. Homma, Y. Ikeuchi, and A. Suzuki. 1993. Effect of high hydrostatic pressure on the conversion of α-connectin to β-connectin. *J. Biochem.* 114:463–467.

Kim, K., Y. Homma,Y. Ikeuchi, and A. Suzuki. 1995. Cleavage of connectin by calpain and cathepsin D. Biosci. *Biotech. Biochem.* 59:896–899.

Kimura, S., T. Matsumura, S. Ohtsuki, Y. Nakauchi, A. Matsuno, and K. Maruyama. 1992. Characterization and localization of α-connectin(titin 1): An elastic protein isolated from rabbit skeletal muscle. *J. Muscle Res. Cell Motil.* 13:39–47.

Kinsella, J. E., and D. M. Whitehead. 1989. Protein in whey: Chemical, physical and functional properties. *Adv. Food. Nutr. Res.* 33:343–438.

Knorr, D. 1996. Advantages, opportunities and challenges of high hydrostatic pressure application to food systems. In *High pressure bioscience and biotechnology,* eds. R. Hayashi and C Balny, 279–287. Amsterdam: Elsevier Science.

Ko, W. C., M. Tanaka, Y. Nagashima, T. Taguchi, and K. Amano. 1990. Effect of high pressure treatment on the thermal gelation of sardine and Alaska pollack meat and myosin paste. *Nippon Shokuhin Kogyo Gakkaishi.* 37:637–642.

Locker, R. H., and D. J. C. Wild. 1984. Tenderization of meat by pressure-heat involves weakening of the gap filaments in myofibrils. *Meat Sci.* 10:207–233.

Lusby, M., J. F. Ridpath, F. C. Parrish, Jr., and R. M. Robson. 1983. Effect of postmortem storage on degradation of the myofibrillar protein titin in bovine longissimus muscle. *J. Food Sci.* 48:1787–1790, 1795.

MacDougall, D. R. 1983. Instrumental assessment of the appearance of foods. In *Sensory quality in foods and beverage,* eds. A. A. Williams and R. K. Atkin, 121–129. Chicester, UK: Ellis Horwood.

Macfarlane, J. J. 1973. Pre-rigor pressurization of muscle: Effects on pH, shear value and taste panel assessment. *J. Food Sci.* 38:294–298.

Macfarlane, J. J. 1985. High pressure technology and meat quality. In *Developments in meat science 3,* ed. R. A. Lawrie, 155–184. London: Elsevier Applied Science.

Macfarlane, J. J., J. J. Mckenzie, R. H. Turner, and P. N. Jones. 1981. Pressure treatment of meat: Effect on thermal transitions and shear values. *Meat Sci.* 5:307–317.

Macfarlane, J. J., and D. J. Morton. 1978. Effects of pressure treatment on the ultrastructure of striated muscle. *Meat Sci.* 2:281–288.

Martino, M. N., L. Otero, P. D. Sanz, and N. E. Zaritzky. 1998. Size and location of ice crystals in pork frozen by high-pressure-assisted freezing as compared to classical methods. *Meat Sci.* 50:303–313.

Maruyama, K., S. Kimura, H. Yoshidomi, H. Sawada, and M, Kikuchi. 1984. Molecular size and shape of β-connectin, an elastic protein of striated muscle. *J. Biochem.* 95:1423–1433.

Maruyama, K., T. Yoshioka, H. Higuchi, K. Ohashi, S. Kimura, and R. Natori. 1985. Connectin filaments link thick fi1aments and Z-lines in frog skeletal muscle as revealed by immunoelectron microscopy. *J. Cell Biol.* 101:2167–2172.

Massaux, C., F. Bera, B. Steyer, M. Cindic, and C. Deroanne. 1999a. High hydrostatic pressure effects on freezing and thawing of pork meat: Quality preservation, processing times and high pressure treatment advantages. In *Advances in pressure bioscience and biotechnology,* ed. H. Ludwig, 485–488. Heidelberg, Germany: Springer.

Massaux, C., F. Bera, B. Steyer, M. Cindic, and C. Deroanne. 1999b. High hydrostatic pressure effects on freezing and thawing processes of pork meat. In *Advances in pressure bioscience and biotechnology,* ed. H. Ludwig, 497–500. Heidelberg, Germany: Springer.

Minerich, P. L., and R. Krug. 2003. Case study in real world: Use of high pressure technology for processing hams. In *2003 IFT annual meeting technical program,* 89. Chicago: IFT.

Nishimura, T., A. Hattori, and K. Takahashi. 1995. Structural weakening of intramuscular connective tissue during conditioning of beef. *Meat Sci.* 39:127–133.

Nishiwaki, T., Y. Ikeuchi, and A. Suzuki. 1996. Effects of high pressure treatment on Mg^{2+}-enhanced ATPase activity of rabbit myofibrils. *Meat Sci.* 43:145–155.

Nose, M., M. Yamagishi, and M. Hattori. 1992. Development of a processing system of meat and meat products by introducing high pressure treatment. In *High pressure and biotechnology,* eds. C. Balny, R. Hayashi, K. Heremans, and P. Masson, 321–323. Montrouge: John Libbey Eurotext.

O'Shea, J. M., D. J. Horgan, and J. J. Macfarlane. 1976. Some effects of pressure treatment on actomyosin systems. *Aust. J. Biol. Sci.* 29:197–207.

Ohmori, T., T. Shigehisa, S. Taji, and R. Hayashi. 1992. Biochemical effects of high hydrostatic pressure on the lysosomal enzymes and proteases. *Agric. Biol. Chem.* 56:1285–1288.

Okamoto, A., and A. Suzuki. 2002. Effects of high hydrostatic-thawing on pork meat. In *Trends in high pressure bioscience and technology,* ed. R. Hayashi, 571–576. Amsterdam: Elsevier Science.

Olson, D. G., F. C. Parrish, Jr., and M. H. Stromer. 1977. Myofibrillar fragmentation and shear resistance of three bovine muscles during postmortem storage. *J. Food Sci.* 41:1036–1041.

Ooi, T. 1994. Effects of pressure on thermal denaturation of proteins. In *High pressure bioscience* (in Japanese), eds. R. Hayashi, S. Kunugi, S. Shimada, and A. Suzuki, 16–24. Kyoto, Japan: Sanei Shuppan.

Orlien, V., and E. Hansen. 2000. Lipid oxidation in high pressure processed chicken breast muscle during chill storage: Critical working pressure in relation to antioxidation mechanism. *Eur. Food Res. Technol.* 211:99–104.

Ouali, A. 1984. Sensitivity to ionic strength of Mg^{2+}-Ca^{2+}-enhanced ATPase activity as an index of myofibrillar ageing in beef. *Meat Sci.* 11:79–88.

Patterson, B. C., and F. C. Parrish, Jr. 1986. A sensory panel and chemical analyses of certain beef chuck muscles. *J. Food Sci.* 51:876–879, 896.

Patterson, M. F., M. Quinn, R. Simpson, and A. Gilmour. 1996. High pressure inactivation in foods of animal origin. In *High pressure bioscience and biotechnology,* eds. R. Hayashi and C. Balny, 267–272. Amsterdam: Elsevier Science.

Ratcliff, D., P. E. Bouton, A. L. Ford, P. V. Harris, J. J. Macfarlane, and J. M. O'Shea. 1977. Pressure-heat treatment of post-rigor muscle: Objective-subjective measurements. *J. Food Sci.* 42:857–859, 865.

Riffero, L. M., and Z. A. Holmes. 1983. Characteristics of pre-rigor pressurized versus conventionally processed beef cooked by microwave and by boiling. *J. Food Sci.* 48:346–350, 372.

Saccani, G., G. Parolari, E. Tanzi, and S. Rabbuti. 2004. Sensory and microbiological properties of dried hams treated with high hydrostatic pressure. In *Proceedings of 50th International Congress of Meat Science and Technology,* 726–729. Helsinki, Finland: University of Helsinki.

Sano, T., S. F. Noguchi, J. J. Matsumoto, and T. Tsuchiya. 1988. Dynamic viscoelastic behaviour of natural actomyosin and myosin during thermal gelation. *J. Food Sci.* 53:924–928.

Seki, N., and T. Watanabe. 1984. Connectin content and its postmortem changes in fish muscle. *J. Biochem.* 95:1161–1167.

Shoji, T., H. Saeki, M. Wakameda, and M. Nonaka. 1990. Gelation of salted paste of Alaska pollack by high hydrostatic pressure and changes in myofibrillar protein in it. *Bull. Jap. Soc. Sci. Fish.* 56:2069–2076.

Suzuki, A. 2002. High pressure-processed foods in Japan and the world. In *Trends in high pressure bioscience and technology,* ed. A. Hayashi, 365–374. Amsterdam: Elsevier Science.

Suzuki, A., N. Homma, A. Fukuda, K. Hirao, T. Uryu, and Y. Ikeuchi. 1994. Effects of high pressure treatment on the flavour-related components in meat. *Meat Sci.* 37:369–379.

Suzuki, A., K. Hoshino, E. Sasaki, N. Sano, M. Nakane, Y. Ikeuchi, and M. Saito. 1987. Postmortem changes in native connectin in rabbit skeletal muscle. *Agric. Biol. Chem.* 52:1439–1444.

Suzuki, A., K. Kim, N. Honma, Y. Ikeuchi, and M. Saito. 1992. Acceleration of meat conditioning by high pressure. In *High pressure and biotechnology,* eds. C. Balny, R. Hayashi, K. Heremans and P. Masson, 219–227. Montrouge: John Libbey Eurotext.

Suzuki, A., K. Kim, and Y. Ikeuchi. 1996. Proteolytic cleavage of connectin/titin. *Adv. Biophys.* 33:53–64.

Suzuki, A., K. Kim, H. Tanji, and Y. Ikeuchi. 1992. Effects of high hydrostatic pressure on postmortem muscle. *Recent Res. Devel. in Agri. Biol. Chem.* 2:307–331.

Suzuki, A., A. Okamoto, Y. Ikeuchi, and M. Saito. 1993. Pressure-induced Ca^{2+} release from rabbit sarcoplasmic reticulum. *Biosci. Biotech. Biochem.* 57:862–863.

Suzuki, A., A. Okamoto, Y. Ikeuchi, and M. Saito. 1994. Effect of high pressure treatment on sarcoplasmic reticulum. *High Pressure Res.* 12:255–261.

Suzuki, A., M. Watanabe, Y. Ikeuchi, M. Saito, and K. Takahashi. 1993. Effects of high pressure treatment on the ultrastructure and thermal behaviour of beef intramuscular collagen. *Meat Sci.* 35:17–25.

Suzuki, A., M. Watanabe, K. Iwamura, Y. Ikeuchi, and M. Saito. 1990. Effect of high pressure treatment on the ultrastructure and myofibrillar protein of beef skeletal muscle. *Agric. Biol. Chem.* 54:3085–3091.

Suzuki, T. 1991. Effect of pressure treatment on the muscle structure and heat-induced gelation of myofibril proteins. In *High pressure science for food,* ed. R. Hayashi, 273–284. Kyoto, Japan: Sannei Shuppan.

Takahashi, K., T. Fukazawa, and T. Yasui. 1967. Formation of myofibrillar fragments and reversible contraction of sarcomere in chicken pectoral muscle. *J. Food Sci.* 32:409–413.

Takahashi, K., and H. Saito. 1979. Post-mortem changes in skeletal muscle connectin. *J. Biochem*. 85:1539–1542.

Tatsumi, R., A. Hattori, and K. Takahashi. 1996. Splitting of connectin/titin filaments into β-connectin/T2 and a 1,200-kDa subfragment by 0.1mM calcium ions. *Adv. Biophys*. 33:65–77.

Ueno, Y., Y. Ikeuchi, and A. Suzuki. 1999. Effect of high pressure treatment on intramuscular connective tissue. *Meat Sci*. 52:143–150.

Wang, K., J. McClure, and A. Tu. 1979. Titin: Major myofibrillar component. *Proc. Natl. Acad. Sci, USA*. 76:3698–3702.

Yamamoto, K., T. Miura, and T. Yasui. 1990. Gelation of myosin filament under high hydrostatic pressure. *Food Struct*. 9:269–277.

Yasui, T., M. Ishioroshi, and K. Samejima. 1980. Heat-induced gelation in the presence of actin. *J. Food Biochem*. 4:61–78.

Yuste, J., M. Cappellas, R. Pla, D. Y. C. Fung, and M. Mor-Mur. 2001. High pressure processing for food safety and preservation: A review. *J. Rapid Meth. and Autom. in Microbiol*. 9:1–10.

Zhao, Y., R. A. Flores, and D. G. Olson. 1998. High hydrostatic pressure effects on rapid thawing of frozen beef. *J. Food Sci*. 63:272–275.

Zipp, A., and W. Kauzmann. 1973. Pressure denaturation of metmyoglobin. *Biochem*. 12:4217–4428.

9 Hydrodynamic Pressure Processing to Improve Meat Quality and Safety

Morse B. Solomon, Martha N. Liu, Jitu R. Patel, Brian C. Bowker, and Manan Sharma
Food Technology and Safety Laboratory, USDA*

CONTENTS

9.1 HDP Technology and Meat Tenderness Enhancement 222
9.2 Alternative Methods to Produce Shock Waves 229
9.3 HDP, Muscle Proteins, and Mechanisms of Meat Tenderization 230
9.4 HDP and Further Processed Meat Products 233
9.5 HDP and Food Safety Enhancements 235
9.5.1 HDP and Microbial Inactivation 236
9.5.2 High-Pressure Processing and Microbial Inactivation 237
9.5.3 Product Shelf Life 238
Acknowledgments 239
References 239

Two of the major concerns in the meat industry today are food safety and inconsistent meat quality, especially tenderness. A means of controlling and assuring a meat product's safety and tenderness level is essential. Tenderness is the major criterion driving consumers' decisions to purchase or repurchase meat. Unfortunately, tenderness has proven to be the most difficult quality factor for meat producers and meat packers to manage. Thus, a commercial method to ensure a consistently tender meat product is of primary importance for enhanced consumer acceptance of meat. A variety of techniques have been utilized for tenderizing meat. Techniques applied individually or in combination include mechanical, chemical, temperature conditioning, aging, electrical stimulation, high pressure heat treatments, and alternative carcass positioning. A number of these techniques require additional holding periods,

* Mention of brand or firm names does not constitute an endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

space, and labor. Furthermore, several of these methods have been criticized for their lack of consistency in tenderizing meat.

The concept of tenderizing meat using shock waves from underwater detonation of explosives, called hydrodynamic pressure processing (HDP), was first patented by Godfrey (1970). *Hydrodynamics* refers to the motion of fluids and the forces acting on solid bodies immersed in these fluids. The HDP process should not be confused with research using high hydrostatic pressure (HHP), which was introduced by Japanese scientists and dates back to the end of the 19th century. Hydrostatics refers to the characteristics of liquids at rest and the pressure in a liquid or exerted by a liquid on an immersed object. In HHP technology, meat products are submerged in fluid in a pressure vessel and pressure is generated by pumping fluid into the closed vessel. In contrast to the instantaneous shock wave generated using HDP, HHP technology tenderizes meat with constant pressure exerted over an extended duration (minutes or hours) and at temperatures as high as 40°C.

The Hydrodyne process (Long 1993) and equipment (Long 1994) patented by John Long of Hydrodyne, Inc., was designed to overcome deficiencies of the original patent by Godfrey (1970) for tenderizing meat using an explosive charge in a tank filled with water. Long explained that Godfrey's principle was sound, but the tank and position of the meat in relation to the tank and explosive charge would present serious difficulties for the commercial tenderization of meat. Long's modifications included a shock-absorbing support system for a suspended vessel with a baffled locking cover to recover water expelled during the detonation.

In 1992, the U.S. Department of Agriculture's Food Technology and Safety Laboratory (FTSL), formerly called the Meat Science Research Laboratory, began testing the HDP concept and developing the HDP technology as part of a Cooperative Research and Development Agreement (CRADA) project with Hydrodyne. Solomon and Long (1995) and Solomon, Long, Eastridge, and Carpenter (1995) were the first to report on the use of HDP to tenderize meat. Improvement in meat tenderness for a variety of muscle foods has been demonstrated using HDP technology (Berry, Solomon, Johnson, et al. 1997; Berry, Solomon, Zuckerman, Eastridge, and Long 1997; Claus et al. 2001a; Eastridge, Solomon, West, Hammond, and Chase 1998; Marriott, Wang, Solomon, and Moody 2001; Meek et al. 2000; Moeller et al. 1999; O'Rourke, Calkins, Rosario, Solomon, and Long 1997a, 1997b, 1998; Solomon 1998a, 1998b, 1999; Solomon, Carpenter, Snowder, and Cockett 1998; Solomon, Long, and Eastridge 1997). Zuckerman and Solomon (1998) and Zuckerman, Berry, Eastridge, and Solomon (1999) were the first to identify a cause of tenderization, namely, disruption of the myofibrillar structure and the structural integrity of the muscle and connective tissue complex. The HDP-treated meat displayed no outward signs of change, but on cooking was found to be significantly more tender than nontreated control samples. Furthermore, a direct relationship between the tenderness of HDP-treated meat and the redistribution of proteins in the homogenate fraction of the HDP-treated samples was reported (Spanier and Romanowski 2000). Spanier and Romanowski (2000) found that the response to HDP was similar to that seen when a myofibrillar fragmentation index is used to correlate the level of meat tenderness during the aging of meat.

HDP involves underwater detonation of a high-energy explosive in a containment vessel to generate a shock wave pressure front at velocities exceeding the speed of sound. The shock wave passes through the liquid medium and vacuum-packaged meat (placed at the bottom of the container). Food products that contain a considerable amount of water and little dissolved gas have compressibility properties similar to that of water. To be an acoustical match with water, the object—in this case, meat—must possess an E/D ratio similar to water, where E is the bulk modulus of elasticity and D is the density of the object (Kolsky 1980). Because it contains approximately 75% water, boneless meat is an acoustical (mechanical impedance) match with water (Kolsky 1980) and is quite suitable for this process. The shock wave in water reflects off any object that is not an acoustical match (Kolsky 1980). Shock waves generated and transmitted through water move equidistant from an explosive source depending on the shape of the explosive (Batsanov 1994). The shock wave, with targeted pressure fronts of 70 MPa to 100 MPa in the HDP process, occurs in fractions of milliseconds. Hugoniot (adiabatic) compression of water (or of aqueous solutions) causes a temperature increase of only 2 to 3°C per 100 MPa, depending on the initial temperature and the rate of pressure increase (Batsanov 1994). Pressure release causes a decrease in temperature of the same order of magnitude. Because the dynamics of the shock wave pressure front in the HDP process occur in fractions of milliseconds and at pressures less than 100 MPa, there is virtually no increase in the temperature of the meat or water.

Shock waves are naturally produced from cosmic explosions, thunderstorms, volcanic eruptions, and meteoroid impacts. In general, a shock wave is defined as a very sharp, thin wave front (Glass 1994). Detonation of an explosive underwater produces both a shock wave and a gas bubble. When a shock wave is generated, the wave front raises the surrounding materials to a high pressure, then induces a flow velocity behind the wave and quickly subsides (Glass 1994). A flow velocity is similar to an aftershock or reverberation of subsiding pressure waves. Cole (1948) defined the shock wave as the largest single source of energy. The first wave front to reach the meat surface in the HDP process is a compression wave (Craig and Rye, personal communication; Gustavson, Lee, Chambers, Solomon, and Berry 2001). Secondary pressure pulses from the resultant gas sphere contractions are apparent in a longer duration than the shock wave, but the energy associated with the gas sphere is typically one third or less of the shock wave energy (Cole 1948) and is dependent on the type and composition of the explosive used. The effects of the gas sphere-generated energy (secondary wave) on the submerged object (meat) depend on the geometry and composition of the explosive charge, target, and shape of the nearby surfaces. As the wave front traveling at hypervelocity initially passes through the meat, a compression force is generated (Hyde 2005). The shock wave generated from the detonation of an explosive decays quickly while expanding. It has a curved front, normally not a perfect sphere, and therefore the pressure jump across its front changes with time and location. As the primary wave front reaches the free unsupported acoustic mismatch surface at the bottom of the container, the wave front is reflected off the bottom of the container by a steel platform. As a result, a near doubling in pressure occurs with the transient pressure wave changing to a tensile wave (Craig and Rye, personal communications; Gustavson et al. 2001; Hyde

2005). The relationship between the wave velocity and the wave period of the ensuing shock wave appear to be critical components of the HDP process for successful meat tenderization.

The explosives used in the HDP develop a very rapid and shattering action when exploded on detonation. This shattering power is referred to as the *brisance* of the explosive. By varying the composition (binary, molecular) and the shape (cylindrical, rectangular) of the explosive, the brisance properties can be adjusted to suit the particular purpose for which it is to be used. The various explosives that can be used in the HDP process differ in the amount of gas that is produced per quantity of explosive, in the amount of heat liberated, in the detonation velocity, in the pressure that gases exert on their immediate surroundings, and in the shattering power developed by the explosive. Thus, a number of these parameters influence the shape and strength of the resultant shock wave.

9.1 HDP TECHNOLOGY AND MEAT TENDERNESS ENHANCEMENT

Research has suggested that although HDP does improve meat tenderness, the magnitude of tenderness improvement is conditional on the configuration and composition of the shock wave container and type of shock absorbers used (Solomon and Berry 2000; Solomon, Berry, Eastridge, Paroczay, et al. 1999; Solomon and Eastridge 1999), quantity of explosive (Solomon 1998a), placement of the explosive (Solomon 1998a, Solomon and Eastridge 1999), shape of the explosive (Eastridge, Solomon, Paroczay, and Callahan 2002; Solomon, Liu, Patel, Paroczay, and Eastridge 2004), boundary conditions of the shock wave container (Eastridge, Solomon, West, and Chase 2000; Solomon and Berry 2002), and geometry of the steel reflecting surface of the container (Callahan, Berry, and Solomon 2004). Further understanding of the properties that affect the response of the muscle that results in the greatest tenderization needs to be evaluated; whether the muscle is treated fresh or frozen/thawed (Berry, Solomon, Zuckerman, et al. 1997; Solomon et al. 2004), conditions after treatment (Callahan et al. 2002; Liu, Callahan, Solomon, Vinyard, and Patel 2004; Solomon, Berry, Paroczay, Callahan, and Eastridge 2002), postmortem time of treatment (Paroczay, Solomon, Berry, Eastridge, and Callahan 2002), and other parameters yet to be determined.

Three types of shock wave containers have been used in research pertaining to the HDP process: (a) plastic explosive containers (PEC; 208-L, 115-L, and 98-L) fitted with a flat, steel reflector plate (figure 9.1); (b) a stationary 1060-L commercial prototype steel vessel (CHU; figure 9.2); and (c) a stationary, small-scale 54-L laboratory model steel vessel (LHU; figure 9.3). The HDP process (Solomon, Berry, Eastridge, Paroczay, et al. 1999) generated in a 115-L PEC resulted in a 43% tenderness improvement for boneless beef strip loins, reducing shear force (8.3 kg to 4.7 kg) compared to samples treated with HDP in the CHU (original commercial prototype Hydrodyne unit with four shock absorbers; figure 9.4), which improved shear force by 33% (8.2 kg to 5.5 kg). As shock-absorbing devices were added (and their composition changed from rubber to steel) to support the CHU vessel, the

FIGURE 9.1 Plastic explosive container (PEC) with steel plate that gets seated inside the bottom of the container.

FIGURE 9.2 Stationary 1060-L commercial prototype steel vessel (CHU) with 16 shock absorbers.

FIGURE 9.3 Stationary 54-L small-scale laboratory model steel vessel (LHU).

FIGURE 9.4 Stationary 1060-L commercial prototype steel vessel with four shock absorbers.

performance of meat tenderization significantly decreased (Solomon and Eastridge 1999). Control samples (Solomon and Eastridge 1999) had shear values of 7.0 kg. Shear values as a result of HDP generated in PECs decreased to 3.7 kg. Shear values in the CHU with four shock-absorbing supports decreased to 4.2 kg (figure 9.4). However, shear values in the CHU with eight shock-absorbing supports (figure 9.5) were 5.9 kg and in the CHU with 16 shock-absorbing supports (figure 9.2) were 6.2 kg, which were not significantly different from the controls. Results from Solomon and Eastridge (1999) suggested that type of container and shock-absorbing system play an important role in the performance of the HDP process to successfully tenderize meat. Solomon and Berry (2000), comparing the plastic containers with the LHU model, reported that HDP generated in 115-L PECs resulted in a 40% improvement in shear force (6.8 to 4.1 kg) compared to samples treated with HDP in the 54-L LHU (figure 9.3), which improved 28% (6.8 to 4.9 kg).

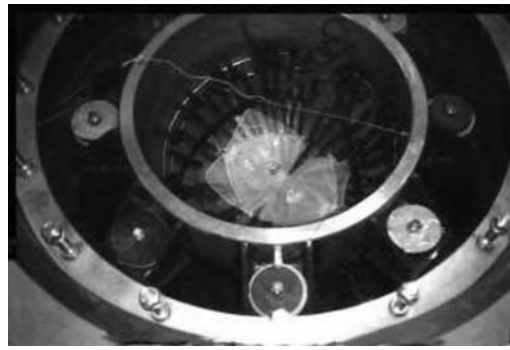


FIGURE 9.5 Stationary 1060-L commercial prototype steel vessel with eight shock absorbers.

In developing parameters for tenderizing meat, the majority of HDP experiments have used a binary explosive composed of a liquid and a solid. Additional trials evaluated the effectiveness of molecular-based explosives (Eastridge et al. 1998; Marriott et al. 2001; Solomon 1998b). Results suggest that at lower pressure fronts (< 100 MPa), binary explosives are more effective at improving tenderness than molecular explosives, but at higher pressure fronts (> 125 MPa) molecular explosives may be more effective than binary ones. The magnitude of tenderness improvement from HDP is also dependent on several additional factors: the quantity of explosive used (Meek et al. 2000; Solomon 1998a; Solomon, Long, and Eastridge 1997), as more is not always better; and the shape of the explosive charge (Eastridge et al. 2002; Solomon et al. 2004), where rectangular (folded) charges were compared to cylindrical-shaped charges. Charge shape affects the dynamics of HDP; a cylindrical-shaped (figure 9.6) charge placed horizontally parallel to meat results in a more uniform tenderizing effect than a rectangular-shaped (figure 9.6) charge (Eastridge et al. 2002). The rectangular-shaped charge resulted in 10% reduction in shear force, and changing to a cylindrical shape reduced shear force by 16%. With the rectangular charge, a higher percentage of tenderness improvement was achieved (up to 38% improvement); however, the degree of improvement among all samples was more variable. The cylindrical-shaped charge had higher mean improvements than the rectangular shape but among samples shear force was less variable. In one study (Solomon et al. 2004), a larger proportion of samples treated by HDP using the rectangular-shaped charge (81%) were successfully tenderized compared to the cylindrical shape (56%). Successful tenderization was defined as 10% or more improvement in shear force values.

The position (distance) of the charge from the meat source (Eastridge et al. 1998; Meek et al. 2000; Solomon 1998b) showed that although peak pressure fronts increase as the explosive charge is moved closer to the meat, the results indicate that tenderness improvements are inconsistent. Different contact boundary conditions for the PEC containers have been evaluated (Eastridge et al. 2000; Solomon and Berry 2002; Spanier, Berry, and Solomon 2000). Eastridge and coworkers (2000) reported that the deeper the air boundary for suspended PEC containers (suspended in air by plastic ropes), the more effective the HDP treatment (figure 9.7). Spanier

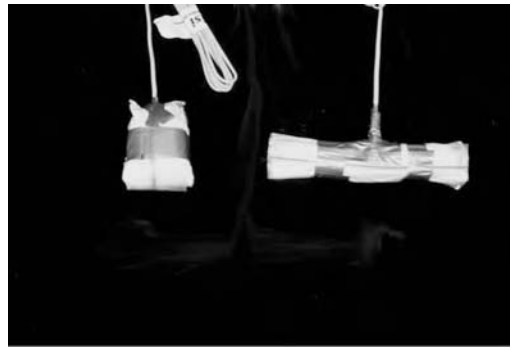


FIGURE 9.6 Shape of charges: Charge on left is rectangular (folded) shape; charge on right is cylindrical shape.

FIGURE 9.7 Plastic explosive container (PEC) suspended in air by plastic ropes.

and coworkers (2000) found that HDP treatment of beef strip loins was better using PEC containers suspended in air by plastic ropes (40% improvement) versus PECs seated on a styrofoam base (21% improvement). Solomon and Berry (2002) reported that type of support surface (soil vs. concrete) and surrounding boundary environment (air vs. water) on soil for placement of the plastic shock wave containers influenced the performance of the HDP process for tenderizing meat. Beef strip loin

shear force was improved 24% (11.7 to 8.9 kg) using a concrete support surface with the container surrounded by air. A 39% shear force improvement (11.9 to 7.3 kg) was found for a soil support surface with the container surrounded by air and a 24% shear force improvement (12.1 to 9.2 kg) with a soil support surface with the container surrounded by water.

Beef eye of rounds were used in a study (Callahan et al. 2004) designed to test the theory that maximum tenderizing effects would occur when the reflector plate has a curved (bowl) shape similar to the shape of the emanating shock wave. A 41-cm diameter weld cap was used as the curved reflector bowl on the bottom of a PEC in place of the standard 1.3-cm-thick, 41-cm-diameter flat steel plate. A 150-g folded, rectangular-shaped charge was detonated at 31 cm above the surface of the meat. Immediately after HDP treatment, 3.2-cm thick steaks were removed from control and HDP samples for shear force evaluation. Cooking time and yield were similar for control and HDP samples. The resulting 5% tenderness improvement, although statistically significant, was highly variable (–9% to 25%) among individual samples, and overall was not considered a successful treatment. The curved reflector bowl did not appear to improve on the tenderization effects of HDP treatment.

Berry, Solomon, Johnson, et al. (1997) conducted two studies to evaluate the sensory and cooking properties from HDP-treated U.S. Select grade strip loins using PECs. The first experiment involved frozen and thawed loins that were treated with HDP. Steaks were cooked to 65°C, 71°C, or 77°C. Steaks were evaluated for sensory properties by a 10-member trained panel. With previously frozen loins, HDP produced significantly higher tenderness ratings and shorter cooking times than controls. Increasing endpoint temperature produced lower cooking yields, longer cooking times, and more well-done cooked color scores, but had no effect on sensory properties. In the second experiment, fresh (never frozen) loins were subjected to HDP. HDP significantly improved tenderness scores compared to controls. In both studies, juiciness and flavor scores were not affected by HDP treatment. Furthermore, Solomon and coworkers (2004) found that HDP improved tenderness in both fresh and frozen and thawed meat samples but the magnitude of instantaneous improvement was higher in fresh meat samples, which was probably due to the intrinsic improvement with the freeze–thaw cycle. After six days of refrigerated storage, tenderness improvement resulting from HDP treatment was sustained for both fresh and frozen and thawed meat samples (shear force values were ≤ 3.9 kg compared to 4.5 kg for control samples). Solomon, Eastridge, Paroczay, and Coleman (2001) reported that the HDP treatment yielded instantaneous improvements in tenderness and these improvements were maintained even after a period of frozen storage. To further evaluate the ability of HDP to tenderize tough meat, intentionally cold-shortened loins with initial shear values of 8.3 kg were treated with HDP and the percentage improvements in tenderness ranged from 37% to 47% for HDP samples (Solomon, Eastridge, and Long 1997).

Variability in instantaneous tenderization using HDP was elucidated to be related to time postmortem of application of the treatment (Paroczay et al. 2002). Fresh beef strip loins were HDP-treated either on Day 2, 4, or 7 postmortem. Instantaneous improvements in tenderness were detected (11% for Day 2, 25% for Day 4, and 28% for Day 7). Shear force values of nontreated control samples tended to improve

(from 7.1 to 6.2 kg) due to aging from two to seven days; however, these differences were not significant. HDP-treated samples showed no additional improvement in tenderness with aging. Solomon and coworkers (2002) found that regardless of time of application of HDP, treated beef ages faster and to a greater degree than nontreated controls. Fresh U.S. Select beef loins (two days postslaughter) were cut into sections; one section was randomly assigned to HDP and the remainder served as control. After HDP, a steak was removed from each section (both control and HDP) representing 0 days of aging. Additional steaks were evaluated at Days 5, 12, 16, 20, 23, 26, and 29 for controls and on Days 5, 12, and 16 for HDP. Tenderness of control steaks improved from Day 0 to Day 12 but no additional improvements were detected beyond Day 12. HDP treatment instantaneously improved tenderness (28% improvement compared to controls) on Day 0 and samples continued to improve through Day 5 to 3.9 kg and through Day 16 to 3.1 kg shear force. Control samples never reached 4 kg shear force although they were aged for 29 days. HDP treatment improved meat tenderness to a greater extent than extended aging alone in nontreated controls. Similar improvements in tenderness with aging were found with pork loins (Callahan et al. 2002). Pork loins that were treated with HDP at two days postslaughter were evaluated for shear force tenderness on the day of the HDP treatment and then after aging for 3, 5, 11, and 18 days. No improvements in tenderness were found on Day 0, but HDP-treated steaks reached maximum tenderness by Day 11 (3.8 kg), whereas the nontreated controls required an additional seven days of aging (18 days total) to reach the same level of tenderness (3.8 kg).

Two studies (O'Rourke et al. 1999; Solomon, Berry, Eastridge, and Paroczay 1999) were conducted to determine whether the orientation of muscle fibers during the HDP process has any effect on the response for successful tenderization. Horizontally (based on muscle fiber orientation) positioned *Semitendinosus* muscle sections resulted in increased improvements in tenderization compared with vertically positioned muscle samples.

In the future, it is likely that HDP may be combined with other processes to provide maximum tenderization improvement. Some of the least tender beef muscles are found in the round, and among beef breeds, Brahman cattle are considered to produce less tender meat. A study was conducted (Liu et al. 2004) to evaluate the efficacy of HDP, blade tenderization (BT), or the combination of BT followed by HDP (BTH) for tenderizing top rounds from Brahman cattle. The top rounds had shear values greater than 6.8 kg determined (prescreened) prior to the study. BT and BTH samples were passed once through a blade tenderizer. Tenderness was determined by shear force measurements on steaks from each treatment and its paired control on Days 0 and 7. Immediately posttreatment, all three treatments significantly improved tenderness compared to controls (18% for BT and HDP alone, 13% for BTH), but no differences were detected among treatments. Treated samples on Day 0 had tenderness values equivalent to control samples that were aged for seven days. Tenderness continued to improve with aging for HDP and BTH treatments (29% and 37% improvement, respectively) but very little for BT-treated (17%) samples. These results show that BT, HDP, and BTH treatments are effective at reducing toughness in top rounds. Furthermore, treatments that include HDP provide greater tenderness after seven days of aging than BT alone.

Although the effectiveness of the HDP process has been repeatedly demonstrated (Berry, Solomon, Johnson, et al. 1997; Marriott et al. 2001; Solomon, Long, and Eastridge 1997; Spanier et al. 2000; Zuckerman, Berry, Eastridge, and Solomon 2002 [beef]; O'Rourke, Calkins, Rosario, Eastridge, et al. 1997; O'Rourke, Calkins, Rosario, Solomon, and Long 1997a, 1997b, 1998 [beef and pork]; Solomon 1998a, 1998b, 1999 [beef, pork, lamb]; Moeller et al. 1999 [pork]; Claus et al. 2001a; Meek et al. 2000 [poultry]), commercialization has been hampered by four technical problems: safety, throughput, packaging, and performance. Aside from the obvious safety concerns when using explosives, the use of explosives necessitates a type of batch processing system that is incompatible with the high throughput of most modern meat processing plants. Meat has to be vacuum packaged and all the air removed from each package to prevent rupturing or tearing during the HDP process. The vacuum-packaging material for the HDP process must be robust enough to withstand the brisance or shattering power of the explosive. Before these issues can be resolved to develop a successful commercialized system, further understanding of how to maximize the shock wave properties and the effects of the shock waves on the muscle system must be determined.

9.2 ALTERNATIVE METHODS TO PRODUCE SHOCK WAVES

Thus, it is evident that because of some of the technical problems, HDP has yet to become successfully commercialized. Meanwhile the search for a novel, nonexplosive, nonpyrotechnic processing technology has become a high priority. A nonexplosive, nonpyrotechnic shock wave meat processing tenderization scheme described in a U.S. patent (Tate, Sellers, Solomon, and Berry 2004) is being investigated under a CRADA with FTSL and Spectra Research. The proposed method and apparatus offers the potential of a meat tenderizing process similar in its effects to HDP, but with several potential advantages. This system does not use explosives. There is no requirement to vacuum package the meat, thus the meat can be processed in air. There is the potential for user-friendly equipment sized and scaled for the end user, whether it is the meat packer, processor, distributor, local market, or even the consumer. Meat is positioned within an interior volume of a decompression chamber. A pressure differential is created across a gas output in communication with the interior volume of the decompression chamber followed by rapid decompression by transferring gas from the interior volume of the decompression chamber through the gas output. This technology is based on the nonpyrotechnic, nonexplosive Audio/Visual Cueing Device and Explosion Simulation Technology by Spectra Research.

A plasma pulse sparking device for tenderizing meat as described in a Canadian patent (Cooper and Solomon 2000) has been studied. This system is similar in principle to the noninvasive electrically induced shock wave lithotripter acoustic field technology used for treatment of kidney stones. In lithotripsy, several thousand focused shock waves are administered to the patient, causing the stone to fragment and subsequently be voided through urine. A typical lithotripter pulse has a positive

peak amplitude of 40MPa to 100 MPa, and is 5 μs long with transient pulses being delivered at a rate of 1 Hz. Temperature rise associated with the lithotripter pressure pulse is negligibly small, and hence the primary effect of the electrically induced shock wave on biological media is of a mechanical nature. Rarefaction properties related to the creation of tensile pressure waves will be examined for successful tenderization of fresh and frozen and thawed meat products.

Claus and coworkers (2001b) evaluated the tenderization of chicken and turkey breasts with an electrically based pulsed plasma (ESW) technology (U.S. patent; Long 2000) for producing hydrodynamic shockwaves. They found that two or more pulse-firing networks from the ESW unit were needed to improve the tenderness of early deboned postrigor chicken (22% improvement) and turkey (12% improvement) breasts. Additional research (Claus, Sagili, and Sammel 2002; Sagili and Claus 2003) further investigated tenderizing beef cuts with the ESW system. Average tenderness improvement using ESW (Claus et al. 2002) for hot-boned beef strip loins ranged from 18% to 24%, whereas eye of rounds improved 22%, bottom rounds 8%, and top rounds by 23%. Average tenderness improvement using ESW (Sagili and Claus 2003) for top rounds was 17% to 19%, whereas a tenderization gradient was observed for strip loins (rib end 16% improvement and sirloin end 32% improvement). Their work (Claus et al. 2002; Sagili and Claus 2003) found that the ESW system has the potential to eliminate the need for aging and could replace the existing BT process used to tenderize meat cuts. A capacitor-discharged pulsed plasma sparking system has begun to be evaluated for tenderizing various muscle foods. The delivery system for continuous treatment through either submersion in noncompressible fluids or a jacketed nonsubmersible system is being developed and evaluated.

A meat tenderization system (Garcia and Woodall 2001) that uses axial planar shockwaves from two-dimensional electro-mechanical transducers located close to the target source will be considered. A doubling of the energy density by having two axially aligned parallel electro-mechanical transducers should achieve a similar reflected energy wave (Garcia and Woodall 2001; Solomon, et al. 1997b) as with the HDP system. The use of electromechanical transducers would allow for an unlimited and continuous firing tunable system without the explosive safety issues.

9.3 HDP, MUSCLE PROTEINS, AND MECHANISMS OF MEAT TENDERIZATION

Meat tenderness is a complex palatability trait that is largely influenced by the interacting effects of the postrigor contraction state, the proteolytic breakdown of muscle proteins, the integrity of the ultrastructural components of muscle fibers, and connective tissue. Due to limited research, however, the specific mechanisms by which HDP shock waves alter such muscle tissue properties to yield meat tenderization are not fully understood.

The ability of high-pressure treatments to alter the properties of muscle proteins and tenderize meat has been well documented using high hydrostatic pressure processing (HHP). Studies using HHP to tenderize meat have shown that high pressure can cause muscle proteins to undergo physicochemical changes such as denaturation,

solubilization, aggregation, and gelation depending on pH, ionic strength, temperature, and pressure level (Fernandez-Martin, Cofrades, Carballo, and Jimenez-Colmenero 2002; Macfarlane 1985; Macfarlane and McKenzie 1976). HHP has been shown to fragment sarcomere structure (Bouton, Harris, Macfarlane, and O'Shea 1977; Macfarlane and Morton 1978; Suzuki, Suzuki, Ikeuchi, and Saito 1991; Suzuki, Watanabe, Iwamura, Ikeuchi, and Saito 1990), disrupt sarcoplasmic reticulum (SR) membranes (Elgasim and Kennick 1982), and depolymerize myofibrillar proteins (Macfarlane 1985). Additionally, high-pressure treatments have been shown to influence calpastatin and calpain activities (Homma, Ikeuchi, and Suzuki 1995; Koohmaraie, Kennick, Elgasim, and Anglemier 1984), cause a release of lysosomal enzymes (Elgasim and Kennick 1982), and enhance proteolytic meat tenderization. Although further research is needed, HDP-induced muscle protein and ultrastructural alterations related to tenderness are thought to potentially have some similarities to those observed with HHP-generated high-pressure treatments.

Because myofibrillar and stromal proteins play such a key role in meat tenderness, the ability of HDP to tenderize meat is most likely due to its effect on these types of proteins.

The instantaneous tenderizing effect that is observed in HDP-treated meat is most likely due to the direct physical disruption that occurs within and between myofibrils due to the high-pressure shock wave. Using transmission electron microscopy on bovine *Longissimus* muscle, Zuckerman and Solomon (1998) and Solomon, Eastridge, Zuckerman, Long, and Johnson (1997) demonstrated that HDP tenderizes meat by fragmenting and disrupting the sarcomere structure within myofibrils. In particular, HDP treatment caused myofibrillar fragmentation in the I-band regions adjacent to Z-lines with fragments of the Z-lines remaining attached to the A-band on both sides of the fractures. Coupled with the knowledge that the I-band region has a flexible molecular structure and the A-band is more rigid in nature due to rigor bond formation, the visual data seem to confirm the theory that the HDP shock waves pass through and physically disrupt the susceptible I-band region more than the A-band. Additionally, Solomon, Eastridge, Zuckerman, et al. (1997) observed increased intramyofibrillar spaces with longitudinal gaps in the myofibril lattice, and a narrowing of the M-line in HDP-treated muscle. Although some of these ultrastructural differences with HDP are similar to the alterations observed with the enzymatic removal of Z-disks and degradation of M-lines by calpains during aging (reviewed by Koohmaraie 1992), the initial tenderness improvement with HDP probably does not occur by the same mechanism as aging. It is not fully understood, however, if this physical disruption explains the entire HDP tenderization mechanism.

Initial studies investigating the effects of HDP on muscle proteins have looked at the redistribution of the various protein fractions. Spanier and Romanowski (2000) found that as HDP-treated beef becomes more tender there is a decrease in the protein content of the myofibrillar protein fraction and an increase in the protein content of the sarcoplasmic fraction with subcellular fractionation by differential centrifugation. Differences in the protein distribution between the isolated sarcoplasmic and myofibrillar protein fractions between control and HDP-treated muscle provides evidence that HDP has an immediate effect on muscle proteins. This redistribution of intracellular proteins with HDP treatment might also account for

the decreased variation and increased uniformity of tenderness that is observed in HDP-treated meat (Spanier et al. 2000).

To investigate further the specific differences in the intracellular muscle protein distribution with isolation, several studies have attempted to identify HDP-induced protein modifications using higher resolution techniques. Using SDS-PAGE on solubilized myofibrillar proteins extracted from beef *Biceps femoris* muscles, Schilling and coworkers (2002) did not observe differences in the electrophoretic protein profiles between HDP and control samples. Similarly, they found no proteolytic differences between the sarcoplasmic protein fractions extracted from HDP and control samples using SDS-PAGE. However, (O'Rourke et al. 1998; O'Rourke et al. 1999; O'Rourke et al. 1997) observed slight differences between the electrophoretic protein profiles of myofibrils isolated from HDP and control muscles from both beef and pork. Varying results between the studies were likely due to differences in the HDP parameters, time postmortem, muscles, and species used in these studies. In the Schilling et al. (2003) study, beef *Biceps femoris* muscles were HDP treated in the 1060-L steel shock wave vessel and showed only a 20% improvement in tenderness over the untreated controls that were already tender at the start of the experiment (3.9 kg shear force). In the O'Rourke et al. (1997) study, however, pork loins were HDP treated in a 208-L plastic container and showed a 33% improvement in tenderness over the untreated controls with starting shear values of 5.6 kg. Thus, the protein differences observed in the O'Rourke et al. (1999) study are probably the result of a more effective HDP treatment than in the Schilling et al. (2002) study.

In addition to the direct effect of the physical disruption of the sarcomere and the fragmentation of the myofibrils, HDP shock waves also seem to have an indirect tenderizing effect on meat. The tenderizing effect of HDP on meat sometimes does not become apparent until a few days after HDP treatment (Paroczay et al. 2002; Solomon et al. 2002). It is hypothesized that this secondary tenderization is the result of the HDP shock waves acting on the muscle proteins in such a way as to influence the activity of proteolytic enzymes or the availability of substrates that play a role in the tenderization of meat with aging. It can be speculated that some of the same proteins affected by postmortem proteolysis and aging may also be altered by HDP treatment. The immediate impact of HDP on muscle proteins and the indirect tenderization effect of HDP are matters of ongoing research.

Zuckerman and coworkers (1999) demonstrated using scanning electron microscopy that HDP influences the arrangement of the collagen fibrillar network of beef *Semimembranosus* muscles. Scanning electron microscopy revealed slight disruption of the endomysial connective tissue in HDP-treated beef (Zuckerman et al. 1999); thus it was hypothesized HDP may impact tenderness of meat by increasing the proportion of soluble collagen in heated meat. Although HDP treatment (Eastridge, Solomon, Pierre, et al. 2002) resulted in 24% lower shear force (7.2 vs. 5.5 kg for control and HDP, respectively), no differences in total collagen (3.23 vs. 3.06 mg/g) or collagen solubility (11.9 vs. 10.5%) were detected for control versus HDP samples. These findings were consistent with those of Schilling et al. (2002) for the *Biceps femoris* muscle and Marriott et al. (2001) who utilized *Longissimus* muscle from older cattle. It appears that with these samples, the tenderizing effect of HDP does not impact the amount of soluble collagen in the muscle. To date, HDP effects

on collagen solubility and its correlation to tenderness are inconclusive. The effects of HDP on collagen solubility are difficult to ascertain given the low number of samples combined with HDP process variances.

Eastridge and coworkers (2005) utilized top rounds from Brahman cattle to determine the effect of BT, HDP and BTH on shear force and collagen solubility. All treated samples (12 top rounds) averaged 23% tenderness improvement compared to controls. Among treatments, there was no difference for total collagen. BT did not affect collagen solubility (6.9 vs. 6.5% for BT vs. control); however, the percentage of soluble collagen was significantly greater for HDP (8.6 vs. 6.5%) and BTH (8.1 vs. 5.5%) treatments compared to their respective controls. The combination BTH treatment did not show synergism; rather, the greatest effect on collagen solubility was due to HDP treatment. Tests of correlation showed that although collagen amount and solubility were negatively correlated ($p < .001$) to tenderness, the correlations were low. The HDP treatment resulted in a significant increase (62%) in collagen solubility compared to BTH (24%) and no difference due to BT treatment. The BT treatment appears to result in an antagonistic response for increasing collagen solubility. Neither Schilling et al. (2002) nor Marriott et al. (2001) detected collagen solubility differences due to HDP treatment. The *Biceps femoris* samples in the Schilling et al. (2002) study were treated with HDP in a 1060-L stationary vessel that was shown to have reduced tenderizing effect (Solomon and Berry 2000; Solomon, Berry, Eastridge, Paroczay, et al. 1999) compared to PECs. Marriott and coworkers (2001) treated *longissimus* muscle samples using HDP in a 98-L plastic vessel; however, those samples were from older animals that likely had mature, less soluble collagen. This led to the conclusion that connective tissue is more resistant to HDP treatment than the myofibrillar structure and that the tenderizing effect of HDP is in large part due to the influence of the shock wave on the myofibrillar proteins and sarcomere structure (Solomon, Eastridge, Zuckerman et al. 1997).

Overall, research on HDP technology has found that it has a significant tenderizing effect on meat, yet the exact mechanism by which this occurs is not well understood. Available data indicate that the physical disruption of the muscle ultrastructure likely is largely responsible for the instantaneous tenderization effect of HDP and it is speculated that any subsequent tenderization with aging is potentially the result of HDP-induced protein alterations that change enzyme–substrate interaction. Further research is needed, however, to determine the key proteins, fragments, and enzymes that play a role in HDP meat tenderization.

9.4 HDP AND FURTHER PROCESSED MEAT PRODUCTS

The effect of HDP on processed meat products has not been fully examined. The application of HDP to finished processed meat products is expected to result in similar improvement to tenderness and consistency as with whole muscle cuts. The mechanisms providing tenderization may result in improvements in protein functionality, which is defined as the ability of proteins to bind to other proteins to form a protein matrix and to bind water or fat, in the case of an emulsion sausage such as frankfurters. Thus, by treating the raw materials with HDP before use in further

processed meat products, it is expected that improved functionality will lead to the development of uniquely textured products and also provide cost savings for the processor and consumer.

During HDP treatment of raw muscles, the tearing of critical bonds and myofibrillar proteins associated with the structural integrity of the muscle complex (Solomon, Eastridge, Zuckerman, et al. 1997) may lead to increased myofibrillar solubilization and protein functionality. The application of HDP prior to freeze-drying beef *Semitendinosus* muscles increased the percentage of rehydration (8.7% higher for Day 0 samples and 9.9% higher for samples stored 60 days), decreased cooking loss (2% to 4%) and improved tenderness (22% to 35% improvement) in comparison to the non-HDP-treated freeze-dried muscles (Senecal, Racicot, Segars, Berry, and Solomon 2001). Thus, besides a more tender final product the improved rehydration and reduced cooking loss would lead to cost savings to food service operators and consumers. Berry, Solomon, and Senecal (2000) noted a greater than 40% tenderness improvement when HDP was applied to beef strip loins either before or after freeze-drying. A decrease in protein solubility was observed for the HDP samples in comparison to controls. Both studies demonstrated that HDP utilization prior to processing has the potential for altering the textural profiles of the finished meat products.

Schilling and coworkers (2002) processed HDP-treated fresh beef into frankfurters and evaluated protein functionality properties. No differences were found between the HDP-processed frankfurters and controls for textural properties, gel strength, myofibrillar or sarcoplasmic protein solubility, cooking yield, or color. Their results suggest that HDP may not lead to beneficial or negative effects for processing frankfurters. One possible explanation for these results is that Schilling and coworkers conducted their HDP treatments in the 1060 L steel containment vessel with 16 shock-absorbing devices, which was shown to have little, if any, effect on tenderizing meat (Solomon and Eastridge 1999). Tests conducted on raw muscle cuts in this containment vessel found that the performance of meat tenderization decreased as the number of shock-absorbing devices was increased (Solomon and Eastridge 1999). Hence, the results of the Schilling et al. (2002) study may be due to problems with the containment vessel and may not be indicative of the HDP process.

Few studies have been conducted to evaluate the effects of HDP and additional ingredients in processed meat products. Beef strip loins injected (8%) with a solution containing 0.25% NaCl and 0.4% sodium tripolyphosphate were combined with electrically generated HDP shock waves (Sagili and Claus 2003). The injected strip loins were found to be more tender than noninjected strip loins. Treatment of beef muscles with electrically generated HDP shock waves before injection (12%) with a solution containing 0.5% NaCl and 0.35% sodium tripolyphosphate resulted in improved meat tenderness, moisture uptake, and moisture retention (Claus et al. 2002). Another study investigated the effect of HDP and the application of salt during the koshering process on beef. Usually the application of coarse salt during the koshering process of whole muscle meats results in surface discoloration due to decreased oxidative stability. HDP in combination with the koshering process reduced the surface discoloration of the salted beef strip loins to the same level as the control after 14 days of refrigerated storage (Holzer, Berry, Campbell, Spanier,

and Solomon 2004). The salted non-HDP samples had greater brown surface discoloration (> 80% of the surface area) than the unsalted controls, HDP, and combination treatments (< 40% discoloration of the surface area). The textural properties of these products were not evaluated in this study.

The effects of HDP treatment on further processed meat products are not fully understood. HDP treatment of commercially processed shelf-stable beef and chicken sticks resulted in a reduction in shear force (tenderness improvement) in comparison to the nontreated controls (Berry et al. 2000). This study indicated that HDP has the ability to tenderize shelf-stable further processed meat products. However, additional studies on further processed meat products of varying textures (i.e., whole muscle, coarse or finely ground, and comminuted products) are necessary to determine the scope effect of HDP.

To date, only a few meat systems have been evaluated and the effects of HDP treatment on further processed meat products are not fully understood. Published results suggest tenderness improvement or no effect when muscles are treated with HDP before processing (Berry et al. 2000; Claus et al. 2002; Sagili and Claus 2003; Schilling et al. 2002; Senecal et al. 2001) and tenderness improvement to the finished processed beef and chicken sticks treated with HDP after processing (Berry et al. 2000). Research is needed to evaluate HDP in further processed meat products ranging from minimally processed (i.e., marinated products and hams) to more complex processed products (i.e., coarsely or finely ground sausages). By increasing the complexity of the processes and ingredients, a better understanding of the effects of HDP can be obtained. Also, where to incorporate the HDP technology in the manufacture of further processed meats needs to be determined, whether it is prior to processing or after the product is produced. Benefits of the HDP technology to the meat processors would be increased protein functionality, reduction in processing time (faster uptake of marinade or brine equilibration), and cost savings. Consumers would benefit from improved quality and development of new further processed meat products.

9.5 HDP AND FOOD SAFETY ENHANCEMENTS

Alternative food preservation and processing technologies, in particular, nonthermal processes, are being developed to meet consumers' demands for high-quality, fresh, convenient, and nutritionally healthier food with natural flavor and taste. Increasingly, consumers prefer safer, natural, or minimally processed food with minimal amounts of chemical preservatives. Most of the currently employed food preservation processes act by either retarding or completely inhibiting the growth of spoilage and pathogenic microorganisms. Often these processes (e.g., heating, chilling, freezing, drying, curing, fermentation, chemical preservatives) affect the freshness and quality of the product. Currently, thermal treatment is the most common microbial inactivation process used in the food industry to produce safe and shelf-stable products. However, severe heat treatments used to inactivate thermotolerant microorganisms adversely affect the nutritional and sensory qualities of food. Therefore, food industries are seeking alternatives to thermal processing for inactivating pathogenic and spoilage microorganisms.

In the last few years, many nonthermal processes have emerged to meet consumers' demands for fresh, high-quality, microbiologically safe foods. These nonthermal techniques must be lethal to pathogenic and spoilage microorganisms, while maintaining the fresh quality of the food product. One nonthermal process currently being developed is HDP. In addition to HDP, oscillating magnetic fields, high-intensity pulsed light, ultraviolet rays, electron beams, and HHP have been shown to be potential food preservation processes for the meat industry.

9.5.1 HDP and Microbial Inactivation

The role of HDP as a nonthermal treatment to reduce bacterial populations in meat remains unclear, as there are conflicting reports on its bactericidal effect. The HDP treatment significantly reduced the parasite *Trichinella spiralis* in pork loins when pressure fronts were in the range of 46 MPa; however the effect was not significant when the pressure fronts were in the range of 50 MPa to 60 MPa (Gamble, Solomon, and Long 1998). There are reports (Williams-Campbell and Solomon 2002b) that HDP treatments caused a 1 to 2.5 $\log_{10}$ cfu/g reduction of spoilage bacteria in ground beef and on beef stew pieces and effected a marginal reduction (< 1 $\log_{10}$ cfu/g) of *Escherichia coli* O157:H7 in intact beef muscle (Patel, Williams-Campbell, Liu, and Solomon 2005b). Holzer and coworkers (2004) reported an immediate 1.5 $\log_{10}$ cfu/g reduction in normal microflora of beef as a result of HDP treatment. Solomon and Williams-Campbell (2000) reported 2 to 3 $\log_{10}$ cfu/g reduction of spoilage bacteria in ground beef, whole muscle beef, and whole muscle pork samples treated with HDP. They also reported that ground beef inoculated with *E. coli* O157:H7 B6914 was nondetectable after being treated with HDP.

The quantity of explosive used in the HDP process influences the bacterial inactivation effect (Williams-Campbell and Solomon 2000b). The magnitude of bacterial inactivation is significantly higher when 50 g or more of explosive is used compared to 25 g. Ground beef treated with 50, 75, or 100 g of explosive reduced indigenous spoilage microorganisms by 3 $\log_{10}$ cfu/g as compared to only a 0.75 $\log_{10}$ cfu/g reduction using 25 g of explosive (Williams-Campbell and Solomon 2000b). Furthermore, using 100 g of explosive for HDP (Williams-Campbell and Solomon 2000b) was also effective in reducing bacterial populations by 2 to 3 $\log_{10}$ cfu/g in three different size packages (11 g, 255 g, and 1.1 kg samples) of ground beef. However, HDP treatment of irradiated minced chicken inoculated with about 2 $\log_{10}$ cfu/g of *Salmonella Typhimurium* resulted in a minimal reduction (0.2–0.3 $\log_{10}$ cfu/g) of the pathogen (Patel, Bhagwat, Sanglay, and Solomon 2005a).

HDP treatment induced the movement of *E. coli* from the surface of intact beef eye of round steaks to a maximum depth of 300 μm in HDP treated steaks and 50 μm to 100 μm beyond the depth of untreated surface inoculated steaks (Lorca et al. 2002a). Thus, Lorca et al. (2002a) concluded that the extent of penetration in HDP-treated steaks did not pose a safety hazard to consumers as most steak-cooking techniques require the exterior heat on the steak surface be sufficient to achieve an internal temperature of 55°C to 82°C.

Hydrodynamic shock waves for controlling food safety can be generated using explosives (HDP), high voltage electric discharges (HVSW; Alvarez, Loske,

Castano-Tostado, and Prieto 2004), an electrically based pulsed plasma (ESW) technology (Long 2000), or a pulsed shock wave (PSW) generated with an electric charge immersed in water (Zuckerman, Krasik, and Felsteiner 2002). *Listeria monocytogenes* cell populations in water were reduced by 3.2 $\log_{10}$ cfu/ml using HVSW with a 350 shock wave dosage (Alvarez et al. 2004). At the same shock wave dosage (Alvarez et al. 2004), 1.7 $\log_{10}$ cfu/ml and 0.6 $\log_{10}$ cfu/ml inactivation was achieved for *S. Typhimurium* and *E. coli* O157:H7, respectively. Because the HVSW and ESW systems generate light pulses in addition to shock waves, theoretically they should be more bactericidal than the HDP system. However, when HDP and ESW treatments were compared (Lorca, Claus, Eifert, Marcy, and Sumner 2002b; Lorca et al. 2002a), neither method reduced the microbial flora or populations of other inoculated pathogens in ground beef. Populations of *E. coli*, *Staphylococcus aureus*, *Lactobacillus* sp., and *Lactococcus* sp. were reduced by ca. 7 $\log_{10}$ cfu/ml by pulsed shockwaves (PSW) generated by electrical discharges in water (Zuckerman, Krasik and Felsteiner 2002). Populations of the yeast *Saccharomyces cerevisiae,* receiving the same number of pulses as the bacteria, were only reduced by about 3 $\log_{10}$ cfu/ml, indicating that yeasts and bacteria may have different sensitivities to PSW due to the different cellular structure of the microorganisms (Zuckerman, Krasik and Felsteiner 2002).

The exact mechanism(s) of the bactericidal effect of HDP are still unknown. One possibility could be the shock wave cavitation interaction during HDP processing. The shock waves initially produce a high positive pressure, which is rapidly (within microseconds) transformed into tensile stress, resulting in the formation of microscopic vapor-filled cavities. These cavities expand momentarily and then violently collapse (cavitation) creating very high energy densities. Cavitation causes high localized temperature and pressure gradients that may kill bacteria. Cavitation depends on the pressure of the medium, the presence of microscopic bubbles in the samples, and the existence of a liquid–air interface (Loske et al. 2002). Besides cavitation, microjets, acceleration, and shearing forces may be responsible for bactericidal effects (Loske et al. 2002).

9.5.2 High-Pressure Processing and Microbial Inactivation

Two principles describe the effect of HHP on chemical reactions and physical changes in food. These principles may provide insight into the mechanisms by which HHP and hydrodynamic pressure (HDP) inactivate bacteria.

1. The Le Chatelier principle states that some phenomenon (phase transition, changes in molecular configuration, chemical reaction) accompanied by a decrease in volume is enhanced by pressure. These changes may adversely affect the viability of bacterial cells. In the case of chemical reactions, pressure shifts the equilibrium toward the system with the lowest volume. When the volume change of activation (ΔV), i.e., the volume of the activated complex, is smaller than that of the initial reactants, a pressure increase and a temperature decrease will increase the reaction rate constant $K\rho$ per the following equation:

$$\ln K\rho = \ln K_0 - \Delta V\ ((p-1)/RT)$$

Increase in temperature also induces a volume increase through dilation. However, an increase in temperature also increases the rate of reaction according to Arrhenius's law.

2. The isotonic principle (Pascal principle) states that pressure is transmitted in a uniform and instantaneous way throughout the sample and therefore the process time is independent of sample volume.

During HHP, several morphological changes in the microbial cell occur, resulting in a bactericidal effect. Some of the changes include compression of gas vacuoles, cell lengthening, separation of the cell membrane from the cell wall, contraction of the cell wall due to the formation of pores, modifications of the cytoskeleton and strand formation, modifications of the nucleus and intracellular organelles, coagulation of cytoplasmic protein, and the release of intracellular constituents from the cell (Shimada, Naito, Yamada, Osumi, and Hayashi 1993). Various microbial enzymatic systems are inhibited or inactivated by pressure (Simpson and Gilmour 1997). Pressure damage to cell membranes denatures or displaces membrane bound ATPase and also may cause intracellular leakage of potassium (Tholozan, Ritz, Jugiau, Federighi, and Tissier 2000). Without the energy normally supplied by the action of ATP hydrolysis by ATPase, active efflux or transport out of the cell of protons cannot occur and therefore cell death results from the acidification of the interior environment of the cell (Cheftel 1995).

9.5.3 Product Shelf Life

The shelf life of HDP-treated ground beef was increased by reducing the nonpathogenic (normal) microbial flora in the sample (Williams-Campbell and Solomon 2002b). Microbial populations of HDP-treated ground beef samples did not increase significantly during the 14 days of storage at 5°C (3.72 $\log_{10}$ cfu/g at Day 0 to 4.58 $\log_{10}$ cfu/g at Day 14), whereas the increase in microbial population of untreated samples was significant (from 5.22 to 9.11 $\log_{10}$ cfu/g) during that storage period. Populations of bacteria reached spoilage levels (7.56 $\log_{10}$ cfu/g) in untreated ground beef within 7 days of storage at 5°C. However, in HDP-treated ground beef, bacterial populations did not reach spoilage levels after 14 days of storage at the same temperature. Surviving lactic acid bacteria were isolated from the meat samples after each storage time and were identified as lactic acid bacteria (Williams-Campbell and Solomon 2000a). Microbiological analysis of ground beef treated with HDP (Williams-Campbell and Solomon 2002a) showed that bacteria groups isolated most frequently in the control ground beef were *Serratia* (23%), *Pseudomonas* (5%), *Hafnia* (5%), *Yersinia* (5%), and *Streptococcus spp.* (5%). For HDP-treated samples (Williams-Campbell and Solomon 2002a), the predominant group detected was *Streptococcus* (15%), and *Serratia*, *Pseudomonas*, *Hafnia*, and *Yersinia spp.* combined for less than 2% of the bacterial population. This suggested that Gram-negative bacteria may be more susceptible to HDP than Gram-positive bacteria and inhibition of Gram-negative spoilage microorganisms should extend the shelf life of fresh meat products.

The bactericidal effect of the HDP treatment is enhanced when combined with other chemical additives and mechanical treatments. High pressure generated during the HDP treatment may sensitize bacteria to antimicrobial peptides or proteins. The shock wave treatment could be combined with new packaging systems, natural

antimicrobials, or used for the development of new meat products based on meat protein changes. A combination of diacetyl and HDP treatment synergistically inhibited the normal spoilage bacteria of ground beef and helped extend its 14-day shelf life (Mudd, Williams-Campbell, and Solomon 2002; Williams-Campbell and Solomon 2001). Holzer and coworkers (2004) found that significant microbial reductions were sustained throughout a 14-day refrigerated storage trial when beef muscles were treated on Day 0 with HDP. O'Rourke and coworkers (1997a) reported less than a 1 $\log_{10}$ cfu/cm^2 reduction in beef muscle when using the HDP treatment (in the commercial prototype CHU unit) and stored in a retail display case up to 21 days. They also found no significant effect using HDP on the rancidity development in the beef samples stored in a retail display case.

Populations of spoilage bacteria and *E. coli* O157:H7 were lower in beef muscles when HDP was combined with a BT treatment compared to the BT treatment alone (Patel, Williams-Campbell, et al. 2005b; Schilling, Marriott, Wang, and Solomon 2003). Although much more work is warranted, HDP or other shock-wave-producing technologies in combination with additional antimicrobial processes, may provide a nonthermal microbiological interaction that preserves the natural and fresh-like attributes of meat products.

ACKNOWLEDGMENTS

We wish to acknowledge Janice Callahan, Janet Eastridge, Cheryl Mudd, Ernest Paroczay, and Gabriel Sanglay. These members of the Food Technology and Safety Laboratory not only provided assistance with various portions of this chapter, but also were instrumental in the numerous research reports that were presented throughout this chapter.

REFERENCES

Alvarez, U. M., A. M. Loske, E. Castano-Tostado, and P. E. Prieto. 2004. Inactivation of *Escherichia coli* O157:H7, *Salmonella Typhimurium* and *Listeria monocytogenes* by underwater shock waves. *Inno. Food Sci. Emerg. Technol.* 5:459–463.

Batsanov, S. S. 1994. *Effects of explosions on materials, modification and synthesis under high pressure shock compression.* New York: Springer-Verlag.

Berry, B. W., M. B. Solomon, W. L. Johnson, J. B. Long, J. S Eastridge, and H. Zuckerman. 1997. Application of the Hydrodyne process to strip loins from U.S. Select grade beef. *J. Anim. Sci.* 75(Suppl. 1):128.

Berry, B. W., M. B. Solomon, and A. G. Senecal. 2000. Application of hydrodynamic pressure processing for further processed meat products. *J. Anim. Sci.* 78(Suppl. 1):161.

Berry, B. W., M. B. Solomon, H. Zuckerman, J. S. Eastridge, and J. B. Long. 1997. Application of Hydrodyne technology for military meat products. In *Proceedings of the annual meeting of Research and Development Associates for Military Food and Packaging,* 279–284. Natick, MA.

Bouton, P. E., P. V. Harris, J. J. Macfarlane, and J. M. O'Shea. 1977. Effect of pressure treatments on the mechanical properties of pre- and postrigor meat. *Meat Sci.* 1:307–318.

Callahan, J. A., B. W. Berry, and M. B. Solomon. 2004. Evaluation of a steel reflector bowl on hydrodynamic pressure processed semitendinosus beef muscles. In *Poster abstracts of the 57th annual Reciprocal Meat Conference, Lexington, KY, June 20–23, 2004, Abstract 23*. Savoy, IL: American Meat Science Association.

Callahan, J. A., M. B. Solomon, E. W. Paroczay, J. S. Eastridge, V. G. Pursel, and A. D. Mitchell. 2002. Enhancement of pork quality using the hydrodynamic pressure process. In *2002 IFT Annual Meeting Book of Abstracts, Anaheim, CA, June 15–19, 2002,* 111. Chicago: Institute of Food Technologists.

Cheftel, J. C. 1995. Review: High pressure microbial inactivation and food preservation. *Good Sci. Technol. Intl.* 1:75–90.

Claus, J., J. Sagili, and L. Sammel. 2002. Tenderization of beef and pork with shock waves produced with a capacitor discharge system. In *Proceedings of the 55th Annual Reciprocal Meat Conference, East Lansing, MI, July 28–31, 2002*; 142. Savoy, IL: American Meat Science Association.

Claus, J. R., J. K. Schilling, N. G. Marriott, S. E. Duncan, M. B. Solomon, and H. Wang. 2001a. Hydrodynamic shockwave tenderization effects using a cylinder processor on early deboned broiler breasts. *Meat Sci.* 58:287–292.

Claus, J. R., J. K. Schilling, N. G. Marriott, S. E. Duncan, M. B. Solomon, and H. Wang. 2001b. Tenderization of chicken and turkey breasts with electrically produced hydrodynamic shockwaves. *Meat Sci.* 58:283–286.

Cole, R. H. 1948. *Underwater explosions*. Princeton, NJ: Princeton University Press.

Cooper, C., and M. B. Solomon. 2000. Meat tenderization by means of plasma sparking device. Canadian Patent #5,432,756.

Eastridge, J. S., M. N. Liu, B. Vinyard, M. B. Solomon, P. Kandhari, and J. A. Callahan. 2005. Effect of hydrodynamic pressure, blade tenderization or their combination on shear force and collagen solubility in top rounds from Brahman cattle. *2005 IFT Annual Meeting Book of Abstracts, New Orleans, LA, July 16–20, 2005,* Abstract #89F-5. Chicago: Institute of Food Technologists.

Eastridge, J. S., M. B. Solomon, E. W. Paroczay, and J. A. Callahan. 2002. Changes in charge shape may improve efficacy of hydrodynamic pressure process. In *Proceedings of the 55th Annual Reciprocal Meat Conference, East Lansing, MI, July 28–31, 2002,* 139. Savoy, IL: American Meat Science Association.

Eastridge, J. S., M. B. Solomon, J. L. Pierre, E. W. Paroczay, and J. A. Callahan. 2002. Collagen solubility in hydrodynamic pressure process-treated beef longissimus muscle. In *2002 IFT Annual Meeting Book of Abstracts,* 208. Chicago: Institute of Food Technologists.

Eastridge, J. S., M. B. Solomon, R. L. West, and C. C. Chase, Jr. 2000. Tenderizing meat from Brahman cattle: Hydrodynamic pressure process and within-muscle effects for bottom round. *J. Anim. Sci.* 78(Suppl. 1):161.

Eastridge, J. S., M. B. Solomon, R. L. West, A. C. Hammond, and C. C. Chase, Jr. 1998. Developing hydrodyne technology parameters for tenderizing meat from Brahman cattle. *J. Anim. Sci.* 76(Suppl. 1):154.

Elgasim, E. A., and W. H. Kennick. 1982. Effect of high hydrostatic pressure on meat microstructure. *Food Micro.* 1:75–82.

Fernandez-Martin, F., S. Cofrades, J. Carballo, and F. Jimenez-Colmenero. 2002. Salt and phosphate effects on the gelling process of pressure/heat treated pork batters. *Meat Sci.* 61:15–23.

Gamble, H., M. B. Solomon, and J. Long. 1998. Effects of hydrodynamic pressure on the viability of *Trichinella spiralis* in pork. *J. Food Prot.* 61:637–639.

Garcia, F., and R. Woodall. 2001. Meat tenderization and sterilization using axial planer shockwaves. U.S. Patent #6,264,543.

Glass, I. I. 1994. *Shock waves and man*. Toronto: University of Toronto Press.

Godfrey, C. S. 1970. Apparatus for tenderizing food. U.S. Patent #3,492,688.

Gustavson, P. K., R. J. Lee, G. P. Chambers, M. B. Solomon, and B. W. Berry. 2001. Tenderizing meat with explosives. In *Shock compression of condensed matter–2001: Proceedings of the Conference of the American Physical Society, Topical Group on Shock Compression of Condensed Matter, Atlanta, GA, June 24–29, 2001,* eds. M. D. Furnish, Y. Horie, and N. N. Thadhani, Abstract A88. Secaucus, NJ: American Institute of Physics.

Holzer, Z., B. W. Berry, A. M. Campbell, A. M. Spanier, and M. B. Solomon. 2004. Effect of koshering and hydrodynamic pressure on beef color, odor, and microbial loads. *J. Muscle Foods*. 15:69–82.

Homma, N., Y. Ikeuchi, and A. Suzuki. 1995. Levels of calpain and calpastatin in meat subjected to high pressure. *Meat Sci*. 41:251–260.

Hyde, J. 2005. *Spall*. Retrieved March 2005 from http://hitf.jsc.nasa.gov/hitfpub/problem/spall.html

Kolsky, H. 1980. *Stress waves in solids*. New York: Dover Publishing.

Koohmaraie, M. 1992. Role of the neutral proteinases in postmortem muscle protein degradation and meat tenderness. In *Proceedings of the 45th Annual Reciprocal Meat Conference, Fort Collins, CO, June 14–17, 1992,* 63–71. Kansas City, MO: American Meat Science Association.

Koohmaraie, M., W. H. Kennick, E. A. Elgasim, and A. F. Anglemier. 1984. Effect of prerigor pressurization on the activity of calcium-activated factor. *J. Food Sci*. 49:680–684.

Liu, M., J. Callahan, M. Solomon, B. Vinyard, and J. Patel. 2004. Tenderization of Brahman top rounds by hydrodynamic pressure processing and blade tenderization. In *Poster abstracts of the 57th Annual Reciprocal Meat Conference, Lexington, KY, June 20–23, 2004,* Abstract 52. Savoy, IL: American Meat Science Association.

Long, J. B. 1993. Tenderizing meat. U.S. Patent #5,273,766.

Long, J. B. 1994. Apparatus for tenderizing meat. U.S. Patent #5,328,403.

Long, J. B. 2000. Treatment of meat by capacitor discharge. U.S. Patent #6,120,818.

Lorca, T. A., M. D. Pierson, J. R. Claus, J. D. Eifert, J. E. Marcy, and S. S. Sumner. 2002a. Penetration of surface-inoculated bacteria as a result of hydrodynamic shock wave treatment of beef steaks. *J. Food Prot*. 65:616–620.

Lorca, T. A., J. R. Claus, J. D. Eifert, J. E. Marcy, and S. S. Sumner. 2002b. Effects of electrically generated hydrodynamic shock waves on the microbial flora of ground beef. Available at http://scholar.lib.vt.edu/theses/available/etd-07292002-153950/unrestricted/jfood-science.pdf

Loske, A. M., U. M. Alvarez, C. Hernandez-Galicia, E. Castano-Tostado, and F. E. Prieto. 2002. Bactericidal effect of underwater shock waves on *Escherichia coli* ATCC 10536 suspensions. *Inno. Food Sci. Emerg. Technol*. 3:321–327.

Macfarlane, J. J. 1985. High pressure technology and meat quality. In *Developments in meat science,* Vol. 3, ed. R. Lawrie, 155–184. New York: Elsevier Applied Science.

Macfarlane, J. J., and I. J. McKenzie. 1976. Pressure-induced solubilization of myofibrillar proteins. *J. Food Sci*. 41:1442–1446.

Macfarlane, J. J., and D. J. Morton. 1978. Effects of pressure treatment on the ultrastructure of striated muscle. *Meat Sci*. 2:281–288.

Marriott, N. G., H. Wang, M. B. Solomon, and W. G. Moody. 2001. Studies of cow beef tenderness enhancement through supersonic-hydrodynamic shock wave treatment. *J. Muscle Foods*. 12:207–218.

Meek, K. I., J. R. Claus, S. E. Duncan, N. G. Marriott, M. B. Solomon, S. J. Kathman, and M. E. Marini. 2000. Quality and sensory characteristics of selected post-rigor, early deboned broiler breast meat tenderized using hydrodynamic shock waves. *Poult. Sci.* 79:126–136.

Moeller, S., D. Wulf, D. Meeker, M. Ndife, N. Sundararajan, and M. B. Solomon. 1999. Impact of the Hydrodyne process on tenderness, microbial load, and sensory characteristics of pork longissimus muscle. *J. Anim. Sci.* 77:2119–2123.

Mudd, C., A. Williams-Campbell, and M. Solomon. 2002. Identification of spoilage microorganisms in ground beef treated with diacetyl and hydrodynamic pressure processing, alone or in combination. *J. Food Prot.* 65(Suppl. A.):94.

O'Rourke, B. M., C. R. Calkins, R. T. Rosario, J. S. Eastridge, M. B. Solomon, and J. B. Long. 1997. Improvement of pork loin tenderness using the Hydrodyne process. *J. Anim. Sci.* 75(Suppl. 1):53.

O'Rourke, B. M., C. R. Calkins, R. T. Rosario, J. S. Eastridge, M. B. Solomon, and J. B. Long. 1999. Relationship of muscle fiber orientation during Hydrodyne treatment to tenderness, aging, and proteolysis of beef. *J. Anim. Sci.* 77(Suppl. 1):45.

O'Rourke, B. M., C. R. Calkins, R. T. Rosario, M. B. Solomon, and J. B. Long. 1997a. Hydrodyne-treated beef: Tenderness, rancidity, and microbial growth. In *Proceedings of the 43rd International Congress of Meat Science and Technology,* ed J. Bass, 588–589. Auckland, New Zealand: International Congress of Meat Science and Technology.

O'Rourke, B. M., C. R. Calkins, R. T. Rosario, M. B. Solomon, and J. B. Long. 1997b. Tenderness and retail stability of Hydrodyne-treated beef. In *Proceedings of the 50th Annual Reciprocal Meat Conference, Ames, IA, June 29–July 2, 1997,* 180–181. Kansas City, MO: American Meat Science Association.

O'Rourke, B. M., C. R. Calkins, R. T. Rosario, M. B. Solomon, and J. B. Long. 1998. Hydrodyne-treated beef: Aging, proteolysis, and tenderness of strip loins. *J. Anim. Sci.* 76(Suppl. 2):44.

Paroczay, E. W., M. B. Solomon, B. W. Berry, J. S. Eastridge, and J. A. Callahan. 2002. Evaluating tenderness improvement using hydrodynamic pressure processing (HDP). In *2002 IFT Annual Meeting Book of Abstracts,* 185. Chicago: Institute of Food Technologists.

Patel, J. R., A. A. Bhagwat, G. C. Sanglay, and M. B. Solomon. 2005a. Rapid detection of *Salmonella* from hydrodynamic pressure-treated poultry using molecular beacon real-time PCR. *Food Microbiol. 23*(1):39–46.

Patel, J. R., A. M. Williams-Campbell, M. N. Liu, and M. B. Solomon. 2005b. Effect of hydrodynamic pressure treatment and cooking on inactivation of *Escherichia coli* O157:H7 in blade tenderized beef steaks. *J. Muscle Foods.* 16:342–353.

Sagili, J. V. V., and J. R. Claus. 2003. Tenderization effects of electrically produced hydrodynamic shock waves on top round and strip loin muscles of beef. In *Proceedings of the 56th Annual Reciprocal Meat Conference, Columbia, MO, June 15–18, 2003,* 122. Savoy, IL: American Meat Science Association.

Schilling, M. W., J. R. Claus, N. G. Marriott, M. B. Solomon, W. N. Eigel, and H. Wang. 2002. No effect of hydrodynamic shock wave on protein functionality of beef muscle. *J. Food Sci.* 67:335–340.

Schilling, M. W., N. G. Marriott, H. Wang, and M. B. Solomon. 2003. Characteristics of USDA utility cow beef subjected to blade tenderization and hydrodynamic shock waves. *J. Muscle Foods.* 14:131–142.

Senecal, A. G., K. Racicot, R. Segars, B. Berry, and M. Solomon. 2001. Effect of hydrodynamic pressure processing on freeze-dried whole muscle semitendinosus. In *2001 IFT Annual Meeting Book of Abstracts, New Orleans, LA, June 23–27, 2001,* 61. Chicago: Institute of Food Technologists.

Shimada, M. A., N. Naito, N. Yamada, M. Osumi, and R. Hayashi. 1993. Effects of hydrostatic pressure on the ultrastructure and leakage of internal substances in the yeast *Saccharomyces cerevisiae*. *Appl. Microbiol. Biotechnol*. 40:121–131.

Simpson, R. K., and A. Gilmour. 1997. The effect of high hydrostatic pressure on the activity of intracellular enzymes of *Listeria monocytogenes*. *Lett. Appl. Microbiol*. 25:48–53.

Solomon, M. B. 1998a. The Hydrodyne process for tenderizing meat: An update. In *Proceedings of the 51st Annual Reciprocal Meat Conference, Storrs, CT, June 28–July 1, 1998,* 171–176. Kansas City, MO: American Meat Science Association.

Solomon, M. B. 1998b. Tenderizing beef using the Hydrodyne process. In *Proc. Int'l. Livestock Congress*. Available at http://ifse.tamu.edu/ilc/beefproceedings.html

Solomon, M. B. 1999. The callipyge phenomenon: Toughness intervention methods. *J. Anim. Sci*. 77(Suppl. 2):238–242.

Solomon, M. B., and B. W. Berry. 2000. Comparison of two different containers for performing the hydrodynamic pressure process. *J. Anim. Sci*. 78(Suppl. 1):161.

Solomon, M., and B. Berry. 2002. Effect of boundary conditions for the hydrodynamic pressure processing plastic shock wave container on meat tenderness improvement. In *Proceedings of the 55th Annual Reciprocal Meat Conference, East Lansing, MI, July 28–31, 2002,* 141. Savoy, IL:American Meat Science Association.

Solomon, M. B., B. W. Berry, J. S. Eastridge, and E. W. Paroczay. 1999. Muscle fiber orientation during Hydrodyne treatment on tenderness improvement in semitendinosus muscles. *J. Anim. Sci*. 77(Suppl. 1):91.

Solomon, M. B., B. W. Berry, J. Eastridge, E. Paroczay, R. Romanowski, and M. George. 1999. A look at two different containers for performing the Hydrodyne process. *J. Anim. Sci*. 77(Suppl. 1):90–91.

Solomon, M. B., B. W. Berry, E. W. Paroczay, J. A. Callahan, and J. S. Eastridge. 2002. Effects of hydrodynamic pressure processing (HDP) and aging on beef tenderness. In *2002 IFT Annual Meeting Book of Abstracts, Anaheim, CA, June 15–19, 2002,* 111. Chicago: Institute of Food Technologists.

Solomon, M. B., C. E. Carpenter, G. D. Snowder, and N. E. Cockett. 1998. Tenderizing callipyge lamb with the Hydrodyne process and electrical stimulation. *J. Muscle Foods*. 9:305–311.

Solomon, M. B., and J. S. Eastridge. 1999. Type of container and quantity of explosive in the Hydrodyne process on beef strip loin tenderness. *J. Anim. Sci*. 77(Suppl. 1):173.

Solomon, M. B., J. S. Eastridge, and J. B. Long. 1997. The Hydrodyne process for tenderizing cold-shortened beef. In *1997 IFT Annual Meeting Book of Abstracts, Orlando, FL, June 14–18, 1997,* 50. Chicago: Institute of Food Technologists.

Solomon, M. B., J. S. Eastridge, E. W. Paroczay, and S. W. Coleman. 2001. Effects of hydrodynamic pressure processing and freezing on beef ribeyes. In *2001 IFT Annual Meeting Book of Abstracts, New Orleans, LA, June 23–27, 2001,* 61. Chicago: Institute of Food Technologists.

Solomon, M. B., J. S. Eastridge, H. Zuckerman, J. B. Long, and W. Johnson. 1997a. Hydrodyne-treated beef: Tenderness and muscle ultrastructure. In *Proceedings of the 43rd International Congress of Meat Science and Technology,* ed. J. Bass, 614–615. Auckland, New Zealand: International Congress of Meat Science and Technology.

Solomon, M. B., M. Liu, J. Patel, E. Paroczay, and J. Eastridge. 2004. Tenderness improvement in fresh and frozen/thawed beef strip loins treated with hydrodynamic pressure processing. *J. Anim. Sci*. 82(Suppl. 1):18.

Solomon, M. B., and J. B. Long. 1995. The Hydrodyne process for tenderizing meat. *J. Anim. Sci*. 73(Suppl. 1):159.

Solomon, M. B., J. B. Long, and J. S. Eastridge. 1997b. The Hydrodyne: A new process to improve beef tenderness. *J. Anim. Sci.* 75:1534–1537.

Solomon, M. B., J. B. Long, J. S. Eastridge, and C. E. Carpenter. 1995. Tenderizing callipyge lamb with the Hydrodyne® process. In *Proceedings of the 41st Annual International Congress of Meat Science and Technology, San Antonio, TX, August 20–25, 1995,* 622–623. Kansas City, MO: American Meat Science Association.

Solomon, M. B., and A. Williams-Campbell. 2000. Hydrodynamic shock waves as a food preservation technique. In *2000 IFT Annual Meeting Book of Abstracts, Dallas, TX, June 10–14, 2000,* 10. Chicago: Institute of Food Technologists.

Spanier, A. M., B. W. Berry, and M. B. Solomon. 2000. Variation in the tenderness of beef strip loins and improvement in tenderness by use of hydrodynamic pressure processing (HDP). *J. Muscle Foods.* 11:183–196.

Spanier, A. M., and R. D. Romanowski. 2000. A potential index for assessing the tenderness of hydrodynamic pressure (HDP)—Treated beef strip loins. *Meat Sci.* 56:193–202.

Suzuki, A., N. Suzuki, Y. Ikeuchi, and M. Saito. 1991. Effects of high pressure treatment on the ultrastructure and solubilization of isolated myofibrils. *Agric. Biol. Chem.* 55:2467–2473.

Suzuki, A., M. Watanabe, K. Iwamura, Y. Ikeuchi, and M. Saito. 1990. Effect of high pressure treatment on the ultrastructure and myofibrillar protein of beef skeletal muscle. *Agri. Biol. Chem.* 54:3085–3091.

Tate, S. E., J. W. Sellers, Jr., M. B. Solomon, and B. W. Berry. 2004. Meat processing scheme. U.S. Patent #6,767,568.

Tholozan, J. L., M. Ritz, F. Jugiau, M. Federighi, and J. P. Tissier. 2000. Physiological effects of high hydrostatic pressure treatments on *Listeria monocytogenes* and *Salmonella typhimurium. J. Appl. Microbiol.* 88:202–212.

Williams-Campbell, A., and M. B. Solomon. 2000a. Extending the shelf-life of ground beef using hydrodynamic pressure process. In *American Society for Microbiology 100th General Meeting, Los Angeles, CA, May 21–25, 2000,* 537. Washington, DC: American Society for Microbiology.

Williams-Campbell, A. M., and M. B. Solomon. 2000b. New non-thermal postharvest technology to improve food safety: Hydrodynamic pressure processing. In *Proceedings of SPIE, Photonic Detection and Intervention Technologies for Safe Food, Boston, MA. November 5–6, 2000,* 167–173. Bellingham, WA: Society of Photo-Optical Instrumentation Engineers.

Williams-Campbell, A., and M. B. Solomon. 2001. Extending the shelf-life of fresh ground beef using diacetyl and hydrodynamic pressure process. In *American Society for Microbiology 101st General Meeting, Orlando, FL, May 20–24, 2001,* 572. Washington, DC: American Society for Microbiology.

Williams-Campbell, A., and M. B. Solomon. 2002a. Microbiological analysis of ground beef treated with hydrodynamic pressure processing. *J. Food Prot.* 65(Suppl. A.):121–122.

Williams-Campbell, A. M., and M. B. Solomon. 2002b. Reduction of spoilage microorganisms in fresh beef using hydrodynamic pressure processing. *J. Food Prot.* 65:571–574.

Zuckerman, H., B. W. Berry, J. S. Eastridge, and M. B. Solomon. 1999. Microstructure changes in connective tissue of bovine semimembranosus muscle caused by hydrodynamic shockwaves. *J. Scanning Microscopy.* 21:128–129.

Zuckerman, H., B. W. Berry, J. S. Eastridge, and M. B. Solomon. 2002. Shear force mapping: A tool for tenderness measurement. *J. Muscle Foods.* 13:1–12.

Zuckerman, H., Y. A. Krasik, and J. Felsteiner. 2002. Inactivation of microorganisms using pulsed high-current underwater discharges. *Inno. Food Sci. Emerg. Technol.* 3:329–336.

Zuckerman, H., and M. B. Solomon. 1998. Ultrastructural changes in bovine longissimus muscle caused by the Hydrodyne process. *J. Muscle Foods.* 9:419–426.

10 Functional Properties of Bioactive Peptides Derived From Meat Proteins

Keizo Arihara
Department of Animal Science, Kitasato University

CONTENTS

10.1 Overview of Bioactive Peptides Derived From Food Proteins 246
10.1.1 ACE Inhibitory and Antihypertensive Peptides 247
10.1.2 Opioid Peptides 249
10.1.3 Immunomodulating Peptides 250
10.1.4 Antimicrobial Peptides 251
10.1.5 Prebiotic Peptides 251
10.1.6 Mineral-Binding Peptides 252
10.1.7 Antithrombotic Peptides 252
10.1.8 Hypocholesterolemic Peptides 252
10.1.9 Antioxidative Peptides 253
10.2 Generation of Peptides From Meat Proteins 253
10.2.1 Aging of Meats 253
10.2.2 Fermented Meats 254
10.2.3 Enzymatic Treatment 254
10.2.4 Identification of Peptides 255
10.3 Antihypertensive Peptides Derived From Meat Proteins 256
10.3.1 ACE Inhibitory Peptides From Meat Proteins 256
10.3.2 Antihypertensive Activity of ACE Inhibitory Peptides 259
10.3.3 Structure–Activity Relationships 259
10.4 Promising Bioactive Peptides From Meat Proteins 260
10.4.1 Opioid Peptides 260
10.4.2 Antioxidative Peptides 261
10.4.3 Peptides With Organoleptic Properties 262
10.5 Conclusions and Future Prospects 262
References 264

Due to increasing concerns about health in recent years, much attention has been paid to the physiological functions of foods, including anticarcinogenicity, antimutagenicity, antioxidative activity and antiaging activity (Arihara 2004; Dentali 2002; Fernández, Fernández-López, Sayas-Barberá, and Pérez-Alvarez 2005; Hasler 1998; Heasman and Mellentin 2001; Playne, Bennett, and Smithers 2003; Pszczola, Katz, and Giese 2000). Efforts have been made by the food industries in many countries (e.g., Japan, the EU nations, the United States) to develop new foods with physiological functions. Foods having these physiological functions are known as functional foods (Rincón-Léon 2003; Roberfroid 2000).

Numerous food components having physiological functions have been isolated and characterized (Hasler 1998). Many vegetables, for example, have been shown to contain a variety of biologically active phytochemicals (Lindsay 2003). There has been an accumulation of scientific findings regarding the roles of such components in the prevention of diseases. Rapid progress has been made in the development of functional foods based on the results of studies on food components that have positive health benefits other than normal nutritional benefits (Arihara 2004; Heasman and Mellentin 2001).

Several attractive meat-based bioactive substances have been studied (Arihara 2004), including carnosine, anserine, L-carnitine, conjugated linoleic acid, glutathione, taurine, and creatine. In addition to these bioactive compounds, protein-derived peptides are another group of promising functional components of meat. Although the activities of these peptides in the sequence of proteins are latent, they are released by proteolytic enzymes (i.e., muscle, microbial, and digestive proteinases). Therefore, meat proteins have possible bioactivities beyond a nutritional source of amino acids alone. It has been shown that enzymatic hydrolysis of food proteins such as milk caseins produces various physiologically functional peptides (Clare and Swaisgood 2000; Korhonen and Pihlanto 2003; Pihlanto and Korhonen 2003). Examples of such peptides include antihypertensive, opioid, immunostimulating, antimicrobial, antithrombotic, hypocholesterolemic, and antioxidative peptides. Although information on bioactive peptides generated from meat proteins is limited, there is a possibility of utilizing such components for developing novel functional meat products and utilizing food ingredients (Arihara 2004).

This chapter includes a brief overview of bioactive peptides generated from food proteins and a discussion of the potential benefits of meat protein-derived peptides. This chapter focuses particularly on angiotension I converting enzyme (ACE) inhibitory (antihypertensive) peptides, the most extensively studied bioactive peptides (Ariyoshi 1993; Yamamoto 1997).

10.1 OVERVIEW OF BIOACTIVE PEPTIDES DERIVED FROM FOOD PROTEINS

Although the main purpose of this chapter is to describe the bioactive peptides derived from meat proteins, information on meat-derived peptides is still insufficient to overview food protein-derived bioactive peptides. For this reason, bioactive

peptides generated from various food proteins are covered briefly at the beginning of this chapter.

Mellander (1950) first reported bioactive peptides generated from food proteins. He suggested that milk 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 (Korhonen and Pihlanto 2003; Pihlanto and Korhonen 2003; Yoshikawa 1996). Although various food proteins contain potential bioactive sequences, the main sources of a wide range of bioactive peptides are milk proteins in the past studies (Clare and Swaisgood 2000; Koldovsky 1989; Mater, LeBlanc, Martin, and Perdigon 2003; Meisel 1998; Meisel and Bockelmann 1999; Pihlanto-Leppälä 2003; Schanbacher, Talhouk, Murray, Gherman, and Willett 1998; Silva and Malcata 2005). Thus, representative bioactive peptides generated from milk proteins are listed in table 10.1.

TABLE 10.1
Examples of Bioactive Peptides Derived From Milk Proteins

Bioactivity	Name	Sequence[a]	Reference
Antihypertensive (ACE inhibitory)	Lactotripeptides	IPP, VPP	Nakamura, Yamamoto, Sakai, Okubo et al. (1995) Nakamura, Yamamoto, Sakai, and Takano (1995)
	Casokinins	FF, FFVAP	Maruyama and Suzuki (1982)
Opioid agonistic	Lactorphins	YGLF, YLLF	Chiba and Yoshikawa (1986)
Immunomodulatory	Immunopeptides	PGPIPN, GLY	Migliore-Samour, Floc'h, and Jollès (1989) Fiat et al. (1993)
Antimicrobial	Isracidin	αs1-casein f(1–23)	Lahov and Regelson (1996)
Prebiotic	PRELP-I	CAVGGCIAL	Lieple et al. (2002)
Mineral-binding	CPP	αs1-casein f(43–58)	Gagnaire et al. (1996)
Antithrombic	Casoplatelin	MAIPPKKNQDK	Jollès et al. (1986)
Hypocholesterolemic	LTH peptide	IIAEK	Nagaoka et al. (2001)
Antioxidative	no name	YFYPEL	Suetsuna, Ukeda, and Ochi (2000)

[a] The one-letter amino acid codes were used.

10.1.1 ACE Inhibitory and Antihypertensive Peptides

The most extensively studied bioactive peptides generated from food proteins are the ACE inhibitory peptides (Ariyoshi 1993; Li, Le, Shi, and Shrestha 2004; Pihlanto-Leppälä 2001; Takano 1998; Vermeirssen, Camp, and Verstraete 2004; Yamamoto 1997; Yamamoto and Takano 1999). ACE inhibitory peptides have attracted particular attention and have been studied extensively because of their ability to prevent hypertension. These peptides could be used as potent functional food additives and would constitute a natural and healthier alternative to ACE inhibitory drugs.

ACE is a dipeptidyl carboxypeptidase, which is widely distributed in mammalian tissues, predominantly as a membrane-bound ectoenzyme in vascular endothelial cells (Ariyoshi 1993; Li et al. 2004). In the renin angiotensin system, ACE plays an important role in the regulation of blood pressure (figure 10.1). ACE is required for converting the inactive decapeptide angiotensin I, by cleaving a dipeptide from the C-terminus, into the potent vasoconstricting octapeptide angiotensin II, resulting in increases in blood pressure and production of aldersterone. Also, ACE inactivates the antihypertensive vasodilator bradykinin, which is a nonapeptide in the kallikrein-kinin system. Therefore, inhibition of ACE results in an antihypertensive effect. Coronary heart disease is a serious form of cardiovascular disease and is the leading cause of death in developed industrialized countries. High blood pressure (hypertension) is a major risk factor for cardiovascular diseases, including coronary heart disease.

Many food protein-derived peptides are known to have an inhibitory effect on ACE. ACE inhibitory peptides derived from a food protein (gelatin) were first reported by Oshima, Shimabukuro, and Nagasawa (1979). Since then, ACE inhibitory peptides have been found in the hydrolyzates of many proteins from foods such as milk (Abubakar, Saito, Kitazawa, Kawai, and Itoh 1998; Gobbetti, Ferranti, Smacchi, Goffredi, and Addeo 2000; Maruyama and Suzuki 1982; Mullally, Meisel, and FitzGerald 1997; Robert, Razaname, Mutter, and Juillerat 2004; Saito, Nakamura, Kitazawa, Kawai, and Itoh 2000), fish (Fujita, Yamagami, and Ohshima 2001; Fujita and Yoshikawa 1999; Kohama et al. 1988; Kohama et al. 1991; Matsufuji, et al. 1994; Seki et al. 1995; Yokoyama, Chiba, and Yoshikawa 1992), meat (Arihara, Nakashima, Mukai, Ishikawa, and Itoh 2001; Fujita, Yokoyama, and Yoshikawa 2000; Jang and Lee 2005; Jang et al. 2004; Katayama, Fuchu, et al. 2003; Katayama, Tomatsu, et al. 2003; Katayama et al. 2004; Nakashima, Arihara, Sasaki, Ishikawa, and Itoh 2002; Saiga, Okamura, et al. 2003), eggs (Yoshii et al. 1999), soybean (Junfeng and Lite 2003; Kuba, Tana, Tawata, and Yasuda 2005), corn (Miyoshi, Kaneko, Ishikawa, Tanaka, and Maruyama 1995), wheat (Matsui, Li, and Osajima 1999), seaweed (Suetsuna 1998; Suetsuna and Nakano 2000), and others (Kim, Byun, Park, and Shahidi 2001; Mito et al. 1996; Saito, Wanezaki, Kawato, and Iwayasu 1994). Some

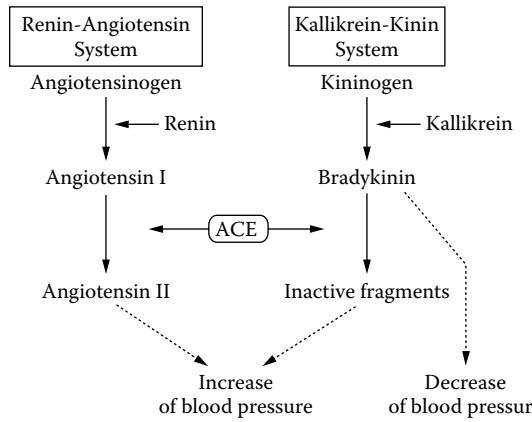


FIGURE 10.1 Role of angiotension I converting enzyme (ACE) in blood pressure regulation.

of these peptides have been reported to show antihypertensive effects in spontaneously hypertensive rats (SHR) by oral administration (Abubakar et al. 1998; Fujita et al. 2001; Fujita et al. 2000; Nakashima et al. 2002; Suetsuna and Nakano 2000). Specific examples of ACE inhibitory peptides derived from meat proteins are given later in this article.

Several products having antihypertensive effects are now commercially available. There are two commercially available dairy products (figure 10.2) containing Ile-Pro-Pro and Val-Pro-Pro, which are generated from milk protein by fermentation with *Lactobacillus helveticus* (Nakamura, Yamamoto, Sakai, Okubo, et al. 1995; Nakamura, Yamamoto, Sakai, and Takano 1995; Seppo, Jauhiaine, Poussa, and Korpela 2003). Calpis Amiel-S drink (figure 10.2, left) has been approved by the Japanese Ministry of Health and Welfare as a Food for Specified Health Use (FOSHU; Arihara 2004). The Finnish fermented milk drink Evolus (figure 10.2, center), developed by Valio Ltd., contains the same tripeptides as those in Amiel-S. Both products have been tested in studies using SHR and in clinical human trials. Another beverage (Casein DP) containing milk protein-derived ACE inhibitory peptides was developed in Japan and has been approved as a FOSHU (Sugai 1998). The thermolysin digest of dried bonito containing antihypertensive peptides has also been used in a soup product in Japan (Fujita et al. 2001).

10.1.2 Opioid Peptides

Opioid peptides are defined as peptides that have an affinity for an opiate receptor as well as opiate-like effects (Pihlanto and Korhonen 2003; Pihlanto-Leppälä 2001). Basically, opioid peptides have effects on the nerve system. They also influence gastrointestinal functions. Examples of typical opioid peptides are endorphins, enkephalin, and prodynorphin. All these typical 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

FIGURE 10.2 Examples of functional foods utilizing bioactive peptides derived from milk proteins (left, "Ameal-S" containing antihypertensive peptides; center, "Evolus" containing antihypertensive peptides; "Kotsukotsu Ca" containing caseinophosphopeptides).

for specific physiological effects (i.e., emotional behavior, suppression of intestinal motility, sedation, 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 protein, are generated from milk casein by enzymatic hydrolysis (Brantl, Teschemacher, Henschen, and Lottspeich 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 SHR as well as in normotensive rats (Nurminen 2000). The antihypertensive 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.

Other than hydrolyzates of milk proteins, opioid peptides were also found in hydrolyzates of wheat gluten (exorphins; Fukudome and Yoshikawa 1992, 1993) and blood hemoglobin (hemorphins; Nyberg, Sanderson, and Glämsta 1997; Zhao, Garreau, Sannier, and Piot 1997). Hemorphins are described later in this chapter.

10.1.3 Immunomodulating Peptides

Many studies have shown that immunomodulatory sequences exist within food proteins, especially milk proteins (Mater et al. 2003; Pihlanto and Korhonen 2003; Schanbacher et al. 1998). Immunomodulatory peptides affect both the immune system and cell proliferation responses.

Although several hydrolyzates 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 and prevented *Klebsielxla pneumoniae* infection in mice (Fiat, Migliore-Samour, et al. 1993; Migliore-Samour, Floc'h, and Jollès 1989). On the other hand, the C-terminal part of milk lactoferrin modulates lymphocyte blastogenesis or differentiation (Nuijens, van Berkel, and Schanbacher 1996). Lactoferricin B, generated from lactoferrin

with pepsin treatment, promoted phagocytic activity of human neutrophils (Miyauchi et al. 1998).

Because opioids can alter the characteristics of cellular components of the immune system (Webster 1998), it has been reported that opioid peptides derived from milk proteins have a modulatory function in the immune system. For example, the opioid peptide β-casomorphin may affect the proliferation of human lamina propria lymphocytes via the opiate receptor (Elitsur and Luk 1991).

Immunomodulating peptides have also been discovered in enzymatic hydrolyzates of proteins from various foods, such as eggs (Tanizaki, Tanaka, and Kato 1997; Watanabe, Tsuji, Shimoyamada, Ogama, and Ebina 1998), soybeans (Yoshikawa et al. 1993), and rice (Matsubayashi and Sakagami 1999; Takahashi et al. 1996). However, to date, meat protein-derived immunomodulating peptides have not been reported.

10.1.4 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 *Staphylococcus aureus* and *Candida albicans* (Lahov and Regelson 1996). Antimicrobial peptides generated by proteolytic digestion of milk lactoferrin have been studied most extensively (Tomita, Takase, Bellamy, and Shimamura 1994). The hydrolyzate of lactoferrin 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 (Hoek, Milne, Grieve, Dionysius, and Smith 1997; Shin et al. 1998). A hen egg ovotransferrin-derived antimicrobial peptide (OTAP-92) that is active against *S. aureus* and *E. coli* has been isolated (Ibrahim, Sugimoto, and Aoki 2000).

10.1.5 Prebiotic Peptides

Several substances are known to enhance the activity of probiotic bacteria, such as intestinal *Lactobacillus* and *Bifidobacterium*. Such substances are called prebiotics (Holzapfel and Schillinger 2002; Teitelbaum and Walker 2002; Tomasik 2003). Oligosaccharides are representative prebiotic substances. In addition to oligosaccharides, the presence of prebiotic peptides has been suggested. Many studies have shown that hydrolyzates 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 (Bezkorovainy, Grohlich, and Nichols 1979; Idota, Kawakami, and Nakajima 1994). Nonglycosylated peptides derived from proteins have recently been identified. Lieple et al. (2002) first reported nonglycosylated peptides that selectively stimulate the growth of bifidobacteria. These peptides (5,584 and 5,801Da) 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. Apart from protein-derived peptides, Etoh, Asamura, Obu, Sonomoto, and Ishizaki, (2000) discovered a growth-stimulating peptide (Ala-Thr-Pro-Glu-Lys-Glu-Glu-Pro-Thr-Ala) for *Bifidobacterium bifidum* from natural rubber serum.

Recently, the authors found that the hydrolyzate of porcine skeletal muscle actomyosin digested by papain enhanced the growth of *Bifidobacterium* strains in media. One of the corresponding prebiotic peptides was purified from the hydrolyzate and identified as a tripeptide (unpublished data).

10.1.6 Mineral-Binding Peptides

Several milk protein-derived peptides generated by enzymatic hydrolysis act as mineral trappers through specific and nonspecific binding sites, resulting in enhancement of the absorption efficiency of minerals (Vegarud, Langsrud, and Svening 2000). Caseinophosphopeptides (CPP) have been studied extensively as mineral-binding peptides (Gagnaire, Pierre, Molle, and Leonil 1996). CPPs function as carriers for calcium and exert an influence on its absorption. Because the calcium-chelating activity of CPPs is attributed to phosphoserine residues in stabilizing the colloidal calcium phosphate, dephosphorylated peptides have no calcium-binding ability (Sato, Noguchi, and Naito 1986). On the other hand, peptides binding different minerals such as iron have been found from nonphosphorylated whey proteins (Vegarud et al. 2000). A FOSHU product utilizing CPP has been developed in Japan (figure 10.2, right).

10.1.7 Antithrombotic Peptides

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, Migliore, and Jollès 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 undecapeptide (casoplatelin) inhibited 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, Molle, and Leonil 1992).

10.1.8 Hypocholesterolemic Peptides

Dietary proteins (e.g., milk and soy proteins) have been shown to lower serum cholesterol level (Nagaoka, Kanamaru, and Kuzuya 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. They discovered a food protein-derived hypocholesterolemic peptide (Ile-Ile-Ala-Glu-Lys) from enzymatic hydrolyzates of β-lactoglobulin. This peptide was found to have 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 are still unknown.

10.1.9 Antioxidative Peptides

Neutralization and reduced release of free radicals occur through the ingestion of diet, such as fruits and vegetables (Lindsay 2003). This beneficial action of food is attributed to the antioxidant potency of various compounds (e.g., vitamin C, vitamin E, polyphenols). The intake of such antioxidants may decrease the risk of diseases, such as cardiovascular disease and certain types of cancer (Hertog 1996).

Several studies have shown that antioxidative peptides can be released from food proteins. Such peptides have been identified from proteins of soybean (Chen, Muramoto, and Yamauchi 1995, 1996), milk (Kudo, Matsuda, Igoshi, and Oki 2001; Rival, Boeriu, and Wichers 2001; Rival, Fornaroli, Boeriu, and Wichers 2001), eggs (Tsuge, Eikawa, Nomura, Yamamoto, and Sugisawa 1991), and meat (Saiga, Tanabe, and Nishimura 2003). For example, milk casein-derived peptides (e.g., Tyr-Phe-Tyr-Pro-Glu-Leu) have been shown to have free radical scavenging activity to inhibit enzymatic and nonenzymatic lipid oxidation (Rival, Boeriu, and Wichers 2001; Rival, Fornaroli, et al. 2001; Suetsuna, Ukeda, and Ochi 2000). Meat protein-derived antioxidative peptides are discussed later.

10.2 GENERATION OF PEPTIDES FROM MEAT PROTEINS

Although most proteins would contain bioactive sequences, those sequences are inactive or incomplete within the parent proteins. Only via proteolytic digestion, active peptide fragments are released from native proteins. Once such peptides are liberated, they can act as regulatory compounds.

During gastrointestinal proteolysis, bioactive peptides would be liberated from food proteins. Ingested proteins are attacked by various digestive enzymes, such as pepsin, trypsin, chymotrypsin, elastase and carboxypeptidase (Pihlanto and Korhonen 2003). Katayama, Fuchu, et al. (2003) reported that ACE inhibitory activity was generated from meat proteins (i.e., myosin, actin, tropomyosin and troponin) by pancreatic protease treatment. In addition to gastrointestinal digestion, there are several ways in which peptides are generated from meat proteins as described below.

10.2.1 Aging of Meats

The content of peptides in meat increases during postmortem aging. Nishimura, Rhue, Okitani, and Kato (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 per g meat at Day 6 (53% increment). Mikami, Nagao, Sekikawa, and Miura, (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 per g meat 2 and 21 days after slaughter, respectively.

During aging or storage, meat proteins are hydrolyzed by muscle endogenous proteases, such as calpains and cathepsins (Etherington 1984; Koohmaraie 1994). Although such enzymatic hydrolysis contributes to improvement of sensory properties of meat (texture, taste, and flavor), there has been no report about the generation of bioactive peptides in meat during postmortem aging. However, our preliminary study showed an increase in ACE inhibitory activity of beef during storage at 4°C (unpublished data). The generation of bioactive peptides occurs by aging or storage.

10.2.2 Fermented Meats

Proteolytic events that occur during fermentation of raw sausages and dry-cured ham have been studied extensively, as components generated from meat proteins are critical for the development of sensory properties of fermented meat products (Ordonez, Hierro, Bruna, and de la Hoz 1998; Toldrá 2004; Toldrá and Flores 1998).

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, Gil, Arnau, Monfort, and Cusso 1989). Because 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, de la Hoz, and Ordonez 1999). However, lactic acid bacteria influence protein degradation by causing a decrease 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 reach about 1% dry matter of products (Dainty and Blom 1995). However, there has been no report on the generation of bioactive peptides in fermented meat products. Recently, Sentandreu et al. (2003) identified several small peptides in dry-cured ham. It would be possible that some generated peptides would have some bioactivity in such products. We measured the ACE inhibitory activities of extracts of several European fermented sausages and found that activity level of all extracts were higher than those of extracts obtained from nonfermented pork products (unpublished data).

10.2.3 Enzymatic Treatment

Utilization of various commercial proteases is one approach for producing bioactive peptides from food proteins. Many bioactive peptides have been experimentally generated in this way (Korhonen and Pihlanto 2003; Pihlanto and Korhonen 2003; Pihlanto-Leppälä 2001). 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 (Bruna, Fernandez, Hierro, Ordonez, and de la Hoz 2000; Diaz, Fernandez, Garcia De Fernando, de la Hoz, and Ordonez 1997). Such treatment would also generate bioactive peptides in meat products.

10.2.4 Identification of Peptides

After generation of bioactive peptides from meat proteins, the next steps are purification and identification of peptides corresponding to bioactivities. Figure 10.3 shows a typical procedure for the study of bioactive peptides. The peptides in hydrolyzates can be fractionated and enriched by various methods, such as precipitation with solvents and chromatography (Gonzalez de Llano and Polo Sánchez 2003). Today, reversed-phase high-performance liquid chromatography (RP-HPLC) is a pivotal technique in the purification of peptides (Aguilar 2004). Applications of HPLC to peptide purification have been expanded rapidly. A combination of HPLC and mass spectrometry (LC-MS) is a useful tool not only for purification of peptides but also

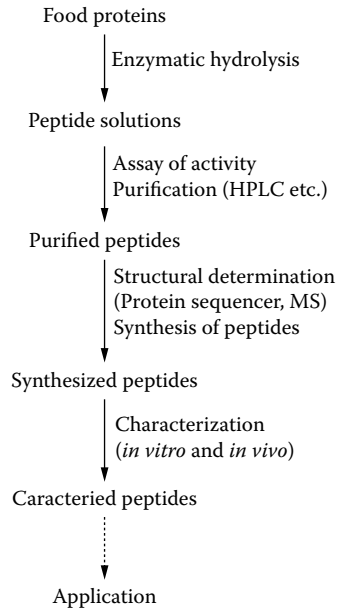


FIGURE 10.3 Typical experimental procedures for the study of bioactive peptides derived from food proteins.

structural identification of peptides (Curtis, Dennis, Waddell, MacGillivary, and Ewart 2002; Schevchenko, Chernushevich, Wilm, and Mann 2000; Sforza, Ferroni, Galaverna, Dossena, and Marchelli 2003; Shen and Noon 2004). Thus, in addition to the Edman degradation method, MS has become a powerful tool for sequencing of peptides (González de Llano and Polo Sánchez 2003).

Utilization of ultrafiltration membranes is another useful technique for producing and enriching specific peptide fractions (Pihlanto-Leppälä 2001). For example, this technique has been utilized for a bioreactor that produces gram-scale bioactive peptides (Bouhallab and Touze 1995). Because the ultrafiltration reactor can be easily upscaled, application to industrial production of bioactive peptides is expected.

10.3 ANTIHYPERTENSIVE PEPTIDES DERIVED FROM MEAT PROTEINS

As stated previously, many ACE inhibitory or antihypertensive peptides derived from food proteins have been reported. Among the several bioactive peptides derived from meat proteins, ACE inhibitory peptides have been studied most extensively.

10.3.1 ACE Inhibitory Peptides From Meat Proteins

Table 10.2 shows a summary of ACE inhibitory peptides generated from meat proteins, including peptides generated not only from the meat of domestic animals and poultry but also that of fish, as both animal and fish muscle proteins have relatively common

TABLE 10.2
ACE Inhibitory Peptides Derived from Meat and Related Proteins

Sequence[a]	Source	IC50[b] (μM)	SHR[c]	Reference
KW	Sardine muscle	1.6	nt	Matsufuji et al. (1994)
VY	Sardine muscle	11.0	nt	Seki et al. (1995)
IY	Dried bonito	2.1	+	Fujita, Yokoyama, and Yoshikawa (2000)
AKK	Sardine muscle	3.1	nt	Matsufuji et al. (1994)
FQP	Dried bonito (actin)	12.0	nt	Yokoyama, Chiba, and Yoshikawa (1992)
GPL	Bovine skin gelatin	2.6	nt	Kim et al. (2001)
GPV	Bovine skin gelatin	4.7	nt	Kim et al. (2001)
IKP	Dried bonito muscle	1.6	+	Fujita, Yokoyama, and Yoshikawa (2000)
IKW	Chicken muscle	0.2	+	Fujita, Yokoyama, and Yoshikawa (2000)
LKA	Chicken muscle (creatine kinase)	8.5	nt	Fujita, Yokoyama, and Yoshikawa (2000)
LKP	Chicken muscle (aldolase)	0.3	+	Fujita, Yokoyama, and Yoshikawa (2000)

TABLE 10.2
ACE Inhibitory Peptides Derived from Meat and Related Proteins (continued)

Sequence[a]	Source	IC50[b] (μM)	SHR[c]	Reference
LAP	Chicken muscle	3.5	+	Fujita, Yokoyama, and Yoshikawa (2000)
VWI	Porcine muscle (actin)	1.1	+	Arihara, Ishikawa et al. (2005)
GWAP	Sardine muscle	3.9	nt	Matsufuji et al. (1994)
ALPHA	Dried bonito (actin)	10.0	nt	Yokoyama, Chiba, and Yoshikawa (1992)
ITTNP	Porcine myosin	549.0	+	Nakashima et al. (2002)
IWHHT	Dried bonito (actin)	5.8	+	Yokoyama, Chiba, and Yoshikawa (1992) Fujita, Yokoyama, and Yoshikawa (2000)
LKPNM	Dried bonito (aldolase)	2.4	+	Fujita, Yamagami, and Ohshima (2001) Fujita, Yokoyama, and Yoshikawa (2000)
MNPPK	Porcine myosin	945.5	+	Nakashima et al. (2002)
DYGLYP	Dried bonito (fibronectin)	62.0	nt	Yokoyama, Chiba, and Yoshikawa (1992)
FQKPKR	Chicken muscle (myosin)	14.0	nt	Fujita, Yokoyama, and Yoshikawa (2000)
FQKVVA	Swine hemoglobin	5.8	nt	Mito et al. (1996)
GKKVLQ	Swine hemoglobin	1.9	nt	Mito et al. (1996)
IKPLNY	Dried bonito (myosin)	43.0	nt	Yokoyama, Chiba, and Yoshikawa (1992)
VLAQYK	Bovine muscle	23.2	+	Jang et al. (2004) Jang et al. (2005)
DMIPAQK	Dried bonito (creatine kinase)	45.0	nt	Yokoyama, Chiba, and Yoshikawa (1992)
FKGRYYP	Chicken muscle (creatine kinase)	0.6	—	Fujita, Yokoyama, and Yoshikawa (2000)
FQKVVAG	Swine hemoglobin	7.4	nt	Mito et al. (1996)
FQKVVAK	Swine hemoglobin	2.1	nt	Mito et al. (1996)
PTHIKWGD	Tuna muscle	0.9	nt	Kohama et al. (1988) Kohama et al. (1991)
VFPMNPPK	Fermented pork (myosin)	66.0	nt	Arihara et al. (2004)
IVGRPRHQG	Chicken muscle (actin)	2.4	—	Fujita, Yokoyama, and Yoshikawa (2000)
RMLGQTPTK	Porcine troponin C	34.0	nt	Katayama, Tomatsu et al. (2003) Katayama et al. (2004)
GFXGTXGLXGF	Chicken muscle (collagen)	42.4	nt	Saiga, Okumura et al. (2003)

[a] The one-letter amino acid codes were used (X, hydroxy-proline).
[b] The concentration of peptide needed to inhibit 50% of the ACE activity.
[c] Antihypertensive activities in spontaneously hypertensive rats (+, positive activity; —, no activity; nt, not tested).

sequences. Many ACE inhibitory peptides have been identified in protein hydrolyzates of fish and fish products, such as sardines (Matsufuji et al. 2004; Seki et al. 1995), tuna (Kohama et al. 1988; Kohama et al. 1991) and dried bonito (Fujita et al. 2001; Fujita and Yoshikawa 1999; Yokoyama et al. 1992).

Arihara et al. (2001) reported ACE inhibitory peptides in enzymatic hydrolyzates of porcine skeletal muscle proteins. Porcine muscle proteins were hydrolyzed by eight kinds of proteases (thermolysin, proteinase K, pronase E, ficin, papain, trypsin, α-chymotrypsin, pepsin). 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 SHR (Nakashima et al. 2002). Also, six tripeptides that have parts of the sequences of the two peptides were orally administered to SHR. Among the six tripeptides, Met-Asn-Pro caused the most significant decrease in systolic blood pressure. Katayama and colleagues (Katayama, Fuchu, et al. 2003; Katayama, Tomatsu, et al. 2003; Katayama et al. 2004) utilized porcine skeletal muscle and respective muscle proteins for proteolytic digestion in a series of studies. All enzymatic hydrolyzates 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 hydrolyzate showed the highest activity (Katayama, Fuchu, et al. 2003). They isolated a corresponding peptide (Arg-Met-Leu-Gly-Gln-Thr-Pro-Thr-Lys) from hydrolyzed porcine troponin C with pepsin (Katayama, Tomatsu, et al. 2003). They also investigated the inhibitory profile of this noncompetitive peptide (Katayama et al. 2004). According to evaluation based on Lineweaver-Burk plots, ACE inhibitory peptides are categorized into competitive, noncompetitive, and uncompetitive inhibitors for ACE (Li et al. 2004). ACE inhibitory activity and antihypertensive effect were also generated from pork muscle by fermentation with lactic acid bacteria (Arihara, Nakashima, Ishikawa, and Itoh 2004). Octapeptide (Val-Phe-Pro-Met-Asn-Pro-Pro-Lys) with ACE inhibitory activity was identified from the fermented pork meat.

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-Gln-Gly, Phe-Lys-Gly-Arg-Tyr-Tyr-Pro, Ile-Lys-Trp) generated from chicken muscle proteins by thermolysin treatment. However, some of them failed to show antihypertensive activity in SHR. This discrepancy was clarified by the authors as described later. Saiga, Okamura, et al. (2003) reported antihypertensive activity of *Aspergillus* protease-treated chicken muscle extract in SHR. Furthermore, they isolated four ACE inhibitory peptides from the hydrolyzate. 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.

More recently, Jang and Lee (2005) assayed ACE inhibitory activities of several enzymatic hydrolyzates of sarcoplasmic protein extracts from beef rump. An ACE inhibitory peptide (Val-Leu-Ala-Gln-Tyr-Lys) was purified from the hydrolyzate with the highest ACE inhibitory activity obtained by using the combination of thermolysin and proteinase A.

10.3.2 Antihypertensive Activity of ACE Inhibitory Peptides

ACE inhibitory peptides from various sources, including meat proteins, have shown antihypertensive effects by oral administration in animal experiments using SHR (Abubakar et al. 1998; Fujita et al. 2001; Fujita et al. 2000; Mito et al. 1996; Nakamura, Yamamoto, Sakai, and Takano 1995; Nakashima et al. 2002; Suetsuna and Nakano 1995; Yoshii et al. 1999). 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 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.

In most studies, ACE inhibitory activity has been measured basically according to the method of Cushman and Cheung (1971). This assay is based on the liberation of hippuric acid from Hip-His-Leu catalyzed by ACE. A sample solution of peptides is mixed with a buffer containing Hip-His-Leu and NaCl and then preincubated. The reaction is initiated by the addition of ACE. After incubation (reaction), the hippuric acid liberated by ACE is photometrically determined. The concentration of an ACE inhibitor needed to inhibit 50% of ACE activity is defined as the IC50 value. The discrepancy of ACE inhibitory activity *in vitro* and antihypertensive effect in vivo was clarified by Fujita et al. (2000). They preincubated ACE inhibitory peptides from several sources, including chicken muscle, with an ACE before measurement of ACE inhibitory activity and classified ACE inhibitory peptides into three groups. One group is inhibitor-type peptides; that is, peptides for which IC50 values are not affected by preincubation with ACE. Another group is prodrug-type peptides; that is, peptides that are converted to true inhibitors by an ACE or other proteases. For example, Ile-Val-Gly-Arg-Arg-Arg-His-Gln-Gly, generated from dried bonito muscle (actin), did not show antihypertensive activity. However, peptide Ile-Val-Gly-Arg-Arg-Arg, generated from Ile-Val-Gly-Arg-Arg-Arg-His-Gln-Gly by trypsin digestion, showed antihypertensive activity. Incidently, the sequence Ile-Val-Gly-Arg-Arg-Arg-His-Gln-Gly also exists in the primary structure of bovine, porcine, and chicken muscle actin. The third group is substrate-type peptides; that is, peptides that are hydrolyzed by an ACE to give peptides with weaker activity. Both inhibitor-type and prodrug-type peptides exert antihypertensive activities after oral administration in SHR. The primary activity of an ACE is to cleave the C-terminal dipeptide of oligopeptide substrates with a wide specificity. Therefore, the substrates for an ACE also show seemingly ACE inhibitory activities in the assay. For discriminating the substrate-type inhibitors from true inhibitors, peptides should be preincubated with an ACE before the measurement of ACE inhibitory activity.

10.3.3 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 seems 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 tryptophan, phenylalanine, tyrosine, or proline at their C-terminus (Cheung, Wang, Ondetti, and Sabe 1980; Cushman 1980; Maruyama et al. 1987; Miyoshi et al. 1991).

As stated earlier, ACE inhibitory peptides without antihypertensive activity may serve as substrates for an ACE and their activity would be inactivated (substrate type inhibitor). Another possibility is that peptides are digested into inactive fragments in the gastrointestinal tract after oral administration. Therefore, resistance of ACE inhibitory peptides to degradation by digestive enzymes is one possible critical feature. For example, ACE inhibitory peptides having a proline residue or Pro-Pro sequence at the C-terminus are resistant to enzymatic degradation (Li et al. 2004).

Several peptides have been reported to have antihypertensive activities without sufficient ACE inhibitory activities (Maeno, Yamamoto, and Takano 1996; Nakashima et al. 2002; Yamamoto and Takano 1999). Generally, di- and tripeptides can be absorbed directly in the intestine, but larger peptides are absorbed after hydrolysis by digestive enzymes in the intestine (Hara, Funabiki, Iwata, and Yamazaki 1984; Saito et al. 1994). Active peptides could be generated from original peptides by gastrointestinal digestion. In fact, Met-Asn-Pro and Pro-Pro-Lys, which have parts of the sequence of Met-Asn-Pro-Pro-Lys (ACE inhibitory and antihypertensive peptide derived from porcine muscle myosin; Nakashima et al. 2002), showed significant antihypertensive activities. Antihypertensive activity of Met-Asn-Pro-Pro-Lys might be caused by these tripeptides generated by gastrointestinal enzymes.

10.4 PROMISING BIOACTIVE PEPTIDES FROM MEAT PROTEINS

Information on meat protein-derived bioactive peptides other than ACE inhibitory peptides is still limited. In this section, several bioactive peptides derived from meat proteins that have been reported and possible bioactive peptides from meat proteins in the future are discussed.

10.4.1 Opioid Peptides

There has been no report on the generation of opioid peptides from muscle 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 hydrolyzate 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 recent study on quantification of hemorphins in Alzheimer's disease brains suggests that these peptides could be candidates for pharmacological prevention of this disease (Poljak, McLean, Sachdev, Brodaty, and Smythe 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).

10.4.2 Antioxidative Peptides

Both carnosine (β-alanyl-L-histidine) and anserine (N-β-alanyl-1-methyl-L-histidine) are endogenous antioxidative dipeptides found in skeletal muscle (Lynch and Kerry 2000). They are known to be the most abundant antioxidants in meats. These peptides have been reported to play many physiological roles, such as prevention of diseases related to oxidative stress (Hipkiss and Brownson 2000; Hipkiss et al. 1998).

Apart from endogenous nonprotein peptides, several antioxidative peptides have been reported to be generated from meat proteins by enzymatic digestion. However, there is still limited information on antioxidative peptides from meat proteins. Saiga, Tanabe, and Nishimura (2003) reported that hydrolyzates obtained from porcine myofibrillar proteins by protease treatment (papain or actinase E) exhibited high antioxidant activity in a linolenic acid peroxidation system induced by Fe^{2+}. These hydrolyzates possessed 1,1 diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity and chelating activity toward metal ions. Antioxidative peptides were isolated from papain hydrolyzate and sequenced as Asp-Ser-Gly-Val-Thr, Ile-Glu-Ala-Glu-Gly-Glu, Asp-Ala-Gln-Glu-Lys-Leu-Glu, Glu-Glu-Leu-Asp-Asn-Ala-Leu-Asn, and Val-Pro-Ser-Ile-Asp-Asp-Gln-Glu-Glu-Leu-Met. Among these five peptides, Asp-Ala-Gln-Glu-Lys-Leu-Glu, corresponding to a part of the sequence of porcine actin, showed the highest level of activity. Acidic amino acids, Asp or Glu, were found in all five peptides. Although it has been reported that peptides having basic amino acids (e.g., His and Lys) show strong antioxidative activity (Chen et al. 1995; Tsuge et al. 1991), it was revealed that acidic peptides as well as basic peptides possess antioxidative activity.

Elastin is a major protein component of elastic tissues such as ligaments. Antioxidative activities of pepsin-solubilized elastin (PSE) and acid-solubilized elastin (ASE) have been reported (Hattori, Yamaji-Tsukamoto, Kumagai, Feng, and Takahashi 1998). Both PSE and ASE were effective inhibitors of the oxidation of oleic acid. Their activity was enhanced in the presence of citric acid as a synergist. Although corresponding antioxidative peptides have not been identified, the mechanism for the antioxidative activity of elastin peptides might include (a) chelating ability for metal ions and (b) good affinity for oil, which would prevent the release of hydrogen or the binding of oxygen.

Recently, Arihara et al. (2005) investigated antioxidative activities of enzymatic hydrolyzates of porcine skeletal muscle actomyosin using a hypoxanthine-xanthine oxidase system as the source of superoxide anion. Three antioxidative peptides were

isolated from a papain-treated hydrolyzate of pork actomyosin and they were sequenced as Asp-Leu-Tyr-Ala, Ser-Leu-Tyr-Ala, and Val-Trp. In addition to antioxidative activity *in vitro*, these peptides showed physiological activity in vivo. Each of these peptides had an antifatigue effect when orally administered to mice in an experiment using a treadmill. Further studies aimed at elucidation of the relationship between antioxidative activity and the physiological effect are now in progress.

10.4.3 Peptides With Organoleptic Properties

Peptides also contribute significantly to organoleptic properties of foods (González de Llano and Polo Sánchez 2003; Nishimura and Kato 1988; Pihlanto and Korhonen 2003). In foods with protein-hydrolysis processes such as fermentation and aging, generation of flavor peptides is an important event. This is discussed here briefly.

Peptides that have a savory flavor are known to be generated from food proteins. For example, some savory peptides (Glu-Asp-Glu, Asp-Glu-Ser, Ser-Glu-Glu) were generated in fish muscle protein hydrolyzates (Noguchi, Arai, Yamashita, Kato, and Fujimaki 1975). An octapeptide with delicious taste (Lys-Gly-Asp-Glu-Glu-Ser-Leu-Ala) was isolated from beef treated with papain (Yamazaki and Maekawa 1978 1980). Later, this peptide was called "beef meaty peptide" or "savory taste-enhancing peptide" (Hau, Cazes, and Fay 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, Ser-Pro-Glu) were found in chicken protein hydrolyzate (Maehashi, Matsuzaki, Yamamoto, and Udaka 1999). Recently, Okumura, Yamada, and Nishimura (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).

10.5 CONCLUSIONS AND FUTURE PROSPECTS

In recent years, efforts have been made to introduce additional physiologically functional properties into meat products (Arihara 2004; Desmond and Troy 2004; Fernández-Ginéz et al. 2005; Jiménez-Colmenero, Carballo, and Cofrades 2001). Utilization of functional ingredients is one approach to the development of functional meat products. Such ingredients include vegetable proteins, fibers, antioxidants, probiotics, and prebiotics. Bioactive peptides described in this article are also promising candidates for ingredients of functional foods, including meat products. Although bioactive peptides, such as ACE inhibitors, have not yet been utilized in the meat industry, meat products with such activity could open up a new market. Because several food products containing antihypertensive peptides, such as sour milk and soup products, have been marketed for hypertensives in Japan (Arihara 2004), hydrolyzates of meat

proteins and their corresponding bioactive peptides might be utilized for physiologically functional foods. It is expected that increasing interest will be shown in basic research and potential applications of bioactive peptides for meat products.

Generation of bioactive peptides in meat products is a possible direction for introducing physiological function, especially suitable for fermented meat products. Bioactive peptides would be generated in fermented meat products as meat proteins are hydrolyzed by proteolytic enzymes during fermentation and storage. Developing functional fermented meat products could be a good strategy in the meat industry. Also, rediscovery of traditional fermented meats as functional foods is an interesting direction. Many traditional fermented foods, such as fermented dairy products, have been rediscovered as functional foods (Farnworth and Mainville 2003; Holzapfel, Schillinger, and Buckenhuskes 2003; Hoshi and Kiuchi 2003; Playne et al. 2003). Numerous physiologically active components including bioactive peptides, have been discovered in these traditional fermented foods. For these reasons, traditional fermented meats are attractive targets for finding new functional meat products. Another attractive approach for developing functional fermented meat products is probiotic lines. Probiotics is defined as "live microorganisms which, when administered in adequate amounts (as part of food), confer a health benefit on the host" (Stanton et al. 2003). In the dairy industry, the main approach for improving the beneficial physiological properties of products has been the development of probiotic lines of traditional fermented products. In recent years, the possibility of development of probiotic meat products has been discussed in the field of meat science and industry (Arihara 2004; Hammes, Haller, and Gänzle 2003; Hugas and Monfort 1997; Incze 1998; Jiménez-Colmenero et al. 2001; Lücke 2000; Työppönen, Petäjä, and Mattila-Sandholm 2003). By using probiotic bacteria, potential health benefits can be introduced to meat products. Technically, it has already become possible to produce probiotic meat products. German and Japanese producers developed meat products containing human intestinal lactic acid bacteria (Arihara 2004). "Breadton" (figure 10.4) is a new range of meat spread products fermented with intestinal lactic acid bacteria (*Lactobacillus rhamnosus* FERM P-15120; Sameshima et al. 1998). In addition, because meat proteins in this product are partially hydrolyzed by proteases prior to fermentation, this product contains relatively high level of peptides. Although further studies of the relationship between ingestion of such products and human health are required, combination of probiotics and peptides would have a great possibility to develop novel functional meat products.

FIGURE 10.4 Fermented meat spread product (Breadon) utilizing the human intestinal lactobacilli.

Food safety is another important aspect for the development of new functional products. Bioactive peptides must be safe when taken orally. Meat bioactive peptides are naturally derived from meat proteins that are consumed daily by humans. Thus, these peptides are considered to be mild and safe without the side effects associated with drugs. However, without sufficient evidence of safety, consumers would probably be reluctant to purchase new functional products. There are still some difficulties in marketing functional meat products: They are unconventional, and consumers in many countries do not recognize meat and meat products as healthy foods, unlike milk and dairy products. Therefore, consumers should be provided with proof of their safety as well as their health benefits.

REFERENCES

Abubakar, A., T. Saito, H. Kitazawa, Y. Kawai, and T. Itoh. 1998. Structural analysis of new antihypertensive peptides derived from cheese whey protein by proteinase K digestion. *J. Dairy Sci.* 81:3131–3138.

Aguilar, M. -I. 2004. Reversed-phase high-performance liquid chromatography. In *HPLC of peptides and proteins,* ed. M.-I. Aguilar, 9–22. Totowa, NJ: Humana.

Arihara, K. 2004. Functional foods. In *Encyclopedia of meat sciences,* eds. W. K. Jensen, C. Devine, and M. Dikeman, 492–499. Oxford, UK: Elsevier.

Arihara, K., S. Ishikawa, R. Itoh, M. Akimoto, Y. Nakashima, and S. Kanai. 2005. Antihypertensive peptides generated from pork proteins. Japan patent (submitted to government).

Arihara, K., Y. Nakashima, S. Ishikawa, and M. Itoh. 2004. Antihypertensive activities generated from porcine skeletal muscle proteins by lactic acid bacteria. In *Abstracts of 50th International Congress of Meat Science and Technology,* 236. Helsinki: Helsinki University Press.

Arihara, K., Y. Nakashima, T. Mukai, S. Ishikawa, and M. Itoh. 2001. Peptide inhibitors for angiotensin I-converting enzyme from enzymatic hydrolysates of porcine skeletal muscle proteins. *Meat Sci.* 67:434–437.

Arihara, K., K. Tomita, S. Ishikawa, M. Itoh, M. Akimoto, and T. Sameshima. 2005. Antifatigue peptides derived from meat proteins. Japan patent (submitted to government).

Ariyoshi, Y. 1993. Angiotensin-converting enzyme inhibitors derived from food proteins. *Trends Food Sci. Technol.* 4:139–144.

Bezkorovainy, A., D. Grohlich, and J. H. Nichols. 1979. Isolation of a glycopolypeptide fraction with *Lactobacillus bifidus* subspecies *pennsylvanicus* growth-promoting activity from whole human milk casein. *Am. J. Clin. Nutr.* 32:1428–1432.

Blishchenko, E., O. Sazonova, A. Surovoy, S. Khaidukov, Y. Sheikine, D. Sokolov, I. Freidlin, M. Philippova, A. Vass, A. Karelin, and V. Ivanov. 2002. Antiproliferative action of valorphin in cell cultures. *J. Peptide Sci.* 8:438–452.

Bouhallab, S., D. Molle, and J. Leonil. 1992. Tryptic hydrolysis of caseinomacropeptide in membrane reactor: Preparation of bioactive peptides. *Biotechnol. Lett.* 14:805–810.

Bouhallab, S., and C. Touze. 1995. Continuous hydrolysis of caseinomacropeptide in a membrane reactor: Kinetic study and gram-scale production of antithrombotic peptides. *Lait.* 75:251–258.

Brantl, V., H. Teschemacher, A. Henschen, and F. Lottspeich. 1979. Novel opioid peptides derived from casein (β-Casomorphins) I. Isolation from bovine casein peptone. Hoppe-Seyler's Z. *Physiol. Chem.* 360:1211–1216.

Brody, E. P. 2000. Biological activities of bovine glycomacropeptide. *Br. J. Nutr.* 84:S39–S46.

Bruna, J. M., M. Fernandez, E. M. Hierro, J. A. Ordonez, and L. de la Hoz. 2000. Combined use of pronase E and a fungal extract (*Penicillium aurantiogriseum*) to potentiate the sensory characteristics of dry fermented sausages. *Meat Sci.* 54:135–145.

Chen, H. -M., K. Muramoto, and F. Yamauchi. 1996. Antioxidant activity of designed peptides based on the antioxidative peptide isolated from digests of a soybean protein. *J. Agric. Food Chem.* 44:2619–2623.

Chen, H. -M., K. Muramoto, and F. Yamauchi. 1995. Structural analysis of antioxidative peptides from soybean β-conglycinin. *J. Agric. Food Chem.* 43:574–578.

Cheung, H. S., F. L. Wang, M. A. Ondetti, E. F. Sabe, and D. W. Cushman. 1980. Binding of peptide substrate and inhibition of angiotensin-converting enzyme: Importance of the COOH-terminal dipeptides sequence. *J. Biol. Chem.* 255:401–407.

Chiba, H., and M. Yoshikawa. 1986. Biologically functional peptides from food proteins: New opioid peptides from milk proteins. In *Protein tailoring for food and medical users,* eds. R. E. Feeney and J. R. Whitaker, 123–153. New York: Marcel Dekker.

Clare, D. A., and H. E. Swaisgood. 2000. Bioactive milk peptides: A prospectus. *J. Dairy Sci.* 83:1187–1195.

Curtis, J. M., D. Dennis, D. S. Waddell, T. MacGillivary, and H. S. Ewart. 2002. Determination of angiotensin-converting enzyme inhibitory peptide Leu-Lys-Pro-Asn-Met (LKPNM) in bonito muscle hydrolysates by LC-MS/MS. *J. Agric. Food Chem.* 50:3919–3925.

Cushman, D. W., and H. S. Cheung. 1971. Spectrophotometric assay and properties of the angiotensin converting enzyme of rabbit lung. *Biochem. Pharmacol.* 20:1637–1648.

Dainty, R., and H. Blom. 1995. Flavor chemistry of fermented sausages. In *Fermented meats,* eds. G. Campbell Plattand P. E. Cook,. 176–193. Glasgow, Scotland: Blackie Academic & Professional.

Dentali, S. 2002. Regulation of functional foods and dietary supplements. *Food Technol.* 56(6):89–94.

Desmond, E., and D. J. Troy. 2004. Nutrient claims on packaging. In *Encyclopedia of meat sciences,* eds. W. K. Jensen, C. Devine, and M. Dikeman, 903–909. Oxford, UK: Elsevier.

Diaz, O., M. Fernandez, G. D. Garcia De Fernando, L. de la Hoz, and J. A. Ordonez. 1997. Proteolysis in dry fermented sausages: The effect of selected exogenous proteases. *Meat Sci.* 46:115–128.

Dransfield, E., and D. Etherington. 1981. Enzymes in the tenderization of meat. In *Enzymes and food processing,* eds. G. G. Birch, N. Blakebrough, and K. J. Parker, 177–194. London: Applied Science Publishers.

Elitsur, Y., and G. D. Luk. 1991. β-casomorphin (BCM) and human colonic lamina propria lymphocyte proliferation. *Clin. Exp. Immunol.* 123:1068–1070.

Etherington, D. J. 1984. The contribution of proteolytic enzymes to postmortem changes in muscle. *J. Anim. Sci.* 59:1644–1650.

Etoh, S., K. Asamura, A. Obu, K. Sonomoto, and A. Ishizaki. 2000. Purification and identification of a growth-stimulating peptide for *Bifidobacterium bifidum* from natural rubber serum powder. *Biosci. Biotechnol. Biochem.* 64:2083–2088.

Farnworth, E. R., and I. Mainville. 2003. Kefir: A fermented milk product. In *Handbook of fermented functional foods,* ed. E. R. Farnworth, 77–111. Boca Raton, FL: CRC Press.

Fernández-Ginéz, J. M., J. Fernández-López, E. Sayas-Barberá, and J. A. Pérez-Alvarez. 2005. Meat products as functional foods: A review. *J. Food Sci.* 70:R37-R43.

Fiat, A.-M., D. Migliore-Samour, P. Jollès, L. Drout, C. Collier, and J. Caen. 1993. Biologically active peptides from milk proteins with emphasis on two examples concerning antithrombotic and immunomodulating activities. *J. Dairy Sci.* 76:301–310.

Fujita, H., T. Yamagami, and K. Ohshima. 2001. Effects of an ACE-inhibitory agent, katsuobushi oligopeptide, in the spontaneously hypertensive rat and in borderline and mildly hypertensive subjects. *Nutr. Res.* 21:1149–1158.

Fujita, H., K. Yokoyama, and M. Yoshikawa. 2000. Classification and antihypertensive activity of angiotensin I-converting enzyme inhibitory peptides derived from food proteins. *J. Food Sci.* 65:564–569.

Fujita, H., and M. Yoshikawa. 1999. LKPNM: A prodrug-type ACE-inhibitory peptide derived from fish protein. *Immunopharmacology.* 44:123–127.

Fukudome, S., and M. Yoshikawa. 1992. Opioid peptides derived from wheat gluten, their isolation and characterization. *FEBS Lett.* 296:107–111.

Fukudome, S., and M. Yoshikawa. 1993. A novel opioid peptide derived from wheat gluten. *FEBS Lett.* 316:17–19.

Gagnaire, V., A. Pierre, D. Molle, and J. Leonil. 1996. Phosphopeptides interacting with colloidal calcium phosphate isolated by tryptic hydrolysis of bovine casein micelles. *J. Dairy Res.* 63:405–422.

Gobbetti, M., P. Ferranti, E. Smacchi, F. Goffredi, and F. Addeo. 2000. Production of angiotensin-I converting-enzyme-inhibitory peptides in fermented milks started by *Lactobacillus delbrueckii* subsp. bulgaricus SS1 and *Lactococcus lactis* subsp. cremoris FT4. *Appl. Environ. Microbiol.* 66:3898–3904.

González de Llano, D., and C. Polo Sánchez. 2003. Peptides. In *Encyclopedia of food science and nutrition,* 2nd ed., eds. B. Caballero, L. C. Trugo, and P. M. Finglas, 4468–4473. London: Academic Press.

Hammes, W. P., D. Haller, and M. G. Gänzle. 2003. Fermented meat. In *Handbook of fermented functional foods,* ed. E. R. Farnworth, 251–275. Boca Raton, FL: CRC Press.

Hara, H., R. Funabiki, M. Iwata, and K. Yamazaki. 1984. Portal absorption of small peptides in rats under unrestrained conditions. *J. Nutr.* 114:1122–1129.

Hasler, C. M. 1998. Functional foods: Their role in disease prevention and health promotion. *Food Technol.* 52(10):63–70.

Hattori, M., K. Yamaji-Tsukamoto, H. Kumagai, Y. Feng, and K. Takahashi. 1998. Antioxidative activity of soluble elastin peptides. *J. Agric. Food Chem.* 46:2167–2170.

Hau, J., D. Cazes, and L. B. Fay. 1997. Comprehensive study of the "beefy meaty peptide". *J. Agric. Food Chem.* 45:1351–1355.

Heasman, M., and J. Mellentin. 2001. *The functional foods revolution.* London: Earthscan Publications.

Henriksen, A. H., and L. H. Stahnke. 1997. Sensory and chromatographic evaluations of water soluble fractions from dried sausages. *J. Agric. Food Chem.* 45:2679–2684.

Hertog, M. G. L. 1996. Epidemiological evidence on potential health properties of flavonoids. *Proc. Nutr. Soc.* 55:385–387.

Hierro, E., L. de la Hoz, and J. A. Ordonez. 1999. Contribution of the microbial and meat endogenous enzymes to the free amino acid and amine contents of dry fermented sausages. *J. Agric. Food Chem.* 47:1156–1161.

Hipkiss, A. R., and C. A. Brownson. 2000. A possible new role for the anti-aging peptide carnosine. *Cell Mol. Life Sci.* 57:747–753.

Hipkiss, A. R., J. E. Preston, D. T. Himsworth, L. Allende, N. J. Abbott, and J. Michaelis. 1998. Pluripotent protective effects of carnosine, a naturally occurring dipeptide. *Ann. NY Acad. Sci.* 854:37–53.

Hoek, K. S., J. M. Milne, P. A. Grieve, D. A. Dionysius, and R. Smith. 1997. Antibacterial activity of bovine lactoferrin-derived peptides. *Antimicrob. Agents Chemother.* 41:54–59.

Holzapfel, W., U. Schillinger, and H. J. Buckenhuskes. Sauerkraut. In *Handbook of fermented functional foods,* ed. E. R. Farnworth, 343–360. Boca Raton, FL: CRC Press.

Holzapfel, W. H., and U. Schillinger. 2002. Introduction to pre- and probiotics. *Food Res. Int.* 35:109–116.

Hoshi, T., and K. Kiuchi. 2003. Natto: A food made by fermenting cooked soybeans with *Bacillus subtilis (natto).* In *Handbook of fermented functional foods,* ed. E. R. Farnworth, 227–250. Boca Raton, FL: CRC Press.

Hugas, M., and J. M. Monfort. 1997. Bacterial starter cultures for meat fermentation. *Food Chem.* 59:547–554.

Ibrahim, H. R., Y. Sugimoto, and T. Aoki. 2000. Ovotransferrin antimicrobial peptide (OTAP-92) kills bacteria through a membrane damage mechanism. *Biochim. Biophys. Acta.* 1523:196–205.

Idota, T., H. Kawakami, and I. Nakajima. 1994. Growth-promoting effects of N-acetyl-neuraminic acid containing substances on bifidobacteria. *Biosci. Biotechnol. Biochem.* 58:1720–1722.

Incze, K. 1998. Dry fermented sausages. *Meat Sci.* 49:S169–S177.

Jang, A., Y. Cho, J. Lee, L. Shin, J. H. Kim, and M. Lee. 2004. The effect of beef peptide on blood pressure and serum lipid concentration of spontaneously hypertensive rat (SHR). *J. Anim. Sci. Technol. (Korea).* 46:107–114.

Jang, A., and M. Lee. 2005. Purification and identification of angiotensin converting enzyme inhibitory peptides from beef hydrolysates. *Meat Sci.* 69:653–661.

Jiménez-Colmenero, F., J. Carballo, and S. Cofrades. 2001. Healthier meat and meat products: Their role as functional foods. *Meat Sci.* 59:5–13.

Jollès, P., and A. Henschen. 1982. Comparison between the clotting of blood and milk. *Trends Biochem. Sci.* 7:325–328.

Jollès, P., S. Levy-Toledano, A. M. Fiat, C. Soria, D. Gillessen, A. Thomaidis, F. W. Dunn, and J. B. Caen. 1986. Analogy between fibrinogen and casein. *Eur. J. Biochem.* 158:379–384.

Junfeng, F., and L. Lite. 2003. Preparation of angiotensin I-converting enzyme inhibiting peptides from soybean protein by enzymatic hydrolysis. *Food Sci. Technol. Res.* 9:254–256.

Katayama, K., H. Fuchu, A. Sakata, S. Kawahara, K. Yamauchi, Y. Kawahara, and M. Muguruma. 2003. Angiotensin I-converting enzyme inhibitory activities of porcine skeletal muscle proteins following enzyme digestion. *Asian-Austral. J. Anim. Sci.* 16:417–424.

Katayama, K., M. Tomatsu, H. Fuchu, M. Sugiyama, S. Kawahara, K. Yamauchi, Y. Kawamura, and M. Muguruma. 2003. Purification and characterization of an angiotensin I-converting enzyme inhibitory peptide derived from porcine troponin C. *Anim. Sci. J.* 74:53–58.

Katayama, K., M. Tomatsu, S. Kawahara, K. Yamauchi, H. Fuchu, Y. Kodama, Y. Kawamura, and M. Muguruma. 2004. Inhibitory profile of nonapeptide derived from porcine troponin C against angiotensin I-converting enzyme. *J. Agric. Food Chem.* 52:771–775.

Kato, T., T. Matsuda, T. Tahara, M. Sugimoto, Y. Sato, and R. Nakamura. 1994. Effects of meat conditioning and lactic fermentation on pork muscle protein degradation. *Biosci. Biotechnol. Biochem.* 58:408–410.

Kayser, H., and H. Meisel. 1996. Stimulation of human peripheral blood lymphocytes by bioactive peptides derived from bovine milk proteins. *FEBS Lett*. 383:18–20.

Kim, S. K., H. G. Byun, P. J. Park, and F. Shahidi. 2001. Angiotensin I converting enzyme inhibitory peptide purified from bovine skin gelatin hydrolysate. *J. Agric. Food Chem*. 49:2992–2997.

Kitamura, S., S. Muroya, S. Tanabe, T. Okumura, K. Chikuni, and T. Nishimura. 2005. Mechanism of production of troponin T fragments during postmortem aging of porcine muscle. *J. Agric. Food Chem*. 53:4178–4181.

Kohama, Y., S. Matsumoto, H. Oka, T. Teramoto, M. Okabe, and T. Miura. 1988. Isolation of angiotensin-converting enzyme inhibitor from tuna muscle. *Biochem. Biophys. Res. Commun*. 155:332–337.

Kohama, Y., H. Oka, Y. Kayamori, K. Tsujikawa, T. Miura, Y. Nagase, and M. Satake. 1991. Potent synthetic analogues of angiotensin-converting enzyme inhibitor derived from tuna muscle. *Agric. Biol. Chem*. 55:2169–2170.

Koldovsky, O. 1989. Critical review: Search for role of milk-borne biologically active peptides for the suckling. *J. Nutr.* 119:1543–1551.

Koohmaraie, M. 1994. Muscle proteinases and meat aging. *Meat Sci*. 36:93–104.

Korhonen, H., and A. Pihlanto. 2003. Food-derived bioactive peptides: Opportunities for designing future foods. *Curr. Pharm. Des*. 9:1297–1308.

Kuba, M., C. Tana, S. Tawata, and M. Yasuda. 2005. Production of angiotensin I-converting enzyme inhibitory peptides from soybean protein with *Monascus purpureus* acid proteinase. *Process. Biochem*. 40:2191–2196.

Kudo, Y., S. Matsuda, K. Igoshi, and T. Oki. 2001. Antioxidative peptide from milk fermented with *Lactobacillus delbrueckii* subsp. *bulgaricus* IFO13953. *Nippon Shokuhin Kogaku Kaishi*. 48:44–50.

Lahov, E., and W. Regelson. 1996. Antibacterial and immunostimulating casein-derived substances from milk: Casecidin, istacidin peptides. *Food Chem. Toxicol*. 34:131–145.

Li, G. H., G. W. Le, Y. H. Shi, and S. Shrestha. 2004. Angiotensin I-converting enzyme inhibitory peptides derived from food proteins and their physiological and pharmacological effects. *Nutr. Res*. 24:469–486.

Lieple, C., K. Adermann, M. Raida, H.-J. Magert, W.-G. Forssman, and H.-D. Zucht. 2002. Human milk provides peptides highly stimulating the growth of bifidobacteria. *Eur. J. Biochem*. 269:712–718.

Lindsay, D. G. 2000. Maximizing the functional benefits of plant foods. In *Functional foods,* eds. G. R. Gibson and C. M. Williams, 183–208. Boca Raton, FL: CRC Press.

Lücke, F. -K. 2000. Utilization of microbes to process and preserve meat. *Meat Sci*. 56:105–115.

Lynch, P. B., and J. P. Kerry. 2000. Utilizing diet to incorporate bioactive compounds and improve the nutritional quality of muscle foods. In *Antioxidants in muscle foods,* eds. E. Decker, C. Faustman, and C. J. Lopez-Bote, 455–480. New York: Wiley.

Maehashi, K., M. Matsuzaki, Y. Yamamoto, and S. Udaka. 1999. Isolation of peptides from an enzymatic hydrolysate of food proteins and characterization of their taste properties. *Biosci. Biotechnol. Biochem*. 63:555–559.

Maeno, M., N. Yamamoto, and T. Takano. 1996. Identification of an antihypertensive peptide from casein hydrolysate produced by a proteinase from *Lactobacillus helveticus* CP790. *J. Dairy Sci*. 79:1316–1321.

Maruyama, S., H. Mitachi, J. Awaya, M. Kurono, N. Tomizuka, and H. Suzuki. 1987. Angiotensin I converting enzyme inhibitory activity of the C-terminal hexapeptide of αs1-casein. *Agric. Biol. Chem*. 51:2557–2561.

Maruyama, S., and H. Suzuki. 1982. A peptide inhibitor of angiotensin I-converting enzyme in the tryptic hydrolysate of casein. *Agric. Biol. Chem.* 46:1393–1394.

Mater, C., J. G. LeBlanc, L. Martin, and G. Perdigon. 2003. Biologically active peptides released in fermented milk: Role and functions. In *Handbook of fermented functional foods,* ed. E. R. Farnworth, 177–201. Boca Raton, FL: CRC Press.

Matsubayashi, Y., and Y. Sakagami. 1999. Characterization of specific binding sites for a mitogenic sulfated peptide, phytosulfokine-α, in the plasma-membrane fraction derived from *Oryza sativa* L. *Eur. J. Biochem.* 262:666–671.

Matsufuji, H., T. Matsui, E. Seki, K. Osajima, M. Nakashima, and Y. Osajima. 1994. Angiotensin I converting enzyme inhibitory peptides in an alkaline protease hydrolyzate derived from sardine muscle. *Biosci. Biotechnol. Biochem.* 58:2244–2245.

Matsui, T., C. H. Li, and Y. Osajima. 1999. Preparation and characterization of novel bioactive peptides responsible for angiotensin I-converting enzyme inhibition from wheat germ. *J. Peptide Sci.* 5:289–297.

Meisel, H. 1986. Chemical characterization and opioid activity of an exorphin isolated from in vivo digests of casein. *FEBS Lett.* 196:223–227.

Meisel, H. 1998. Overview on milk protein-derived peptides. *Int. Dairy J.* 8:363–373.

Meisel, H., and W. Bockelmann. 1999. Bioactive peptides encrypted in milk proteins: Proteolytic activation and thropho-functional properties. *Antonie van Leeuwenhoek.* 76:207–215.

Mellander, O. 1950. The physiological importance of the casein phosphopeptide calcium salts II: Peroral calcium dosage of infants. *Acta Soc. Med. Uppsala.* 55:247–255.

Migliore-Samour, D., F. Floc'h, and P. Jollès. 1989. Biologically active casein peptides implicated in immunomodulation. *J. Dairy Res.* 56:357–362.

Mikami, M., M. Nagao, M. Sekikawa, and H. Miura. 1995. Changes in peptide and free amino acid contents of different bovine muscle homogenate during storage. *Anim. Sci. Technol. (Japan).* 66:630–638.

Mito, K., M. Fujii, M. Kuwahara, N. Matsumura, T. Shimizu, S. Sugano, and H. Karaki. 1996. Antihypertensive effect of angiotensin I-converting enzyme inhibitory peptides derived from hemoglobin. *Eur. J. Pharmacol.* 304:93–98.

Miyauchi, H., S. Hashimoto, M. Nakajima, I. Shinoda, Y. Fukuwatari, and H. Hayasawa. 1998. Bovine lactoferrin stimulates the phagocytic activity of human neutrophils: Identification of its active domain. *Cell. Immunol.* 187:34–37.

Miyoshi, S., H. Ishikawa, T. Kaneko, F. Fukui, H. Tanaka, and S. Maruyama. 1991. Structures and activity of angiotensin-converting enzyme inhibitors in α-zein hydrolysate. *Agric. Biol. Chem.* 55:1313–1318.

Miyoshi, S., T. Kaneko, H. Ishikawa, H. Tanaka, and S. Maruyama. 1995. Production of peptides from corn endosperm proteins by some proteases. *Ann. NY Acad. Sci.* 750:429–431.

Mullally, M. M., H. Meisel, and R. J. FitzGerald. 1997. Identification of a novel angiotensin-I-converting enzyme inhibitory peptide corresponding to a tryptic fragment of bovine beta-lactoglobulin. *FEBS Lett.* 402:99–101.

Nagaoka, S., Y. Futamura, K. Miwa, T. Awano, K. Yamauchi, Y. Kanamaru, T. Kojima, and T. Kuwata. 2001. Identification of novel hypocholesterolemic peptides derived from bovine milk β-lactoglobulin. *Biochem. Biophys. Res. Commun.* 281:11–17.

Nagaoka, S., Y. Kanamaru, and Y. Kuzuya. 1992. Comparative studies on the serum cholesterol lowering action of whey protein and soyprotein in rats. *Biosci. Biotechnol. Biochem.* 56:1484–1485.

Nakamura, Y., N. Yamamoto, K. Sakai, A. Okubo, S. Yamazaki, and T. Takano. 1995. Purification and characterization of angiotensin I-converting enzyme inhibitors from sour milk. *J. Dairy Sci.* 78:777–783.

Nakamura, Y., N. Yamamoto, K. Sakai, and T. Takano. 1995. Antihypertensive effect of sour milk and peptides isolated from it that are inhibitors to angiotensin I-converting enzyme. *J. Dairy Sci.* 78:1253–1257.

Nakashima, Y., K. Arihara, A. Sasaki, S. Ishikawa, and M. Itoh. 2002. Antihypertensive activities of peptides derived from porcine skeletal muscle myosin in spontaneously hypertensive rats. *J. Food Sci.* 67:434–437.

Nishimura, T., and H. Kato. 1988. Mechanisms involved in the improvement of meat taste during postmortem aging. *Food Sci. Technol. Int. Tokyo.* 4:241–249.

Nishimura, T., M. R. Rhue, A. Okitani, and H. Kato. 1988. Components contributing to the improvement of meat taste during storage. *Agric. Biol. Chem.* 52:2323–2330.

Noguchi, M., S. Arai, M. Yamashita, H. Kato, and M. Fujimaki. 1975. Isolation and identification of acidic oligopeptides occurring in a flavor potentiating fraction from a fish protein hydrolysate. *J. Agric. Food Chem.* 23:49–53.

Nuijens, J. H., P. H. C. van Berkel, and F. L. Schanbacher. 1996. Structure and biological actions of lactoferrin. *J. Mammary Gland Biol. Neoplasia.* 1:285–294.

Nurminen, M.-L., M. Sipola, H. Kaarto, A. Pihlanto-Leppälä, K. Piilola, R. Korpela, O. Tossavainen, H. Korhonen, and H. Vapaatalo. 2000. α-Lactophin lowers blood pressure via radiotelemetry in normotensive and spontaneously hypertensive rats. *Life Sci.* 66:1535–1543.

Nyberg, F., K. Sanderson, and E.-L. Glämsta. 1997. The hemorphins: A new class of opioid peptides derived from the blood protein hemoglobin. *Biopolymers.* 43:147–156.

Okumura, T., R. Yamada, and T. Nishimura. 2004. Sourness-suppressing peptides in cooked pork loins. *Biosci. Biotechnol. Biochem.* 68:1657–1662.

Ondetti, M. A., and D. W. Cushman. 1982. Enzymes of the renin-angiotensin system and their inhibitors. *Ann. Rev. Biochem.* 51:283–308.

Ordonez, J. A., E. M. Hierro, J. M. Bruna, and L. de la Hoz. 1998. Changes in the components of dry fermented sausages during ripening. *Crit. Rev. Food Sci. Nutr.* 39:329–367.

Oshima, G., H. Shimabukuro, and K. Nagasawa. 1979. Peptide inhibitors of angiotensin I-converting enzyme in digests of gelatin by bacterial collagenase. *Biochim. Biophy. Acta.* 566:128–137.

Otani, H., and I. Hata. 1995. Inhibition of proliferative responses of mouse spleen lymphocytes and rabbit Peyer's patch cells by bovine milk caseins and their digests. *J. Dairy Res.* 62:339–348.

Pihlanto, A., and H. Korhonen. 2003. Bioactive peptides and proteins. *Adv. Food and Nutr. Res.* 47:175–276.

Pihlanto-Leppälä, A. 2001. Bioactive peptides derived from bovine whey proteins: Opioid and ACE inhibitory peptides. *Trends Food Sci. Technol.* 11:347–356.

Pihlanto-Leppälä, A. 2003. Bioactive peptides. In *Encyclopedia of dairy sciences,* eds. H. Roginski, J. W. Fuquay, and P. F. Fox, 1960–1967. Amsterdam: Elsevier.

Playne, M. J., L. E. Bennett, and G. W. Smithers. 2003. Functional dairy foods and ingredients. *Aust. J. Dairy Technol.* 58:242–264.

Poljak, A., C. A. McLean, P. Sachdev, H. Brodaty, and G. A. Smythe. 2004. Quantification of hemorphins in Alzheimer's disease brains. *J. Neurosci. Res.* 75:704–714.

Pszczola, D. E., F. Katz, and J. Giese. 2004. Research trends in healthful foods. *Food Technol. 54*(3):54–66.

Rincón-León, F. 2003. Functional foods. In *Encyclopedia of food science and nutrition,* 2nd ed., eds. B. Caballero, L. C. Trugo, and P. M. Finglas, 2827–2832. London: Academic Press.

Rival, S. G., C. G. Boeriu, and H. J. Wichers. 2001. Caseins and casein hydrolysates: 2. Antioxidative properties and relevance to lipoxygenase inhibition. *J. Agric. Food Chem.* 49:295–302.

Rival, S. G., S. Fornaroli, C. G. Boeriu, and H. J. Wichers. 2001. Caseins and casein hydrolysates: 1. Lipoxygenase inhibitory properties. *J. Agric. Food Chem.* 49:287–294.

Roberfroid, M. B. 2000. Defining functional foods. In *Functional foods,* eds. G. R. Gibson and C. M. Williams, 9–27. Boca Raton, FL: CRC Press.

Robert, M.-C., A. Razaname, M. Mutter, and M. A. Juillerat. 2004. Identification of Angiotensin-I converting enzyme inhibitory peptides derived from sodium caseinate hydrolysates produced by *Lactobacillus helveticus* NCC 2765. *J. Agric. Food Chem.* 52:6923–6931.

Saiga, A., T. Okumura, T. Makihara, S. Katsuta, T. Shimizu, R. Yamada, and T. Nishimura. 2003. Angiotensin I-converting enzyme inhibitory peptides in a hydrolyzed chicken breast muscle extract. *J. Agric. Food Chem.* 51:1741–1745.

Saiga, A., S. Tanabe, and T. Nishimura. 2003. Antioxidant activity of peptides obtained from porcine myofibrillar proteins by protease treatment. *J. Agric. Food Chem.* 51:3661–3667.

Saito, T., T. Nakamura, H. Kitazawa, Y. Kawai, and T. Itoh. 2000. Isolation and structural analysis of antihypertensive peptides that exist naturally in Gouda cheese. *J. Dairy Sci.* 83:1434–1440.

Saito, Y., K. Wanezaki, A. Kawato, and S. Iwayasu. 1994. Structure and activity of angiotensin I converting enzyme inhibitory peptides from sake and sake lees. *Biosci. Biotechnol. Biochem.* 58:1767–1771.

Sameshima, T., C. Magome, K. Takeshita, K. Arihara, M. Itoh, and Y. Kondo. 1998. Effect of intestinal *Lactobacillus* starter cultures on the behaviour of *Staphylococcus aureus* in fermented sausage. *Int. J. Food Microbiol.* 41:1–7.

Sarraga, C., M. Gil, J. Arnau, J. M. Monfort, and R. Cusso. 1989. Effect of curing salt and phosphate on the activity of porcine muscle proteases. *Meat Sci.* 25:241–249.

Sato, R., T. Noguchi, and H. Naito. 1986. Casein phosphopeptide (CPP) enhances calcium absorption from the ligated segment of rat small intestine. *J. Nutr. Sci. Vitaminol.* 32:67–76.

Schanbacher, F. L., R. S. Talhouk, F. A. Murray, L. I. Gherman, and L. B. Willett. 1998. Milk-borne bioactive peptides. *Int. Dairy J.* 8:393–403.

Schevchenko, A., I. Chernushevich, M. Wilm, and M. Mann. 2000. De novo peptide sequencing by nanoelectrospray tandem mass spectrometry using tripe quadrupole and quadrupole/time-of-flight instruments. In *Mass spectrometry of proteins and peptides,* ed. J. R. Chapman, 1–16. Totowa, NJ: Humana Press.

Seki, E., K. Osajima, H. Matsufuji, T. Matsui, and Y. Osajima. 1995. Val-Tyr, an angiotensin I converting enzyme inhibitor from sardines that have resistance to gastrointestinal proteases. *Nippon Nogeikagaku Kaishi.* 69:1013–1020.

Sentandreu, M. A., S. Stoeva, M. C. Aristoy, K. Laib, W. Voelter, and F. Toldrá. 2003. Identification of small peptides generated in Spanish dry-cured ham. *J. Food Sci.* 68:64–69.

Seppo, L., T. Jauhiaine, T. Poussa, and R. Korpela. 2003. A fermented milk high in bioactive peptides has a blood-pressure lowering effect in hypertensive subjects. *Am. J. Clin. Nutr.* 77:326–330.

Sforza, S., L. Ferroni, G. Galaverna, A. Dossena, and R. Marchelli. 2003. Extraction, semi-quantification, and fast on-line identification of oligopeptides in Grana Padano cheese by HPLC-MS. *J. Agric. Food Chem.* 51:2130–2135.

Shen, T.-L., and K. R. Noon. 2004. Liquid chromatography-mass spectrometry and tandem mass spectrometry of peptides and proteins. In *HPLC of peptides and proteins,* ed. M.-I. Aguilar, 111–139. Totowa, NJ: Humana Press.

Shin, K., K. Yamauchi, S. Teraguchi, H. Hayasawa, M. Tomita, Y. Otsuka, and S. Yamazaki. 1998. Antibacterial activity of bovine lactoferrin and its peptides against enterohaemorrhagic *Escherichia coli* O157:H7. *Lett. Appl. Microbiol.* 6:407–411.

Silva, S. V., and F. X. Malcata. 2005. Caseins as source of bioactive peptides (review). *Int. Dairy J.* 15:1–15.

Stanton, C., C. Desmond, M. Coakley, J. K. Collins, G. Fitzgerald, and P. Ross. 2003. Challenges facing development of probiotic-containing functional foods. In *Handbook of fermented functional foods,* ed. E. R. Farnworth, 27–58. Boca Raton, FL: CRC Press.

Suetsuna, K. 1998. Separation and identification of angiotensin I-converting enzyme inhibitory peptides from peptic digest of *Hizikia fusiformis* protein. *Nippon Suisan Gakkaishi*. 64:862–866.

Suetsuna, K., and T. Nakano. 2000. Identification of antihypertensive peptide from peptic digest of wakame (*Undaria pinnatifida*). *J. Nutr. Biochem.* 11:450–454.

Suetsuna, K., H. Ukeda, and H. Ochi. 2000. Isolation and characterization of free radical scavenging activities peptides derived from casein. *J. Nutr. Biochem.* 11:128–131.

Sugai, R. 1998. ACE inhibitors and functional foods. *IDF Bull.* 336:17–20.

Sugano, M., S. Goto, Y. Yamada, K. Yoshida, Y. Hashimoto, T. Matsuo, and M. Kimoto. 1990. Cholesterol-lowering activity of various undigested fraction of soybean protein in rats. *J. Nutr.* 120:977–985.

Takahashi, M., S. Moriguchi, M. Ikeno, S. Kono, K. Ohata, H. Usui, K. Kurahashi, R. Sasaki, and M. Yoshikawa. 1996. Studies on the ileum-contracting mechanisms and identification as a complement C3a receptor antagonist of oryzatensin, a bioactive peptide derived from rice albumin. *Peptides*. 17:5–12.

Takano, T. 1998. Milk derived peptides and hypertension reduction. *Int. Dairy J.* 8:375–381.

Tanizaki, H., H. Tanaka, and A. Kato. 1997. Activation of macrophages by sulfated glycopeptides in ovomucin, yolk membrane, chalazae in chicken eggs. *Biosci. Biotechnol. Biochem.* 61:1883–1889.

Teitelbaum, J. E., and W. A. Walker. 2002. Nutritional impact of pre- and probiotics as protective gastrointestinal organisms. *Ann. Rev. Nutr.* 22:107–138.

Toldrá, F. 2004. Dry. In *Encyclopedia of meat sciences,* eds. W. K. Jensen, C. Devine, and M. Dikeman, 360–365. Oxford, UK: Elsevier.

Toldrá, F., and M. Flores. 1998. The role of muscle proteases and lipases in flavor development during the processing of dry-cured ham. *Crit. Rev. Food Sci. Nutr.* 38:331–352.

Tomasik, P. J. 2003. Probiotics and prebiotics. *Cereal Chem.* 80:113–117.

Tomita, M., M. Takase, W. R. Bellamy, and S. Shimamura. 1994. A review, the active peptide of lactoferrin. *Acta Paediatr. Japan.* 36:585–591.

Tsuge, N., Y. Eikawa, Y. Nomura, M. Yamamoto, and K. Sugisawa. 1991. Antioxidative activity of peptides prepared by enzymatic hydrolysis of egg-white albumin. *Nippon Nogeikagaku Kaishi*. 65:1635–1641.

Työppönen, S., E. Petäjä, and T. Mattila-Sandholm. 2003. Bioprotectives and probiotics for dry sausages. *Int. J. Food Microbiol.* 83:233–244.

Vegarud, G. E., T. Langsrud, and C. Svening. 2000. Mineral-binding milk proteins and peptides; occurrence, biochemical and technological characteristics. *Br. J. Nutr.* 84:91–98.

Vermeirssen, V., J. V. Camp, and W. Verstraete. 2004. Bioavailability of angiotensin I converting enzyme inhibitory peptide (review article). *Br. J. Nutr.* 92:357–366.

Watanabe, K., Y. Tsuji, M. Shimoyamada, N. Ogama, and T. Ebina. 1998. Antitumor effects of pronase treated fragments, glycopeptides, form ovomucin in hen egg white in a double grafted tumor system. *J. Agric. Food Chem.* 46:3033–3038.

Webster, N. R. 1998. Opioids and immune system. *Br. J. Anesth.* 81:835–836.

Yamamoto, N. 1997. Antihypertensive peptides derived from food proteins. *Biopolymers.* 43:129–134.

Yamamoto, N., and T. Takano. 1999. Antihypertensive peptides derived from milk proteins. *Nahrung.* 43:159–164.

Yamazaki, Y., and K. Maekawa. 1978. Peptide with delicious taste. *Agric. Biol. Chem.* 42:1761–1765.

Yamazaki, Y., and K. Maekawa. 1980. Synthesis of a peptide with delicious taste. *Agric. Biol. Chem.* 44:93–97.

Yokoyama, K., H. Chiba, and M. Yoshikawa. 1992. Peptide inhibitors for angiotensin I-converting enzyme from thermolysin digest of dried bonito. *Biosci. Biotechnol. Biochem.* 56:1541–1545.

Yoshii, H., N. Tachi, O. Sakamura, H. Takeyama, R. Ohba, and T. Itani. 1999. Antihypertensive effect of oligo-peptide derived from hen's eggs. *Nippon Shokuhin Kagaku Kogaku Kaishi.* 46:45–50.

Yoshikawa, M. 1996. Selection of proteases for production of biologically active peptides from food proteins. *Jpn. J. Dairy Food Sci.* 45:A51–A60.

Yoshikawa, M., K. Kishi, M. Takahashi, A. Watanabe, T. Miyamura, M. Yamazaki, and H. Chiba. 1993. Immunostimulating peptide derived from soybean protein. *Ann. NY Acad. Sci.* 685:375–376.

Zhao, Q., I. Garreau, F. Sannier, and J. M. Piot. 1997. Opioid peptides derived from hemoglobin: Hemorphins. *Biopolymers.* 43:75–98.

11 New Approaches for the Development of Functional Meat Products

Francisco Jiménez-Colmenero
Instituto del Frío, Ciudad Universitaria

Milagro Reig and Fidel Toldrá
Instituto de Agroquímica y Tecnología de Alimentos

CONTENTS

11.1 A Global Perspective on Functional Foods 276
11.2 Options to Design Functional Meat and Meat Products 277
11.3 Animal Production Strategies for Improving the Nutritional Profiles of Raw Meat 279
11.3.1 Genetics for the Reduction of Animal Fats 279
11.3.2 Dietary Feed Formulation for the Modification of Animal Fats 280
11.3.2.1 Manipulation of Fatty Acids in Pigs 280
11.3.2.2 Manipulation of Fatty Acids in Ruminants 283
11.3.3 Dietary Enrichment in Compounds of Specific Nutritional Interest 285
11.3.3.1 Vitamins and Antioxidants 285
11.3.3.2 Minerals 285
11.4 Processing Strategies for Developing Functional Meat Products 286
11.4.1 Reduction of Specific Unhealthy Compounds 287
11.4.1.1 Fat and Cholesterol 287
11.4.1.2 Sodium 288
11.4.1.3 Allergens 291
11.4.2 Promoting the Presence of Specific Healthy Compounds 291
11.4.2.1 Fat and Fatty Acid Profile 291
11.4.2.2 Proteins, Peptides, and Amino Acids 293
11.4.2.3 Prebiotics and Probiotics 294

11.4.2.4 Antioxidants 295
11.4.2.5 Minerals 298
11.4.2.6 Plant Sterols and Stanols 298
11.4.2.7 Other Compounds 298
11.5 Safety and Scientific Criteria for Functional Effects 299
References 300

11.1 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 diet. In this sense, an adequate diet cannot 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. In 1991 Japan was the first country to introduce specific regulatory approval. 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 labelling 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, Bloch, Thomson, Enrione, and Manning 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, previously unknown, or dispelled old dogma about potential adverse health effects (Hasler 2000).

The use of biomarkers has pointed to some benefits from the consumption of functional foods. This is true of the enhancement of physiological functions such as gastrointestinal physiology (better mechanisms for nutrient absorption and digestion), improved immune system, better overall physical performance, and behavioral (influence on appetite and satiety) and psychological (influence on stress, mental performance, sleep) functions (Verschuren 2002). However, there are also biomarkers indicating reductions in the risk of certain disorders such as cardiovascular diseases (CVDs; Hornstra et al. 1998), obesity, and type 2 diabetes, a linked disorder (Hill and Peters 2002), osteoporosis prevention (Weaver 2003), and some types of cancer (Rafter 2002).

11.2 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 (Ashwell 2002; Holm 2003; Roberfroid 2000): (a) to raise the concentration of the beneficial natural compound (either nutrient or nonnutrient) in the food up to the desired effective level, which 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); (b) to add a compound that is not normally present in the food but has proven benefits (e.g., a prebiotic in meat products); (c) to replace a compound, usually a macronutrient (e.g., a fat) whose intake is usually excessive and hence harmful to health, with a compound with demonstrated health benefits; (d) to remove specific compounds, either naturally present or produced by processing, to reduce adverse health effects (e.g., the reduction of saturated fatty acids, trans fatty acids [TFAs], a toxic compound, or a food allergen); (e) 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 (f) 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 11.1). The more promising target functions for these foods concern (a) growth, development, and differentiation; (b) substrate metabolism; (c) defense against reactive oxidative species; (d) cardiovascular system; (e) intestinal physiology; and (f) behavioral and psychological functions (Diplock et al. 1999).

TABLE 11.1
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, carnitine, carnosine, anserine, etc.)
Prebiotic	Dietary fibers (oats, soy, citrus, etc.), oligosaccharides
Probiotic	Lactic acid bacteria (*Lactobacillus casei*, *L. 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), and so on

Meat and meat products are generally recognized as being contributors to nutrition because they constitute an important source of high-biological-value proteins, group B vitamins, minerals, trace elements, and other bioactive compounds. On the other hand, they also contribute to the intake of fat, saturated fatty acids, 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 considered as a serious risk factor (through various of their different constituents) involved in some of the most prevalent diseases of Western society, such as CVD, hypertension, obesity and 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 (Westsrate, van Poppel, and Verschuren 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 (World Health Organization 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, these types of foods 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 (Jiménez Colmenero 2005).

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 (Jiménez-Colmenero 2005). 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, and examples of currently commercialized meat-based functional foods.

11.3 ANIMAL PRODUCTION STRATEGIES FOR IMPROVING THE NUTRITIONAL PROFILES OF RAW MEAT

11.3.1 Genetics for the Reduction of Animal Fats

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 backfat thickness. Sometimes the meat is poorer in quality even though grading traits are better (Hovenier, Kanis, Asseldonk, and Westerink 1992). Some studies with cattle breeds have been directed toward achieving more tender meat (Johnson, Calkins, Huffman, Johnson, and Hargrove 1990; Wheeler, Savell, Cross, Lunt, and Smith 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, Pursel, and Mitchell 2002). More extensive information on the use of biotechnology for the improvement of growth and composition of farm animals can be found in chapter 1.

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, 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, and so on. 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 g per 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 2002; Toldrá, Reig, Hernández, and Navarro 1996). 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, Claeys, and Demeyer 2002). Moreover, some changes in lipids have been reported depending on the sire genetic type (Armero, Navarro, Nadal, Baselga, and Toldrá 2002). Enzyme fingerprints, based on the reported activity of muscle endo- and exoproteases, lipases, and esterases, constitute a useful tool to understand the biochemical activity (expected proteolysis and lipolysis) during meat processing and to predict its sensory and nutritional quality (Armero, Barbosa, Toldrá, Baselga, and Pla 1999).

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, Flores, et al. 1999). 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 (Rosell and Toldrá 1998; Toldrá, Flores, Aristoy, Virgili, and Parolari 1996).

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, Barbosa, et al. 1999). 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, Jones, Bayntun, and Dransfield 1985). The elimination of castration would help reduce fat content in boars to close to that of gilts (Bass, Butler-Hogg, and Kirton 1990).

11.3.2 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 saturated fatty acids (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 to reduce the adverse effect of arachidonic acid and its eicosanoid products (Simopoulos, Leaf, and Salem 2000). This n-6:n-3 ratio constitutes a risk factor in coronary heart diseases and cancers (Enser et al. 1998), so that an 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 later. 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, Mourot, Mounier, and Ecolan 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, Cross, Pond, and Mersmann 1993).

11.3.2.1 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 (Jakobsen 1999; Toldrá, Reig,

et al. 1996). 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, although only half of the effect was achieved in 14 days (Warnants, Van Oeckel, and Boucqué 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, Noble, Cocchi, and McCartney 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), an n-6 fatty acid typically present in soy, maize, sunflower, and barley, significantly increase the concentration of this fatty acid in meat (see table 11.2). When these oils are used in feeds, the oleic acid content is partly replaced by linoleic acid (Hernández, Navarro, and Toldrá 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, Turner, Schoenherr, Coffey, and Pilkington 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), an 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, Richardson, Wood, Gill, and Sheard 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 11.3), have reported even higher PUFA:SFA ratios, around 0.6 to 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 leanest and fattest animals are usual (Enser et al. 1998).

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% to 6% fish oil to pigs for 2 to 4 weeks before slaughter produced a substantial increase in EPA and DHA (see table 11.2) and reduced the n-6:n-3 ratio to 2.2 (Irie and Sakimoto 1992). However, feeding with 6% unrefined fish 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 the 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 to 42 days was reported to have no adverse effects on sensory attributes (Marriott,

TABLE 11.2
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]**
Myristic acid (C 14:0)	—	—	1.37	1.55	1.6	1.93
Palmitic acid (C 16:0)	23.86	27.82	24.15	25.1	20.6	26.89
Estearic acid (C 18:0)	10.16	12.53	11.73	12.62	9.8	16.30
Palmitoleic acid (C 16:1)	3.0	3.56	3.63	2.79	3.6	2.56
Oleic acid (C 18:1)	39.06	37.81	46.22	36.47	45.9	37.31
C 20:1	-	0.01	0.29	0.47		—
Linoleic acid (C 18:2)	17.15	14.6	8.95	16.49	12.3	7.78
C 20:2	—	0.01	0.44	0.49	0.4	0.59
Linolenic acid (C 18:3)	0.91	0.01	0.26	1.14	3.0	2.08
C 20:3	0.21	0.01	0.25	0.3	0.1	0.08
Arachidonic acid (C 20:4)	4.26	2.14	2.13	0.25	0.74	0.46
Eicosapentanoic acid (C 22:5)	0.64	0.01	—	—	—	0.91
Hexadecanoic acid (C 22:6)	0.75	0.01	—	—	—	1.13
Total saturated fatty acid (SFA)	34.02	40.35	37.83	39.42	33.6	45.12
Total monounsaturated fatty acid (MUFA)	42.06	42.38	50.26	39.74	49.5	39.87
Total polyunsaturated fatty acid (PUFA)	23.92	16.79	11.91	20.84	16.6	13.03
PUFA:SFA ratio	0.7	0.42	0.32	0.53	0.49	0.29
n-6:n-3 ratio	9.4	559	45.3	16.6	4.50	2.2

[a] Morgan, Noble, Cocchi, and McCartney (1992)
[b] Larick, Turner, Schoenherr, Coffey, and Pilkington (1992)
[c] Leszczynski et al. (1992
[d] Toldrá, Rubio, Navarro, and Cabrerizo (2004)
[e] Miller, Shackelford, Hayden, and Reagan (1990)
[f] Irie and Sakimoto (1992)

Garrett, Sims, Wang, and Abril 2002). Dietary conjugated linoleic acid has been reported to enrich the CLA isomer content to around 0.35 g/100 g total fatty acids, and to reduce the intramuscular cholesterol (Lauridsen, Mu, and Henckel 2005).

The n-6:n-3 ratio of poultry meat is similar to the pork ratio. In fact, the feeds used for poultry are typically rich in linoleic acid as one of the main compounds in grain, maize, plant seeds, and oils. Higher concentrations of n-3 fatty acids may be attained by feeding 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).

11.3.2.2 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 because more than 90% of the polyunsaturated fatty acids 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. 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 ruminants are shown in table 11.3. Dietary full canola altered the fatty acid composition in beef meat, especially by decreasing palmitic acid and increasing stearic and oleic acids (Rule, Busboom, and Kercher 1994). Comparison of a maize-based concentrated feed with soybean oil revealed than 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 soybean oil increased the trans-10, cis-12 CLA 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, O'Kiely, Monahan, Stanton, and Moloney 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 increases substantially (see table 11.3) 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 saturated fatty acids, especially palmitic acid (Castro, Manso, Mantecón, Guirao, and Jimeno 2005). It has

TABLE 11.3
Examples of the Effect of Type of Feed on Fatty Acid Composition (Expressed as % of Total Fatty Acids) of Beef and Lamb Meat Lipids

	Feed Enriched in Beef						Feed Enriched in Lamb	
Fatty Acid	Maize-Based Concentrate[a]	Pasture[b]	Pasture[c]	Maize-Based Concentrate[c]	Soybean Oil[a]	Canola Meal[d]	Barley, Maize, Wheat, and Soybean Meal[e]	Concentrate + Forage[f]
Myristic acid (C 14:0)	2.9	2.9	1.6	2.2	3.3	2.8	2.2	3.7
Myristic acid (C 15:0)	1.3	—	—	—	1.5	0.5	—	—
Palmitic acid (C 16:0)	26.7	23.9	21.6	24.3	26.0	24.8	21.1	23.2
Palmitic acid (C 17:0)	1.6	—	—	—	1.6	1.0	1.3	—
Estearic acid (C 18:0)	9.7	19.4	17.7	15.8	11.0	11.4	11.6	16.3
Myristoleic (C 14:1)	—	0.4	0.2	0.4	—	—	—	—
Palmitoleic acid (C 16:1)	4.9	2.4	2.5	3.4	3.9	4.8	2.1	2.3
Oleic acid (C 18:1)	40.0	35.4	31.5	37.3	33.4	44.8	38.7	40.4
Oleic acid (C 20:1)	—	—	—	—	—	0.9	—	—
Linoleic acid (C 18:2)	6.0	1.8	3.3	2.8	7.4	5.7	10.2	7.5
Linoleic acid (C 20:2)	—	—	—	—	—	—	—	—
Linolenic acid (C 18:3)	0.5	0.6	1.3	0.3	0.6	0.5	0.56	1.0
Linolenic acid (C 20:3)	2.4	—	—	—	2.1	—	—	—
Conjugated linoleic acid (CLA)	0.23	1.3	0.53	0.25	0.3	—	—	—
Arachidonic acid (C 20:4)	—	—	—	—	—	1.5	—	2.1
Total saturated fatty acid (SFA)	42.2	48.1	49.1	47.6	42.4	40.5	42.7	43.2
Total monounsaturated fatty acid (MUFA)	44.9	38.2	41.0	46.4	37.3	50.5	46.0	42.7
Total polyunsaturated fatty acid (PUFA)	9.1	4.3	10.0	6.0	10.4	7.7	4.8	10.6
PUFA:SFA ratio	0.22	0.09	0.20	0.13	0.24	0.19	0.11	3.31
n-6:n-3 ratio	16.8	3.0	1.44	3.0	15.8	11.4	18.1	9.32

[a] Dhiman et al. (2005); [b] Realini, Duckett, and Windham (2004); [c] Realini, Duckett, Brito, et al. (2004); [d] Rule, Busboom, and Kercher (1994); [e] Castro et al. (2005); [f] Salvatori et al. (2004)

been reported that the fatty acid composition is somehow affected by genotype (Salvatori et al. 2004).

11.3.3 Dietary Enrichment in Compounds of Specific Nutritional Interest

11.3.3.1 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 is dependent on its concentration and time of supplementation (Mercier, Gatellier, Viau, Remignon, and Renerre 1998). Usual levels are around 100 to 200 mg/kg in the feed for several weeks prior to slaughter. For instance, vitamin E content of 12.9 mg per kg dry matter may be reached after feeding 200 mg of α-tocopheryl acetate per kg diet in pigs between 60 kg 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, Kerry, Buckley, Lynch, and Morrisey 1997). Dietary supplementation with this lipid-soluble antioxidant improves the oxidative stability of the meat (Buckley, Morrisey, and Gray 1995), which is important because phospholipids are located in subcellular membranes such as mitochondria, microsomes, and so on. Their PUFA content is relatively high and this makes them susceptible to peroxidation due to the proximity of prooxidants like myoglobin, cytochromes, nonhaem 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 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).

11.3.3.2 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 haem 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).

Supplementation with sodium selenite or selenium-rich yeast makes for high selenium content in meat.

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 sulphate 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, Warner, Dunshea, and Leury 1999).

11.4 PROCESSING STRATEGIES FOR DEVELOPING FUNCTIONAL MEAT PRODUCTS

Meat technology plays a number of major roles in the development of functional meat products. In addition to animal production strategies, processing strategies can be used to alter the composition of meat products. For the production of functional meat products (as with other functional foods) there are a number of different technological approaches that can be adopted to remove, reduce, increase, add, 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 or reduce the risk of disease (Ashwell 2002; Jiménez Colmenero 2005; Roberfroid 2000). 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) that may have specific positive and negative implications for health. In some cases these substances may occur naturally in the product (in meat raw materials, ingredients, or additives used in their preparation), whereas 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 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 (Jiménez Colmenero, Carballo, and Cofrades 2001), aimed at reducing the concentration or limiting the formation of unhealthy compounds or promoting the presence of healthy compounds.

Several basic approaches can be adopted to successfully induce such effects (Jiménez Colmenero 2005). These focus on aspects relating to selection and preparation (postslaughter) of meat ingredients 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 to limit the concentration and formation of unhealthy components.

The development of functional meat products poses some technological challenges, chiefly as regards optimizing the formulation, processing, and subsequent storage 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, that is, whether it is composed of identifiable pieces of meat, coarsely or finely ground, emulsions, cooked, cured, and so on. To help optimize the desired beneficial properties there are a number of technologies (conventional and emerging) that promise friendly processing conditions.

11.4.1 Reduction of Specific Unhealthy Compounds

Meat and meat derivatives, like any other food, may contain a number of components (of highly diverse origins) that 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, saturated fatty acids, cholesterol, and salt.

There are a number of steps in the processing and storage of meat products, and also in their preparation and consumption, that can modify the concentration and bioavailability of some of their components. These may 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 that 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 (Cassens 1999; Goldberg et al. 2004; Hotchkiss and Parker 1990; 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, and so on. 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 of such unhealthy nonintrinsic substances, at least in excess of established limits. From a practical point of view, any approach to promoting potential functional meat products must involve reducing the concentration and formation of those compounds to within acceptable limits.

11.4.1.1 Fat and Cholesterol

Recent years have seen the development of numerous low-fat meat products, 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 (World Health Organization 2003), many consumers are currently limiting the amount of fat and 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% to 40%, almost a quarter of which derives from the consumption of meat and meat products (Chizzolini, Zanardi, Dorigoni, and Ghidini 1999; Sheard, Wood, Nute, and Ball 1998).

Although 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% fat), there are various meat products to which the same statement 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, which would improve their poor image in terms of health benefits. 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, for which purpose protein- or carbohydrate-based fat replacers are used. The feasibility of developing low-fat 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, etc.).

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. 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 (Jiménez Colmenero 2005; Sandrou and Arvanitoyannis 2000).

Some meat products are currently being marketed with claims relating to fat and cholesterol contents (see table 11.4 and figure 11.1).

11.4.1.2 Sodium

Sodium intake generally exceeds nutritional recommendations in industrialized countries (Antonios and MacGregor 1997; World Health Organization 2003). Excessive

TABLE 11.4
Examples of Nutritional Claims Marketing Meat Products With Reduction of Specific Components

Component	Products	Company
Less fat	Frankfurter, turkey breast, dry sausage	Campofrio (Spain)
Low fat	Turkey breast	Cárnicas Serrano (Spain)
Low fat	Beef and pork sausage	Albrights (U.K.)
30% lower fat	Bacon	Oscar Mayer (U.S.)
Fat-free	Bologna, wiener	Oscar Mayer (U.S.)
Fat-free	Turkey breast	Farmland (U.S.)
Fat-free	Cooked ham, smoked ham, turkey breast, ham, wiener	Schneiders (Canada)
No animal fat	Sausage (mortadela), turkey bacon (bacon de pavo)	Cárnicas Serrano (Spain)
0% saturated fat	Meat snack	Protos Foods (U.S.)
Trans fat free	Breaded chicken and turkey products	House of Raeford Farms (U.S.)[a]
Less cholesterol	Sausage, turkey breast	Campofrio (Spain)
Low cholesterol	Pastrami de pavo	Tello (Spain)
Cholesterol-reducing food wrap	Sausage	Devro (U.K.)
Less salt	Frankfurter, turkey breast, dry sausage, cooked ham	Campofrio (Spain)
Less (35%) salt	Luncheon meats and dinner ham	Schneiders (Canada)
Free-salt added	Cooked ham	ElPozo (Spain)
Less (25%) sodium	Bacon	Smithfield (U.S.)
Lower (28-50%) sodium	Bologna, ham, turkey breast, roast beef	Boar's Head (U.S.)
Lower sodium	Bacon	Oscar Mayer (U.S.)
Lower sodium	Bacon	Farmland (U.S.)
No phosphate added	Turkey breast, cooked ham	Campofrio (Spain)
Gluten-free	Chicken franks, turkey burgers, uncured chicken bologna	Shelton's Poultry (U.S.)
Gluten-free	Sausages and burgers	Graig Farm Organics (U.K.)
Gluten-free and lactose-free	Turkey breast	Tello (Spain)
Gluten-free and lactose-free	Sausages, ham, poultry specialties	Freybe Gourmet Food (Canada)
Allergen-free	Pork sausage, hamburger steak, meatballs (FOSHU)	Nipponham (Japan)[b]

[a] Anonymous (2005). [b] Arihara (2004).

intake of sodium has been associated with high blood pressure, one of the major risk factors for CVD (Antonios and MacGregor 1997). Because approximately 20% to 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.

FIGURE 11.1 Examples of some marketed meat products with specific nutritional claims.

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. How far the salt level can be reduced depends on aspects relating to the type of product, its composition, the type of processing required, and the preparation conditions. A number of binding agents 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; Jiménez Colmenero, Ayo, and Carballo 2005; Mohahan and Troy 1997).

The salt content of meat products has decreased in recent years, and some products are now marketed as salt-free. Similarly, meat products are being marketed without added phosphates (see table 11.4 and figure 11.1).

11.4.1.3 Allergens

Some of the ingredients used in the preparation of meat products, like vegetable, egg, or milk proteins, contain substances that cause allergic reactions in some consumers. Such nonmeat ingredients can be excluded to produce "allergen-free" meat products such as pork sausages, hamburger steak, or meatballs, recently approved (FOSHU) in Japan (Arihara 2004). The industry also produces gluten-free and lactose-free meat products (turkey breast, sausages, ham, etc.; see table 11.4 and figure 11.1).

11.4.2 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 purposes (technological, sensory, nutritional, microbiological, economic) 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.

11.4.2.1 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 (Jiménez Colmenero 2005). 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 saturated fatty acid levels, raising mono- and polyunsaturated fatty acid 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 MUFA or n-3 PUFA contents and lower n-6:n-3 PUFA ratio) achieved by animal production practices (discussed earlier). Frankfurters, low-fat sausages, dry fermented sausages, cooked ham, and pork liver pate have been manufactured using materials (backfat and meat) obtained from pigs fed diets

enriched with safflower, sunflower, canola, linseed, or fish oils (D'Arrigo et al. 2004; Hoz, D'Arrigo, Camberro, and Ordoñez 2004; Jeun-Horng, Yuan-Hui, and Chun-Chin 2002; Santos, Ordoñez, Camberro, D'Arrigo, and Hoz 2004; Shackelford, Miller, Haydon, and Reagan 1990; St. John, Buyck, Keeton, Leu, and Smith 1986).

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 (e.g., 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 backfat or beef fat) in normal and low-fat meat products such as frankfurters (Lurueña-Martinez, Vivar-Quintana, and Revilla 2004; Marquez, Ahmed, West, and Johnson 1999; Paneras, Bloukas, and Filis 1998), cooked sausages (Yılmaz, Simsek, and Isıkli 2002), ground beef patties (Liu, Huffman, and Egbert 1991), fermented sausages (Ansorena and Astiasarán 2004; Bloukas, Paneras, and Fournitzis 1997; Muguerza, Ansorena, Gimeno, and Astiasarán 2002), and others. Other nonmeat ingredients such as walnuts have been used to achieve healthier fatty acid profiles in frankfurters and restructured beef steaks (Jiménez 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, and so on (Ahola 2001). Fish oils (n-3 polyunsaturated oil) have been used in low-fat frankfurters (Park, Rhee, Keeton, and Rhee 1989). Interesterified vegetable oils have been used to modify the fatty acid profile of salami (Javidipour, Vural, Özbas, and Tekin 2005).

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 (World Health Organization 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; Yılmaz 2004).

Fatty acid profiles of meat products have been improved by direct addition of CLA to pork patties (Joo, Lee, Hah, Ha, and Park 2000) or beef patties (Chae, Keeton, and Smith 2004; Hur et al. 2004). 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 (Arihara 2004; Mulvihill 2001).

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 (see table 11.5 and figure 11.1).

TABLE 11.5
Examples of Nutritional Claims of Marketed Meat Products With Specific Components Added

Component	Product	Company
n-3 PUFA	Cooked ham	Cárnicas Serrano (Spain)
n-3 PUFA	Sliced meat	Salumificio Fratelli Veroni (Italy)[a]
n-3, n-6 PUFA	Frankfurters	El Acueducto (Spain)
Rapeseed oil products (high in n-3,6,9 PUFAs)	Frankfurters, sandwich meats, sausages, meatballs	Huittestern Lihapojat (Finland)[b]
Soy protein	Pork frankfurter (FOSHU)	Nipponham (Japan)[c]
Fiber	Frankfurter, turkey breast, dry sausage, cooked ham	Campofrio (Spain)
Fiber (dextrin)	Vienna-type sausage	Itoham Foods (Japan)
Bifido-pro Activo (fiber)	BioYork, Biopavo	Casademont (Spain)
Lactobacillus rhamnosus FERM P-15120	Fermented meat spread products	Prima Meat Packers (Japan)[c]
Living organism (as used in yogurt)	Salami	Reinert (Germany)
Vitamins E–C	Frankfurter, turkey breast, cooked ham	Campofrio (Spain)
8 vitamins	Sausage (mortadella), turkey bacon (bacon de pavo)	Cárnicas Serrano (Spain)
Plant sterol	Frankfurters, sausages, cold meats	Pouttu Ltd (Finland)
Stanol ester	Frankfurters, chicken, meatballs	Atria Oyj (Finland)
Calcium	Poultry sausage	Reinert (Germany)
Calcium	Turkey breast	Cárnicas Serrano (Spain)
L-carnitine	Wiener sausage, salami	Kupfer (Germany)[d]

[a] Desmond and Troy (2004). [b] Sloan (2000). [c] Arihara (2004). [d] Anonymous (2002).

11.4.2.2 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, Costello, Carlson, Greaser, and Jones 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 the 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 health physiological functions in the organism. More detailed information

on bioactive peptides is given in chapter 10. L-carnitine (a derivative of the amino acid, lysine), 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; in Germany the first L-carnitine-enriched meat products (wiener sausage and salami) were introduced in 2002 (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). Some also contain health-enhancing components that 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 in combating 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).

11.4.2.3 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 favoring regulation of blood glucose levels, and preventing 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 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, sugarbeet, soy, rice, apples, peas, citrus, (lemons, oranges), and other foods have been incorporated in the formulations of several meat products including ground meat and sausages (Fernández-López et al. 2004; Jiménez Colmenero et al. 2001; Keeton 1994; Kim, Godber, and Prinaywiwatkul 2000). 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 (Archer, Johnson, Devereux, and Baxter 2004; Pszczola 1998; Sloan 2000). Intake of oligosaccharides improves beneficial intestinal microflora, reduces blood glucose levels, and reduces the production of carcinogens in the intestine (Anandh, Lakshmanan, and Anjaneluyu 2003). Inulin has been used as a fat replacer in sausage, where it presented interesting satiating properties (Archer et al. 2004).

Microbial systems associated with functional foods include probiotics and microorganisms producing functional foods or functional constituents (Knorr 1998). 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. Target products in meat processing include various kinds of dry sausage that are processed by fermentation without heating, which could be suitable vehicles for probiotic intake to the human gastrointestinal tract. 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, Petäjä, and Mattila-Sandlhom 2003). 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 $10^{6\text{-}10}$ viable microbes (Anandh et al. 2003; Työppönen et al. 2003). Meat products with added prebiotics and probiotics have been marketed in different countries (see table 11.5).

11.4.2.4 Antioxidants

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/pro-oxidants/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). Because 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 (discussed earlier) have been used to promote their presence in intact muscle. Reformed and restructured cured turkey products (Walsh, Kerry, Buckley, Arendt, and Morrissey 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; Jensen, Lauridsen, and Bertelsen 1998; Jiménez Colmenero 2005). 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-brand 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 and Vitamina 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,

Torrescano, Djenane, Beltran, and Roncales 2003), dry-cured sausages (Fernández-López et al. 2004), roasted chicken (Pszczola 1998), and cooked pork (O'Connell, Kerry, Fannin, and Gilroy 2002).

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 or liver loaves have been prepared with plant-derived ingredients such as tomato pulp or juice (lycopene rich; Sánchez-Escalante, Djenane, Torrescano, Beltrán, and Roncalés 2001; Yılmaz et al. 2002), carrot and sweet potato (rich in provitamin A; Devatkal, Mendiratta, and Kondaiah 2004; Saleh and Ahmed 1998), or spinach (rich in lutein and zeaxanthin; Pizzocaro, Senesi, Veronese, and Gasparoli 1998). The ready availability of carotenoids (e.g., lycopene and lutein) as food ingredients means that industrial use is likely to grow fast (Sloan 2000).

Phytate (myoinositol hexaphosphate), a natural plant antioxidant present in numerous vegetables (most cereals, nuts, legumes, etc.), presents some health-promoting effects. At one time it was considered an undesirable substance because of its antinutritional properties with respect to mineral absorption (Lee, Hendricks, and Cornforth 1998). Now, however, it is prized because it exerts strong anticarcinogenic effects, reduces the incidence of kidney stones, lowers blood cholesterol, and improves the glycemic index in humans. Phytates have been incorporated in meat products in the form either of a commercial preparation (sodium phytate) in restructured beef (Lee et al. 1998), or as components of any of a number of phytate-rich plants added to different meat products, such as rice fiber in beef roast (Kim et al. 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 (Nissen, Byrne, Bertelsen, and Skibsted 2004; Sánchez-Escalante et al. 2001; Turubatovic and Milatovic-Stevanovic 2001). Other compounds like taurine or carnosine have been added to meat products during processing to limit lipid oxidation (Morrissey, Sheehy, Galvin, Kerry, and Buckley 1998; Sánchez-Escalante et al. 2001).

Some of the compounds just cited have also been used in meat products for purposes other than enhancing lipid stability. For instance, antioxidants (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 or suppress HA biotransformation or 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).

11.4.2.5 Minerals

Some meat products (e.g., pork sausages and turkey sausages) have been made with additional calcium (Arihara 2004; Harris 2000) and fluorated salt. They are aimed at children and are intended to help bone and tooth development. Recent scientific evidence has associated dietary calcium with osteoporosis, hypertension, and cancer (Weaver and Liebman 2002).

At the same time, some nonmeat ingredients can promote the presence in processed meat of a number of minerals (e.g., copper, magnesium, or manganese), with implications for human health. The magnesium and manganese contents of restructured beef steak increased with the addition of walnut (Serrano et al. 2005). In this way meats, which are thought of as foods with an intermediate level of magnesium, can be made to contribute more of a micronutrient that is reported to have potential antiarrhythmic effects (Albert, Gaziano, Willett, and Manson 2002).

Some meat products are marketed with extra calcium (see table 11.5 and figure 11.1); others are advertised as rich in phosphorous or iron.

11.4.2.6 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 and 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 (table 11.5) 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 or their esterified forms and mineral salts has been used to prepare plant sterol-enriched frankfurters, sausage, and cold cuts (Scientific Committee on Food 2003).

Vegetable oils (and other plant products) used as animal fat replacements or in frying processes (either industrially or in domestic food preparation) promote the presence of sterols in meat derivatives.

11.4.2.7 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 (Anandh et al. 2003, Fista, Bloukas, and

Siomos 2004; Gibis, Schoch, and Fisher 1999; Sadler 2004), including anticarcinogenic, antihypertensive, antihypercholesterolaemic, antibacterial, and antioxidant properties.

11.5 SAFETY AND SCIENTIFIC CRITERIA FOR FUNCTIONAL EFFECTS

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: (a) altered consumption behavior, (b) the negative effect of this on particular highly sensitive groups of the population, (c) interactions between components and medicaments, (d) effects of low micronutrient intakes, (e) effects of excessive micronutrient intakes, and (f) 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. However, such simple changes do not assure the functional effect. 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 improvement of physiological functions or reduced risk of certain diseases. Thus, further studies are needed to demonstrate a functional effect before a health claim can be made about the efficacy of meat and meat components for human health. It is absolutely essential to focus on a scientific basis from which exact functional values can be attributed to meats. This means understanding the mechanisms involved in the modulation of certain functions (physiological, biochemical, cellular, genomic, etc.) by these functional components and the relevance of this for health enhancement and well-being or for reduction of the risk of certain diseases.

It is most important that regulations be introduced to protect consumers from misleading claims, but such regulations are still lacking in many countries (Katan and De Roos 2004). 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.

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 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 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.

REFERENCES

Ahola, M. 2001. Innovation in foods: Health, quality and well being links. *Finnish Food Ind. and Res. Scandinavia. 10,* 56–57.

Albert, C. M., J. M. Gaziano, W. C. Willett, and J. E. Manson. 2002. Nut consumption and decreased risk of sudden cardiac death in the physicians health study. *Arch. Int. Med.* 162:1382–1387.

Anandh, M. A., V. Lakshmanan, and A. S. R. Anjaneluyu. 2003. Designer meat foods. *Indian Food Indust. 22*(4):40–45.

Anonymous. 2002. L-carnitine: Bringing health to functional foods. *Innv. Food Technol.* 17:14–15.

Anonymous. 2005. Healthier chicken. Retrieved February 16, 2005, from www.meatprocess.com

Ansorena, D., and I. Astiasarán. 2004. The use of linseed oil improves nutritional quality of the lipid fraction of dry-fermented sausages. *Food Chem.* 87:69–74.

Antonios, T. F. T., and G. A. MacGregor. 1997. Scientific basis for reducing the salt (sodium) content in food products. In *Production and processing of healthy meat, poultry and fish products,* eds. A. M. Pearson and T. R. Dutson, 84–100. London: Blackie Academic & Professional.

Archer, B. J., S. K. Johnson, H. M. Devereux, and A. Baxter. 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. *Br. J. Nutr.* 91:591–599.

Arihara, K. 2004. Functional foods. In *Encyclopedia of meat sciences,* Vol. 1, eds. W. Jensen, C. Devine, and M. Dikemann, 492–499. London: Elsevier Science.

Aristoy, M. C., and F. Toldrá. 1998. Concentration of free amino acids and dipeptides in porcine skeletal muscles with different oxidative patterns. *Meat Sci.* 50:327–332.

Armero, E., J. A. Barbosa, F. Toldrá, M. Baselga, and M. Pla. 1999. Effect of the terminal sire and sex on pork muscle cathepsin (B, B+L and H), cysteine proteinase inhibitors and lipolytic enzyme activities. *Meat Sci.* 51:185–189.

Armero, E., M. Flores, F. Toldrá, J. A. Barbosa, J. Olivet, M. Pla, and M. Baselga. 1999. Effects of pig sire types and sex on carcass traits, meat quality and sensory quality of dry-cured ham. *J. Sci. Food Agric.* 79:1147–1154.

Armero, E., J. L. Navarro, M. I. Nadal, M. Baselga, and F. Toldrá. 2002. Lipid composition of pork muscle as affected by sire genetic type. *J. Food Biochem.* 26:91–102.

Ashwell, M. 2002. *Concepts of functional foods* (International Life Science Institute Europe Concise Monograph Series). Brussels, Belgium: International Life Science Institute.

Bass, J. J., B. W. Butler-Hogg, and A. H. Kirton. 1990. Practical methods of controlling fatness in farm animals. In *Reducing fat in meat animals,* eds. J. D. Wood and A. V. Fischer, 398–436. London: Elsevier Applied Science.

Belury, M. A. 2002. Dietary conjugated linoleic acid in health: Physiological effects and mechanisms of action. *Ann. Rev. Nutr.* 22:505–531.

Bloukas, J. G., E. D. Paneras, and G. C. Fournitzis. 1997. Effect of replacing pork backfat with olive oil on processing and quality characteristics of fermented sausages. *Meat Sci.* 45:133–144.

Buckley, D. J., P. A. Morrisey, and J. I. Gray. 1995. Influence of dietary vitamin E on the oxidative stability and quality of pig meat. *J. Anim. Sci.* 73:3122–3130.

Carbajal, A. 2004. Consumo de carne y tendencias: Calidad de vida y epidemiología de enfermedades asociadas. In *La Carne y Productos Cárnicos como Alimentos Funcionales,* eds. F. Jiménez Colmenero, F. J. Sánchez-Muniz, and B. Olmedilla, 13–38. Madrid, Spain: editec@red.

Cassens, R. G. 1999. Contribution of meat to human health. In *Proceedings of the 45th International Congress of Meat Science and Technology, Vol. II,* 642–648. Yokohama, Japan: ICOMST.

Castro, T., T. Manso, A. R. Mantecón, J. Guirao, and V. Jimeno. 2005. Fatty acid composition and carcass characteristics of growing lamb fed diets containing palm oil supplements. *Meat Sci.* 69:757–764.

Chae, S. H., J. T. Keeton, and S. B. Smith. 2004. Conjugated linoleic acid reduces lipid oxidation in aerobically stored, cooked ground beef patties. *J. Food Sci.* 69:306–309.

Chizzolini, R., E. Zanardi, V. Dorigoni, and S. Ghidini. 1999. Calorific value and cholesterol content of normal and low-fat meat and meat products. *Trends Food Sci. Technol.* 10:119–128.

Clarke, A. D. 1997. Reducing cholesterol levels in meat, poultry and fish products. In *Production and processing of healthy meat, poultry and fish products,* eds. A. M. Pearson and T. R. Dutson,101–117. London: Blackie Academic & Professional.

Collins, J. E. 1997. Reducing salt (sodium) levels in processed meat, poultry and fish products. In *Production and processing of healthy meat, poultry and fish products,* eds. A. M. Pearson and T. R. Dutson,282–297. London: Blackie Academic & Professional.

D'Arrigo, M., L. Hoz, I. Cambero, C. J. Lopez-Bote, C. Pin, and J. A. Ordoñez. 2004. Production of n-3 enriched pork liver paté. *Lebensm-Wiss u-Technol.* 37:585–591.

Decker, E. A., and Z. Xu. 1998. Minimizing rancidity in muscle foods. *Food Technol.* 52:54–59.

De Smet, S., E. Claeys, and D. Demeyer. 2002. Muscle enzymes in relation to meat quality and muscularity. In *Research advances in the quality of meat and meat products,* ed. F. Toldrá, 123–142. Trivandrum, India: Research Signpost.

Desmond, E., and D. Troy. 2004. Nutrient claims on packaging. In *Encyclopedia of meat science,* Vol. II, eds. W. K. Jensen, C. Devine, and M. Dikeman, 903–910. Oxford, UK: Elsevier Academic.

Devatkal, S., S. K. Mendiratta, and N. Kondaiah. 2004. Quality characteristics of loaves from buffalo meat, liver and vegetables. *Meat Sci.* 67:377–383.

Dhiman, T. R., S. Zaman, K. C. Olson, H. R. Bingham, A. L. Ure, and M. W. Pariza. 2005. Influence of feeding soybean oil on conjugated linoleic acid content in beef. *J. Agric. Food Chem.* 53:684–689.

Diplock, A.T., P. J. Aggett, M. Ashwell, F. Bornet, E. B. Fern, and M. B. Roberfroid. 1999. Scientific concept of functional foods in Europe: Consensus document. *Br. J Nutr.* 81:S1–S27.

D'Souza, D. N., R. D. Warner, F. R. Dunshea, and B. J. Leury. 1999. Comparison of different dietary magnesium supplements on pork quality. *Meat Sci.* 51:221–225.

Dye, L., and J. Blundell. 2002. Functional foods: Psychological and behavioural functions. *Br. J. Nutr.* 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. *EFSA Journal.* 81:1–49.

Elmore, J. S., S. L. Cooper, M. Enser, D. S. Mottram, L. A. Sinclair, R. G. Wilkinson, and J. D. Wood. 2005. Dietary manipulation of fatty acid composition in lamb meat and its effect on the volatile aroma compounds of grilled lamb. *Meat Sci.* 69:233–242.

Enser, M., K. G. Hallet, B. Hewett, G. A. Fursey, J. D. Wood, and G. Harrington. 1998. Fatty acid content and composition of UK beef and lamb muscle in relation to production system and implications for human nutrition. *Meat Sci.* 49:329–341.

Enser, M., R. I. Richardson, J. D. Wood, B. P. Gill, and P. R. Sheard. 2000. Feeding linseed to increase the n-3 PUFA of pork: Fatty acid composition of muscle, adipose tissue, liver and sausages. *Meat Sci.* 55:201–212.

EU Commission. 2003. Proposal for a Regulation of the European Parliament and of the Council on nutrition and health claims made on food. COM (2003) 424, Commission of the European Communities 2003/0165. Brussels, Belgium: Author.

Feldman, L. B. 2002. The scientific evidence for a beneficial health relationship between walnuts and coronary disease. *J. Nutr.* 132:1062S–1101S.

Fernández, X., J. Mourot, A. Mounier, and P. Ecolan. 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 Sci.* 41:335–343.

Fernández-López, J., J. M. Fernández-Ginés, L. Aleson-Carbonell, E. Sendra, E. Sayas-Barberá, and J. A. Pérez-Alvarez. 2004. Application of functional citrus by-products to meat products. *Trends Food Sci. Technol.* 15:176–185.

Fista, G. A., J. G. Bloukas, and A. S. Siomos. 2004. Effect of leek and onion on processing and quality characteristics of Greek traditional sausages. *Meat Sci.* 68:163–172.

Gibis, M., A. Schoch, and A. Fisher. 1999. The effect of spices on the reduction of the formation of mutagenic/carcinogenic heterocyclic amines in beef patties. In *Proceedings of 45th International Congress of Meat Science and Technology, Vol. II,* 716–717. Yokohama, Japan: ICOMST.

Gnanasambandam, R., and J. F. Zayas. 1992. Functionality of wheat germ protein in comminuted meat products as compared with corn germ and soy proteins. *J. Food Sci.* 57:829–833.

Goldberg, T., W. Cai, M. Peppa, V. Dardaine, B. S. Baliga, J. Uribarri, and H. Vassara. 2004. Advanced glycoxidation end products in commonly consumed foods. *J. Amer. Diet. Assoc.* 104:1287–1291.

Harris, C. 2000. Meat products are perfect as functional foods. *Meat Proc. Inter.* 19.

Harris K. B., H. R. Cross, W. G. Pond, and H. J. Mersmann. 1993. Effect of dietary fat and cholesterol level on tissue cholesterol concentrations of growing pigs selected for high or low serum cholesterol. *J. Anim. Sci.* 71:807–810.

Hasler, C. M. 1998. Functional foods: Their role in disease prevention and health promotion. *Food Technol.* 52:63–70.

Hasler, C. M. 2000. The changing face of functional foods. *J. Am. Coll. Nut.* 19:499S–506S.

Hasler, C. M., A. S. Bloch, C. A. Thomson, E. Enrione, and C. Manning. 2004. Position of the American Dietetic Association: Functional foods. *J. Am. Diet. Assoc.* 104:814–826.

Hernández, P., J. L. Navarro, and F. Toldrá. 1998. Lipid composition and lipolytic enzyme activities in porcine skeletal muscles with different oxidative pattern. *Meat Sci.* 49:1–10.

Higgs, J. D. 2000. The changing nature of red meat: 20 years of improving nutritional quality. *Trends Food Sci. Technol.* 11:85–95.

Hill, J. O., and J. C. Peters. 2002. Biomarkers and functional foods for obesity and diabetes. *Br. J. Nutr.* 88:S213–S218.

Holm, F. 2003. New functional foods ingredients cardiovascular health. Project No. QLK1-CT-2000-00040, SMEs No. 5). Retrieved October 2004 from www.nutrition.org.uk

Hornstra, G., C. A. Barth, C. Galli, R. P. Mensink, M. Mutanen, R. A. Riemersma, M. Roberfroid, K. Salminen, G. Vansant, and P. M. Verschuren. 1998. Functional food science and the cardiovascular system. *Br. J. Nutr.* 80:S113–S146.

Hotchkiss, J. H., and R. S. Parker. 1990. Toxic compounds produced during cooking and meat processing. In *Advances in meat research,* eds. A. M. Pearson and T. R. Dutson, Vol. 6, 105–134. London: Elsevier Applied Science.

Hovenier, R., E. Kanis, T. H. Asseldonk, and N. G. Westerink. 1992. Genetic parameters of pig meat quality traits in a halothane negative population. *Meat Sci.* 32:309–321.

Hoz, L., M. D. D'Arrigo, I. Camberro, and J. A. Ordoñez. 2004. Development of an n-3 fatty acid and α-tocopherol enriched dry fermented sausage. *Meat Sci.* 67:485–495.

Hoz, L., C. J. López-Bote, M. I. Cambero, M. D'Arrigo, C. Pin, C. Santos, and J. A. Ordóñez. 2003. Effect of dietary linseed oil and α-tocopherol on pork tenderloin (*Psoas major*) muscle. *Meat Sci.* 65:1039–1044.

Hur, S. J., B. W. Ye, J. L. Lee, Y. L. Ha, G. B. Park, and S. T. Joo. 2004. Effects of conjugated linoleic acid on color and lipid oxidation of beef patties during cold storage. *Meat Sci.* 66:771–775.

Irie, M., and M. Sakimoto. 1992. Fat characteristics of pigs fed fish oil containing eicosapentaenoic and docosahexanoic acids. *J. Anim. Sci.* 70:470–477.

Isabel, B., C. J. López-Bote, L. Hoz, M. Timón, C. García, and J. Ruiz. 2003. Effects of feeding elevated concentrations of monounsaturated fatty acids and vitamin E to swine on characteristics of dry-cured hams. *Meat Sci.* 64:475–482.

Jakobsen, K. 1999. Dietary modifications of animal fats: Status and future perspectives. *Fett/Lipid.* 101:475–483.

Javidipour, I., H. Vural, Ö. Ö. Özbas, and A. Tekin. 2005. Effect of interesterified vegetable oils and sugar beet fibre on the quality of turkey-type salami. *Int. J. Food Sci. Technol.* 40:177–185.

Jensen, C., C. Lauridsen, and G. Bertelsen. 1998. Dietary vitamin E: Quality and storage stability of pork and poultry. *Trends Food Sci. Technol.* 9:62–72.

Jeun-Horng, L., L. Yuan-Hui, and K. Chun-Chin. 2002. Effect of dietary fish oil on fatty acid composition, lipid oxidation and sensory property of chicken frankfurters during storage. *Meat Sci.* 60:161–167.

Jiménez Colmenero, F. 2004. Chemistry and physics of comminuted meat products: Non-meat proteins. In *Encyclopedia of meat science,* eds. W. K. Jensen, C. Devine, and M. Dikeman, Vol. I, 271–278. Oxford, UK: Elsevier Academic.

Jiménez Colmenero, F. 2005. Estrategia technológicas de optimización de componentes para el desarrollo de derivados cárnicos funcionales. In *Derivados cárnicos funcionales: Estrategias y perspectivas,* eds. F. Sánchez Muniz, F. Jiménez Colmenero and B. Olmedilla. 65–76. Fundación Española de la Nutrición. Madrid, Spain.

Jiménez Colmenero, F., M. J. Ayo, and J. Carballo. 2005. Physicochemical properties of low sodium frankfurter with added walnut: Effect of transglutaminase combined with caseinate, KCl and dietary fibre as salt replacers. *Meat Sci.* 69:781–788.

Jiménez Colmenero, F., J. Carballo, and S. Cofrades. 2001. Healthier meat and meat products: Their role as functional foods. *Meat Sci.* 59:5–13.

Jiménez Colmenero, F., A. Serrano, J. Ayo, M. T. Solas, S. Cofrades, and J. Carballo. 2003. Physicochemical and sensory characteristics of restructured beef steak with added walnuts. *Meat Sci.* 65:1391–1397.

Johnson, M. H., C. R. Calkins, R. D. Huffman, D. D. Johnson, and D. D. Hargrove. 1990. Differences in cathepsin B+L and calcium-dependent protease activities among breed type and their relationship to beef tenderness. *J. Anim. Sci.* 68:2371–2379.

Johnston, C. S. 2003. Vitamina C. In *Conocimientos Actuales sobre Nutrición,* eds. B. A. Bowman and R. M. Russell, 191–200. Washington, DC: ILSI Press.

Joo, S. T., J. I. Lee, K. H. Hah, Y. L. Ha, and G. B. Park. 2000. Effect of conjugated linoleic acid additives on quality characteristics of pork patties. *Korean J. Food Sci. Technol.* 32:62–68.

Katan, M. B., and N. M. De Roos. 2004. Promises and problems of functional foods. *Crit. Rev. Food Sci. Nut.* 44:369–377.

Keeton, J. T. 1994. Low-fat meat products: Technological problems with processing. *Meat Sci.* 36:261–276.

Kim, J. S., J. S. Godber, and W. Prinaywiwatkul. 2000. Restructured beef roasts containing rice bran oil and fiber influences cholesterol oxidation and nutritional profile. *J. Muscle Foods.* 11:111–127.

Knorr, D. 1998. Technology aspects related to microorganisms in functional foods. *Trends Food Sci. Technol.* 9:295–306.

Larick, D. K., B. E. Turner, W. D. Schoenherr, M. T. Coffey, and D. H. Pilkington. 1992. Volatile compound contents and fatty acid composition of pork as influenced by linoleic acid content of the diet. *J. Anim Sci.* 70:1397–1403.

Lauridsen, C., G. Andersen, M. Andersson, V. Danielsen, R. Engberg, and K. Jakobsen. 1999. Effect of dietary fish oil supplied to pigs from weaning to 60kg liveweight on performance, tissue fatty acid composition and palatability of pork when slaughtered at 100 kg liveweight. *J. Anim Feed Sci.* 8:441–456.

Lauridsen, C., H. Mu, and P. Henckel. 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 Sci.* 69:393–399.

Leclerq, B. 1990. Facteurs influençant la teneur en lipids des viands de porc et de volailles. *Viandes Prod. Carnées.* 11:250–254.

Lee, B. J., D. G. Hendricks, and D. P. Cornforth. 1998. Effect of sodium phytate, sodium pyrophosphate and sodium tripolyphosphate on physico-chemical characteristics of restructured beef. *Meat Sci.* 50:273–283.

Leino, M. 2001. Health trends in meat production. Paper presented at the IFAJ 45th Annual Congress, Savonlinna, Finland. Retrieved October 5, 2004 from http://www.maataloustoimittajat.fi/ifaj-2001/speeches/monday/leino_en.htm

Leszczynski, D. E., J. Pikul, R. A. Easter, F. K. McKeith, D. G. McLaren, J. Novakofski, P. J. Bechtel, and D. E. Jewell. 1992. Characterization of lipid in loin and bacon from finishing pigs fed full-fat soybeans or tallow. *J. Anim. Sci.* 70:2175–2181.

Liu, M. N., D. L. Huffman, and W. R. Egbert. 1991. Replacement of beef fat with partially hydrogenated plant oil in lean ground beef patties. *J. Food Sci.* 56:861–862.

Lurueña-Martinez, M. A., A. M. Vivar-Quintana, and I. Revilla. 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 Sci.* 68:383–389.

Lynch, P. B., and J. P. Kerry. 2000. Utilizing diet to incorporate bioactive compounds and improve the nutritional quality of muscle foods. In *Antioxidants in muscle foods,* eds. E. Decker, C. Faustman, and C. J. López-Bote, 455–480. New York: Wiley Interscience.

Marquez, E. J., E. M. Ahmed, R. L. West, and D. D. Johnson. 1989. Emulsion stability and sensory quality of beef frankfurters produced at different fat and peanut oil levels. *J. Food Sci.* 54:867–870, 873.

Marriott, N. G., J. E. Garrett, M. D. Sims, H. Wang, and J. R. Abril. 2002. Characteristics of pork with docosahexanoic acid supplemented in the diet. *J. Muscle Foods.* 13:253–263.

Mercier, Y., P. Gatellier, M. Viau, H. Remignon, and M. Renerre. 1998. Effect of dietary fat and vitamin E on colour stability and on lipid and protein oxidation in turkey meat during storage. *Meat Sci.* 48:301–318.

Miller, M. F., S. D. Shackelford, K. D. Hayden, and J. O. Reagan. 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. *J. Anim. Sci.* 68:1624–1631.

Mohahan, F. J., and D. J. Troy. 1997. Overcoming sensory problems in low fat and low salt products. In *Production and processing of healthy meat, poultry and fish products,* eds. A. M. Pearson and T. R. Dutson, 257–281. London: Blackie Academic & Professional.

Morgan, C. A., R. C. Noble, M. Cocchi, and R. McCartney. 1992. Manipulation of the fatty acid composition of pig meat lipids by dietary means. *J. Sci. Food Agric.* 58:357–368.

Morrissey, P. A., P. J. A. Sheehy, K. Galvin, J. P. Kerry, and D. J. Buckley. 1998. Lipid stability in meat and meat products. *Meat Sci.* 49:S73–S86.

Muguerza, E., D. Ansorena, O. Gimeno, and I. Astiasarán. 2002. Nutritional advantages of dry fermented sausages elaborated with vegetable oils. In *Proceedings of 48th International Congress of Meat Science and Technology, Vol. II,* 1012–1013. Rome, Italy: ICOMST.

Mulvihill, B. 2001. Ruminant meat as a source of conjugated linoleic acid (CLA). *Nutrit. Bull.* 26:295–299.

Nissen, L. R., D. V. Byrne, G. Bertelsen, and L. H. Skibsted. 2004. The antioxidative activity of plant extracts in cooked pork patties as evaluated by descriptive sensory profiling and chemical analysis. *Meat Sci.* 68:485–495.

Noci, F., P. O'Kiely, F. J. Monahan, C. Stanton, and A. P. Moloney. 2005. Conjugated linoleic acid concentration in M. Longissium dorsi from the heifers offered sunflower oil-based concentrates and conserved forages. *Meat Sci.* 69:509–518.

O'Connell, M., J. P. Kerry, S. Fannin, and D. Gilroy. 2002. Assessment of honey as a functional ingredient in cooked meat pork. In *Proceedings of the 48th International Congress of Meat Science and Technology, Vol. II,* 1016–1017. Rome, Italy: ICOMST.

Ohr, L. M. 2004. Nutraceutical & functional foods. *Food Technol. 58*(8):71–76

O'Sullivan, M. G., J. P. Kerry, D. J. Buckley, P. B. Lynch, and P. A. Morrisey. 1997. The distribution of dietary vitamin E in the muscles of the porcine carcass. *Meat Sci.* 45:297–305.

Paneras, E. D., J. G. Bloukas, and D. G. Filis. 1998. Production of low-fat frankfurters with vegetable oils following the dietary guidelines for fatty acids. *J. Muscle Foods.* 9:111–126.

Park, J., K. S. Rhee, J. T. Keeton, and K. C. Rhee. 1989. Properties of low-fat frankfurters containing monounsaturated and omega-3 polyunsaturated oils. *J. Food Sci.* 54:500–504.

Pennington, J. A. T. 2002. Food composition databases for bioactive food components. *J. Food Comp. Anal.* 15:419–434.

Pizzocaro, F., E. Senesi, P. Veronese, and A. Gasparoli. 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 Technol*. 52:38–46.

Rafter, J. J. 2002. Scientific basis of biomarkers and benefits of functional foods for reduction of disease risk: Cancer. *Br. J. Nutr.* 88:S219–S224.

Realini, C. E., S. K. Duckett, G. W. Brito, M. Dalla Rizza, and D. De Mattos. 2004. Effect of pasture vs. concentrate feeding with or without antioxidants on carcass characteristics, fatty acid composition, and quality of Uruguayan beef. *Meat Sci*. 66:567–577.

Realini, C. E., S. K. Duckett, and W. R. Windham. 2004. 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 Sci*. 68:35–43.

Reig, M., and F. Toldrá. 1998a. Pork meat as a source of vitamins. *Recent Res. Devel. Nutr. Res*. 2:29–34.

Reig, M., and F. Toldrá. 1998b. Protein nutritional quality of muscle foods. *Recent Res. Devel. Agric. Food Chem*. 2:71–78.

Roberfroid, M. B. 2000. Concept and strategies of functional food science: The European perspective. *Am. J. Clin. Nutr.* 71:S1660–S1664.

Romans, J. R., W. J. Costello, C. W. Carlson, M. L. Greaser, and K. W. Jones. 1994. *The meat we eat*. Danville, IL: Interstate Publisher.

Rosell, C. M., and F. Toldrá. 1998. Comparison of muscle proteolytic and lipolytic enzyme levels in raw hams from Iberian and White pigs. *J. Sci. Food Agric*. 76:117–122.

Ruiz-Capillas, C., and F. Jiménez Colmenero. 2004. Biogenic amines in meat and meat products. *Crit. Rev. Food Sci. Nutrit*. 44:489–599.

Rule, D. C., J. R. Busboom, and C. J. Kercher. 1994. Effect of dietary canola on fatty acid composition of bovine adipose tissue, muscle, kidney, and liver. *J. Anim. Sci*. 72:2735–2744.

Sadler, M. J. 2004. Meat alternatives: Market developments and heath benefits. *Trends Food Sci. Technol*. 15:250–260.

Saleh, N. T., and Z. S. Ahmed. 1998. Impact of natural sources rich in provitamin A on cooking characteristics, color, texture and sensory attributes of beef patties. *Meat Sci*. 50:285–293.

Salvatori, G., L. Pantaleo, C. Di Cesare, G. Maiorano, F. Filetti, and G. Oriani. 2004. Fatty acid composition and cholesterol content of muscles as related to genotype and vitamin E treatment in crossbred lambs. *Meat Sci*. 67:45–55.

Sánchez-Escalante, A., D. Djenane, G. Torrescano, J. A. Beltrán, and P. Roncalés. 2001. The effects of ascorbic acid, taurine, carnosine and rosemary powder on colour and lipid stability of beef patties packaged in modified atmosphere. *Meat Sci*. 58:421–429.

Sánchez-Escalante, A., G. Torrescano, D. Djenane, J. A. Beltran, and P. Roncales. 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 Sci. Technol. Inter.* 9:77–84.

Sandrou, D. K., and I. S. Arvanitoyannis. 2000. Low-fat/calorie foods: Current state and perspectives. *Crit. Rev. Food Sci. Technol*. 40:427–447.

Santos, C., J. A. Ordoñez, I. Camberro, M. D'Arrigo, and L. Hoz. 2004. Physicochemical characteristics of an α-linolenic acid and α-tocopherol-enriched cooked ham. *Food Chem*. 88:123–128.

Scientific Committee on Food. 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). 15 Brussels, Belgium: Health and Consumer Protection Directorate-General, European Commission.

Serrano, A., S. Cofrades, C. Ruiz-Capillas, B. Olmedilla-Alonso, C. Herrero-Barbudo, and F. Jiménez-Colmenero. 2005. Nutritional profile of restructured beef steak with added walnuts. *Meat Sci*. 70:647–654.

Shackelford, S. D., M. F. Miller, K. D. Haydon, and J. O. Reagan. 1990. Effects of feeding elevated levels of monounsaturated fats to growing-finishing swine on acceptability of low-fat sausage. *J. Food Sci*. 55:1497–1500.

Sheard, P. R., M. Enser, D. J. Wood, G. R. Nute, B. P. Gill, and R. I. Richardson. 2000. Shelf life and quality of pork and pork products with raised n-3 PUFA. *Meat Sci*. 55:213–221.

Sheard, P. R., J. D. Wood, G. R. Nute, and R. C. Ball. 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 Sci*. 49:193–204.

Simopoulos, A. P., A. Leaf, and N. Salem. 2000. Workshop on the essentiality of and recommended dietary intakes for omega-6 and omega-3 fatty acids. *Food Rev. Int*. 16:113–117.

Sloan, A. E. 2000. The top ten functional food trends. *Food Technol*. 54:33–62.

Solomon, M. B., V. G. Pursel, and A. D. Mitchell. 2002. Biotechnology for meat quality enhancement. In *Research advances in the quality of meat and meat products,* ed. F. Toldrá, 17–31. Trivandrum, India: Research Signpost.

St. John, L. C., M. J. Buyck, J. T. Keeton, R. Leu, and S. B. Smith. 1986. Sensory and physical attributes of frankfurters with reduced fat and elevated monounsaturated fats. *J. Food Sci*. 51:1144–1146, 1179.

Suray, P. F. 2003. Natural antioxidants: From the animal feed industry to human health. In *Natural antioxidants in avian nutrition and reproduction,* ed. P. F. Suray,565–604. Nottingham, UK: Nottingham University Press.

Toldrá, F. 2002. *Dry-cured meat products*. Trumbull, CT: Food & Nutrition Press.

Toldrá, F., and M. Flores. 2004. Meat quality factors. In *Handbook of food analysis,* ed. L. M. L. Nollet,1961–1977. New York: Marcel Dekker.

Toldrá, F., M. Flores, M. C. Aristoy, R. Virgili, and G. Parolari. 1996. Pattern of muscle proteolytic and lipolytic enzymes from light and heavy pigs. *J. Sci. Food Agric*. 71:124–128.

Toldrá, F., and M. Reig. 2004. Analysis of meat in food. In *Handbook of food analysis,* ed. L. M. L. Nollet,1941–1959. New York: Marcel Dekker.

Toldrá, F., M. Reig, P. Hernández, and J. L. Navarro. 1996. Lipids from pork meat as related to a healthy diet. *Recent Res. Devel. Nutr.* 1:79–86.

Toldrá, F., M. A. Rubio, J. L. Navarro, and L. Cabrerizo. 2004. Quality aspects of pork and its nutritional impact. In *Quality of fresh and processed foods: Advances in experimental medicine and biology,* eds. F. Shahidi, A. M. Spanier, C.-T. Ho, and T. Braggins, Vol. 542, 25–32. New York: Kluwer Academic/Plenum.

Turubatovic, L., and M. Milatovic-Stevanovic. 2001. Bioactive components: New aspects of applying supplements in the meat industry. *Tehnologija Mesa*. 42:377–397.

Työppönen, S., E. Petäjä, and T. Mattila-Sandlhom. 2003. Bioprotective and probiotics for dry sausages. *Int. J. Food Microbiol*. 83:233–244.

Ulrich, C. M., and J. D. Potter. 2004. Diet, genes and cancer risk. In *Functional foods: Safety aspects,* ed. Senate Commission on Food Safety, 37–49. Wheinheim, Germany: Wiley-Vch Verlang.

Verschuren, P. M. 2002. Summary report: Functional foods: Scientific and global perspectives. *Br. J. Nutr.* 88:S125–S130.

Vitaglione, P., and V. Fogliano. 2004. Use of antioxidants to minimize the human health risk associated to mutagenic/carcinogenic heterocyclic amines in food. *J. Chromatog.* 8002:189–199.

Walsh, M. M., J. F. Kerry, D. J. Buckley, E. K. Arendt, and P. A. Morrissey. 1998. Effect of dietary supplementation with α-tocopheryl acetate on stability of reformed and restructured low nitrite cured turkey products. *Meat Sci.* 50:191–201.

Warnants, N., M. J. Van Oeckel, and C. V. Boucqué. 1999. Incorporation of dietary polyunsaturated fatty acids into pork fatty tissues. *J. Anim. Sci.* 77:2478–2490.

Weaver, C. M., and M. Liebman. 2002. Biomarkers of bone health appropriate for evaluating functional foods designed to reduce risk of osteoporosis. *Br. J. Nutr.* 88:S225–S232.

Weaver, M. C. 2003. Calcio. In *Conocimientos Actuales sobre Nutrición,* eds. B. A. Bowman and R. M. Russell, 297–305. Washington, DC: ILSI Press.

Westsrate, J. A., G. van Poppel, and P. M. Verschuren. 2002. Functional foods, trends and future. *Br. J. Nutr.* 88:S233–S235.

Wheeler, T. L., J. W. Savell, H. R. Cross, D. K. Lunt, and S. B. Smith. 1990. Mechanisms associated with the variation in tenderness of meat from Brahman and Hereford cattle. *J. Anim. Sci.* 68:4206–4220.

Williams, C. M. 2000. Dietary fatty acids and human health. *Ann. Zootech.* 49:165–180.

Wirth, F. 1991. Reducing the fat and sodium content of meat products. What possibilities are there? *Fleischwirtsch* 7:294–297.

Wood, J. D., R. C. D. Jones, J. A. Bayntun, and E. Dransfield. 1985. Backfat quality in boars and barrows at 90 kg live weight. *Animal Prod.* 40:481–487.

Wood, J. D., R. I. Richardson, G. R. Nute, A. V. Fisher, M. M. Campo, E. Kasapidou, P. R. Sheard, and M. Enser. 2003. Effects of fatty acids on meat quality: A review. *Meat Sci.* 66:21–32.

World Health Organization. 2003. *Diet, nutrition and the prevention of chronic diseases* (WHO Technical Report Series 916). Geneva, Switzerland.

Yılmaz, I. 2004. Effects of rye bran addition on fatty acid composition and quality characteristics of low-fat meatballs. *Meat Sci.* 67:245–249.

Yılmaz, I., O. Simsek, and M. Isıkli. 2002. Fatty acid composition and quality characteristics of low-fat cooked sausages made with beef and chicken meat, tomato juice and sunflower oil. *Meat Sci.* 62:253–258.

12 Processing of Nitrite-Free Cured Meats

Ronald B. Pegg
Department of Applied Microbiology and Food Science, University of Saskatchewan

Fereidoon Shahidi
Department of Biochemistry, Memorial University of Newfoundland

CONTENTS

12.1 Origins of Nitrite Curing of Meats 309
12.2 *N*-Nitrosamines 311
12.3 Processing Options When Curing Meats 311
12.3.1 Formulating 311
12.3.2 Curing Methods 312
12.3.2.1 Dry Curing 312
12.3.2.2 Brine Curing 313
12.3.2.3 Multiple-Needle Pumping 314
12.3.2.4 Tumbling or Massaging 314
12.3.2.5 Chopping or Blending 314
12.4 Benefits and Drawbacks of Nitrite Curing 315
12.4.1 Color Characteristics 315
12.4.2 Antioxidant Properties 318
12.4.3 Flavor Characteristics 320
12.4.4 Antimicrobial Properties 321
12.5 Tailor Designing Nitrite-Free Meat Products 322
References 324

12.1 ORIGINS OF NITRITE CURING OF MEATS

The origin of salting meats is lost in antiquity, but it is believed that the ancient Sumerian civilization, which flourished in the southern part of Mesopotamia during the fourth and third millenniums BC, was the first to practice this process. 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 products could he 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, Forrest, Gerrard, and Mills 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 long before the Christian era, as it was routinely employed for muscle food preservation in ancient China, Babylonia, and Sumeria (Jensen 1953). As the use of salt as a meat preservative spread, it was found that high concentrations of salt would promote 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 19th and the early 20th century when the growing meat packing 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).

12.2 *N*-NITROSAMINES

Despite all of its desirable effects in processed meat products, nitrite is a source of concern due to its role, under certain conditions, in the formation of *N*-nitrosamines at trace quantities (i.e., parts-per-billion levels). Typical volatile *N*-nitrosamines detected in cured products after heat processing 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, Barbour, and Scanlan 1997). These compounds are known to be 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 to 200 mg/kg in most products, with lower maximum addition levels for bacon (about 120 mg/kg). The meat industry 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, Henderson, and Boice 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).

12.3 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.

12.3.1 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.

12.3.2 Curing Methods

There are a few means by which meats can be cured. The basic methods include the following.

12.3.2.1 Dry Curing

This 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 (2–4°C) for 20 to 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).

12.3.2.2 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° to 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 to 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).

12.3.2.3 Multiple-Needle Pumping

The practice of pumping or injecting meat with a perforated needle originated in the late 19th 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.

12.3.2.4 Tumbling or Massaging

Although strictly not a basic curing technique, tumbling or massaging of pickle-injected meat cuts is 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. Nowadays practically all units 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. Without the presence of tumblers and massagers in meat processing operations, the higher processing yields and lower production costs associated with a number of value-added meat products could not be achieved.

12.3.2.5 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 employ-

ment 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.

12.4 BENEFITS AND DRAWBACKS OF NITRITE CURING

Today it is recognized that to cure meat, two ingredients must be added: salt and nitrite. 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 as unique: This one food additive can afford to meat a characteristic cured color by means of a heat-stable nitrosylprotoheme pigment, a typical cured flavor, an extended period of refrigerated storage to 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 non-nitrite curing mixtures.

12.4.1 Color Characteristics

From a health standpoint, the bacteriostatic action of nitrite is paramount, but from a sales perspective, it is the color of the meat product that 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.

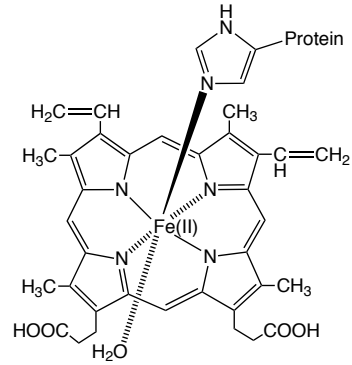
A number of colorants to replace nitrite such as nicotinic acid, 3- and 4-acylpyridine, *N*,*N*-diethylnicotinamide, erythrosine, protoporphyrin-IX, cochineal, dried radish chip extracts, betalain pigments from beet root extract, and angkak from *Monascus purpureus* have been tested in meat systems. 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 composite nitrite-free curing system, which bestow 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, Fedorova, Prizenko, and Loginova 1975; Palmin and Prizenko 1974; Palmin, Prizenko, Fedorova, and Loginova 1973).

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, 1991d; Shahidi, Pegg, and Sen 1994; Shahidi, Rubin, Diosady, Chew, and Wood 1984; Shahidi, Rubin, Diosady, and Wood 1985); the preparation of the latter process is depicted in figure 12.1. 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 to 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 12.1), colorimetric data (i.e., Hunter color values) demonstrated that pigment-treated pork samples at a 12 to 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, Zlender, Abram, and Kumperger 1997). Whether there is an interaction between myoglobin and the added CCMP is uncertain. Nevertheless, meats richer in myoglobin require greater addition levels of CCMP to attain an attractive cured color in the final product (Pegg and Shahidi 1990).

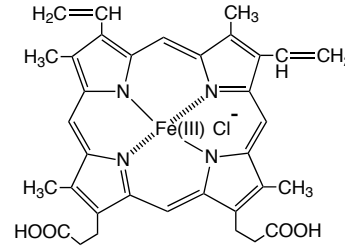
In a pilot-scale study, nitrite-free cured frankfurter and salami products were prepared. Industry panel members were unable to distinguish the nitrite-cured control (i.e., 200 mg/kg sodium nitrite addition based on formulation) from the nitrite-free test samples (i.e., 21–27 mg/kg CCMP) based on visual 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, Pegg, and Sen 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). The optimal



[One of the four heme moieties from bovine red blood cells, red, Fe^{2+}]

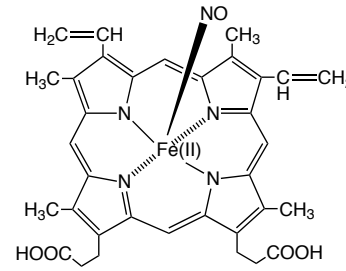
Hemin isolation by Schalfejew method using acetic acid/salt



Hemin chloride
[green/black color in basic solution, Fe^{3+}]

[NO] addition

Dissolution in an acetate buffer containing a reductant and polyphosphate



Mononitrosyl protoheme
[Cooked cured-meat pigment, pink, Fe^{2+}]

FIGURE 12.1 Preparation and chemical structure of the preformed cooked cured-meat pigment from hemin chloride after its isolation from bovine red blood cells.

TABLE 12.1
Concentration Effect of the Preformed Cooked Cured-Meat Pigment (CCMP) on Hunter Color Values of Cooked Ground Pork[a]

Treatment (mg/kg)	Hunter Values			Hue Angle (arctan b/a)
	L	a	b	
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

[a]All pork systems were prepared with 20% (w/w) distilled water and 550 mg/kg sodium ascorbate.
Reprinted from Pegg (1993) with permission.

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.

12.4.2 Antioxidant Properties

It is well known that nitrite functions as a strong antioxidant in cured meats and thus prevents lipid oxidation. Yet, 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, Rubin, and Wood 1987a), sequestrants (Shahidi, Rubin, Diosady, Kassam, and Li Sui Fong 1986), and their combinations (Shahidi, Rubin, and Wood 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 the synthetic antioxidants tested, butylated hydroxyanisole (BHA) and tert-butylhydroquinone (TBHQ) were the most effective, even at 30 mg/kg, in

TABLE 12.2
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, 3,000	1.86
6	Tetrasodium pyrophosphate, 3,000	1.66
7	Sodium hexametaphosphate, 3,000	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, 3,000	0.28
17	(15) + Sodium hypophosphate, 3,000	0.21

[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.
Adapted from Shahidi and Pegg 1992. Reprinted with permission from *Food Chemistry*. Copyright held by Elsevier Ltd., United Kingdom.

retarding oxidation during a 5-week storage period at 4°C. This was confirmed by monitoring the production of 2-thiobarbituric reactive substances (TBARS) over this period and comparing to those for the nitrite-cured control (table 12.2). 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 (3,000 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 above levels did not have any further effect in controlling lipid oxidation (Shahidi et al. 1987b).

In the food industry there has been an ever increasing trend toward the use of natural ingredients due to greater sensitivity of consumers to synthetic additives, and especially since BHA and BHT are suspected to have carcinogenic activity (Valentão et al. 2002). Research has shown that constituents of aromatic plants can

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, Pegg, Rahimi-Moghaddam, Barl, and Weil 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, Pegg, and Saleemi 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 3-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.

12.4.3 Flavor Characteristics

The National Academy of Sciences (NAS 1982) reported that the generation of cured meat flavor was 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, Shahidi, Rubin, and Diosady (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 3,000 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

TABLE 12.3
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

[a] Pork system contained 20% (w/w) distilled water.
[b] Pork system contained 20% (w/w) distilled water; sodium nitrite, 150 mg/kg; and 550 mg/kg sodium ascorbate.
[c] Pork system contained 20% (w/w) distilled water; the preformed cooked cured-meat pigment, 12 mg/kg; sodium tripolyphosphate, 3,000 mg/kg; sodium ascorbate, 550 mg/kg; and tert-butylhydroquinone, 30 mg/kg.
Adapted from Shahidi and Pegg 1992. Reprinted with permission from *Food Chemistry*. Copyright held by Elsevier Ltd., United Kingdom.

antioxidant/chelating agent combinations. The concentration of volatiles in these systems was depressed almost to the level of the nitrite-cured control (table 12.3).

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 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.

12.4.4 Antimicrobial Properties

Nitrite exerts a concentration-dependent antimicrobial effect in cured-meat products, including, but not limited to, 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 (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 micro-organisms of public health significance, delay product spoilage, and not interfere with beneficial micro-organisms such as lactic acid-producing cultures, required in the manufacturing of fermented meat products. Furthermore, the compound of choice must 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 3,000 mg/kg alone or at 1,000 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, Collins-Thompson, Usborne, and Picard (1986) evaluated the antibotulinal activity of sodium hypophosphite, potassium sorbate, and monomethyl fumarate in nitrite-free curing systems. The treatment containing 3,000 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 outgrowth and toxin production (table 12.4). Monomethyl fumarate at 1,250 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 3,000 mg/kg of it had a flavor as desirable as that of its conventionally cured counterpart.

12.5 TAILOR DESIGNING NITRITE-FREE MEAT PRODUCTS

Employment 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, it has been found when formulating meat products that 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

TABLE 12.4
Effect of Treatment Composition on Gas and Toxin Production by *Clostridium botulinum* in Cooked Ground Pork[a]

		Incubation at 27°C (days)								
Exp No.	**Treatment[b]**	**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 –

[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); monomethyl fumarate, (MMF).

Adapted from Wood, Collins-Thompson, Usborne, and Picard (1986). Reprinted with permission from the *Journal of Food Protection*. Copyright held by the International Association for Food Protection, Des Moines, Iowa, USA.

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 meatloaf, 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 were found to be successful in reproducing 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, 1995b). 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 [HACCP]) 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.

REFERENCES

Aberle, E., J. C. Forrest, D. E. Gerrard, and E. W. Mills. 2001. *Principles of meat science* (4th ed). Dubuque, IA: Kendall/Hunt.

Amarowicz, R., R. B. Pegg, P. Rahimi-Moghaddam, B. Barl, and J. A. Weil. 2004. Free-radical scavenging capacity and antioxidant activity of selected plant species from the Canadian prairies. *Food Chem.* 84:551–562.

Banner, R. J. 1981. Sodium hypophosphite: New nitrite replacer? Food Eng. 53(10):130.

Binkerd, E. F., and O. E. Kolari. 1975. The history and use of nitrate and nitrite in the curing of meat. *Food Cosmet. Toxicol.* 13:655–661.

Blot, W. J., B. E. Henderson, and J. D. Boice, Jr. 1999. Childhood cancer in relation to cured meat intake: Review of the epidemiological evidence. *Nutr. Cancer* 34:111–118.

Cross, C. K., and P. Ziegler. 1965. A comparison of the volatile fractions from cured and uncured meat. *J. Food Sci.* 30:610–614.

Dziezak, J. D. 1986. Preservatives: Antioxidants. *Food Technol.* 40(9):94–97, 100–102.

Flores, J., and F. Toldrá. 1993. Curing. In *Encyclopedia of food science, food technology and nutrition,* eds. R. Macrae, R. K. Robinson, and M. J. Sadlor, 1277–1282. London: Academic Press.

Fox, J. B., Jr. 1974. Role of cure accelerators. In *Proceedings of the Meat Industry Research Conference,* 17–21. Arlington, VA: American Meat Institute Foundation.

Glória, M. B. A., J. F. Barbour, and R. A. Scanlan. 1997. Volatile nitrosamines in fried bacon. *J. Agric. Food Chem.* 45:1816–1818.

Haldane, J. 1901. The red colour of salted meat. *J. Hygiene.* 1:115–122.

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 F. M. Clydesdale. 1978. The psychophysical relationships between color and flavor. *CRC Crit. Rev. Food Sci. Nutr.* 10:303–321.

Ledward, D. A. 1992. Colour of raw and cooked meat. In *The chemistry of muscle-based foods,* eds. D. W. Johnston, M. K. Knight, and D. A. Ledward, 128–144. Cambridge, UK: The Royal Society of Chemistry.

Livingston, D. J., and W. D. Brown. 1981. The chemistry of myoglobin and its reactions. *Food Technol.* 35:244–252.

Loeppky, R. N. 1994. Nitrosamine and *N*-nitroso compound chemistry and biochemistry. In *Nitrosamines and related N-nitroso compounds: Chemistry and biochemistry,* eds. R. N. Loeppky and C. J. Michejda, 1–18. Washington, DC: American Chemical Society.

Nakatani, N. 1997. Antioxidants from spices and herbs. In *Natural antioxidants: Chemistry, health effects, and applications,* ed. F. Shahidi, 64–75. Champaign, IL: AOCS Press.

National Academy of Sciences. 1982. *Alternatives to the current use of nitrite in foods.* Washington, DC: National Academy Press.

Palmin, V., T. Fedorova, V. Prizenko, and O. Loginova. 1975. The manufacture of nitroso-haemochromogen and its application as meat product colorant. *Myasnaya Industriya SSSR.* 2:40–41.

Palmin, V. V., and V. K. Prizenko. 1974. Stabilization of colour of cured meat using nitric oxide. Izvestia Vysshikh Uchebnykh Zavedenii, *Pishchevaya Teknologiya.* 3:51–53.

Palmin, V. V., V. K. Prizenko, T. A. Fedorova, and O. A. Loginova. 1973. *Some aspects of colour formation in meat products.* Proceedings of the 19th European Meeting of Meat Research Workers, Part IV, 1457–1472. Paris.

Pegg, R. B. 1993. Development of nitrite-free meat curing systems. PhD thesis, Memorial University, St. John's, NF, Canada.

Pegg, R. B., and F. Shahidi. 1990. *Effects of myoglobin and nitrite or preformed cooked cured-meat pigment concentrations on the colour of cooked meats.* Paper presented at the 33rd Annual Conference of the Canadian Institute of Food Science and Technology, Saskatoon, SK.

Pegg, R. B., and F. Shahidi. 2000. *Nitrite curing of meat: The N-nitrosamine problem and nitrite alternatives.* Trumbull, CT: Food & Nutrition Press.

Peters, J. M., S. Preston-Martin, S. J. London, J. D. Bowman, J. D. Buckley, and D. C. Thomas. 1994. Processed meats and risk of childhood leukaemia (California, USA). *Can. Causes Contr.* 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. *Arb. K. GesundhAmt.* 7:471–474.

Preussmann, R., and B. W. Stewart. 1984. *N*-nitroso carcinogens. In *Chemical carcinogens* (2nd ed.), ed. C. E. Searle, Vol. 2, 643–838. Washington, DC: American Chemical Society.

Rhodehamel, E. J., and M. D. Pierson. 1990. Sodium hypophosphite inhibition of the growth of selected Gram negative foodborne pathogenic and spoilage bacteria. *J. Food Prot.* 53:56–63.

Sebranek, J. G. 1979. Advances in the technology of nitrite use and consideration of alternatives. *Food Technol. 33*(7):58–62, 93.

Shahidi, F. 1989. Flavor of cooked meats. In *Flavor chemistry: Trends and developments,* eds. R. Teranishi, R. G. Buttery, and F. Shahidi, 188–201. Washington, DC: American Chemical Society.

Shahidi, F., and R. B. Pegg. 1991a. Effect of the preformed cooked cured-meat pigment (CCMP) on color parameters of muscle foods. *J. Muscle Foods.* 2:297–304.

Shahidi, F., and R. B. Pegg. 1991b. Encapsulation of the pre-formed cooked cured-meat pigment. *J. Food Sci.* 56:1500–1504, 1518.

Shahidi, F., and R. B. Pegg. 1991c. Novel synthesis of cooked cured-meat pigment. *J. Food Sci.* 56:1205–1208.

Shahidi, F., and R. B. Pegg. 1991d. Safety and sensory aspects of nitrite alternatives in meat curing. *Can. Chem. News.* 43(2):12–15.

Shahidi, F., and R. B. Pegg. 1992. Nitrite-free meat curing systems: Update and review. *Food Chem.* 43:185–191.

Shahidi, F., and R. B. Pegg. 1993. Powdered cooked cured-meat pigment which is a non-nitrite meat preservative. U.S. Patent No. 5,230,915.

Shahidi, F., and R. B. Pegg. 1995a. Proteinaceous food product containing stabilized cooked cured-meat pigment. U.S. Patent 5,443,852.

Shahidi, F., and R. B. Pegg. 1995b. Stabilized cooked cured-meat pigment. U.S. Patent 5,425,956.

Shahidi, F., R. B. Pegg, and Z. O. Saleemi. 1995. Stabilization of meat lipids with ground spices. *J. Food Lipids.* 2:145–153.

Shahidi, F., R. B. Pegg, and N. P. Sen. 1993. Novel nitrite-free processed meats. In *Impact of food research on new product development,* eds. R. Ali and P. J. Barlow, 221–245. Karachi, Pakistan: University of Karachi.

Shahidi, F., R. B. Pegg, and N. P. Sen. 1994. Absence of volatile *N*-nitrosamines in cooked nitrite-free cured muscle foods. *Meat Sci.* 37:327–336.

Shahidi, F., L. J. Rubin, L. L. Diosady, V. Chew, and D. F. Wood. 1984. Preparation of dinitrosyl ferrohemochrome from hemin and sodium nitrite. *Can. Inst. Food Sci. Technol. J.* 17:33–37.

Shahidi, F., L. J. Rubin, L. L. Diosady, N. Kassam, and J. C. Li Sui Fong. 1986. Effect of sequestering agents on lipid oxidation in cooked meats. *Food Chem.* 21:145–152.

Shahidi, F., L. J. Rubin, L. L. Diosady, and D. F. Wood. 1985. Preparation of the cooked cured-meat pigment, dinitrosyl ferrohemochrome, from hemin and nitric oxide. *J. Food Sci.* 52:564–567.

Shahidi, F., L. J. Rubin, and D. F. Wood. 1987a. Control of lipid oxidation in cooked ground pork with antioxidants and dinitrosyl ferrohemochrome. *J. Food Sci.* 52:564–567.

Shahidi, F., L. J. Rubin, and D. F. Wood. 1987b. Control of lipid oxidation in cooked meats by combinations of antioxidants and chelators. *Food Chem.* 23:151–157.

Shahidi, F., L. J. Rubin, and D. F. Wood. 1988. Stabilization of meat lipids with nitrite-free curing mixtures. *Meat Sci.* 22:73–80.

Sofos, J. N., and F. F. Busta. 1980. Alternatives to the use of nitrite as an antibotulinal agent. *Food Technol.* 34:244–251.

Stevanović, M., B. Zlender, V. Abram, and U. Kumperger. 1997. The influence of pork muscle's type and addition of pigment CCMP on the colour of meat emulsion coagulates. In *Proceedings of the 43rd International Congress of Meat Science and Technology, Auckland, New Zealand, July 27–August 1, 1997,* ed. J. Bass, 462–463. Hamilton, NZ: Rice Printers Ltd.

Sweet, C. W. 1975. Additive composition for reduced particle size meats in the curing thereof. U.S. Patent 3,899,600.

Toldrá, F., and M. Flores. 1998. The role of muscle proteases and lipases in flavor development during the processing of dry-cured ham. *CRC Crit. Rev. Food Sci. Nutr.* 38:331–352.

Townsend, W. E., and D. G. Olson. 1987. Cured meats and cured meat products processing. In *The science of meat and meat products* (3rd ed.), eds. J. F. Price and B. S. Schweigert, 431–456. Trumbull, CT: Food & Nutrition Press.

Tricker, A. R., and R. Preussmann. 1991. Carcinogenic *N*-nitrosamines in the diet: Occurrence, formation, mechanisms and carcinogenic potential. *Mutat. Res.* 259:277–289.

Valentão, P., E. Fernandes, F. Carvalho, P. B. Andrade, R. M. Seabra, and M. L. Bastos. 2002. Antioxidative properties of cardoon (*Cynara cardunculus* L.) infusion against superoxide radical, hydroxyl radical, and hypochlorous acid. *J. Agric. Food Chem.* 50:4989–4993.

Wood, D. S., D. L. Collins-Thompson, W. R. Usborne, and B. Picard. 1986. An evaluation of antibotulinal activity in nitrite-free curing systems containing dinitrosyl ferrohemochrome. *J. Food Prot.* 49:691–695.

Yun, J. J. 1984. Alternative meat curing system: Flavour and oxidative stability. MASc. thesis, University of Toronto, Toronto, ON, Canada.

Yun, J., F. Shahidi, L. J. Rubin, and L. L. Diosady. 1987. Oxidative stability and flavour acceptability of nitrite-free meat-curing systems. *Can. Inst. Food Sci. Technol. J.* 20:246–251.

13 Biochemical Proteolysis Basis for Improved Processing of Dry-Cured Meats

Fidel Toldrá
Instituto de Agroquímica y Tecnología de Alimentos

CONTENTS

13.1 The Muscle Proteolytic System 330
13.1.1 Endopeptidases or Proteinases 331
13.1.2 Exopeptidases 332
13.2 The Microbial Proteolytic System 333
13.2.1 Endopeptidases 335
13.2.2 Exopeptidases 335
13.2.3 Transformation of Amino Acids 337
13.2.3.1 Degradation Reactions 337
13.2.3.2 Decarboxylation 338
13.2.3.3 Oxidative Deamination 338
13.2.3.4 Transamination 338
13.3 Proteolysis in Dry-Cured Meats 338
13.3.1 Proteolysis in Dry-Cured Ham and Its Effect on Quality 339
13.3.2 Proteolysis in Dry-Fermented Sausages and Its Effect on Quality 341
13.4 Enhancement and Control of Proteolysis in Dry-Cured Meats 342
References 345

The origin of dry-cured meats is lost in ancient times. They probably originated when primitive man used salting as a useful preservation tool for times of scarcity. Since then, knowledge has been orally transmitted from generation to generation through the centuries. As a consequence, processing was very empirical and technology received little attention. It was in the second half of the 20th century that the numerous chemical and biochemical changes taking place during processing received increased attention and thus, scientific and technical literature started to be

published (Flores and Toldrá 1993; Toldrá 1992; Toldrá, Flores, Navarro, Aristoy, and Flores 1997). These advances had rapid applications in processing technology and resulted in sensible improvement in the quality of the final product (Toldrá 2002).

A wide number of meat products are denominated by the term dry-cured meats. These products, which use salt and most times nitrate or nitrite, may consist of either an entire piece (dry-cured ham or dry-cured loin), which is ripened, dried, or smoked, or a mixture (dry- and semidry-fermented sausage) of minced meat and fat, which is stuffed into a casing, fermented, and dried or smoked (Demeyer and Toldrá 2004; Toldrá 2004a). In general, most of these products are usually eaten raw with no need for further smoking or cooking. Therefore, a variety of processing technologies (with different drying, ripening, and smoking conditions) and raw materials (genetics, feed, rearing system, etc.) are typically found and reflected in a wide variety of products.

Mediterranean sausages are generally ripened at mild temperatures for long periods of time and are not smoked. Typical examples are French saucisson, Spanish chorizo, and Italian salami (Toldrá 2004c). On the other hand, northern European products are typically ripened for a short time and smoked. Typical examples are the German and Hungarian style salamis (Leistner 1992). They are classified as dry-fermented sausages when weight loss exceeds 30% and semidry when weight loss is lower than 20% (Sebranek 2004).

Hams from certain pig crossbreeds constitute the raw material for typical dry-cured hams such as Spanish Iberian and Serrano, Italian Parma and San Danielle, and French Bayonne. Most of these hams are controlled by consortiums that control the accomplishment of requirements like type of crossbreeds, type of feed, slaughter age, processing technology, and so on. Processing time is generally long (at least 6 months or even 1–2 years) and weight loss is high, around 32% to 34% (Toldrá 2004b). Other hams are salted and ripened for shorter periods (a few weeks) and then smoked. This is the case of the Kentucky and Virginia country-style hams, the traditional German Westphalian ham, the Finnish "sauna" hams and Chinese Ching Hua or Yunnan hams (Campbell-Platt 1995). Dry-cured loins are produced from salted pork loins that are ripened for a few weeks (Hernández, Navarro, and Toldrá 1999).

Dry-cured meats experience intense biochemical changes during processing, with proteolysis being one of the most important and relevant for final quality. In fact, proteolysis has an important effect on texture and taste and, indirectly, on aroma development (Toldrá and Flores 1998). Good control of proteolysis is of primary importance to produce a product with consistent, regular, high quality. Muscle and microbial proteases are the main elements responsible for the proteolytical changes and thus a good knowledge of its properties and mode of action is essential for controlled proteolysis. The proteolysis phenomena, the involved muscle and microbial proteases, and the resulting products are described in this chapter.

13.1 THE MUSCLE PROTEOLYTIC SYSTEM

Skeletal muscle contains a good number of enzymes involved in multiple metabolic pathways. Some of the most important are related to protein changes. Endopeptidases (calpains and cathepsins) are responsible for protein breakdown, tri- and dipeptidylpeptidases are involved in the generation of small tri- and dipeptides, and, finally,

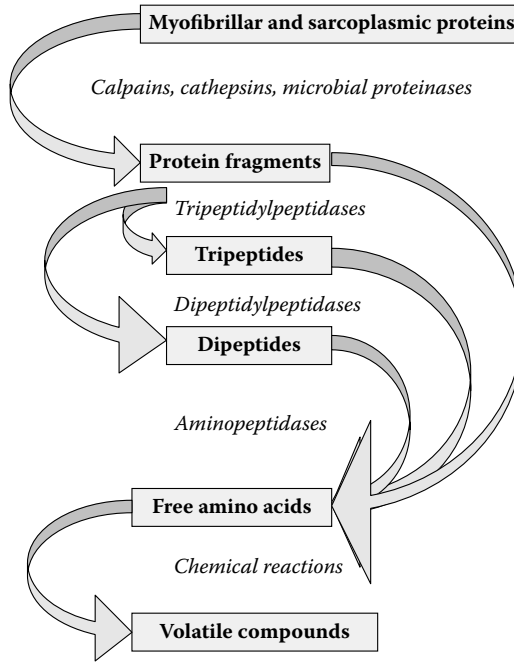


FIGURE 13.1 General scheme of the proteolytical chain in dry-cured meats.

aminopeptidases and carboxypeptidases release free amino acids (Toldrá 1992). A general scheme of the proteolytical chain is shown in figure 13.1. Most of these enzymes remain very active in postmortem muscle, playing important roles in proteolysis; thus they have an important relevance for the development of meat quality. Some of these proteases, having optimal acid pH, are located in organules like lysosomes whereas others are bound to membranes or free in the cytosol (Haard 1990).

13.1.1 Endopeptidases or Proteinases

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 of 20 to 40 kDa (see table 13.1) that allow them to penetrate into the myofibrillar structure and hydrolyze important proteins like myosin and troponins that experience important changes during dry-curing. Substrate preferences for these enzymes are compiled in table 13.1. Optimal pH for cathepsins B and L is around 6.0, for cathepsin H it is near 6.8, and for cathepsin D it is in the range from 3.0 to 5.0 (Rico, Toldrá, and Flores 1990, 1991). 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 they differ in their Ca^{2+} requirement for activation, 50 μM to 70 μM of Ca^{2+} for calpain I or μ-calpain and 1 mM to 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

TABLE 13.1
Main Muscle Proteinases: Location and Substrate Preferences

Enzyme	EC Number	Location	Substrates	Optimal pH Range
Cathepsin B	EC 3.4.22.1.	Lysosome	Myosin heavy chain, actin, collagen	5.5–6.0
Cathepsin D	EC 3.4.23.5.	Lysosome	Myosin heavy chain, titin, M and C proteins, tropomyosin, troponins T and I	3.0–5.0
Cathepsin L	EC 3.4.22.15.	Lysosome	Myosin heavy chain, titin, M and C proteins, tropomyosin, troponins T and I	5.5–6.0
Cathepsin H	EC 3.4.22.16.	Lysosome	Myofibrillar proteins	6.5–7.0
μ-calpain	EC 3.4.22.17.	Z-line	Titin, nebulin, troponins T and I, tropomyosin, C protein, filamin, desmin, and vinculin	7.0–7.5
m-calpain	EC 3.4.22.17.	Z-line	Titin, nebulin, troponins T and I, tropomyosin, C protein, filamin, desmin, and vinculin	7.0–7.5
20S proteasome		Cytosol	Myofibrillar proteins	7.0–7.5

Note: Adapted from Goll et al. (1983); Hughes, Healy, McSweeney, and O'Neill (2000); Hughes, O'Neill, McSweeney, and Healy (1999); Matsukura, Okitani, Nishimura, and Katoh (1981); Okitani, Nishimura, and Katoh (1981); Schwartz and Bird (1977); Toldrá 2002).

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, Seideman, Schollmeyer, Dutson, and Grouse 1987). The third group of proteases is the proteasome complex, a large protease with multiple catalytic sites. This enzyme is able to exhibit different activites like chymotrypsin-like activity, trypsin-like activity, and peptidyl-glutamyl hydrolyzing activity (Coux, Tanaka, and Goldberg 1995). The 20S proteasome may have an effect on 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 substrate preferences for these enzymes are shown in table 13.1 and the mode of action in figure 13.2.

13.1.2 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, whereas DPP III and IV have optimal pH around 7.8 to 8.0 (Sentandreu and Toldrá 1998, 2000, 2001b, 2001c). Dipeptidases are enzymes that are able to hydrolyze dipeptides and their names can vary depending on the preference for certain amino acids. Aminopeptidases, which

Endopeptidases	Lys-Ala-Arg-Leu-Gly-His-Gly-Phe-.....
Tripeptidylpeptidases	Ala-Ala-Phe-Leu-Tyr-Phe-Gly-Leu-....
Dipeptidylpeptidases	Ala-Ala-Lys-Phe-Arg-Ala-Trp-Tyr-....
X-Pro-dipeptidylpeptidases	Gly-Pro-Lys-Arg-Tyr-Ala-Arg-Lys-....
Tripeptidases	Ala-Ala-Phe
Dipeptidases	Ala-Arg
General aminopeptidases	Ala-Arg-Ala-Tyr-Leu-Gly-His-Phe-....
Arginyl aminopeptidases	Lys-Ala-Arg-Tyr-Leu-Gly-His-Phe-....
Carboxypeptidases	.....-Phe-Leu-Tyr-Ala-Arg-Phe-Arg-Leu

FIGURE 13.2 Example of peptide bonds hydrolysis by different types of proteases.

have large molecular masses, are able to release a free amino acid from the amino terminus of peptides and proteins. 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, whereas arginyl aminopeptidase, or aminopeptidase B, is only able to hydrolyze arginine and lysine (Toldrá 2005b). A minor role is expected for leucyl and pyroglutamyl aminopeptidases due to their optimal basic pH (Toldrá, Rico, and Flores 1992).

There are two carboxypeptidases, both located in the lysosomes, with optimal activity at acid pH. They generate free amino acids from the carboxy 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, Rawlings, and Woessner 2004). The mode of action of these proteases against different substrates is shown in figure 13.2 and the substrate preferences are given in table 13.2.

13.2 THE MICROBIAL PROTEOLYTIC SYSTEM

The proteolytic system is essential for a number of cellular processes involved in major physiological processes like the 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 13.1 and 13.2). These enzymes are also endopeptidases or proteinases and peptidases. 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

TABLE 13.2
Main Muscle Peptidases: Location and Substrate Preferences

Enzyme	EC Number	Location	Substrates	Optimal pH Range
DPP I	EC 3.4.14.1	Lysosome	Ala-Arg-peptide, Gly-Arg-peptide	5.0–6.0
DPP II	EC 3.4.14.2	Lysosome	Gly-Pro-peptide	5.0–6.0
DPP III	EC 3.4.14.4	Cytosol	Arg-Arg-peptide, Ala-Arg-peptide	7.5–8.0
DPP IV	EC 3.4.14.5	Membrane	Gly-Pro-peptide	7.5–8.0
TPP I	EC 3.4.14.9	Lysosome	Gly-Pro-Phe-peptide Ala-Ala-Phe-peptide	3.0–5.0
TPP II	EC 3.4.14.10	Cytosol	Gly-Pro-Phe-peptide, Ala-Ala-Phe-peptide	6.5–7.5
Alanyl aminopeptidase	EC 3.4.11.14	Cytosol	Ala-peptide, amino acids-peptide	6.0–7.0
Arginyl aminopeptidase	EC 3.4.11.6	Cytosol	Arg-peptide, Lys-peptide	6.0–7.0
Methionyl aminopeptidase	EC 3.4.11.18	Cytosol	Met-peptide, Ala-peptide, Lys-peptide, Leu-peptide	7.0–7.5
Leucyl aminopeptidase	EC 3.4.11.1	Cytosol	Leu-peptide	9.0
Pyroglutamyl aminopeptidase	EC 3.4.19.3	Cytosol	Pyroglu-peptide	8.0–8.5
Carboxypeptidase A	EC 3.4.16.1	Lysosome	Peptide-hydrophobic amino acids	5.0–5.5
Carboxypeptidase B	EC 3.4.18.1	Lysosome	Peptide-amino acid	5.0–5.5

Note: Adapted from Barrett, Rawlings, and Woessner (2004); Flores, Aristoy, and Toldrá (1993, 1996); Flores, Marina, and F. Toldrá (2000); Sentandreu and Toldrá (1998, 2000, 2001a, 2001b); Toldrá (1992, 2002); Sentandreu et al. (2000).

mode of action may differ substantially 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, Poolman, and Konings 1993). The proteolytic activity associated with the cell wall 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, dipeptidylpeptidases, and aminopeptidases, to small peptides and free amino acids (Bockelmann 1995). Many micro-organisms have been used as starters for fermented meats. Some of the most important are *Lactobacillus sakei, L. curvatus, L. carnosus, L. plantarum, Kocuria varians, Staphylococcus xylosus,* and the yeast *Debaryomices hansenii* (Toldrá 2004d, 2006).

Lactic acid bacteria constitutes a group of micro-organisms 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.

13.2.1 Endopeptidases

Lactic acid bacteria have some endopeptidases or proteinases associated with the cell envelope. These proteinases are responsible for the 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, 1999b; Sanz et al. 1999a, 1999b). This *in vitro* observation agrees with other reports that bacteria proteinases are less effective than muscle proteinases in hydrolyzing myofibrillar proteins (Molly et al. 1997). However, these *Lactobacilli* strains are able to hydrolyze the 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 with the existence of a single cell-wall-associated proteinase in dairy lactic acid bacteria that 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 hydrophylic peptides is important because they are correlated to desirable cured-meat flavors, whereas hydrophobic peptides are correlated to bitterness (Aristoy and Toldrá 1995; Henriksen and Stahnke 1997). Some hydrophobic peptides are generated, but 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 13.3) and has homology with PrB (protease B) from *S. cerevisiae* (Bolumar, Sanz, Aristoy, and Toldrá 2005). This protease 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 expectations for an important role in meat fermentation where pH may easily reach 5.0 or even lower.

13.2.2 Exopeptidases

There are important exopeptidases in *Lactobacillus sakei,* the most prevalent species in European sausages (see table 13.4). The first is the major or general aminopeptidase, which has an optimal neutral pH (around 7.5) and is similar to PepL from *L. 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). Second is the arginine aminopeptidase, which has optimal acid pH, is activated by salt, and has preference for basic residues like arginine and lysine (Sanz and Toldrá 2002). No similar enzymes have ben purified from dairy lactic acid bacteria. Another important exopeptidase is 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,

TABLE 13.3
Main Purified Peptidases 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, Seronine, Talon, and Hebraud (1995)
Tripeptidase	Pep T		Ala-Ala-Ala	40	7.0	Sanz, Mulholland, and Toldrá (1998)

TABLE 13.4
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, Sanz, Aristoy, and Toldrá (2005)
Prolyl aminopeptidase	370	—	—	Pro-peptide	45	7.5	Bolumar, Sanz, Aristoy, and Toldrá (2003a)
Arginyl aminopeptidase	101	ApY	Ca^{2+}, Mg^{2+}, Co^{2+}	Arg-peptide, Lys-peptide	37	7.0	Bolumar, Sanz, Aristoy, and Toldrá (2003b)

Seronine, Talon, and Hebraud 1995). Another is a tripeptidase with optimal neutral pH, able to hydrolyze a wide spectrum of tripeptides except those with Pro in the second position and similarity to PepT from lactococci (Sanz, Mulholland, and Toldrá 1998). Finally, there is an X-prolyl-dipeptidylpeptidase, which has optimal neutral pH, ability to hydrolyze X-Pro dipeptides from the amino terminus from different peptides, and with similarity to PepX from dairy lactic acid bacteria (Sanz and Toldrá 2001). The addition of cell-free extracts from *L. sakei, L. curvatus,* 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, 1999b). 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 *Debaryomices hansenii*, there are also several exopeptidases (see table 13.4). Prolyl aminopeptidase has an optimal neutral pH, restricted to the hydrolysis of Pro at the amino terminus of peptides (Bolumar, Sanz, Aristoy, and Toldrá 2003a). An arginyl aminopeptidase has an optimal neutral pH, maximum specificity for basic residues like arginine and lysine, and homology to ApY from *S. cerevisiae* (Bolumar, Sanz, Aristoy, and Toldrá 2003b). Experiences *in vitro* with whole cells and cell-free extracts of *Debaryomices 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).

13.2.3 Transformation of Amino Acids

There are several reactions such as decarboxylation, degradation, deamination, transamination, and so on, that can transform amino acids into different compounds that can affect the sensory characteristics of the final product (Demeyer and Toldrá 2004; Ordoñez, Hierro, Bruna, and de la Hoz 1999; Toldrá, Flores, and Sanz 2001). Microbial enzymes are involved in most of these reactions that use free amino acids, generated from proteolysis, as substrate (see figure 13.1).

13.2.3.1 Degradation Reactions

Some branched aldehydes may be produced through Strecker degradation of certain amino acids. This is the case for 3-methylbutanal, 2-methylbutanal, and phenylacetaldehyde, which are produced in dry-fermented sausages from leucine, isoleucine, and phenylalanine, respectively (Ordoñez et al. 1999). Other amino acids like methionine, cysteine, and cystine that contain sulfur can also experience Strecker degradation and produce sulfur compounds that have low threshold values and a high aromatic impact (Flores, Spanier, and Toldrá 1998). An excess of proteolysis and generation of these volatile sulfur compounds may produce undesirable flavors. The microbial degradation of the amino acid side chain is another reaction present

in fermented products. Phenol and indole may be formed from tyrosine and tryptophan, respectively (Molimard and Spinnler 1996).

13.2.3.2 Decarboxylation

These reactions produce biogenic amines by microbial decarboxylation of certain amino acids (Ordoñez et al. 1999). Tyrosine, tryptophane, and phenylalanine can be decarboxylated to generate tyramine, tryptamine, and phenylethylamine, respectively. In the same way, lysine, histidine, and ornithine can generate cadaverine, histamine, and putrescine, respectively. Amines constitute a serious risk for consumers and their generation must be avoided through careful selection of raw materials, starter cultures, and processing conditions.

13.2.3.3 Oxidative Deamination

This type of reaction is produced by several bacteria generating ammonia (Ordoñez et al. 1999). Some of the involved enzymes are glutamate dehydrogenase and alanine dehydrogenase. These enzymes can generate α-ketoglutarate and pyruvate, respectively, and ammonia. Another pathway, carried out by deaminases, consists in the nonoxidative deamination of amino acids.

13.2.3.4 Transamination

Amino transferases and transaminases present in micro-organisms catalyze the conversion of a given amino acid into another one. For instance, a glutaminase from *Debaryomices hansenii* was recently purified and characterized. This enzyme was able to transform glutamine into glutamic acid and ammonia (Durá, Flores, and Toldrá 2002).

13.3 PROTEOLYSIS IN DRY-CURED MEATS

Proteolysis constitutes an important biochemical mechanism in dry-cured meats that needs to be controlled to exert beneficial effects on the final quality of the product. Proteolysis is the progressive breakdown of major meat proteins (sarcoplasmic and myofibrillar proteins), contributing to a weakening of the myofibrillar network and the subsequent generation of peptides and free amino acids that contribute to flavor development (Toldrá 2004d). However, an excess of proteolysis may negatively affect the sensory characteristics for the following reasons (Toldrá 2005a, 2006):

1. A noticeable bitter and metallic taste due to the excessive accumulation of certain low molecular weight nitrogen compounds (peptides and free phenylalanine and triptophane).
2. The presence of white crystals of tyrosine distributed randomly through the product, making it less attractive.
3. Poor texture (excessive softness) due to an excessive breakdown of myofibrillar proteins.

The main aspects concerning the influence of proteolysis on the quality of dry-cured hams and dry and semidry fermented sausages are given next.

13.3.1 Proteolysis in Dry-Cured Ham and Its Effect on Quality

Proteolysis is quite extensive because of the length of the process, which can last up to 2 years. Myosin and other important myofibrillar proteins are broken down during the process (Toldrá, Rico, and Flores 1993). The result is an important generation of small peptides and free amino acids with a direct contribution to taste and indirect effect on aroma through different reactions. Proteolysis depends on many factors that affect the muscle enzyme profile like the original crossbreeds (Armero, Barbosa, Toldrá, Baselga, and Pla 1999; Armero, Baselga, Aristoy, and Toldrá 1999; Armero, Flores, et al. 1999; Cava, Ferrer, Estévez, Morcuende, and Toldrá 2004), the age of the pigs (Rosell and Toldrá 1998; Toldrá, Flores, Aristoy, Virgili, and Parolari 1996), and the type of muscle (Aristoy and Toldrá 1998). Muscle enzymes exhibit a great stability during the processing of dry-cured hams and some activity is still found after a good number of months (Toldrá 1998; Toldrá and Etherington 1988; Toldrá and Flores 1998). Other important factors are related to the processing technology, like the temperature and time of ripening, which have a direct effect on the enzymes' activity and the salt present in the product, exerting a great effect on muscle proteases (Rico et al. 1990, 1991; Rosell and Toldrá 1996; Sentandreu and Toldrá 2001a; Toldrá, Cerveró, and Part 1992; Toldrá, Rico, and Flores 1992). Some studies have been done with reduced salt content (Martin, Córdoba, Antequera, Timón, and Ventanas 1998). However, an excessive softness has been reported in hams with initial high cathepsin B activity and low salt content (García-Garrido, Quiles, Tapiador, and Luque 2000; Parolari, Virgili, and Schivazzappa 1994). Texture is an important attribute clearly related to cathepsin activity (Monin et al. 1997; Tabilo, Flores, Fiszman, and Toldrá 1999).

As mentioned earlier, there is a large generation of small peptides, especially within the range between 2,700 and 4,500 Da, or even below 2,700 Da along the process (Aristoy and Toldrá 1995; Flores, Aristoy, Spanier, and Toldrá 1997; Rodríguez-Núñez, Aristoy, and Toldrá 1995). Very small peptides, like several tri- and dipeptides generated by tripeptidyl and dipeptidylpeptidases, have been recently isolated and sequenced (Sentandreu et al. 2003). Some of these peptides give characteristic tastes that depend on their particular composition in amino acids as shown in table 13.5 (Aristoy and Toldrá 1995). The final amounts of peptides depend on the factors previously mentioned, mainly temperature, processing length, and amount of added salt. The generation of free amino acids by aminopeptidases, as final products of proteolysis, is very intense and can reach several hundreds of milligrams per 100 g of ham (Córdoba et al. 1994; Toldrá, Aristoy, and Flores 2000; Toldrá, Flores, and Aristoy 1995; Toldrá, Flores, and Sanz 1997). A typical profile of generated amino acids and respective amounts is shown in table 13.6. The generation rate is affected by processing parameters and the amount of salt. It is well known that free amino acids can contribute to specific tastes when above their perception thresholds (Kato, Rhue, and Nishimura 1989; Nishimura and Kato 1988). The main contribution of amino acids to the taste

TABLE 13.5
Main Enzymatic Routes for Peptides and Free Amino Acids Generation During the Processing of Dry-Cured Meats and Its Contribution to Flavor

Compounds	Enzymes Involved	Contribution to Flavor
Tri-peptides	Muscle tripeptidyl peptidases	Taste will depend on the specific amino acid composition
Di-peptides	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 *L. sakei*	Bitter taste will increase as the concentration of these amino acids rises; 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 *D. 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

Note: Adapted from Toldrá (2002, 2004d, 2006).

of dry-cured meats is shown in table 13.5. Glutamic and aspartic acids are generated in large amounts and may impart an acid taste although their sodium salts contribute to taste enhancement. Phenylalanine, tryptophane, and tyrosine are also generated in large amounts and may contribute to bitter taste if not controlled. On the other hand, other amino acids like alanine, serine, proline, glycine, and hydroxyproline modulate taste through their sweetness (Aristoy and Toldrá 1995). The final taste depends on the balance among all the amino acids and its relative contribution. Therefore, the aged taste of Parma ham has been correlated to its lysine and tyrosine content (Careri et al. 1993) although minimal effect has been observed in French-type dry-cured ham (Buscailhon, Berdagué, Gandemer, Touraille, and Monin 1994). An unpleasant bitter-like or metal aftertaste is associated with excessive proteolysis (a proteolysis index higher than 29%–30%) and can be found in hams with low salt content or in certain uncontrolled processes (Careri et al. 1993; Parolari et al. 1994). Some free amino acids can also constitute a source of volatile compounds during further ripening or heating (McCain, Blumer, Craig, and Steel 1968). For instance, methionine and cysteine may give sulfur volatile compounds, whereas leucine, valine, and isoleucine

TABLE 13.6
Net Generation of Free Amino Acids (Expressed in mg/100g) in Dry-Cured Ham and Dry-Fermented Sausage

Amino Acids	Dry-Cured Ham (Ripened for 7 Months)	Dry-Cured Ham (Ripened for 15 Months)	Dry-Fermented Sausage
Aspartic acid	197.0	301.1	43.4
Glutamic acid	354.0	498.6	180.6
Serine	183.6	250.3	49.4
Asparagine	46.9	27.1	22.5
Glycine	155.9	216.1	71.9
Glutamine	46.9	24.6	32.5
Tyrosine	127.2	127.2	4.4
Proline	195.9	288.6	47.8
Alanine	270.7	389.3	137.7
Arginine	236.8	230.8	64.4
Threonine	193.9	279.9	50.1
Valine	221.4	315.5	94.0
Methionine	90.8	133.7	54.2
Isoleucine	155.7	218.3	75.1
Leucine	246.9	342.7	138.9
Phenylalanine	149.3	209.2	83.3
Tryptophan	25.7	31.4	13.3
Histidine	70.0	98.0	28.9
Lysine	508.9	734.6	58.1

Note: Adapted from Flores, Sanz, Spanier, Aristoy, and Toldrá (1998); Toldrá, Flores, and Aristoy (1995); Toldrá, Flores, Navarro, Aristoy, and Flores (1997).

can generate branched-chain aldehydes by Strecker degradation reations (Flores, Sanz, Spanier, Aristoy, and Toldrá 1998).

13.3.2 Proteolysis in Dry-Fermented Sausages and Its Effect on Quality

Dry-fermented sausages are fermented for 1 or 2 days and ripened for several weeks. The extent of ripening and drying depends on the type of product and its diameter (Demeyer and Toldrá 2004). In general, short processes require higher fermentation temperatures. Semidry sausages are typically fermented to a low pH and heated but not dried. This is the reason why their moisture content is relatively high (80%–85%) and the texture softer (Sebranek 2004).

During fermentation and ripening and drying, muscle and microbial proteases exert a combined action, but their relative contribution depends on the micro-organisms used as starter cultures. From different studies based on the use of antibiotics and other protease inhibitors, it appears that muscle cathepsin D, very active at pH 4.5, starts the degradation of myosin and actin, whereas cathepsins B and L would be more

restricted to actin and its degradation products (Molly et al. 1997; Sanz et al. 1999a). Myosin, α-actinin, and actin are the most degraded proteins (García de Fernando and Fox 1991). In the case of sarcoplasmic proteins, lactobacilli have shown good ability for its degradation (Fadda et al. 1999a, 1999b; Sanz et al. 1999a).

After the initial breakdown of meat proteins, different peptides are generated through the action of peptidases. Muscle and bacterial peptidases and exopeptidases would have a shared role in the last steps of proteolysis generating peptides and free amino acids (Toldrá 2004d). In general, more or less enzyme activity will be observed depending on the applied temperature and extent of the process. Some of these peptides, hydrophylic and relatively short, have been sequenced and its parent protein identified by comparison of homologies. Two peptides were originated from myoglobin and one from creatin kinase, and three other peptides were originated from the myofibrillar proteins troponin T, troponin I, and myosin light chain 2 (Hughes et al. 2002).

The generation of small peptides by muscle and microbial tri- and dipeptidylpeptidases may be somehow depressed by the level of salt, which inhibits this type of enzymes (Sentandreu and Toldrá 2001a). This is the reason for wide variations in the increases in nonprotein nitrogen, from twofold to twelvefold. The highest nonprotein nitrogen value has been reported in sausages with low pH value, below 4.7 (Flores, Marcus, Nieto, and Navarro 1997). Some of these peptides give characteristic tastes (see table 13.6). X-Pro dipeptides can be generated through the action of bacterial X-prolyl dipeptidylpeptidase (Sanz and Toldrá 2001). Final proteolysis steps onvolve the generation of free amino acids by aminopeptidases. An example of the generated free amino acids is shown in table 13.5. Some of them, like valine, alanine, leucine, isoleucine, phenylalanine, and methionine, reach the highest levels (Sanz, Sentandreu, and Toldrá 2002). Alanyl and methionyl aminopeptidases together with major aminopeptidase from *L. sakei* are very important in this generation. Some amino acids like valine, methionine, and leucine could also be generated from tri- and dipeptides by the action of microbial tri- and dipeptidases (Sanz and Toldrá 1999). Arginine and lysine would be generated by muscle aminopeptidase B and arginyl and microbial arginyl aminopeptidases (Toldrá, in press-b).

Taste descriptors like spicy, beefy, sweet, bitter, and astringent have been correlated with certain amino acids (Talon, Leroy-Sétrin, and Fadda 2004). Other amino acids and peptides have been identified as contributing to taste (Henriksen and Stahnke 1997; see table 13.5). Branched-chain aldehydes and corresponding secondary products such as acids, alcohols, and esters can be generated by bacterial metabolism of leucine, valine, and isoleucine mainly by species from the *Micrococcaceae* family (*Staphylococcus* and *Kocuria*) and *Debaryomices hansenii,* and to a lesser extent by *Lactobacilli* (Demeyer et al. 2000; Demeyer and Stahnke 2002). Other volatile compounds like 3-methylbutanoic acid and α-hydroxy isocaproic acid have been identified after incubation of leucine with *Staphylococcus xylosus* and *S. carnosus* (Olesen, Stahnke, and Talon 2004).

13.4 ENHANCEMENT AND CONTROL OF PROTEOLYSIS IN DRY-CURED MEATS

An important trend has been the addition of enzymes to accelerate the process, especially flavor development. Some of the assayed proteinases were a lactobacillus

serine proteinase (Naes, Holck, Axelsson, Andersen, and Blom 1995) a proteinase (alcalase) from *Bacillus licheniformis,* and a proteinase from *L. paracasei* subspecies *paracasei* (Hagen, Berdagué, Holck, Naes, and Blom 1996); neutral proteinase (neutrase) from *Bacillus subtilis* (Zapelena, Ansorena, Zalacaín, Astiasarán, and Bello 1998); neutrase, alcalase, and HT proteolytic from *B. subtilis* var. *amiloliquefaciens;* fungal protease from *Aspergillus oryzae* (Ordoñez et al. 1999; Zapelena, Zalacaín, Paz de Peña, Astiasarán, and Bello 1997); and pronase E from *Streptomyces griseus* (Díaz, Fernández, García de Fernando, de la Hoz, and Ordoñez 1993). In certain cases, some of these proteinases have shown an improvement in sensory scores for the sausages, especially in overall acceptability. Other proteases like papain and bromelain may give undesirable flavor and texture (Ansorena, Astiasarán, and Bello 2002). However, the addition of commercial proteinases to accelerate the process is rather difficult to control because the amounts used in other foods are not adequate for these meats. Selection of the accurate amount of commercial proteinase is essential to obtain the desired effects in the sausage but avoiding texture defects, mainly softening. The addition of proteases alone is not enough for shortening the processing time, as the generated amino acids need additional time to be transformed into volatile compounds with desirable aroma properties. Another option is the addition of whole cells or cell-free extracts trying to accelerate and improve the sensory quality of the sausages (Durá, Flores, and Toldrá 2004a, 2004b, 2004c).

The control of raw materials is essential for dry-cured ham and important for fermented sausages, as there are some variations in the activity of muscle proteases depending on genetics and age of the animals (Armero, Barbosa, et al. 1999; Armero, Baselga, et al. 1999; Armero, Flores, et al. 1999; Toldrá 2002). The choice of starter cultures is also essential for fermented sausages. Two groups of micro-organisms are typically used. The first group is lactic acid bacteria (*Lactobaccillus* or *Pediococcus*) to provide acidulation to the product, and a second group is either *Micrococcaceae* or yeasts (*Debaryomices hansenii*) to provide nitrate reduction and flavor development (Demeyer and Stahnke 2002). Finally, processing conditions are important, as they can increase or reduce the enzyme activity. The control of proteolytic activity in the hams can be achieved through different ways as summarized in table 13.7 and table 13.8. The easiest way is by controlling the relative humidity and temperature in the curing rooms because they have an important effect on enzymatic activity (Toldrá 1998; Toldrá and Flores 1998). The pH achieved in the product during the process, near neutral pH in ham and acid pH in fermented sausages, will modulate the enzyme activity. Furthermore, proteolysis can be controlled by adding an excess of salt because it has an inhibitory effect on cathepsins and other proteases and thus reduces softening (Toldrá 2002). Other curing agents have lower effects on enzyme activity as reflected in table 13.8. Finally, time for ripening (see table 13.7) is important when intense flavor development is required, as most of the generated amino acids need some time to experience further reactions to aroma volatile compounds (Toldrá, Gavara, and Lagarón 2004). In summary, the ability to steer the proteolysis into the meat products is very important from the economic point of view because reproducible and steerable production processes are needed to produce dry-cured meats of consistently high quality (Toldrá and Verplaetse 1995).

TABLE 13.7
Control of Proteolysis in Dry-Cured Meats: Effect of Process Parameters

Process Parameter	Trend	Product	Effect on Proteases
pH	Acid	Dry-fermented sausages	Good activity of cathepsins D, B, H, and L, DPP I, DPP II, TPP I, and arginine aminopeptidase from *L. sakei*.
			Reduced activity of calpains, DPP III and IV, TPP II and muscle aminopeptidases, major aminopeptidase and Pro-DPP from *L. sakei*, protease B, prolyl and arginyl aminopeptidases from *D. hansenii*
	Neutral	Dry-cured ham	Good activity of calpain on first days, cathepsins B and L, DPP III and IV, TPP II, and aminopeptidases; reduced activity of cathepsin D
Time of ripening	Short	Semidry-fermented sausages	Poor enzyme action
	Medium	Dry-fermented sausages	Biochemical changes due to enzyme action will depend on the extent of ripening (usually several weeks)
	Long	Dry-cured ham	Very long time (up to 1 or 2 years) for important biochemical changes, especially affecting flavor; calpain activity restricted to initial weeks
Temperature	Mild	Dry-fermented sausages and dry-cured ham	Enzyme activity is not enhanced but have time enough for important biochemical changes, especially during ripening (when temperature increases above 10°C) and fermentation (temperatures above 20°C)
Reduction in water activity	Mild	Semidry-fermented sausages	Good activity of all proteases
	Intense	Dry-fermented sausages and dry-cured ham	Reduced enzyme activity as a_w drops, especially below 0.90
Redox potential	Decrease	All dry-cured meats	Good enzyme activity under reducing conditions.

Note: Adapted from Toldrá (2004d, 2005, 2006).

TABLE 13.8
Control of Proteolysis in Dry-Cured Meats: Effect of Curing Agents

Curing Agent	Amount	Product	Effect on Proteases
NaCl	High (5–6%)	Dry-cured ham	Strong inhibiton of cathepsins and partial inhibiton of other proteases; partial activation of calpain, muscle aminopeptidase B, and arginine aminopeptidase from *L. sakei* that are chloride-activated at low NaCl concentration
NaCl	Medium (2–3%)	Dry- and semidry-fermented sausages	Partial inhibiton of cathepsins and other proteases; activation of calpain and aminopeptidase B that are chloride-activated at low NaCl concentration
Nitrate and nitrite	Normal (125 ppm)	All dry-cured meats	Slight inhibition of the enzyme activity
Ascorbic acid	Normal (500 ppm)	Dry-fermented sausages	No significant effect on most proteases; slight inhibition of m-calpain, cathepsin H, leucyl aminopeptidase, and aminopeptidase B; inhibition of major aminopeptidase from *L. sakei*
Glucose	Up to to 2 gL^{-1}	Dry-fermented sausages	Slight activation of leucyl aminopeptidase and cathepsins B, H, and D

Note: Adapted from Toldrá (2004d, 2005, 2006).

REFERENCES

Ansorena, D., I. Astiasarán, and J. Bello. 2002. Use of exogenous enzymes in dry fermented sausages. In *Research advances in the quality of meat and meat products,* ed. F. Toldrá, 157–174. Trivandrum, India: Research Signpost.

Aristoy, M. C., and F. Toldrá. 1998. Concentration of free amino acids and dipeptides in porcine skeletal muscles with different oxidative patterns. *Meat Sci.* 50:327–332.

Aristoy, M. C., and F. Toldrá. 1995. Isolation of flavor peptides from raw pork meat and dry-cured ham. In *Food flavors: Generation, analysis and process influence,* ed. G. Charalambous, 1323–1344. Amsterdam: Elsevier Science.

Armero, E., J. A. Barbosa, F. Toldrá, M. Baselga, and M. Pla. 1999. Effect of the terminal sire and sex on pork muscle cathepsin (B, B+L and H), cysteine proteinase inhibitors and lipolytic enzyme activities. *Meat Sci.* 51:185–189.

Armero, E., M. Baselga, M. C. Aristoy, and F. Toldrá. 1999. Effects of sire types and sex on pork muscle exopeptidase activity and the content of natural dipeptides and free amino acids. *J. Sci. Food Agric.* 79:1280–1284.

Armero, E., M. Flores, F. Toldrá, J. A. Barbosa, J. Olivet, M. Pla, and M. Baselga. 1999. Effects of pig sire types and sex on carcass traits, meat quality and sensory quality of dry-cured ham. *J. Sci. Food Agric.* 79:1147–1154.

Barrett, A. J., N. D. Rawlings, and J. F. Woessner, eds. 2004. *Handbook of proteolytic enzymes* (2nd ed.). Amsterdam: Elsevier/Academic Press.

Bockelmann, W. 1995. The proteolytic system of starter and non-starter bacteria: Components and their importance for cheese ripening. *Int. Dairy J.* 5:977–994.

Bolumar, T., Y. Sanz, M. C. Aristoy, and F. Toldrá. 2003a. Purification and characterization of a prolyl aminopeptidase from *Debaryomyces hansenii*. *Appl. Environ. Microbiol.* 69:227–232.

Bolumar, T., Y. Sanz, M. C. Aristoy, and F. Toldrá. 2003b. Purification and properties of an arginyl aminopeptidase from *Debaryomyces hansenii*. *Int. J. Food Microbiol.* 86:141–151.

Bolumar, T., Y. Sanz, M. C. Aristoy, and F. Toldrá. 2005. Protease B from *Debaryomyces hansenii:* Purification and biochemical properties. *Int. J. Food Microbiol.* 98:167–177.

Buscailhon, S., J. L. Berdagué, G. Gandemer, C. Touraille, and G. Monin. 1994. Effects of initial pH on compositional changes and sensory traits of French dry-cured hams. *J. Muscle Foods.* 5:257–270.

Campbell-Platt, G. 1995. Fermented meats: A world perspective. In *Fermented meats,* eds. G. Campbell-Platt & P. E. Cook, 39–52. London: Blackie Academic & Professional.

Careri, M., A. Mangia, G. Barbieri, L. Bolzoni, R. Virgili, and G. Parolari. 1993. Sensory property relationships to chemical data of Italian type dry-cured ham. *J. Food Sci.* 58:968–972.

Cava, R., J. M. Ferrer, M. Estévez, D. Morcuende, and F. Toldrá. 2004. Composition and proteolytic and lipolytic enzyme activity in muscle *Longissimus dorsi* from Iberian pigs and industrial genotype pigs. *Food Chem.* 88:25–33.

Córdoba, J. J., T. Antequera, C. García, J. Ventanas, C. López-Bote, and M. A. Asensio. 1994. Evolution of free amino acids and amines during ripening of Iberian cured ham. *J. Agric. Food Chem.* 42:2296–2301.

Coux, O., K. Tanaka, and A. Goldberg. 1995. Structure and function of the 20S and 26S proteasomes. *Ann. Rev. Biochem.* 65:801–847.

Demeyer, D. I., M. Raemakers, A. Rizzo, A. Holck, A. De Smedt, B. Ten Brink, B. Hagen, C. Montel, E. Zanardi, E. Murbrek, F. Leroy, F. Vanderdriessche, K. Lorentsen, K. Venema, L. Sunesen, L. Stahnke, L. De Vuyst, R. Talon, R. Chizzolini, and S. Eerola. 2000. Control of bioflavor and safety in fermented sausages: First results of a European project. *Food Res. Int.* 33:171–180.

Demeyer, D. I., and L. Stahnke. 2002. Quality control of fermented meat products. In *Meat processing: Improving quality,* eds. J. Kerry, J. Kerry, and D. Ledward, 359–393. Cambridge, UK: Woodhead.

Demeyer, D. I., and F. Toldrá. 2004. Fermentation. In *Encyclopedia of meat sciences,* eds. W. Jensen, C. Devine, and M. Dikemann, 467–474. London: Elsevier Science.

Díaz, O., M. Fernández, G. D. García de Fernando, L. de la Hoz, and J. A. Ordoñez. 1993. Effect of the addition of pronase E on the proteolysis in dry-fermented sausages. *Meat Sci.* 34:205–211.

Durá, M. A., M. Flores, and F. Toldrá. 2002. Purification and characterisation of a glutaminase from *Debaryomices* spp. *Int. J. Food Microbiol.* 76:117–126.

Durá, M. A., M. Flores, and F. Toldrá. 2004a. Effect of *Debaryomyces* spp on aroma formation and sensory quality of dry-fermented sausages. *Meat Sci.* 68:439–446.

Durá, M. A., M. Flores, and F. Toldrá. 2004b. Effect of *Debaryomyces spp* on the proteolysis of dry-fermented sausages. *Meat Sci.* 68:319–328.

Durá, M. A., M. Flores, and F. Toldrá. 2004c. Effect of growth phase and dry-cured sausage processing conditions on Debaryomyces spp. generation of volatile compounds from branched-chain amino acids. *Food Chem.* 86:391–399.

Fadda, S., Y. Sanz, G. Vignolo, M. C. Aristoy, G. Oliver, and F. Toldrá. 1999a. Characterization of muscle sarcoplasmic and myofibrillar protein hydrolysis caused by *Lactobacillus plantarum*. *Appl. Environ. Microbiol.* 65:3540–3546.

Fadda, S., Y. Sanz, G. Vignolo, M. C. Aristoy, G. Oliver, and F. Toldrá. 1999b. Hydrolysis of pork muscle sarcoplasmic proteins by *Lactobacillus curvatus* and *Lactobacillus sake*. *Appl. Environ. Microbiol.* 65:578–584.

Flores, J., J. R. Marcus, P. Nieto, and J. L. Navarro. 1997. Effect of processing conditions on proteolysis and taste of dry-cured sausages. *Z. Lebensm Unters Forsch. A.* 204:168–172.

Flores, J., and F. Toldrá. 1993. Curing: Processes and applications. In *Encyclopedia of food science, food technology and nutrition,* eds. R. Macrae, R. Robinson, M. Sadle, and G. Fullerlove, 1277–1282. London: Academic Press.

Flores, M., M. C. Aristoy, A. M. Spanier, and F. Toldrá. 1997. Non-volatile components effects on quality of Serrano dry-cured ham as related to processing time. *J. Food Sci.* 62:1235–1239.

Flores, M., M. C. Aristoy, and F. Toldrá. 1993. HPLC purification and characterization of porcine muscle aminopeptidase *B*. *Biochimie*. 75:861–867.

Flores, M., M. C. Aristoy, and F. Toldrá. 1996. HPLC purification and characterization of soluble alanyl aminopeptidase from porcine skeletal muscle. *J. Agric. Food Chem.* 44:2578–2583.

Flores, M., M. Marina, and F. Toldrá. 2000. Purification and characterization of a soluble methionyl aminopeptidase from porcine skeletal muscle. *Meat Sci.* 56:247–254.

Flores, M., Y. Sanz, A. M. Spanier, M. C. Aristoy, and F. Toldrá. 1998. Contribution of muscle and microbial aminopeptidases to flavor development in dry-cured meat products. In *Food flavor: Generation, analysis and process influence,* eds. E. T. Contis, C. T. Ho, C. J. Mussinan, T. H. Parliament, F. Shahidi and A. M. Spanier, 547–557. Amsterdam: Elsevier Science.

Flores, M., A. M. Spanier, and F. Toldrá. 1998. Flavour analysis of dry-cured ham. In *Flavor of meat, meat products and seafoods,* ed. F. Shahidi, 320–341. London: Blackie Academic & Professional.

García de Fernando, G., and P. F. Fox. 1991. Study of proteolysis during the processing of a dry fermented pork sausage. *Meat Sci.* 30:367–383.

García-Garrido, J. A., R. Quiles, J. Tapiador, and M. D. Luque. 2000. Activity of cathepsin B, D, H and L in Spanish dry-cured ham of normal and defective texture. *Meat Sci.* 56:1–6.

Goll, D. E., Y. Otsuka, P. A. Nagainis, J. D. Shannon, S. K. Sathe, and M. Muguruma. 1983. Role of muscle proteinases in maintenance of muscle integrity and mass. *J. Food Biochem.* 7:137–177.

Haard, N. F. 1990. Enzymes from food myosystems. *J. Muscle Foods.* 1:293–338.

Hagen, B. F., J. L. Berdagué, A. L. Holck, H. Naes, and H. Blom. 1996. Bacterial proteinase reduces maturation time of dry fermented sausages. *J. Food Sci.* 61:1024–1029.

Henriksen, A. P., and L. H. Stahnke. 1997. Sensory and chromatographic evaluations of water soluble fraction from dried sausages. *J. Agric. Food Chem.* 45:2679–2684.

Hernández, P., J. L. Navarro, and F. Toldrá. 1999. Lipolytic and oxidative changes in two Spanish pork loin products: Dry-cured loin and pickled-cured loin. *Meat Sci.* 51:123–128.

Hughes, M. C., A. Healy, P. L. H. McSweeney, and E. E. O'Neill. 2000. Proteolytic specificity of cathepsin D on bovine F-actin. *Meat Sci.* 56:165–172.

Hughes, M. C., J. P. Kerry, E. K. Arendt, P. M. Kenneally, P. L. H. McSweeney, and E. E. O'Neill. 2002. Characterization of proteolysis during the ripening of semidry fermented sausages. *Meat Sci*. 62:205–216.

Hughes, M. C., E. E. O'Neill, P. L. H. McSweeney, and A. Healy. 1999. Proteolysis of bovine F-actin by cathepsin B. *Food Chem*. 64:525–530.

Kato, H., M. R. Rhue, and T. Nishimura. 1989. Role of free amino acids and peptides in food taste. In *Flavor chemistry: Trends and developments,* eds. R. Teranishi, R. G. Buttery, and F, Shahidi, 158–174. Washington, DC: ACS Symposium Series.

Koohmaraie, M., S. C. Seideman, J. E. Schollmeyer, T. R. Dutson, and J. D. Grouse. 1987. Effect of postmortem storage on Ca^{2+} dependent proteases, their inhibitor and myofibril fragmentation. *Meat Sci*. 19:187–196.

Kunji, E. R. S., G. Fang, C. M. Jeronimus-Stratingh, A. P. Bruins, B. Poolman, and W. N. Konings. 1996. The proteolytic systems of lactic acid bacteria. *Antonie van Leeuwenhoek*. 70:187–221.

Leistner, L. 1992. The essentials of producing stable and safe raw fermented sausages. In *New technologies for meat and meat products,* eds. F. J. M. Smulders, F. Toldrá, J. Flores, and M. Prieto, 1–19. Nijmegen, The Netherlands: Audet.

Martin, I., J. J. Córdoba, T. Antequera, M. L. Timón, and J. Ventanas. 1998. Effects of salt and temperature on proteolysis during ripening of Iberian ham. *Meat Sci*. 49:145–153.

Matsukura, U., A. Okitani, T. Nishimura, and H. Katoh. 1981. Mode of degradation of myofibrillar proteins by an endogenous protease, cathepsin L. *Biochim. Biophys. Acta*. 662:41–47.

McCain, G. R., T. N. Blumer, H. B. Craig, and R. G. Steel. 1968. Free amino acids in ham muscle during successive aging periods and their relation to flavor. *J. Food Sci*. 33:142–146.

Molimard, P., and H. E. Spinnler. 1996. Review: Compounds involved in the flavor of surface mold-ripened chesses: Origins and properties. *J. Diary Sci*. 79:169–184.

Molly, K., D. I. Demeyer, G. Johansson, M. Raemaekers, M. Ghistelinck, and I. Geenen. 1997. The importance of meat enzymes in ripening and flavor generation in dry fermented sausages: First results of a European project. *Food Chem*. 54:539–545.

Monin, G., P. Marinova, A. Talmant, J. F. Martin, M. Cornet, D. Lanore, and F. Grasso. 1997. Chemical and structural changes in dry-cured hams (Bayonne hams) during processing and effects of the dehairing technique. *Meat Sci*. 47:29–46.

Montel, M. C., M. P. Seronine, R. Talon, and M. Hebraud. 1995. Purification and characterization of a dipeptidase from *Lactobacillus sake*. *Appl. Environ. Microbiol*. 61:837–839.

Naes, H., A. L. Holck, L. Axelsson, H. J. Andersen, and H. Blom. 1995. Accelerated ripening of dry-fermented sausage by addition of a Lactobacillus proteinase. *Int. J. Food Sci. Technol*. 29:651–659.

Nishimura, T., and H. Kato. 1988. Taste of free amino acids and peptides. *Food Rev. Int*. 4:175–194.

Okitani, A., T. Nishimura, and H. Katoh. 1981. Characterization of hydrolase H, a new muscle protease possessing aminoendopeptidase activity. *Eur. J. Biochem*. 115:269–274.

Olesen, P. T., L. H. Stahnke, and R. Talon. 2004. Effect of ascorbate, nitrate and nitrite on the amount of flavour compounds produced from leucine by Staphylococcus xylosus and Staphylococuus carnosus. *Meat Sci*. 68:193–200.

Ordoñez, J. A., E. M. Hierro, J. M. Bruna, and I. de la Hoz. 1999. Changes in the components of dry-fermented sausages during ripening. *Crit. Rev. Food. Sci. Nutr.* 39:329–367.

Ouali, A., and M. A. Sentandreu. 2002. Overview of muscle peptidases and their potential role in meat texture development. In *Research advances in the quality of meat and meat products,* ed. F. Toldrá, 33–63. Trivandrum, India: Research Signpost.

Parolari, G., R. Virgili, and C. Schivazzappa. 1994. Relationship between cathepsin B activity and compositional parameters in dry-cured hams of normal and defective texture. *Meat Sci.* 38:117–122.

Pritchard, G. G., and T. Coolbear. 1993. The physiology and biochemistry of the proteolytic system of lactic acid bacteria. *FEMS Microb. Rev.* 12:179–206.

Rico, E., F. Toldrá, and J. Flores. 1990. Activity of cathepsin D as affected by chemical and physical dry-curing parameters. *Z. Lebensm. Unters. Forsch.* 191:20–23.

Rico, E., F. Toldrá, and J. Flores. 1991. Effect of dry-curing process parameters on pork muscle cathepsins B, H and L activities. *Z. Lebensm. Unters. Forsch.* 193:541–544.

Rodríguez-Núñez, E., M. C. Aristoy, and F. Toldrá. 1995. Peptide generation in the processing of dry-cured ham. *Food Chem.* 53:187–190.

Rosell, C. M., and F. Toldrá. 1996. Effect of curing agents on m-calpain activity throughout the curing process. *Z. Lebensm. Unters. Forchs.* 203:320–325.

Rosell, C. M., and F. Toldrá. 1998. Comparison of muscle proteolytic and lipolytic enzyme levels in raw hams from Iberian and White pigs. *J. Sci. Food. Agric.* 76:117–122.

Santos, N. N., R. C. Santos, Y. Sanz, T. Bolumar, M. C. Aristoy, and F. Toldrá. 2001. Hydrolysis of muscle sarcoplasmic proteins by Debaryomyces hansenii. *Int. J. Food Microbiol.* 68:199–206.

Sanz, Y., S. Fadda, G. Vignolo, M. C. Aristoy, G. Oliver, and F. Toldrá. 1999a. Hydrolysis of muscle myofibrillar proteins by *Lactobacillus curvatus* and *Lactobacillus sake*. *Int. J. Food Microbiol.* 53:115–125.

Sanz, Y., S. Fadda, G. Vignolo, M. C. Aristoy, G. Oliver, and F. Toldrá. 1999b. Hydrolytic action of *Lactobacillus casei* CRL 705 on pork muscle sarcoplasmic and myofibrillar proteins. *J. Agric. Food Chem.* 47:3441–3448.

Sanz, Y., F. Mulholland, and F. Toldrá. 1998. Purification and characterization of a tripeptidase from *Lactobacillus sake*. *J. Agric. Food Chem.* 46:349–353.

Sanz, Y., M. A. Sentandreu, and F. Toldrá. 2002. Role of muscle and bacterial exopeptidases in meat fermentation. In *Research advances in the quality of meat and meat products,* ed. F. Toldrá, 143–155. Trivandrum, India: Research Signpost.

Sanz, Y., and F. Toldrá. 1997. Purification and characterization of an aminopeptidase from *Lactobacillus sake*. *J. Agric. Food Chem.* 45:1552–1558.

Sanz, Y., and F. Toldrá. 1999. The role of exopeptidases from Lactobacillus sake in dry fermented sausages. *Recent Res. Devel. Agric. & Food Chem.* 3:11–21.

Sanz, Y., and F. Toldrá. 2001. Purification and characterization of an X-prolyl-dipeptidyl peptidase from *Lactobacillus sake*. *Appl. Environ. Microbiol.* 67:1815–1820

Sanz, Y., and F. Toldrá. 2002. Purification and characterization of an arginine aminopeptidase from *Lactobacillus sakei*. *Appl. Environ. Microbiol.* 68:1980–1987.

Schwartz, W. N., and J. W. C. Bird. 1977. Degradation of myofibrillar proteins by cathepsins B and D. *Biochem. J.* 167:811–820.

Sebranek, J. G. 2004. Semidry fermented sausages. In *Handbook of food and beverage fermentation technology,* eds. Y. H. Hui, L. M. Goddik, J. Josephsen, P. S. Stanfield, A. S. Hansen, W. K. Nip, and F. Toldrá, 385–396. New York: Marcel Dekker.

Sentandreu, M. A., G. Coulis, and A. Ouali. 2002. Role of muscle endopeptidases and their inhibitors in meat tenderness. *Trends Food Sci. Technol.* 13:398–419.

Sentandreu, M. A., S. Stoeva, M. C. Aristoy, K. Laib, W. Voelter, and F. Toldrá. 2003. Identification of taste related peptides in Spanish Serrano dry-cured hams. *J. Food Sci.* 68:64–69.

Sentandreu, M. A., and F. Toldrá. 1998. Biochemical properties of dipeptidylpeptidase III purified from porcine skeletal muscle. *J. Agric. Food Chem.* 46:3977–3984.

Sentandreu, M. A., and F. Toldrá. 2000. Purification and biochemical properties of dipeptidylpeptidase I from porcine skeletal muscle. *J. Agric. Food Chem.* 48:5014–5022.

Sentandreu, M. A., and F. Toldrá. 2001a. Dipeptidylpeptidase activities along the processing of Serrano dry-cured ham. *Eur Food Res Technol.* 213:83–87.

Sentandreu, M. A., and F. Toldrá. 2001b. Dipeptidylpeptidase IV from porcine skeletal muscle: Purification and biochemical properties. *Food Chem.* 75:159–168.

Sentandreu, M. A., and F. Toldrá. 2001c. Importance of dipeptidylpeptidase II in postmortem pork muscle. *Meat Sci.* 57:93–103.

Tabilo, G., M. Flores, S. Fiszman, and F. Toldrá. 1999. Postmortem meat quality and sex affect textural properties and protein breakdown of dry-cured ham. *Meat Sci.* 51:255–260.

Talon, T., S. Leroy-Sétrin, and S. Fadda. 2004. Dry fermented sausages. In *Handbook of food and beverage fermentation technology,* eds. Y. H. Hui, L. M. Goddik, J. Josephsen, P. S. Stanfield, A. S. Hansen, W. K. Nip, and F. Toldrá, 397–416. New York: Marcel Dekker.

Tan, P. S. T., B. Poolman, and W. S. Konings. 1993. Proteolytic enzymes of *Lactococcus lactis. J. Dairy Res.* 60:269–286.

Toldrá, F. 1992. The enzymology of dry-curing of meat products. In *New technologies for meat and meat products,* eds. J. M. Smulders, F. Toldrá, J. Flores, and M. Prieto, 209–231. Nijmegen, The Netherlands: Audet.

Toldrá, F. 1998. Proteolysis and lipolysis in flavour development of dry-cured meat products. *Meat Sci.* 49:S101–S110.

Toldrá, F. 2002. *Dry-cured meat products*. Trumbull, CT: Food & Nutrition Press.

Toldrá, F. 2004a. Curing: Dry. In *Encyclopedia of meat sciences,* eds. W. Jensen, C. Devine, and M. Dikemann, 360–365. London: Elsevier Science.

Toldrá, F. 2004b. Dry-cured ham. In *Handbook of food and beverage fermentation technology,* eds. Y. H. Hui, L. M. Goddik, J. Josephsen, P. S. Stanfield, A. S. Hansen, W. K. Nip, and F. Toldrá, 369–384. New York: Marcel Dekker.

Toldrá, F. 2004c. Ethnic meat products: Mediterranean. In *Encyclopedia of meat sciences,* eds. W. Jensen, C. Devine, and M. Dikemann, 451–453. London: Elsevier Science.

Toldrá, F. 2004d. Fermented meats. In *Food processing: Principles and applications,* eds. Y. H. Hui and J. S. Smith, 399–415. Ames, IA: Blackwell.

Toldrá, F. 2005a. Dry-cured ham. In *Handbook of food science, technology and engineering,* eds. Y. H. Hui, J. D. Culbertson, S. Duncan, I. Guerrero-Legarreta, E. C. Y. Li-Chan, C. Y. Ma, C. H. Manley, T. A. McMeekin, W. K. Nip, L. M. L Nollet, M. S. Rahman, F. Toldrá, and Y. L. Xiong, Vol. 4. 164-1–164-11. Boca Raton, FL: Marcel Dekker.

Toldrá, F. 2005b. Meat: Chemistry and biochemistry. In *Handbook of food science, technology and engineering,* eds. Y. H. Hui, J. D. Culbertson, S. Duncan, I. Guerrero-Legarreta, E. C. Y. Li-Chan, C. Y. Ma, C. H. Manley, T. A. McMeekin, W. K. Nip, L. M. L Nollet, M. S. Rahman, F. Toldrá, and Y. L. Xiong, Vol. 1. 28-1–28-14. Boca Raton, FL: Marcel Dekker/CRC Press.

Toldrá, F. 2006. Biochemistry of fermented meat. In *Food biochemistry and food processing,* eds. Y. H. Hui, W. K. Nip, M. L. Nollet, G. Paliyath, and B. K. Simpson. 641–658. Ames, IA: Blackwell Publishing.

Toldrá, F., M. C. Aristoy, and M. Flores. 2000. Contribution of muscle aminopeptidases to flavor development in dry-cured ham. *Food Res. Int.* 33:181–185.

Toldrá, F., M. C. Cerveró, and C. Part. 1992. Porcine aminopeptidase activity as affected by curing agents. *J. Food Sci.* 58:724–726, 747.

Toldrá, F., and D. J. Etherington. 1988. Examination of cathepsins B, D, H and L activities in dry cured hams. *Meat Sci.* 23:1–7.

Toldrá, F., and M. Flores. 1998. The role of muscle proteases and lipases in flavor development during the processing of dry-cured ham. *CRC Crit. Rev. Food Sci. Nutr.* 38:331–352.

Toldrá, F., M. Flores, and M. C. Aristoy. 1995. Enzyme generation of free amino acids and its nutritional significance in processed pork meats. In *Food flavors: Generation, analysis and process influence,* ed. G. Charalambous, 1303–1322. Amsterdam: Elsevier Science.

Toldrá, F., M. Flores, M. C. Aristoy, R. Virgili, and G. Parolari. 1996. Pattern of muscle proteolytic and lipolytic enzymes from light and heavy pigs. *J. Sci. Food Agric.* 71:124–128.

Toldrá, F., M. Flores, J. L. Navarro, M. C. Aristoy, and M. Flores. 1997. New developments in dry-cured ham. In *Chemistry of novel foods,* eds. H. Okai, O. Mills, A. M. Spanier, and M. Tamura, 259–272. Carol Stream, IL: Allured.

Toldrá, F., M. Flores, and Y. Sanz. 1997. Dry-cured ham flavour: Enzymatic generation and process influence. *Food Chem.* 59:523–530.

Toldrá, F., M. Flores, and Y. Sanz. 2001. Meat fermentation technology. In *Meat science and applications,* eds. Y. H. Hui, W. K. Nip, R. W. Rogers, and O. A. Young, 537–561. New York: Marcel Dekker.

Toldrá, F., R. Gavara, and J. M. Lagarón. 2004. Packaging and quality control. In *Handbook of food and beverage fermentation technology,* eds. Y. H. Hui, L. M. Goddik, J. Josephsen, P. S. Stanfield, A. S. Hansen, W. K. Nip, and F. Toldrá, 445–458. New York: Marcel Dekker.

Toldrá, F., E. Rico, and J. Flores. 1992. Activities of pork muscle proteases in cured meats. *Biochimie.* 74:291–296.

Toldrá, F., E. Rico, and J. Flores. 1993. Cathepsin B, D, H and L activity in the processing of dry-cured-ham. *J. Sci. Food Agric.* 62:157–161.

Toldrá, F., and A. Verplaetse. 1995. Endogenous enzyme activity and quality for raw product processing. In *Composition of meat in relation to processing, nutritional and sensory quality,* eds. K. Lündstrom, I. Hansson, and E. Winklund, 41–55. Uppsala, Sweden: Ecceamst.

Visser, S. 1993. Proteolytic enzymes and their relation to cheese ripening. *J. Dairy Sci.* 76:329–350.

Zapelena, M. J., D. Ansorena, I. Zalacaín, I. Astiasarán, and J. Bello. 1998. Dry fermented sausages manufactured with different amounts of commercial proteinases: Evolution of total free α-NH_2-N-groups and sensory evaluation of the textura. *Meat Sci.* 49:213–221.

Zapelena, M. J., I. Zalacaín, M. Paz de Peña, I. Astiasarán, and J. Bello. 1997. Addition of a neutral proteinase from bacillus subtilis (Neutrase) together with a starter to a dry-fermented sausage elaboration and its effect on the amino acid profiles and the flavor development. *J. Agric. Food Chem.* 45:472–475.

14 Vacuum Salting Treatment for the Accelerated Processing of Dry-Cured Ham

José M. Barat, Raul Grau, Pedro Fito, and Amparo Chiralt
Food Science and Technology Department, Food Engineering Institute for Development, Polytechnical University of Valencia

CONTENTS

14.1 General Procedure for Obtaining Dry-Cured Ham 355
14.2 Use of Vacuum Impregnation in Food Salting 356
14.2.1 Basic Principles of the Vacuum Impregnation 356
14.2.1.1 Process Variables Affecting the Results Obtained in Brine Salting by Vacuum Impregnation 357
14.3 Use of Brine Vacuum Impregnation in Ham Salting 360
14.3.1 Use of Fresh Hams as Raw Material 361
14.3.2 Use of Frozen Hams as Raw Material 363
14.3.2.1 Postsalting Studies With Frozen Hams 365
14.4 Future Studies 366
Nomenclature 366
Acknowledgment 367
References 367

The most ancient data about the use of NaCl in food preservation corresponds to the Bronze Age (Ismail and Wooton 1992). There is no doubt that the initial reason for using NaCl in food processing was its preservation activity. Nevertheless, its relationship with flavor perception is also very well known. Although preservation was the initial reason for using NaCl in the processing of dry-cured ham, NaCl has other important roles like imparting its characteristic salty taste, and influencing the lipolytic and proteolytic enzymatic activity in meat during its processing, which are strongly related to the characteristic texture and flavors in the final product (Aristoy

and Toldrá 1995; Belletti, Dazzi, Chizzolini, Palmia, and Parolai 1983; Hansen-Moller, Hinrichsen, and Jacobsen 1997; MacCain, Blumer, Craig, and Steel 1968; Sárraga, Gil, Arnau, Monfort, and Cussó 1989; Toldrá, Rico, and Flores 1993).

The usual salting procedure to obtain dry-cured ham is the use of dry salting, and more specifically pile salting with drainage. Nevertheless, because this chapter focuses on the use of an alternative salting method, some considerations about the different possible salting methods are described.

First of all, it must be noted that in the case of the dry-cured ham, the NaCl target concentration at the end of the salting step, whatever the used method, should be expressed as the NaCl concentration in the ham muscle on a dry basis (X^{NaCl}), as this value is constant throughout the following processing steps, and so is in the final product, which should result in equal amount of moisture for all hams to obtain the same product from the composition point of view.

The main information required for the characterization of a new salting method for any food product, and more specifically dry-cured hams, depends on the type of salting procedure to be used. In the case of a salting procedure with kinetic control (the equilibrium being far away from the desired final point), the main information needed is the time required to reach the same salting level as in the traditional salting process (with pile salting). In the case of salting procedures with thermodynamic control (e.g., brine injection) with the equilibrium point fixed in the target value, the main information needed is the quantity of the salting agent to be added to reach the desired equilibrium point.

There are different procedures to accomplish the meat salting (FOA 1981; Ismail and Wooton 1992), the main classification being dry and wet salting. The use of dry salting without drainage with a small food:salt ratio could imply that at the end of the salting period the product could be surrounded by a brine. In this former case it would be considered pickling. The salting of food by placing the product in plastic bags with salt, after which the bag was evacuated and sealed, has been reported (Orr 1967). The use of vacuum packaging could contribute to the water release from the food.

There are two main possibilities when using dry salting, with or without overpressure. When pile salting is used, an overpressure is generated inside the pile, differing depending on the location inside the pile. This variability in the pressure supported by each of the piled products can contribute to the variability observed during pile salting. Another possibility is the existence of drainage, if any, during dry salting. Another alternative is to propel salt crystals at high speed that penetrate the product (Doe 1998). Sometimes NaCl or the curing agents are applied on the meat surface by massaging or tumbling, increasing the NaCl gain rate, probably due to the pressure changes during salting.

There are many possibilities for the brine salting. One of the more important variables is the NaCl concentration of the brine, which affects way the product behavior in a very important way, because low NaCl concentration contributes to an increase in the water holding capacity and, consequently, to the NaCl and water gain, whereas the saturated brines imply the product dehydration while NaCl goes into the meat (Barat, Rodríguez-Barona, Andrés, and Fito 2002; Offer and Trinick 1983; Wilding, Hedges, and Lillford 1986). Another possibility when using brines

during food salting is the modification of the pressure, increasing (Messens, Van Camp, and Huyghebart 1997) decreasing, or alternating the pressure (Barat, Grau, Montero, Chiralt, and Fito 2001; Barat, Grau, Pagán-Moreno, and Fito 2004; Deumier, Bohuon, Trystram, Saber, and Collignan 2003; Ghavimi, Rogers, Althen, and Ammerman 1986; Marriott, Graham, Boling, and Collins 1984) or even the use of ultrasounds (Sanchez, Simal, Femenia, Benedito, and Rosello 1999). Finally, we note the used of brine injection (Varnam and Sutherland 1995), which allows a very fast salting process, increasing the product weight with the injected brine. Nevertheless, injection can imply the contamination of the product by the injection needles or the solution (Townsend and Olson 1987) and the marks of the needles in meat can remain in the final product.

Temperature is a very important process variable during salting, because it is strongly related with the rate of the mass transport. Nevertheless, it is usually fixed to values slightly above 0°C (between 2°C and 4°C), because higher temperatures could imply a fast microbial growth and the alteration of the product as a consequence of that, mainly at the beginning of the process.

The optimal salting process should reach the desired NaCl gain as fast as possible, making it easy to control the NaCl concentration of the salted product, with a narrow variability among the salted products, and with the minimum environmental impact. In the case of the salting procedures, this is equivalent to the minimum waste volume generation.

14.1 GENERAL PROCEDURE FOR OBTAINING DRY-CURED HAM

Spanish dry-cured ham is one of the most traditional cured meat products in Spain. It is a nonsmoked meat product, manufactured according to ancient traditions.

The manufacturing process can vary slightly according to the tradition of each production area, but these three stages are fundamental:

- *Salting*. Hams are completely covered with a mixture of the curing ingredients (nitrate or nitrite and sugars) and salt, and maintained under refrigeration (at 1°C–3.3°C). The duration of this stage is usually determined by defining the days:kg ratio, typically from 0.7 for thawed hams to 1.5 for fresh hams (Toldrá 2002). Generally, the dry-curing is applied without any added water. Consequently, the curing agents are solubilized in the native liquid present in the meat and they mainly penetrate by diffusion. The dry-curing process is applied to pieces and minced meats (Flores 1997).
- *Postsalting*. Salt penetrates from the ham surface to the interior, allowing a more homogeneous concentration at the end of this stage. Salted hams are kept at low temperatures (approximately 3.3°C), to avoid microbial growth in the inner zones (Pérez-Álvarez et al. 1996), for approximately 50 days.

- *Dry maturation or ripening period*. This involves time–temperature combinations. The homogenized pieces are placed in natural or air-conditioned drying chambers where the relative humidity usually varies between 90% and 70% with a temperature range from 5°C to 26°C (Toldrá 2004). The ripening period varies according to the type of ham, from a minimum of 6 months (quick process) to a maximum of 36 months (slow process). Proteolysis and lipolysis take place during this stage. These reactions are responsible for the sensory characteristics of the final product.

14.2 USE OF VACUUM IMPREGNATION IN FOOD SALTING

14.2.1 Basic Principles of the Vacuum Impregnation

Vacuum impregnation (VI) can be applied to products submerged in a liquid, which would be brined for salting purposes (figure 14.1). The key point in using VI (Fito and Chiralt 1997; Fito et al. 1994) to increase the salting rate when working with porous products (e.g., cheese, meat) is the introduction of brine into the pores by the promotion of the hydrodynamic mechanism (HDM; Fito, Andrés, Chiralt, and Pardo 1996) using alternating pressures. VI can be applied either in a single cycle or a combination of several cycles. Each cycle consists of three steps. The first step begins when the vacuum is applied to the system and the pressure decreases to the equilibrium value (t_1). Once the equilibrium pressure is achieved, the system is maintained for a time (t_2), after which the atmospheric pressure is restored (t_3). During the vacuum period, the gas occluded in the porous structure of the food expands and partially flows out, allowing a more intense capillary penetration. In the third period, the restoring of the atmospheric pressure promotes the residual gas compression and the external brine penetration by an HDM (Deumier, Trystram, Collignan, Guédider, and Bohuon 2003; Fito et al. 1996). In addition to the promotion of the HDM, the changing pressures could imply the sample massaging and, thus, an additional reason for the increased mass transfer rate.
The process variables affecting the effectiveness of vacuum impregnation are explained next.

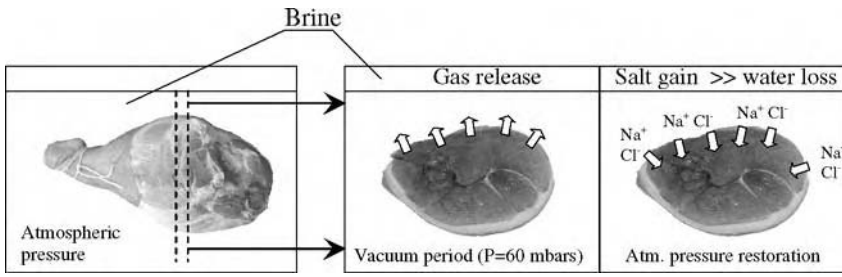


FIGURE 14.1 Scheme of the first and third step in ham brine vacuum impregnation.

14.2.1.1 Process Variables Affecting the Results Obtained in Brine Salting by Vacuum Impregnation

14.2.1.1.1 Influence of Compression Ratio: Mathematical Modeling

One of the mass transfer phenomena related to the compression ratio is the brine entrance by capillarity, which has been stated as influencing the salting process when working with porous foods such as cheese (Geurts, Walstra, and Mulder 1974). Capillary penetration in a pore occurs, coupled with the compression of the occluded internal gas. The volume fraction of the sample penetrated by the external liquid due to capillary forces (X_c) is a function of the capillary pressure (p_c), the pressure in system (p) and the effective porosity of the product ($\varepsilon_{\varepsilon_e}$) (Andújar, Argüelles, Barat, Andrés, and Fito 2001a), with greater capillary entrance the lower the p value.

$$X_c = \varepsilon_e\left(\frac{p_c}{p + p_c}\right) \tag{14.1}$$

When atmospheric pressure (p_2) is restored in the system, the external brine penetrates as a consequence of the HDM action. For rigid matrices, the total volume fraction of the sample impregnated by the external brine (X) can be determined by using Equation 14.2 (Fito. 1994), being dependent on the compression ratio r (Equation 14.3). By the analysis of Equation 14.2, it can be deduced that the greater the vacuum level applied in the first step, the greater the impregnation degree when the system is taken to atmospheric pressure.

$$X = \varepsilon_e\left(1 - \frac{1}{r}\right) \tag{14.2}$$

$$r = \frac{p_2}{p_1} + \frac{p_c}{p_1} \tag{14.3}$$

In viscoelastic matrices, pressure changes can promote sample deformations coupled with impregnation. A sample expansion can be observed when vacuum is applied, and the compression of the sample appears when the atmospheric pressure is restored (Fito et al. 1996). The HDM action is mainly dependent on the atmospheric pressure restoration step. The brine penetration and the sample shrinkage are coupled, so higher shrinkage would reduce the brine gain and lower shrinkage would increase the brine entrance (Chiralt et al. 1999).

Fito et al. (1996) proposed Equation 14.4 to describe the equilibrium for the HDM coupled with deformation–relaxation phenomena in VI operations of porous food.

$$\varepsilon_e = \frac{(X - \gamma)r + \gamma_1}{r - 1} \tag{14.4}$$

TABLE 14.1
Mass Transport Phenomena and Structural Changes Occurring Throughout Meat Brining at the Different Steps in the Vacuum Impregnation Processes

Process Step	Mass Transport Phenomena	Structural Changes
Immersion at atmospheric pressure before the vacuum pulse	Capillary penetration of brine. Salt and water diffusion in the product liquid phase near the sample surface.	Changes near the sample surface: conformational changes of proteins.
Application of vacuum pressure to the system	Gas and free internal liquid flow out. Advance of the salting front due to the more intense capillary effects. Development of salt-water concentration profiles due to diffusion phenomena coupled with brine penetration.	Expansion of the pores occupied by gas. Progression of conformational changes of biopolymers and changes in their water bonding capacity.
Restoring of the atmospheric pressure	Advance of brine penetration due to the hydrodynamic mechanism. Coupling with the diffusional transport of water and salt.	Volume reduction of matrix pores: expulsion of free liquid phase can occur. Progression of conformational changes of biopolymers and changes in their water bonding capacity.

Note: Adapted from Chiralt and Fito (1997).

The study of the compression ratio working with turkey meat fillets (Deumier, Bohuon, et al. 2003) showed a marked influence depending on the vacuum pressure applied to the system. The lower the vacuum pressure, the higher the salt uptake and the lower the water loss, even being positive for very low pressures. Those effects on the mass transfer implied higher processing yields when lower vacuum pressures were used.

Table 14.1 schematically shows the different phenomena occurring at the different steps in the brining process.

14.2.1.1.2 Influence of Brine Concentration

Apart from the well-known influence of brine concentration on the salting driving force (Andres, Rodríguez-Barona, Barat, and Fito 2002) and the water holding capacity of meat (Offer and Trinick 1983; Wilding et al. 1986), the brine concentration also affects the total NaCl gain as a consequence of the entrance by HDM. The overall NaCl mass fraction reached in the product liquid phase due to impregnation promoted by the vacuum pulse (z_{HDM}^{NaCl}), without considering the coupled diffusional gain, can be calculated by applying Equation 14.5. This equation takes into account the characteristic penetration and deformation volume ratios (X and γ), sample initial NaCl and water mass fractions (x_0^{NaCl}, x_0^{w}) and the brine density (ρ_b) and NaCl mass fraction

(y^{NaCl}). As deduced from Equation 14.5, the greater the y^{NaCl} value, the greater the process effectiveness in terms of salt uptake by impregnation (Fito and Chiralt 1997).

$$z_{HDM}^{NaCl} = \frac{M_0^0 x_0^{NaCl} + (1+\gamma) V_0^0 X \rho_b y^{NaCl}}{M_0^0 x_0^{NaCl} + M_0^0 x_0^{NaCl} (1+\gamma) V_0^0 X \rho_b} \tag{14.5}$$

14.2.1.1.3 Influence of the Number of Vacuum Pulses

Deumier, Bohuon, et al. (2003), working with turkey meat fillets, observed that the number of VI cycles also had a significant influence on water loss and salt gain. As the number of cycles increased, salt gain increased and water loss decreased. In a treatment with 10 vacuum pulses (VPs), the first VP was responsible for 71% of the water loss and 17% of the salt gain over a total of 10 cycles. Brine VI thus had a very early effect on water loss, but this effect decreased from cycle to cycle, whereas the VP effect on salt gain was slower and more gradual, with each cycle accounting for 7% to 17% of the salt gain.

14.2.1.1.4 Influence of the Length of Vacuum Period in the Brine Vacuum Impregnation Process

Kinetics of hydrodynamic gain of an external solution in the product pores when the atmospheric pressure is restored is very fast as compared with diffusional transport, and is only dependent on the pressure drop during the liquid flow determined by the liquid viscosity and pore diameter and tortuosity (Chiralt et al. 1999). When the impregnated pieces are larger and the pores are smaller, more time could be necessary to complete sample equilibration in mechanical terms because of difficulties in gas release. In the brine VI (BVI) process of meat, pores in the structure contain small gas phase volume entrapped in a free liquid phase, making the mechanical equilibration process in the vacuum period difficult. The length of this period could affect the impregnation level if no mechanical equilibrium is reached when restoring atmospheric pressure.

One of the meat products salted using VI is tasajo (Andújar et al. 2001a). Tasajo is a traditional Cuban intermediate-moisture product made from beef sheets that are soaked in brine, drained, rubbed in salt, and layered with salt in vats for several days, then washed and sun-dried. The production method is strongly artisan and the introduction of new technologies could be helpful to optimize the process. It was observed that the longer the vacuum time, the smaller the weight loss at a determined salting time, this being coherent with a greater impregnation level (Andújar et al. 2001a, 2001b). The parallel linear relationships between ΔM^0 and the square root of time working with meat fillets for 1 and 2 hours of vacuum application indicated that no notable differences in mass transfer rate behavior were induced by VP, except the amount of liquid impregnated by HDM, which was greater when vacuum was applied for 2 hours.

The influence of the cycle ratio (time under vacuum pressure/time at atmospheric pressure after the vacuum period) on the mass transfer was observed working with turkey meat (Deumier, Bohuon, et al. 2003). Water loss tended to decrease and salt

gain tended to increase as the cycle ratio increased. For high cyclic ratios, water loss was minimal and salt gain maximal, indicating that these cyclic ratios enhanced the pulse vacuum salting performance. When the stationary phases under vacuum and atmospheric pressures were eliminated, after 1 and 2 hours of processing, water loss and salt gain levels obtained with VI without a stationary phase were midway between those obtained under working at atmospheric pressures and by using the complete VI process described earlier.

14.2.1.1.5 Influence of Temperature

Temperature does not affect the effectiveness of VI with brine. Viscoelastic properties of solid matrix are influenced by temperature, and a softening of the structure is observed when temperature increases. Pressure changes in soft matrices will cause deformation more than impregnation effects, thereby decreasing VI effectiveness (Chiralt and Fito 1987; Chiralt et al. 2001).

14.2.1.1.6 Influence of Sample Microstructure

Porosity of the solid matrix is the most relevant structural property in VI effectiveness. In meat products, the muscle structure and vascular network are responsible for the VI response of muscle pieces during BVI. The gas phase is practically absent in the muscle structure. Nevertheless, some gas could remain in the vascular network as a consequence of animal slaughtering and quartering, acting as channels for HDM in VI operations. Nevertheless, the way in which muscle is impregnated by an external solution has not yet been clarified.

14.3 USE OF BRINE VACUUM IMPREGNATION IN HAM SALTING

The study of the use of BVI as an alternative to pile salting for accelerating the processing of dry-cured ham has been performed working with fresh and frozen hams as raw materials (Barat et al. 2004; Barat, Grau, Ibáñez, and Fito 2005; see figure 14.2).

FIGURE 14.2 Salting tank employed in the brine vacuum salting experiments.

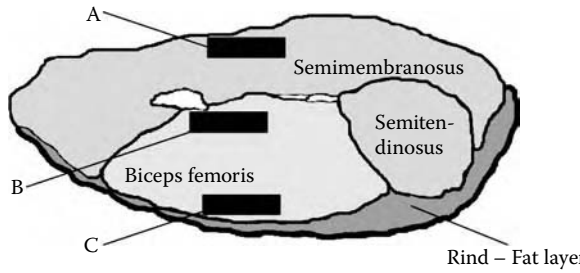


FIGURE 14.3 Samples analyzed from the widest ham section.

In addition, the effectiveness of the VI was determined by comparison with brine salting at atmospheric pressure. In all hams, the analyses were carried out using four samples; three of them were obtained from the widest section of the ham (A, B, and C points in figure 14.3) and the fourth from the whole homogenized ham muscles (R; Barat et al. 2004).

14.3.1 Use of Fresh Hams as Raw Material

An initial study was done by using the traditional salting method (pile salting) working with fresh hams to define the objective NaCl concentration in the ham muscle expressed in dry basis (X^{NaCl}) and the total weight loss (ΔM^{o}_{t}) after the salting step, expressed as in Equation 14.6 (Barat et al. 1998, 2001).

$$\Delta M^{\circ}_{t} = \frac{M^{\circ}_{t} - M^{\circ}_{0}}{M^{\circ}_{0}} \tag{14.6}$$

A total of 45 fresh hams with an average weight of 10.06 ± 0.75 kg with the pH within the range of 5.9 to 6.2 were used in the study. The obtained X^{NaCl} value for the fresh hams employed was 0.079 ± 0.0012 (w/w), which was defined as the value to be reached when using BVI for the other salting techniques, being the ΔM^{o}_{t} value –0.054 ± 0.006 (w/w).

The brine concentration used in the salting experiments was 24% (w/w) and the salting temperature was 3°C. Many combinations of time and VP length were employed to determine the effectiveness of VI in fresh ham salting (FBSV), with the vacuum pressure at 50 mbars. The vacuum period ranged from 1 to 216 hours, and the atmospheric period ranged from 4 to 48 hours. The maximum time employed in the brine atmospheric salting (FBS) was 360 hours, and the average time employed to characterize the traditional pile salting was 228 hours (Barat et al. 2001).

At the different salting times the whole hams were taken out and placed for 24 hours in a chamber where the temperature and relative humidity were controlled at 3°C and 85%, respectively, to avoid drying and to allow the porous matrix to absorb the penetrated brine before being analyzed.

The obtained X^{NaCl} plotted as a function of the square root of total salting time for all samples salted by the different procedures is shown in figure 14.2. For FBS

and FBSV experiments, a linear relationship between X^{NaCl} and $t^{0.5}$ was observed, coherent with the diffusion control of the salt uptake. A greater slope of the fitted straight line for BVI samples is observed due to an additional brine flow by HDM promoted by vacuum. However, short BVI treatments (about 24 hours) did not suppose significant differences in salt uptake as compared with BI treatments. This result is in accordance with that obtained by Deumier, Bohuon, et al. (2003) working with turkey meat. Those authors observed that the effect of the VP was higher for longer processing times, and no significant differences were observed between brine salting at atmospheric pressure and by using VI for short processing time. From 48 hours and onward of vacuum application the effects of the HDM appeared notable in salt gain.

The horizontal line drawn in figure 14.4 corresponds to the X^{NaCl} value obtained with pile salting, and the point defined by the cross of each of the adjusted lines with the horizontal line determines the time needed to reach the same X^{NaCl} value in the case of using pile salting.

Brining of ham implied a reduction in salting time as compared with the use of dry salt when working both at atmospheric and under vacuum pressure. The use of VP for a short time at the beginning of the process seemed to be less effective than continuous vacuum action throughout the brining. Therefore, capillary action promoted at low pressure seems to be the mechanism responsible for the faster salt gain. To obtain the usual salt content reached after industrial salting (X^{NaCl}~0.079), the required time in FBS and FBSV would be 6.9 and 3.7 days, according to the linear predictions, which implies a time reduction in salting time for FBS and FBSV of 27% and 61%, respectively, as compared to the traditional procedure.

The salt distribution in the wider section ham, just after salting, for the longer salting times can be seen in figure 14.5. The inner NaCl concentration in the ham liquid phase (z^{NaCl}) was higher for the FBSV salted hams even for shorter processing times (10 days for FBSV and 15 days for FBS). A deeper salt penetration occurs in

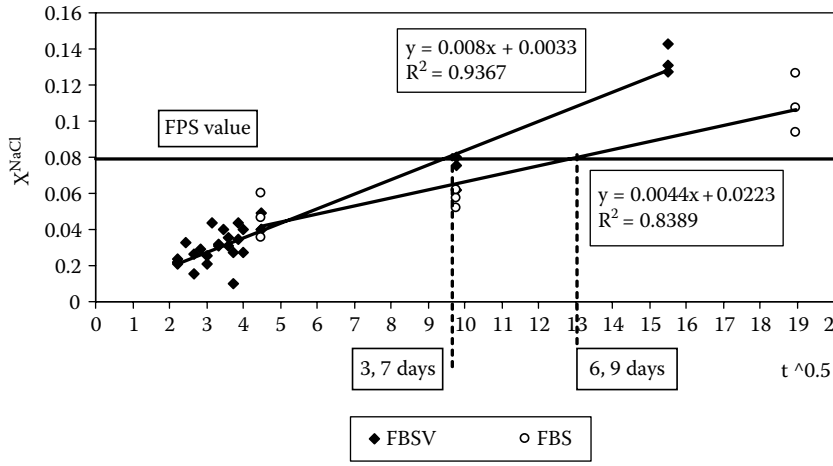


FIGURE 14.4 Salt concentration in dry basis (X^{NaCl}) obtained in the different treatments (FBSV, FBS, and FPS) for different salting times.

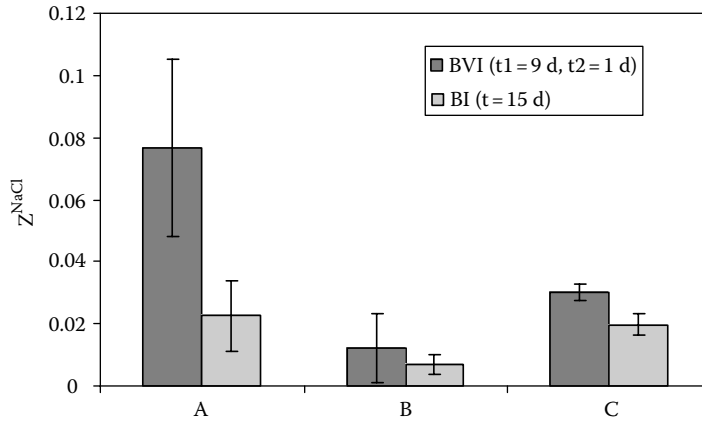


FIGURE 14.5 Salt distribution in the internal parts (A, B and C) of ham (Batch 1) immediately after salting at different times for BVI and BI treatments.

BVI, mainly in the zone around the bone joint, which is an important aspect concerning safety of product during curing.

Regarding the total weight loses, when both the dry salted and BVI hams had the target XNaCl concentration, the total weight loss for FPS hams was –0.054 ± 0.006 (w/w) and for the VI hams was –0.012 ± 0.009 (w/w). These results indicated a lower dehydration and a higher NaCl gain when using BVI, as observed by Deumier, Bohuon, et al. (2003) working with turkey meat.

14.3.2 Use of Frozen Hams as Raw Material

A similar study was performed with frozen hams. In this study, the use of brine salting with VI was used as an alternative to the traditional pile salting, as was simultaneous ham thawing and salting (Barat, Grau, Montero, Chiralt, and Fito 1997; Ngapo, Babare, and Mawson 1998), which could imply a significant decrease in processing time as compared to the traditional method.

The use frozen hams implies some changes in traditional Spanish cured ham manufacturing. One of the changes is the addition of a thawing step in a temperature-controlled chamber at 3°C for around 4 to 5 days (Ngapo et al. 1998). Another of the known modifications in the traditional process is the shortening of the salting step as a result of the faster salt uptake when working with thawed hams (Toldrá 2002). The introduction of a thawing step in a cold chamber implies an increase in processing costs compared to the traditional method, although the reduction in processing time contributes to reduced costs.

The time employed in the salting experiments working with the reference fresh raw material was calculated by using a ratio of 1.4 day/kg, which was within the range of those employed in industrial processes (1–1.5 days/kg; Toldrá 2002). In the case of the thawed hams, a ratio of 0.96 days/kg was employed, thus reducing the time/kg ratio according to recommendations from other authors (Bañon, Cayuela,

Granados, and Garrido 1999). These ratios lead to a total time of 13 days for the fresh hams and 9 days for the thawed hams.

The study consisted not only in the characterization of the time needed to reach the same NaCl concentration on a dry basis as in the traditional procedure (Barat et al. 2004), but also the characterization of the postsalting time needed when using a simultaneous brine thawing and salting process to obtain the same NaCl concentration in the liquid phase (z^{NaCl}) that is obtained in the critical point by the traditional manufacturing procedure (Barat et al. 2005).

A total of 63 fresh hams with an average weight of 9.4 ± 0.8 kg with the pH within the 5.7 to 6.3 range were used in this study. Some of them were processed fresh following the traditional procedure and the others were frozen for the comparative studies.

The VP consisted of submitting the system (the hams immersed in the saturated brine) to vacuum for 3 hours, and restoring the atmospheric pressure after that time, promoting the action of the HDM (Chiralt et al. 2001). All the salting experiments were carried out at 3°C.

The X^{NaCl} value obtained for the fresh hams, which would be defined as the target for the other salting techniques, was 0.067±0.014, and the total weight change was –0.082 ± 0.019 for fresh and thawed hams (including in the case of the thawed hams the dripping losses during thawing in a cold chamber at 3°C for 5 days).

Once the traditional salting process was characterized for the used raw material, and the required final X^{NaCl} value was defined, the thawing and salting experiments at atmospheric pressure and with a VP after the hams were thawed were performed for two salting times (3 and 5 days) to determine the time needed to reach the desired X^{NaCl} value (0.067).

The experimental data obtained in the study are shown in figure 14.6. It can be seen that the thawed and salted hams treated for a total of 5 days reached an X^{NaCl} value similar to the traditionally processed, whereas the thawed and salted hams treated with a VP, even for 3 days of processing, had a slightly higher X^{NaCl} concentration than the reference hams, which demonstrates a clear influence of the VP on the NaCl gain. This meant, approximately, a 79% and 64% reduction in the total salting time working with and without a VP, respectively (around 3 and 5 days for the simultaneous thawing and salting with and without a VP, and 5 and 9 days for the traditional method).

Total ham weight changes for the thawed dry salted, BTS, and BTS-AP hams when the target X^{NaCl} concentration was reached were –0.082 ± 0.019, 0.001 ± 0.002, and 0.035 ± 0.017 (w/w), respectively. A clear influence of the salting method can be seen, as the VI hams had a lower weight loss, which in this case is equivalent to having a lower water loss, in accordance with results obtained in other studies (Deumier, Bohuon, et al. 2003).

The influence of the moment at which the VP was applied throughout the process was studied for a total salting time of 5 days. Three combinations for the VP application were studied: VP application just at the beginning of the experiment (BTS-BP), once the most unfavorable part of the sample had reached 0°C (2.3 days of processing; BTS-AP), and two pulses, one at the beginning and the other after

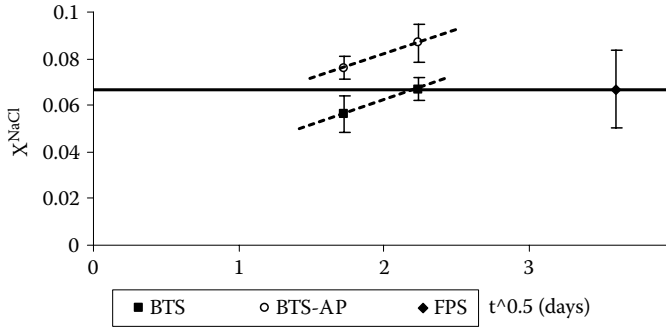


FIGURE 14.6 NaCl concentration on a dry basis (X^{NaCl} w/w) (with standard deviation bars) at the end of the brine thawing and salting experiments with (BTS-AP) and without (BTS) vacuum impregnation and fresh hams pile salting (FPS).

2.3 days of processing (BTS-BTP). The VP consisted of a vacuum period of 3 hours at 60 mbar pressure.

The X^{NaCl} concentrations for the three VP combinations, BTS-BP, BTS-AP, and BTS-BTP, were 0.0896 ± 0.017, 0.0866 ± 0.008, and 0.0815 ± 0.015 (w/w), respectively. All values were higher when compared to those hams not vacuum impregnated and treated for 5 days. There were no significant differences among all the treatments studied, although the use of the VP at the beginning of the treatment seemed to be the most effective, as it was the one that enabled higher X^{NaCl} contents for all points (A, B, C, and R). Nevertheless, the use of VP with thawed hams (2.3 days) seemed to be more interesting because of the release of the inner blood through the hole of the femoral vein during the vacuum period.

14.3.2.1 Postsalting Studies With Frozen Hams

The postsalting stage was carried out in a chamber with controlled temperature (3°C) and relative humidity (90%). The fresh pile salted hams were analyzed at the end of the salting period and after 50 days of postsalting conditions. In the case of the brine thawed and salted hams, three different postsalting periods and conditions were studied using three hams in each case (Barat et al. 2005).

The results obtained with the fresh hams allowed us to define the NaCl concentration in the inner zones of the ham (Points B and C of the widest ham section) that would allow increasing the chamber temperature in the following drying stage without microbial risk. The unit of the NaCl concentration that was used to determine the target value was the concentration referred to the meat liquid phase (z^{NaCl}), which can be estimated from the water and NaCl content determinations according to Equation 14.7 and considering that nearly all sodium and chloride ions and water were free in the ham.

$$z^{NaCl} = \frac{x^{NxCl}}{x^{NaCl} + x^{w}} \tag{14.7}$$

The use of the z^{NaCl} values is strongly recommended because it is closely related to the water activity values in the ham during the salting and postsalting periods (Barat et al. 2005).

The NaCl concentration of the ham liquid phase in the fresh pile salted hams at the A, B, and C points of the widest section were 0.043 ± 0.004, 0.0205 ± 0.002, and 0.0224 ± 0.001, respectively. It must be noted that the z^{NaCl} value at Point A was greater than at Points B and C, and the z^{NaCl} value at Point C was higher than for Point B. In addition, Point B is the riskiest area in the ham due to its location in the central ham section near the femoral vein. Considering that the fresh ham pile salting employing 1.3 days of salting for each initial kilogram and 50 days of postsalting (as in our study) is a reference processing method to obtain Spanish cured ham (Guerrero, Gou, Alonso, and Armau 1996), the postsalting stage can be considered finished when the z^{NaCl} value at Point B is around 0.02. The experimental results lead to the conclusion that for the brine thawed and salted hams, the postsalting stage could be reduced to half the time employed with fresh hams pile salted (from 50 to 25 days).

14.4 FUTURE STUDIES

As shown, BVI can be used to accelerate the salting and postsalting stages during dry-cured ham production. Nevertheless, the influence of BVI on the dry maturation stage and the sensory and microbiological characteristics of the final product must be clarified.

The lack of pressing during the brine salting experiments as compared with pile salting implied a change in the conformation of the salted hams. An increase in the characteristic dimension is expected, which could influence the following mass transfer processes (water outflow during drying). In fact, water diffusivity in the meat is 20 times lower than the NaCl diffusivity (Wood 1966). The other phenomenon that could affect the drying stage length would be the initial higher water content of the BVI hams due to the lower dehydration suffered during the salting stage.

NOMENCLATURE

γ	Relative sample volume deformation at the end of the VI process
γ_1	Relative sample volume deformation at the end of the first VI step
ρ_b	Density of brine
ε_e	Sample effective porosity
z_t^i	Mass fraction of component *i* in the food liquid phase at time *t* of the process
x_t^i	Mass fraction of component *i* in the sample at time *t*
$z_{t,HDM}^i$	Value of z_t^i reached in the sample after VI with the external solution
BTS	Brine thawing and salting at atmospheric pressure
BTS-AP	Brine thawing and salting using VI after thawing
BTS-BP	Brine thawing and salting using VI at the beginning of the thawing and salting experiment

BTS-BTP	Brine thawing and salting using VI at the beginning and after thawing
BVI	Brine vacuum impregnation
FBS	Fresh hams brine salting at atmospheric pressure
FBSV	Fresh hams brine salting with VI
FPS	Fresh hams pile salting
HDM	Hydrodynamic mechanism
M^o_0	Sample initial mass
M^o_t	Sample mass at time *t*
p	Pressure in the system
p_1	Vacuum pressure in the first VI process step
p_2	Atmospheric pressure in the second VI process step
p_c	Capillary pressure
r	Compression ratio in the VI process
TPS	Thawed hams pile salting
V^o_0	Sample initial volume
VI	Vacuum impregnation
VP	Vacuum pulse
X	Sample volume fraction impregnated by the solution at the end of the VI process
X_c	Sample volume fraction penetrated by the external liquid due to capillary forces
y^i	Mass fraction of the component *i* in the impregnating solution (brine)

ACKNOWLEDGMENT

We thank the Comisión Interministerial de Ciencia y Tecnología (Spain) for its financial support in projects PETR1995-0014-OP and PETR1995-0403-OP-02-01.

REFERENCES

Andres, A., S. Rodríguez-Barona, J. M. Barat, and P. Fito. 2002. Mass transfer kinetics during cod salting operation. *Food Sci. Technol. Int.* 8:309–314.

Andújar, G., A. Argüelles, J. M. Barat, A. Andrés, and P. Fito. 2001a. Salting studies during Tasajo making. In *Osmotic dehydration and vacuum impregnation: Applications in food industries,* eds. P. Fito, A. Chiralt, J. M. Barat, D. Beshnilian, and W. Spiess, 171–183. Lancaster, PA: Technomic Publishing.

Andújar, G., A. Argüelles, J. M. Barat, A. Andrés, and P. Fito. 2001b. Tasajo salting by brine immersion: Influence of vacuum impregnation. In *8th international conference on engineering and food,* eds. J. Welti-Chanes, G. V. Barbosa-Cánovas, and J. M. Aguilera, 1319–1323. Lancaster, PA: Technomic Publishing.

Aristoy, M. C., and F. Toldrá. 1995. Isolation of flavor peptide from raw pork meat and dry cured ham. In *Food flavors: Generation, analysis and process influence,* ed. G. Charalambous, 1323–1344. Amsterdam: Elsevier Science.

Bañon, S., J. M. Cayuela, M. V. Granados, and M. D. Garrido. 1999. Pre-cure freezing affects proteolysis in dry-cured hams. *Meat Sci.* 51:11–16.

Barat, J. M., R. Grau, J. B. Ibáñez, and P. Fito. 2005. Post-salting studies in Spanish cured ham manufacturing: Time reduction by using brine thawing-salting. *Meat Sci.* 69:201–208.

Barat, J. M., R. Grau, A. Montero, A. Chiralt, and P. Fito. 2001. Salting time reduction of Spanish hams by brine salting. In *Osmotic dehydration and vacuum impregnation: Applications in food industries,* eds. P. Fito, A. Chiralt, J. M. Barat, D. Beshnilian, and W. Spiess, 155–169. Lancaster, PA: Technomic Publishing.

Barat, J. M., R. Grau, A. Montero, A. Chiralt, and P. Fito. 1997. Procedimiento de descongelación y salado simultaneo de piezas de carne o pescado. Spanish patent P9701702.

Barat, J. M., R. Grau, A. Montero, A. Chiralt, and P. Fito. 1998. Feasibility of brining of ham for curing. In *Meat consumption and culture: Congress proceedings, 44th International Congress of Meat Science and Technology,* eds. A. Driesde and J. M. Monfort, 970–971. Madrid: Estrategias Alimentarias S.L.-EUROCARNE.

Barat, J. M., R. Grau, M. J. Pagán-Moreno, and P. Fito. 2004. Simultaneous brine thawing-salting operation during spanish cured ham manufacturing. *Meat Sci.* 66:603–608.

Barat, J. M., S. Rodríguez-Barona, A. Andrés, and P. Fito. 2002. Influence of increasing brine concentration in the cod salting process. *J. Food Sci.* 65:1922–1925.

Belletti, M., G. Dazzi, R. Chizzolini, F. Palmia, and G. Parolai. 1983. Physical and chemical changes occurring in proteins during the maturation of Parma ham: I. Biochemical and functional changes. *Ind. Conserve.* 58:143–146.

Chiralt, A., and P. Fito. 1997. Salting of manchego-type cheese by vacuum impregnation. In *Food engineering 2000,* eds. P. Fito, E. Ortega, and G. Barbosa, 215–230. New York: Chapman & Hall.

Chiralt, A., P. Fito, A. Andrés, J. M. Barat, J. Martínez-Monzó, and N. Martínez-Navarrete. 1999. Vacuum impregnation: A tool in minimally processing of foods. In *Processing of foods: Quality optimization and process assessment,* eds. F. A. R. Oliveira and J. C. Oliveira, 341–356. Boca Raton, FL: CRC Press.

Chiralt, A., P. Fito, M. J. Barat, A. Andrés, C. González-Martínez, I. Escriche, and M. M. Camacho. 2001. Use of vacuum impregnation in food salting process. *J. Food Eng.* 49:141–151.

Deumier, F., P. Bohuon, G. Trystram, N. Saber, and A. Collignan. 2003. Pulsed vacuum brining of poultry meat: Experimental study on the impact of vacuum cycles on mass transfer. *J. Food Eng.* 58:75–83.

Deumier, F., G. Trystram, A. Collignan, L. Guédider, and P. Bohuon. 2003. Pulsed vacuum brining of poultry meat: Interpretation of mass transfer mechanisms. *J. Food Eng.* 58:85–93.

Doe, P. E. 1998. Fish drying and smoking. Lancaster, PA: Technomic Publishing.

FAO. 1981. The preservation of losses in cured fish (Rep. No. 279). FAO Fish.

Fito, P. 1994. Modelling of vacuum osmotic dehydration of foods. In *Water in foods: Fundamental aspects and their significance in relation to processing of foods,* eds. P. Fito, A. Mulet, and B. Mckenna, 313–328. London: Elsevier Applied Science.

Fito, P., A. Andrés, A. Chiralt, and P. Pardo. 1996. Coupling of hydrodynamic mechanism and deformation-relaxation phenomena during vacuum treatments in solids porous food-liquid systems. *J. Food Eng.* 27:229–240.

Fito, P., and A. Chiralt. 1997. An approach to the modelling of solid food-liquid operations: Application to osmotic dehydration. In *Food engineering 2000,* eds. P. Fito, E. Ortega, and G. Barbosa, 231–252. New York: Chapman & Hall.

Fito, P., A. Chiralt, J. Serra, M. Mata, R. Pastor, A. Andrés, and X. Q. Shi. 1994. An alternating flow procedure to improve liquid exchanges in food products and equipment for carrying out said procedure. Spanish patent 94500071.9; European patent 0,625,314,A2.

Flores, J. 1997. Mediterranean vs. northern European meat products: Processing technologies and main differences. *Food Chem.* 59:505–510.

Geurts, T. J., P. Walstra, P., and H. Mulder. 1974. Transport of salt and water during salting of cheese: 1. Analysis of the processes involved. *Netherl. Milk Dairy J.* 28:102–129.

Ghavimi, B., R. W. Rogers, T. G. Althen, and G. R. Ammerman. 1986. Effects of non-vacuum, vacuum and nitrogen back-flush tumbling on various characteristics of restructured cured beef. *J. Food Sci.* 51:1166–1168.

Guerrero, L., P. Gou, P. Alonso, and J. Armau. 1996. Study of the physicochemical and sensorial characteristics of dry-cured hams in three pig genetic types. *J. Sci. Food Agr.* 70:526–530.

Hansen-Moller, J., L. Hinrichsen, and T. Jacobsen. 1997. Evaluation of peptides generated in Italian-style dry-cured ham processing. *J. Agr. Food Chem.* 45:3123–3128.

Ismail, N., and M. Wooton. 1992. Fish salting and drying: A review. *ASEAN J.* 7:175–183.

MacCain, G. R., T. N. Blumer, H. B. Craig, and R. G. Steel. 1968. Free amino acids in ham muscle during successive aging periods and their relation to flavor. *J. Food Sci.* 33:142–148.

Marriott, N. G., P. P. Graham, J. W. Boling, and W. F. Collins. Vacuum tumbling of dry-cured hams. *J. Animal Sci.* 58:1376–1381.

Messens, W., J. Van Camp, and A. Huyghebart. 1997. The use of high pressure to modify the functionality of food proteins. *Trends Food Sci. Technol.* 8:107–112.

Ngapo, T. M., I. H. Babare, and R. F. Mawson. 1998. Simultaneous thawing and curing of whole porcine muscle. In *Meat consumption and culture: Congress proceedings 44th International Congress of Meat Science and Technology,* eds. A. Driesde and J. M. Monfort, 406–407. Madrid: Estrategias Alimentarias S.L.-EUROCARNE.

Offer, G., and J. Trinick. 1983. On the mechanism of water holding in meat: The swelling and shrinking of myofibrils. *Meat Sci.* 8:245–281.

Orr, A. T. 1967. Method and product for curing and packaging fish. British Patent 1,077,644.

Pérez-Álvarez, J. A., J. Fernández-López, M. A. Gago-Gago, M. J. Pagan-Moreno, C. Ruiz-Peludo, M. R. Rosmini, F. López-Santobeña, and V. Aranda-Catalá. 1996. Properties of dry-cured ham: Temperature and pH influence during salting stage. *J. Muscle Food.* 88:315–328.

Sanchez, E. S., S. Simal, A. Femenia, J. Benedito, and C. Rosello. 1999. Influence of ultrasounds on mass transport during cheese brining. *Eur. Food Res. Technol.* 209:215–219.

Sárraga, C., M. Gil, J. Arnau, J. M. Monfort, and R. Cussó. 1989. Effect of curing salt and phosphate on the activity of porcine muscle proteases. *Meat Sci.* 25:241–249.

Toldrá, F. 2002. *Dry-cured meat products*. Trumbull, CT: Food & Nutrition Press.

Toldrá, F. 2004. Curing: Dry. In *Encyclopedia of meat sciences,* eds. W. Jensen, C. Devine, and M. Dikemann, 360–365. London: Elsevier Science.

Toldrá, F., E. Rico, and J. Flores. 1993. Cathepsin B, D, H and L activities in the processing of dry-cured ham. *J. Sci. Food Agr.* 62:157–161.

Townsend, W. E., and D. G. Olson. 1987. Cured meats and cured meat products processing. In *The science of meat and meat products,* eds. J. F. Price and B. S. Schweigert, 431–456. Westport, CT: Food and Nutrition Press.

Varnam, A. H., and J. P. Sutherland. 1995. *Meat and meat products*. London: Chapman & Hall.

Wilding, P., N. Hedges, and P. J. Lillford. 1986. Salt-induced swelling of meat: The effect of storage time, pH, ion-type and concentration. *Meat Sci.* 18:55–75.

Wood, F. W. 1966. The diffusion of salt in pork muscle and fat tissue. *J. Sci. Fd. Agric.* 17:138–140.

15 The Use of Bacteriocins Against Meat-Borne Pathogens

Teresa Aymerich, Margarita Garriga, Anna Jofré, Belén Martín, and Joseph M. Monfort
Institute for Food Research and Technology,
Meat Technology Center

CONTENTS

15.1 Characteristics of the Most Suitable Bacteriocins for Meat and Meat Products 373
15.2 Application of Bacteriocins and/or Bacteriocinogenic Cultures to Improve the Microbial Safety of Meat Products 374
15.2.1 Raw Meat Products 374
15.2.2 Cooked and Cured-Cooked Meat Products 375
15.2.3 Fermented Sausages 383
15.3 Active Packaging: A Promising New Antimicrobial Technology Approach 384
15.4 Bacteriocins and New Preservation Technologies to Be Applied to Meat Products 386
15.5 Considerations About the Application of Bacteriocins or Their Producers in Foods 387
15.5.1 Interaction of Bacteriocins and Bacteriocinogenic Strains Through Meat Compounds or Additives 387
15.5.2 Bacteriocin-Resistant Bacteria 389
15.5.3 Commercial Availability and Industrial Applications 389
15.5.4 Regulation Aspects 390
References 391

Meat is a rich nutrient matrix that provides a suitable environment for proliferation of meat spoilage micro-organisms and common food-borne pathogens. Food safety is a top priority for the European Union, as indicated in the White Paper on Food Safety (Commission of European Communities 2002). It is controlled by Regulation (EC) No. 178/2002, modified by Regulation (EC) No. 1642/2003 (European Council

2002, 2003). Modern technologies and safety concepts, like hazard analysis and critical control point, food safety objective, and the appropriate level of protection (Stringer 2004) have been introduced to improve food safety. Nevertheless, the prevalence of food-borne pathogens and the reported number of food-borne zoonoses and intoxications is still increasing and the frequency of reported outbreaks has also grown (Center for Science in the Public Interest 2004), in part due to the success of new detection technologies and better epidemiological surveillance programs.

The major microbial hazards of meat products are *Salmonella*, *Listeria monocytogenes*, enterohemorragic *Escherichia coli, Campylobacter,* and *Staphylococcus aureus*. There are some other minor microbial hazards that have to be taken into account like *Yersinia enterocolitica*, *Clostridium*, bacteria-forming biogenic amines, and also molds producing mycotoxins. Considering the data available in the reports of the U.S. Centers for Disease Control and Prevention (CDC) and the FoodNet surveillance database during 1983 and 1997, salmonellosis and listeriosis represent 31% and 28% of total food-related deaths, respectively, whereas *Campylobacter* and *E. coli* represent 5% and 4.3%, and *Staphylococcus aureus* only 0.8% (Mead et al. 1999).

L. monocytogenes is a pathogen that causes a severe illness, listeriosis. The disease primarily affects the population most at risk: pregnant women, newborns, and adults with weakened immune systems. Although its infective dose is not known because it varies depending on the strain and susceptibility of the individual, the International Commission on Microbiological Specification for Foods (1996) concluded that 100 CFU/g of *L. monocytogenes* in food at the time of consumption is not problematic for people not at risk. *L. monocytogenes* is widely distributed in nature (Kathariou 2002; Vázquez-Boland, Domínguez-Bernal, González-Zorn, Kreft, and Goebel 2001) and meat products are a major source (Ojeniyi, Wegener, Jensen, and Bisgaard 1996; Peccio, Autio, Korkeala, Rosmini, and Trevisani 2003; Samelis and Metaxopoulos 1999; Wing and Gregory 2002). In Denmark, the regulatory policy on *L. monocytogenes* in ready-to-eat products requires zero tolerance in food that supports growth, although the presence of *L. monocytogenes* is accepted as long as the level does not exceed 100 CFU/g (Norrung, Andersen, and Schlundt 1999). The United States has the strictest regulation, demanding zero tolerance. Accordingly, companies stipulate absence of *L. monocytogenes* in their products.

Salmonella is more frequent than *Listeria*, but salmonellosis is not as severe as listeriosis. It is responsible for 25.6% of food-borne illness-related hospitalizations (Mead et al. 1999). According to European Union directive 94/65/EC for minced meat and meat preparations (Eurpoean Council 1994), *Salmonella* spp. must be absent in 10 g. Salmonellosis causes a high incidence of food poisoning worldwide due to the consumption of contaminated food products of animal origin (Thorns 2000). In England and Wales, 43 of the 70 *Salmonella* outbreaks were attributed to meat and meat products (Cowden et al. 1995).

It is clear that traditional methods of preservation will not preclude microbial hazard. Moreover, consumers demand more natural or mild processed products, stable and safe, with a longer shelf life, and without chemical preservatives. So, alternative control measures like biopreservation (e.g., bacteriocinogenic lactic acid bacteria cultures, their bacteriocins, or both), reducing storage temperature to lower

than 2°C, and new postprocessing technologies (high hydrostatic pressure, electromagnetic waves, irradiation, ohmic heating) have received increasing attention.

Lactic acid bacteria (LAB) have a long history of safe use in foods as indigenous natural microflora of meat, milk, vegetable, and fish products. They can produce several antimicrobial compounds, including organic acids, carbon dioxide, hydrogen peroxide, diacetyl, ethanol, and bacteriocins that may play an important role in food preservation to combat microbial contamination of foodstuffs as an alternative to chemical preservative agents. Among these natural antimicrobials, bacteriocins, which are low-molecular-mass peptides or proteins with an antibacterial mode of action against closely related bacteria, have been reported to increase the competitiveness of the producer strain in the food matrix (Chen and Hoover 2003; Cleveland, Montville, Nes, and Chikindas 2001) and contribute to the prevention of food spoilage and pathogenic bacteria such as *L. monocytogenes*. Several studies have demonstrated the use of LAB and bacteriocins to minimize the risk of outgrowth of *L. monocytogenes* in different types of meats and meat-related products discussed in this chapter.

15.1 CHARACTERISTICS OF THE MOST SUITABLE BACTERIOCINS FOR MEAT AND MEAT PRODUCTS

Bacteriocins produced by LAB are, in general, cationic, hydrophobic, or amphiphilic molecules composed of 20 to 60 amino acid residues (Nes and Holo 2000). They are classified into three groups (Klaenhammer 1993; Nes, Diep, Håvarstein, and Brurberg 1996). The bacteriocins of interest to the meat industry mainly belong to Class IIA except for nisin, which belongs to Class IA. Class IA bacteriocins are small, cationic, elongated lantibiotic peptides (<5 KDa) containing the unusual amino acids lanthionine (Lan), α-methyllanthionine (MeLan), dehydroalanine, and dehydrobutyrine. It is represented by nisin from *Lactococcus lactis* (Hurst 1981). Class IIA bacteriocins are small (<10 KDa), heat-stable, cationic, nonlantibiotic peptides being the reference bacteriocin pediocin PA-1, hence the group is named pediocin-like. They are one-peptide bacteriocins with a double-glycine leader peptide and a dedicated secretion and processing machinery. This subgroup has attracted much of the interest due to their inhibition capacity against *Listeria* (Chen and Hoover 2003; Ennahar, Sashihara, Sonomoto, and Ishizaki 2000). The activity of bacteriocins is exerted through the formation of pores in the bacterial membrane and the anionic lipids of the cytoplasmic membrane are their primary receptors (Abee, Krockel, and Hill 1995; Moll, Konings, and Driessen 1999). Conductivity and stability of pores induced by lantibiotics may be heightened by docking to molecules such as lipid II (the peptidoglycan precursor), whereas in the case of Class II bacteriocins, receptors in the target membrane apparently act to determine specificity (Venema, Venema, and Kok 1995). Class I bacteriocins may induce pore formation according to a wedge-like model, and Class II bacteriocins may function by creating barrel stave-like pores or carpet mechanisms whereby peptides orient parallel to the membrane surface and interfere with mem-

brane structure (Moll et al. 1999). Class I bacteriocins have a broader inhibitory spectrum when compared to Class II.

15.2 APPLICATION OF BACTERIOCINS AND/OR BACTERIOCINOGENIC CULTURES TO IMPROVE THE MICROBIAL SAFETY OF MEAT PRODUCTS

Bacteriocinogenic starter cultures and their bacteriocins can act as extra hurdles to food pathogens, thus enhancing preservation and wholesomeness of meat and meat products. They can be included in the meat batter, sprayed onto the surface, or added through active packaging, depending on the type of meat product to be applied. When they are applied as starter cultures, adjuncts of fermentation, or bioprotective cultures, their success depends on the ability of the culture to grow and to produce the antibacterial factors in the food under the technological and physicochemical environment (temperature, pH, ingredients, additives, water activity, etc.). Moreover, in fresh and fermented meat, the applied bacteria must be able to compete with the endogenous microflora. This approach offers an indirect way to add bacteriocins to a meat product and seems more acceptable for producers and consumers in foods like fermented sausages and vacuum-packed meat with a relatively short shelf life, where high initial inoculum numbers (10^6–10^7 CFU/g) would not affect sensorial quality. When the bioprotective starter culture is not competitive enough or not able to produce the antimicrobial substances, or may affect the sensorial quality, bacteriocins may be applied as fermentation liquor or as purified antagonistic substances. In the latter case, the bacteriocin dosage is more precise and predictable but its application is limited by national regulations on food additives.

Nisin, pediocin, plantaricin, sakacins, enterocins, and leucocins and their producer strains have been used alone or in combination with other hurdles to control *Listeria* outgrowth in meat and meat products. Table 15.1 and table 15.2 summarize the bacteriocins and the bioprotective cultures used until now in the biopreservation of meat products.

15.2.1 Raw Meat Products

Raw meat products are prone to the growth of meat spoilage micro-organims and common food-borne pathogenic bacteria such as *Salmonella, S. aureus*, and *L. monocytogenes*. Vacuum and modified atmosphere packaging (MAP) with several CO_2 and N_2 rates is the most widely used packaging system to increase shelf life and safety of meat products. The chilled storage in a modified atmosphere has an effect on the meat microflora, triggering a change from Gram-negative bacilli to LAB. Thus LAB strains may be good candidates for application as bioprotective cultures on raw meat products, reducing the risk of growth and survival of food-borne pathogenic bacteria (Holzapfel, Geisen, and Schillinger 1995). Inoculum size and selected strains must be balanced to achieve the desired antimicrobial effect while not affecting the sensorial properties of the tested food (Kröckel 1997).

Schillinger, Kaya, and Lücke (1991) reported the bioprotective effect of *Lb. sakei* Lb 706 inoculated in pasteurized minced meat. A decrease in the *L. monocytogenes* counts by 1 log cycle was observed when compared to an isogenic nonbacteriocin strain. Differences in *L. monocytogenes* growth in German-type fresh Mettwurst inoculated with *Lb. sakei* Lb706 (sakacin A producer) were observed depending on the pH of the pork meat used. The same strain at low dose (3 log CFU/g) was not capable of inhibiting *Listeria* on chilled, vacuum-packaged, raw beef (Buncic, Avery, and Moorhead 1997).

Lb. sakei CTC494 (1×10^6 CFU/g) or sakacin K (800 AU/g) inhibited the growth of *Listeria* inoculated at 2×10^2 CFU/g to different extents in raw minced pork and chicken breast stored at 7°C (Hugas, Pagés, Garriga, and Monfort 1998; see figures 15.1 and 15.2). The greatest inhibition was observed in the vacuum-packaged samples of poultry and in the MAP samples of raw minced pork. The addition of sakacin K resulted in an immediate bactericidal action against *L. innocua* in every product and atmosphere studied. Other authors observed enhanced inhibition of *L. monocytogenes* when *Lb. sakei* 2a was used in combination with MAP of Brazilian sausages (Liserre, Landgraf, Destro, and Franco 2002).

In raw minced pork meat inoculated with a high level of a three-strain cocktail of *L. monocytogenes* (10^5 CFU/g), the most effective treatment in limiting the outgrowth of the pathogen was *Lb. sakei* CTC494 plus 800 AU/g of sakacin K (2.37 log CFU/g decrease). The treatments containing 800 AU/g sakacin K and 800 AU/g of enterocins A and B showed 1.25 log CFU/g decrease of the pathogen, whereas *E. faecium* CTC492 plus enterocins recorded 1 log CFU/g decrease (unpublished results).

Nisin has been used in several applications, sprayed to sanitize the surface of red meat carcasses (Cutter and Siragusa 1994), to decontaminate artificially contaminated pieces of raw pork (Murray and Richard 1997), and combined with 2% sodium lactate to control *S. aureus* MMPR3 and *Salmonella* Kentucky AT1 in fresh pork sausages (Scannell, Hill, Buckley, and Arendt 1997; Schlyter, Glass, Loeffelholz, Degnan, and Luchansky 1993). The combined effect of sodium diacetate (0.3% and 0.5%) and pediocin (5,000 AU/ml) against *L. monocytogenes* in turkey slurries was reported by Schlyter et al. (1993). In batches with pediocin (5,000 AU/ml) and 0.5% diacetate at 25°C or with pediocin and 0.3% diacetate at 4°C, counts of *L. monocytogenes* were approximately 7 log CFU/ml lower when compared to the control batch. The antilisterial activity of nisin in minced raw buffalo meat at concentrations of 400 and 800 IU/g and in combination with 2% sodium chloride was reported by Pawar, Malik, Bhilegaonkar, and Barbuddhe (2000). Batches with nisin at 800 IU/g achieved a 2.4 log cycle reduction when compared to the control batch on Day 16. The use of 2% of sodium chloride increased the bacteriocin effect.

15.2.2 Cooked and Cured-Cooked Meat Products

Cooked meat products offer low protection against the growth of pathogenic bacteria during postprocessing (slicing and packaging). The low salt content (>2%), a pH of around 6.0, a water activity higher than 0.95, the small amount of residual nitrite, and the lack of endogenous microbiota are only small obstacles to inhibit the usual

TABLE 15.1
Bacteriocins That Have Been Successfully Applied to Biopreservation of Meat Products

Bacteriocin	Producer	Dosage	Application Method	Product	References
Sakacin A/K	*Lb. sakei* CTC494	400–800 AU/g	Meat batter	Fermented sausages	Hugas, Garriga, Aymerich, and Monfort (1995)
		400 AU/g	Meat surface/batter	Cooked meat products, poultry breast, minced pork meat	Hugas, Pagés, Garriga, and Monfort (1998)
		800 AU/g	Meat batter/surface	Frankfurter sausages	Hugas, Garriga, Aymerich, and Monfort (2002)
		17 AU/g	Cellulose casings		
Sakacin P	*Lb. sakei* LB790 (pMLS114)	12 ng/g, 3.5 μg/g	Meat surface	Chicken cold cuts	Katla et al. (2002)
Enterocin CCM4231	*E. faecium* CCM4231	12,800 AU/g	Meat batter	Dry fermented hornád	Lauková, Czikková, Laczková, and Turek (1999)
Enterocin 416K1	*E. casseliflavus* IM416K1	10 AU/g	Meat batter	Italian sausage "cacciatore"	Sabia, de Niederhäusern, Messi, Manicardi, and Bondi (2003)
Enterocin A & B	*E. faecium* CTC492	1,600–4,800 AU/g	Meat surface	Cooked ham	Aymerich et al. (2000)
		3,200–4,800 AU/g	Meat surface	Chicken breasts	
		1,600–3,200–4,800 AU/g	Meat batter	Minced pork meat	
		1,600–4,800 AU/g	Meat batter	Pâté	
		221–648 AU/g		Fermented sausages	
		3,500 AU/g	Meat batter	Frankfurter sausages	Hugas, Garriga, Aymerich, and Monfort (2002)
		1,750 AU/g	Meat surface		
		73 AU/g	Cellulose casings		
Nisin	*Lc. lactis* ssp *lactis*	16 AU/g	Cellulose casings	Frankfurter sausages	Hugas, Garriga, Aymerich, and Monfort (2002)
		800 AU/g	Meat batter		
		400 AU/g	Meat surface		
		10,000 IU/ml	Immersed	Cooked tenderloin pork	Fang and Lin (1994a)
		1,000 and 10,000 IU/ml	Immersed	Tenderloin pork meat	Fang and Lin (1994b)

		400 and 800 IU/g	Meat batter	Minced raw buffalo	Pawar, Malik, Bhilegaonkar, and Barbuddhe (2000)
		5,000 AU/ml	Meat surface	Red meat carcasses	Cutter & Siragusa (1994)
		2,560 AU/cm^2	Immobilized onto cellulose-based bioactive inserts	Sliced cooked ham	Scannell et al. (2000)
		100 μg/ml	Packaging films treated with nisin	Fresh boiler drumstick skin	Natrajan and Sheldon (2000a)*
		500 μg/ml	Agar/polysaccharide-based films	Poultry skin	Natrajan and Sheldon (2000b)*
		7,500–10,000 IU/ml	Coated-barrier films	Hot dogs	Franklin, Cooksey, and Getty (2004)
Lactocin 705	*Lb. casei* CRL 705	4,800–8,400 and 16,800 AU/ml	Meat batter	Minced beef	Vignolo, Fadda, de Kairuz, de Ruiz Holgado, and Oliver (1996)
Lacticin 3147	*Lc. lactis* ssp. *lactis* DPC3147	2,560 AU/cm^2	Immobilized onto cellulose-based bioactive inserts	Sliced cooked ham	Scannell et al. (2000)
Leucocin 4010	*Leuc. carnosum* 4010	20 ml/kg	Meat surface	Sliced pork saveloys	Jacobsen, Budde, and Koch (2003)
Pediocin PA-1	*Pd. acidilactici*	10 AU/g	Cellulose casings	Frankfurter sausages	Hugas, Garriga, Aymerich, and Monfort (2002)
		800 AU/g	Meat batter		
		400 AU/g	Meat surface		
Pediocin	*Pd. acidilactici*	6 and 5 AU/cm^2	Coated onto cellulose casings and plastic bags	Ham and beef	Ming, Weber, Ayres, and Sandine (1997)
Pediocin AcH	*Lb. plantarum* WHE 92	—	Meat surface	Sliced cooked sausages	Mattila, Saris, and Työppönen (2003)
		—	Meat batter	Minced raw pork	Murria and Richard (1997)
Reuterin	*Lb. reuteri*	—	Immersed	Beef sausage	Kuleasan and Cakmakci (2002)
Plantaricin UG1	*Lb. plantarum* UG1	22,880 AU/ml	Mixing in stomacher	Minced beef meat	Enan, Alalyan, Abdel-salam, and Debevere (2002)
Bacteriocin 32Y	*Lb. curvatus* 32Y	6,400 AU/ml	Soaking, coating, and sprayed	Hamburgers	Mauriello, Ercolini, La Storia, Casaburi, and Villani (2004)

Note: All the in situ assays were performed against *Listeria* unless indicated by * where the target strain was *Salmonella typhymurium*.

TABLE 15.2
Bacteriocinogenic Cultures That Have Been Successfully Applied to Biopreservation of Meat Products

Bacteriocinogenic Starter Culture	Bacteriocin	Inocula (log CFU/g)	Product	References
Lb. bavaricus MN	Bavaricin MN	3–5	Heat-treated beef cubes	Winkowski, Crandall, and Montville (1993)
Lb. sakei Lb790 (pMLS114)	Sakacin P	4	Chicken cold cuts	Katla et al. (2002)
Lb. sakei Lb706	Sakacin A	6	Cured raw pork	Schillinger, Kaya, and Lücke (1991)
		6	Beef minced meat	Schillinger, Kaya, and Lücke (1991)
		6	Fermented sausages	Hugas, Neumeyer, Pagés, Garriga, and Hammes (1997)
Lb. sakei CTC494	Sakacin K	6	Cooked meat products, minced pork meat, poultry breast	Hugas, Pagés, Garriga, and Monfort (1998)
		6	Fermented sausages	Hugas, Garriga, Aymerich, and Monfort (1995); Hugas, Neumeyer, Pagés, Garriga, and Hammes (1997)
Lb sakei 2a	—	5	Brazilian sausages	Liserre, Landgraf, Destro, and Franco (2002)
Lb. sakei Lb674	Sakacin P	5–6	Vacuum-packaged sliced bologna-type sausage	Kröckel (1997)
Lb. curvatus LBPE	—	5g/kg lyophilized culture	Dry fermented sausages	Benkerroum et al. (2005)
Lb. curvatus LTH1174	Curvacin A	6	Fermented sausages	Hugas, Neumeyer, Pagés, Garriga, and Hammes (1997)
Lb. plantarum MCS	—	7	Salami	Campanini, Pedrazzoni, Barbuti, and Baldini (1993)
Lb. plantarum UG1	Plantaricin UG1	7	Beef meat	Enan, Alalyan, Abdel-salam, and Debevere (2002)
Leuc. carnosum 4010	Leucocin A and Leucocin C	6	Pork saveloys	Jacobsen, Budde, and Koch (2003)
		5.08 and 6.78	Cooked, vacuum-packaged, and sliced pork meat sausages	Budde, Hornbaek, Jacobsen, Barkholt, and Koch (2003)

TABLE 15.2
Bacteriocinogenic Cultures That Have Been Successfully Applied to Biopreservation of Meat Products (continued)

Bacteriocinogenic Starter Culture	Bacteriocin	Inocula (log CFU/g)	Product	References
Lc. lactis LMG21206	—	5–6	Dry-fermented sausages	Benkerroum et al. (2005)
Pd. acidilactici JBL1095	Pediocin AcH	7	Turkey summer sausages	Luchansky et al. (1992)
Pd. acidilactici		3 and 5	Chicken summer sausages	Baccus-Taylor, Glass, Luchansky, and Maurer (1993)
Pd. acidilactici	Pediocin AcH	4.7	Vacuum-packaged all-beef wieners	Degnan, Yousef, and Luchansky (1992)
Pd. acidilactici PA-2 *Lb. bavaricus* MI-401	—	2–5	Dry-fermented sausages	Lahti, Johansson, Honkanen-Buzalski, Hill, and Nurmi (2001)
E. faecium RZS C5	Enterocins A, B and P	6	Fermented sausages	Callewaert, Hugas, and De Vuyst (2000)
E. casseliflavus IM416K1	Enterocin 416K1	5	Italian sausages (cacciatore)	Sabia, de Niederhäusern, Messi, Manicardi, and Bondi (2003)
Carnobacterium piscicola L103	—	—	Vacuum-packed meat	Schöbitz, Zaror, León, and Costa (1999)

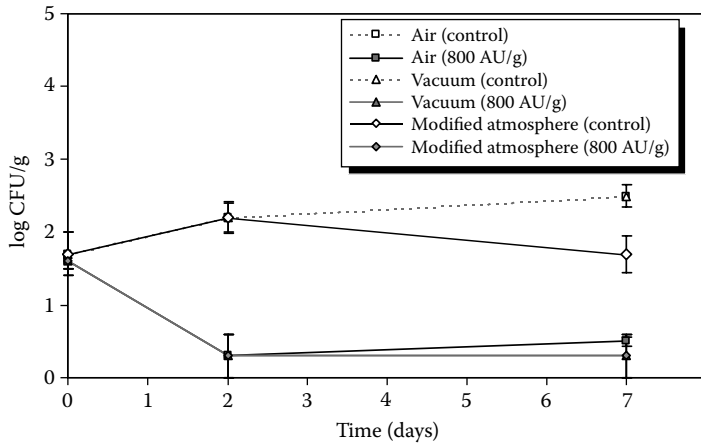


FIGURE 15.1 Growth of *Listeria* in minced pork packed in air, vacuum, and modified atmosphere under the presence of 800 AU/g of sakacin K and in control batches.

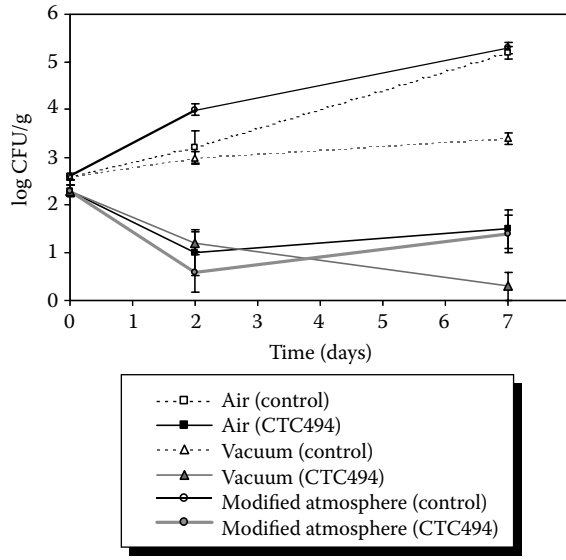


FIGURE 15.2 Growth of *Listeria* on chicken breasts packed in air, vacuum, and modified atmosphere under the effect of the bioprotective culture, *Lb. sakei* CTC494 and in control conditions.

types of micro-organisms associated with postprocessing contamination. The occurrence of *L. monocytogenes* in these products was found to be connected with cross-contamination after heat treatment (Genigeorgis 1989; Luchansky et al. 1992; Sheridan, Duffy, McDowell, and Blair 1994). If contamination with pathogens occurs after postprocessing, slicing of cooked ham, peeling and handling of frankfurter type sausages, or during packaging, bacteria will grow exponentially even at refrigeration temperatures, reaching very high numbers within days. In fact, vacuum- or gas-packed cooked sliced meat products have little or no inherent stability against the growth of *L. monocytogenes*, even under proper refrigeration (Blom et al. 1997; Buchanan, Stahl, and Whiting 1989; Duffy, Vanderlinde, and Grau 1994; Farber, Cai, and Ross 1996; Thomas and Wimpenny 1996).

The chilled storage in a modified atmosphere has a selective effect on the meat microbiota triggering a predominance of fermentative LAB toward Gram-negative bacteria (Dainty and Mackey 1992). Thus LAB strains may also be good candidates for application as bioprotective cultures in these products, thereby reducing the risk of growth and survival of food-borne pathogenic bacteria (Holzapfel 1995). Moreover the double effect of bacteriocins over pathogenic and spoilage bacteria may increase food safety while better preserving the sensorial characteristics. 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 inocula of 2×10^2 CFU/g to 2 MPN/g in cooked ham after 7 days of storage at 7°C (Hugas et al. 1998). Sakacin K effectiveness was demonstrated earlier during storage in both MAP systems, although at the end of storage there were no significant differences between

batches. In vacuum-packed bologna-type sausage, Kröckel (1997) reported 1 log cycle reduction on *L. monocytogenes* counts in the presence of *Lb. sakei* Lb674 and 3 log cycle reduction when sakacin P was added, compared to the control batch. Katla et al. (2002) demonstrated a 3 log CFU/g reduction of *L. monocytogenes* inoculated in chicken cold cuts when a high concentration (12 ng/g) of purified sakacin P was applied. *Listeria* counts were 2 log CFU/g below the control when a low bacteriocin concentration (3.5 ng/g) or sakacin K producing strains were used. The high dosage of bacteriocin had a bacteriostatic effect throughout the storage period of 4 weeks. However a low concentration or the addition of sakacin P producer or the nonsakacin P producer isogenic strain, permitted initial growth although at a slower rate than in the control batch.

Enterocins (4,800 AU/g) also proved to be effective against *Listeria* in cooked ham, showing a 7.98 log cycle reduction compared to the control batch after 37 days of chilled storage (Aymerich, Garriga, et al. 2000). The same concentration of enterocins in pâté completely avoids *Listeria* outgrowth, keeping the counts below 3 MPN/g throughout the whole storage period (figure 15.3). The antilisterial effect of the bacteriocins enterocin A, sakacin K, and two commercial biopreservatives based on the bacteriocins nisin and pediocin (Nisaplin and ALTA 2351, respectively) was demonstrated in frankfurter sausages when added to meat batter before cooking (Hugas, Garriga, Aymerich, and Monfort 2002). All the bacteriocins were active when dosed at the established concentrations: 3,500 AU/g for enterocin A and 800 AU/g for sakacin K, Nisaplin, and ALTA 2351, reducing the *Listeria* counts significantly during the 60 days of storage at 3.5°C. The batch with enterocin and sakacin gave significantly better results than the batch with Nisaplin, whereas no significant

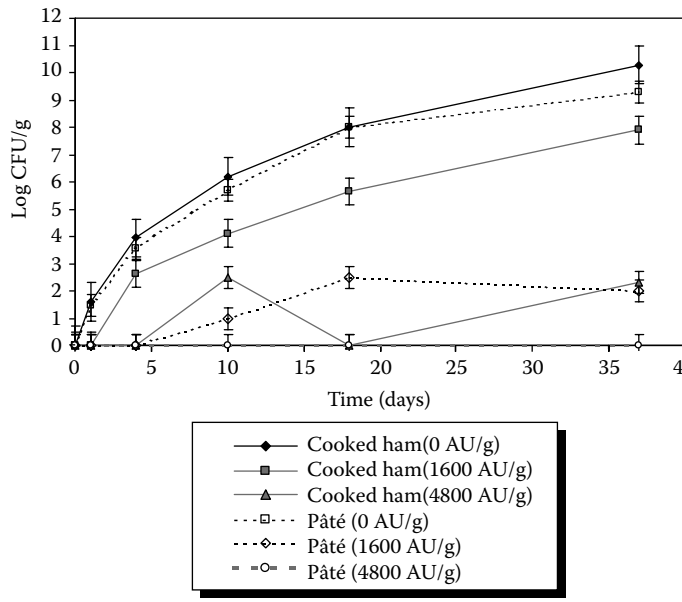


FIGURE 15.3 Antilisterial effect of enterocins applied to cooked ham and pâté.

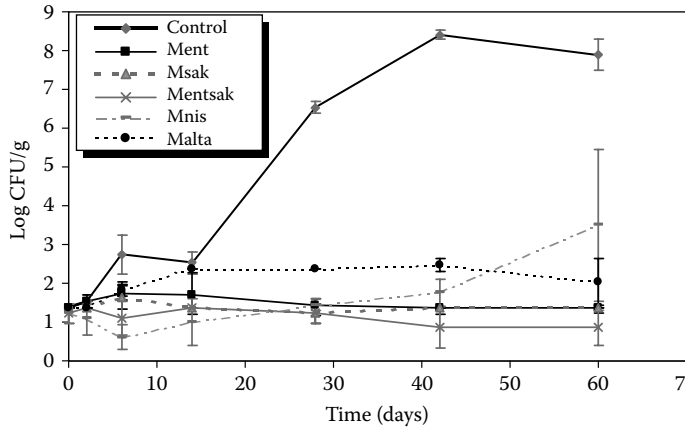


FIGURE 15.4 Behavior of *L. monocytogenes* in vacuum-packed frankfurter sausages stored at 3.5°C. Control batch (control batch), batch with enterocins (Ment), sakacin K (Msak), nisin (Mnis), pediocin (Malta), and a combination of sakacin and enterocins (Mentsak).

differences could be established with the other batches in which sakacin and enterocin were applied separately or with ALTA. A 7.61-log decrease in *Listeria* counts was observed in the batch with enterocin plus sakacin when compared to the control batch (figure 15.4). When the bacteriocins were sprayed onto the surface of the peeled frankfurters, a significant reduction in the *Listeria* counts, probably due to physical contact, was immediately observed. *Listeria* counts under ≤ 3 MPN/g were maintained until the 14th day of storage in the batches with enterocin, sakacin, and ALTA. In the nisin batch those low levels were kept until the end of the storage period (60 days).

In sliced cooked sausages treated with pediocin AcH, Mattila, Saris, and Työppönen (2003) observed a *L. monocytogenes* decrease from the initial level of 2.7 log CFU/g to < 2 log CFU/g after 6 days of storage, remaining at this level until the end of the storage period (21 days).

Jacobsen, Budde, and Koch (2003) reported that the use of living cells of *Leuconostoc carnosum* 4010 was better than the partially purified bacteriocins 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 4 weeks of storage at 10°C.

The antilisterial effect of nisin in autoclaved tenderloin pork meat was described by Fang and Lin (1994a). The growth of *L. monocytogenes* Scott A was prevented when samples were treated with 10,000 IU/ml either in air or packed in MAP, the effect being more pronounced at 4°C than at 20°C. Gill and Holley (2002) reported no increase in antimicrobial activity against Gram-negative bacteria when chrisin (2.5% pure nisin) was used together with EDTA or lysozyme in processed ham and bologna. In contrast, several authors (Carneiro de Melo, Cassar, and Miles; Cutter and Siragusa 1995; Stevens, Sheldon, Klapes, and Klaenhammer 1991, 1992) have reported additional increasing effects in organisms suspended in buffer. Screening of antimicrobials for use in food systems in nutrient-deficient systems seems to be inappropriate.

15.2.3 Fermented Sausages

Fermented sausages have a long history of safe traditional products and most pathogenic bacteria can be prevented through good manufacturing practice. However, the control of *L. monocytogenes* in these products is difficult due to (a) its tolerance to pH (4.8–9.6), (b) its tolerance to high sodium chloride levels (< 10%) and to sodium nitrite (< 1,000 ppm; Buchanan et al. 1989; Duffy et al. 1994; Thomas and Wimpenny 1996), and (c) its ability to grow at a wide range of temperatures (3°C–45°C). Although *L. monocytogenes* growth does not occur generally in these products, an increase of nearly 1 log CFU/g has been observed in low-acid type sausages (pH > 5.3; Garriga et al. 2005), thus potential contamination from raw materials cannot be ruled out. This pathogen is ubiquitous in the environment and it is often isolated in manufacturing plants as an in-house bacterium (Chasseignaux 1999; van den Elzen and Snijders 1993). Salvat, Toquin, Michel, and Colin (1995) reported that as many as 68% of environmental samples in a curing plant were positive for *L. monocytogenes* and that after cleaning, 17% of the samples remained positive. Stuffing machines were found to be contaminated with this pathogen, with 20% of the samples being positive after cleaning (Fadda, Aymerich, Hugas, and Garriga 2005). Recent European investigations reported 12% to 16% *Listeria*-positive isolations in industrial fermented meat products (Agence Française de Sécurité Alimentaire des Aliments 2000). When the raw meat is contaminated with *Listeria*, even at low levels, the final product could be positive for *L. monocytogenes,* although the counts are usually low (< 10–100 CFU/g; Aymerich, Martín, Garriga, and Hugas 2003; Johnson, Doyle, Cassens, and Schoeni 1988; Junttila, Hirn, Hill, and Nurmi 1989).

In fermented meat products, starter and biopreservative LAB strains are very competitive, and largely dominate other bacterial communities. They are generally recognized as safe (GRAS) micro-organisms and produce antimicrobials such as lactic acid and bacteriocins. Moreover, bacteriocins do not seem to affect the growth of selected strains of *Kocuria* (Sobrino et al. 1991; Tichaczek, Nissen-Meyer, Nes, Vogel, and Hammes 1992) and *Staphylococcus carnosus* (Schillinger and Lücke 1989; Tichaczek et al. 1992), which are essential for the flavor development of the fermented sausages.

Several authors have reported the antilisterial effect of bacteriocin-producing strains in these types of products. *Lb. sakei* CTC494, sensorially proved to be a suitable starter culture (Garriga et al. 1996), was able to suppress the growth of *Listeria* initially spiked at 9×10^3 CFU/g and to diminish their number by 2.64 log in dry sausages compared to the initial counts (Hugas, Garriga, Aymerich, and Monfort 1995). The inhibition observed was shown to be due to the bacteriocin produced by *Lb. sakei* CTC494, because the batches inoculated with the nonbacteriocinogenic starter strain (with lower pH and higher production of lactic acid) were unable to diminish *Listeria* to the same extent as the Bac^+ strain (bacteriocinogenic strain; Hugas et al. 1995). When different strains producing the same bacteriocin were assayed in fermented sausages, manufactured under different formula or technology, their activity against *Listeria* was different (Hugas, Neumeyer, Pagés, Garriga, and Hammes 1997). In batches of series A (Spanish nitrate–nitrite formulation including abundant addition of ingredients), containing curvacin A producing *Lb. curvatus* LTH1174 and *Lb. sakei* CTC494 (sakacin K), the counts

of *Listeria* were reduced by more than 1.5 to 2 log cycles when compared to the nonbacteriocinogenic control batch. In series B (German formulation with only glucose, nitrate, and sodium chloride) 1 log reduction of *Listeria* count was observed in batches with *Lb. sakei* Lb706 (sakacin A producer) and *Lb. curvatus* LTH1174. Benkerroum and Sandine (1988) reported a decrease in *Listeria* counts below the detection limit achieved after 15 days of drying when using an inocula of 10^6 to 10^7 CFU/g of *Lc. lactis* LMG21206 (Bac^+). Although *Lb. sakei* and *Lb. curvatus* have the higher capacity to grow and produce bacteriocin in fermented sausages, *Lc. lactis* should not be excluded as a possible application for fermented sausages.

In dry-fermented sausages inoculated with the pediocin producer *Pd. acidilactici* PAC1.0, absence of *L. monocytogenes* at the end of the drying process could only be achieved by pH under 4.9, although a 10- to 100-fold reduction of a five-strain cocktail of *Listeria* was observed when compared to the results obtained using its nonbacteriocin producer isogenic strain (Foegeding, Thomas, Pilkington, and Klaenhammer 1992). Pediocins produced by *Pd. acidilactici* during fermentation provide an additional protection against *Listeria* proliferation in turkey summer sausages (Luchansky et al. 1992), in chicken summer sausages (Baccus-Taylor, Glass, Luchansky, and Maurer 1993), and in pork and beef fermented sausages (Lahti, Johansson, Honkanen-Buzalski, Hill, and Nurmi 2001). Lahti et al. (2001) observed a rapid decrease of *L. monocytogenes* when a starter culture, composed of *S. xylosus* DD-34 and the bacteriocinogenic strains, *Pd. acidilactici* PA-2 and *Lb. bavaricus* MI-401, was used. During the maturation of artificially contaminated salami, *L. monocytogenes* counts tended to decrease and no significant differences were observed between samples inoculated with *Lb. plantarum* MCS or with the bacteriocin negative mutant strain (Campanini, Pedrazzoni, Barbuti, and Baldini 1993).

The enterocins (648 AU/g) included in the meat mixture spiked with 3 log CFU/g of *Listeria* were able to decrease and keep their number to 6 MPN/g from the third day of fermentation until the end of the drying period (Aymerich, Artigas, Garriga, Monfort, and Hugas 2000). The higher efficiency of semipurified enterocins compared with the Bac^+ strain (*E. faecium* CTC492) may be explained by the inhibition of the bacteriocin production due to salt, pepper, and the low pH of this type of dry-fermented sausages (Aymerich, Artigas, et al. 2000). Thus, bacteriocins may be considered as an additional antilisteria hurdle that could act cooperatively with the other additives and the global drying process typical in sausage manufacture. Other authors have reported the antilisterial effect in several types of fermented sausages by enterocins CCM4231 (Lauková, Czikková, Laczková, and Turek 1999; Lauková, Turek, Mareková, and Nagy 2003), enterocins 416K1 (Sabia, de Niederhäusern, Messi, Manicardi, and Bondi 2003), enterocin AS-48 (Ananou et al., in press), or by *E. faecium* RZS C5 (Callewaert, Hugas, and De Vuyst 2000) and *E. casseliflavus* IM416K1 (Sabia et al. 2003).

15.3 ACTIVE PACKAGING: A PROMISING NEW ANTIMICROBIAL TECHNOLOGY APPROACH

In recent years, the incorporation of bacteriocins into packaging films as a bacteriocin-delivery mechanism to control spoilage and pathogenic bacteria has been an

area of active research. Several advantages over other methodologies should be considered: (a) only the exact amount of bacteriocin would be used, (b) gradual antimicrobial activity can be considered, (c) there is no direct addition of the additive to the food product, and (d) there is an environmental advantage if the film is edible or biodegradable. Following vacuum packaging or MAP, the combination of obstacles from MAP, refrigerated storage, and bacteriocin inhibition would provide a product with enhanced microbial stability.

Nisin is the most widely studied bacteriocin in packaging and several solvents and polymer structures have been assayed. The incorporation of nisin in the film-forming solution of edible cellulose films made with hydroxypropylmethylcellulose produced films with inhibitory effect against *L. innocua* and *S. aureus*. However, film additives such as stearic acid, used to improve the water vapor barrier of the film, significantly reduced inhibitory activity. It was noted that desadsorption from the film and diffusion into the food required further optimization for nisin to function more effectively as a preservative agent in food packaging (Coma, Sebti, Pardon, Deschamps, and Pichavant 2001). Antimicrobial activity against *L. innocua* and *S. aureus* was found in cellulose-based bioactive inserts (Perganorm^r Dresden Papier, Germany) and polyethylene or polyamide pouches (70:30 PE:PA, Cryovac, Dublin, Ireland) containing immobilized lacticin 3147 and nisin at 2,560 AU/cm^2. However, the adsorption to the film is bacteriocin-dependent and whereas nisin bonded well to the film and was active for 3 months at both room temperature and under refrigeration, adsorption of lacticin 3147 to plastic bags was unsuccessful. In MAP packed and refrigerated sliced cooked ham nisin-adsorbed films were able to reduce *L. innocua* counts by 1.5 log CFU/g after 12 days (starting inocula of 3×10^5 CFU/g) and *S. aureus* was reduced by 2.8 log CFU/g (starting inocula of 3×10^5 CFU/g; Scannell et al. 2000). In vacuum-packaged hot dogs, the effectiveness of coated-barrier films with a methylcellulose/hydroxypropyl methylcellulose-based solution containing 10,000, 7,500, 2,500, or 156.3 IU/ml of nisin was evaluated. A cocktail of five *L. monocytogenes* was spiked on the surfaces of hot dogs at 5 log CFU/package that were subsequently vacuum packaged and stored at 4°C. In batches with 10,000 and 7,500 IU/ml, the reduction was larger than 2 log CFU/package throughout the 60 days of storage (Franklin, Cooksey, and Getty 2004).

Although nisin's spectrum of inhibitory activity is limited to Gram-positive bacteria, when combined with food-grade chelating agents such as EDTA, citrate, and phosphate, nisin also inhibits Gram-negative bacteria including *Salmonella* serotypes and *E. coli* O157:H7 (Stevens et al. 1992). Natrajan and Sheldon (2000a) reported the efficacy against *Salmonella typhymurium* in fresh broiler drumstick skin samples packed with films of varying hydrophobicities (polyvinyl chloride, linear low-density polyethylene and nylon) and protein- and polysaccharide-based (calcium alginate and agar). Both kinds of films contained nisin and varying concentrations of citric acid, EDTA, and tween 80. When the plastic and nylon films were applied to fresh broiler drumstick skin samples, a reduction of 0.4 to 2.1 log cycles was observed in *S. typhymurium* counts after 24 hours at 4°C (Natrajan and Sheldon 2000a). The same pathogen was inhibited on poultry skin using protein and polysaccharide-based films. When nisin was included in calcium alginate gels, *Salmonella* counts decreased by 1.98 to 3.01 log units after 72 hours at 4°C. When nisin was

included in agar films at 500 μg/ml, *Salmonella* counts were reduced by 1.8 log cycles (1.25% agar gel) and 4.6 log cycles (0.75% agar gel), respectively, after 72 to 96 hours at 4°C (Natrajan and Sheldon 2000b).

In addition to nisin, the effectiveness of other bacteriocin-containing films has been tested in meat products. Pediocin coated onto cellulose casings (9.30 μg/cm^2) and plastic bags (7.75 mg/cm^2 = 5 AU/cm^2) completely inhibited the growth of *L. monocytogenes* in beef, ham, and turkey breasts during 12 weeks of storage at 4°C. Compared with nontreated samples, *L. monocytogenes* counts decreased by almost 2 log units in ham and beef and by almost 3 log units in turkey breasts (Ming, Weber, Ayres, and Sandine [1997]). Enterocin A, sakacin K, and two commercial biopreservatives, based on the bacteriocins nisin and pediocin applied through cellulose casing in frankfurter sausages, were able to delay the outgrowth of *L. monocytogenes* (artificially spiked at 1.7 log CFU/g) during 6 days of storage at 3.5°C. Enterocins and pediocin extended the antimicrobial effect until the 14th day of storage (Hugas, Garriga, Aymerich, et al. 2002). Active polythene films with partially purified antilisterial bacteriocin 32Y were used for industrial coating of meat products artificially contaminated with *L. monocytogenes* V7 (PE-OPA film coated with 6,400 AU/ml). No significant reduction was observed in pork steaks, whereas an approximately 0.5 log reduction could be observed with hamburgers after 3 days of storage at 4°C when compared with nonactivated packaging (Mauriello, Ercolini, La Storia, Casaburi, and Villani 2004).

15.4 BACTERIOCINS AND NEW PRESERVATION TECHNOLOGIES TO BE APPLIED TO MEAT PRODUCTS

The application of bacteriocins in meat and meat products as well as in other types of foods has several limitations that may hamper their application. Bacteriocins do not have a broad host range. They are only active against closely related strains and they are generally ineffective against spoilage and pathogenic Gram-negative bacteria. Thus, from the point of view of safety, it is not possible to rely solely on the antimicrobial effect of bacteriocins. To overcome this, a hurdle combination technology is necessary. Several studies have reported additive or synergistic effects between bacteriocins, several antimicrobials, and emergent processing technologies like high hydrostatic pressure (HHP), electric pulses, magnetic fields, irradiation, and so on. These preservation technologies enhance the antibacterial activity of bacteriocins and Gram-negative bacteria, normally insensitive to bacteriocins such as *E. coli* or *Salmonella* become sensitive (Kalchayanand, Sikes, Dunne, and Ray 1994), thus improving food safety in the context of the hurdle concept proposed by Leistner (2000). HHP is a very promising technology for the preservation of meat products such as cooked and dry-cured meat products. It is well accepted by European consumers (Baron et al. 1999) but until now only a few reports have dealt with the antimicrobial combined effect of HHP and bacteriocins in food matrices and model meat products under refrigeration. The applied pressure (400–900 MPa) is uniform and it is not time or mass dependent like the thermal treatments. HHP is able to destroy the microbial vegetative cells by destabilizing the structural and functional integrity of the cytoplasmic membrane, by inducing

protein denaturation, and by inhibiting genetic mechanisms (Hoover, Metrick, Papineau, Farkas, and Knorr 1989).

Kalchayanand, Sikes, Dunne, and Ray (1998) reported the effectiveness of pediocin AcH associated with a high hydrostatic treatment in a suspension of bacterial cells (10^8 CFU/ml) in peptone solution. A pressurization treatment of 345 MPa for 5 minutes at 25°C plus pediocin AcH (3,000 AU/ml) increased the viability loss versus a treatment of only pressurization. When compared with nonpediocin treated batches a higher 1.7 log cycle decrease for *S. aureus*, 2.1 for *L. monocytogenes,* and 0.6 log cycle for *E. coli* O157:H7 was reported.

In a meat model system, Garriga, Aymerich, Costa, Monfort, and Hugas (2002) reported the combined effect of antimicrobials (enterocins A and B, sakacin K, pediocin AcH, and nisin) with a high hydrostatic pressure treatment of 400 MPa for 10 minutes at 17°C. Although *Staphylococcus* was the least sensitive genera to pressurization, the samples including nisin displayed lower and significantly different counts during the 4°C storage than the rest of the treatments. A greater inactivation of *E. coli* was recorded in the presence of nisin, and the number of survivors remained unchanged during storage at 4°C for 61 days. *L. monocytogenes* were kept under 10^2 CFU/g in batches containing sakacin K, enterocins A and B, or pediocin (ALTA 2351™, Quest International) until the end of the storage time (61 days).

In sliced cooked ham, Aymerich, Jofré, Garriga, and Hugas (2005) reported an additive effect between the HHP treatment (400 MPa for 10 minutes at 17°C) and nisin (800 AU/ml, 44.85 ppm). Although the most efficient treatment for decreasing *Salmonella enterica* counts was HHP, absence of the food-borne pathogen in 50% of the samples could only be achieved when a combined treatment of HHP and nisin was applied. Nisin also enhanced the antilisterial effect of the HHP treatment when a three-strain cocktail of *L. monocytogenes* was inoculated in sliced cooked ham vacuum stored at 6°C for 3 months. At the end of the storage period, a significant decrease in *L. monocytogenes* counts (2 log cycle reduction) was achieved in HHP batches with nisin when compared to non-nisin HHP batches.

In frankfurters, the effectiveness of a combined treatment comprising pediocin (ALTA 2351) and postpackaging irradiation to control *L. monocytogenes* was reported by Chen, Sebranek, Dickson, and Mendonca (2004). The combination of pediocin (6,000 AU/link) with postpackaging irradiation (1.2 KGy) was necessary to achieve a 50% reduction of initial counts. The use of pediocin at 6,000 AU/link and an irradiation treatment of 2.3 KGy were able to inhibit the pathogen for 12 weeks at 4°C or 10°C.

15.5 CONSIDERATIONS ABOUT THE APPLICATION OF BACTERIOCIN OR THEIR PRODUCERS IN FOODS

15.5.1 Interaction of Bacteriocins and Bacteriocinogenic Strains Through Meat Compounds or Additives

The production of a certain bacteriocin or its bacteriocinogenic strain in a laboratory media does not imply its effectiveness in foodstuffs. When evaluating a bacteriocinogenic culture, we have to take into account that meat and meat products are

complex systems with a number of factors influencing microbial growth and metabolite production. Therefore, the influence of formula and technology on the performance of the bacteriocinogenic cultures needs to be assayed. The main factors affecting the efficacy and production of bacteriocins in meat are: (a) adequate environment for growth or bacteriocin production, (b) bioavailability and stability of bacteriocins over time, and (c) development of bacteriocin-resistant organisms.

Leroy and De Vuyst (1999) reported that the temperature and the acidity conditions that prevail during the fermentation process of dry-fermented sausages was optimal for the production of sakacin K by *Lb. sakei* CTC494. In addition, low temperatures decreased the inactivation rate of sakacin K and extended its bioavailability. The use of *Lb. sakei* CTC494 Bac^+ in Spanish-style fermented sausages containing nitrate, nitrite, pepper, and glucose was shown to be effective. The addition of black pepper, a spice containing manganese that has been shown to stimulate lactobacilli starter cultures growth (Hagen and Holck 1999), did not increase the production of sakacin K but enhanced the inhibitory activity of sakacin K against *L. monocytogenes*. A synergistic antilisterial effect between pepper, salt, and nitrite and *Lb. sakei* CTC494 Bac^+ was observed (Hugas, Garriga, Pascual, Aymerich, and Monfort 2002). *Lb. curvatus* LTH 1174, curvacin A producer, showed a wide range of pH growth and the optimum growth temperature for curvacin production between 20°C and 27°C. Thus it could be a promising bacteriocin-producing strain for fermentations performed near 25°C (Messens, Verluyten, Leroy, and De Vuyst 2003). The growth of *E. faecium* CTC492 was not affected by the concentration of the ingredients used in the sausage manufacture, although sodium chloride, sodium nitrite, and pepper, important ingredients for the salt–spice taste of this kind of sausage, were detrimental to bacteriocin production (Aymerich, Artigas, et al. 2000). In addition, enterocin production is maximal at neutral pH. Therefore, the production of enterocins *in vitro* and its use as an additive appears to be the best alternative.

When bacteriocins are used as additives, their effectiveness may be affected by many factors acting simultaneously, like adsorption to food macromolecules such as fat (Jung, Bodyfelt, and Daeschel 1992) and degradation by proteolitic enzymes (Hurst 1981). The presence of ethanol and emulsifiers may partially prevent adsorption losses (Jung et al. 1992). The physicochemical parameters of the food to matrix may also influence their activity. Thomas and Wimpenny (1996) reported increased antimicrobial activity of nisin against *L. monocytogenes* and *S. aureus* by NaCl and pH down to 5.0. Yang, Johnson, and Ray (1992) also showed an increased antilisterial activity of sakacin P at low pH. At acidic pH (5.5) the addition of salt increased the activity of sakacin P, whereas divalent and trivalent cations, such as magnesium and calcium, may decrease their activity (Gänzle, Hertel, and Hammes 1996). Aesen et al. (2003) studied the interactions of sakacin P and nisin with salmon and chicken. More than 80% of the added bacteriocin was quickly adsorbed to proteins in the food matrix. In non-heat-treated foods, proteolytic activity caused a rapid degradation of the bacteriocins and less than 1% of the total activity could be detected after 1 week. However, in heat-treated foods, the bacteriocin activity was stable for more than 4 weeks. Mixing triglyceride oils with bacteriocin solutions caused a considerable loss of activity but high fat content in salmon compared with chicken had no

adverse effect. Nisin was less adsorbed to muscle proteins at low pH and the negative effect of oil was less pronounced than in sakacin P.

15.5.2 Bacteriocin-Resistant Bacteria

Besides the effect of the inoculum size (Gay, Cerf, and Davey 1996) and stressful conditions (Augustin, Brouillaud-Delattre, Rosso, and Carlier 2000) that may affect the sensibility or resistance of *L. monocytogenes* to bacteriocins, a major concern regarding the use of bacteriocins on food is the natural or developed resistance in the target bacteria. Natural resistance to Class IIA bacteriocins and to nisin has been reported (Ennahar et al. 2000; Larsen and Norrung 1993; Rasch and Knochel 1998; Ukuku and Shelef 1997). Resistance, although not always stable, of *L. monocytogenes* to bacteriocins develops at frequencies ranging from less than 10^{-9} to 10^{-5} (Bouttefroy and Millière 2000; Dykes and Hastings 1998; Ming and Daeschel 1993). In some cases, bacteriocin resistance may impair the growth of the resistant strain. Gravesen, Axelsen, Mendes da Silva, Hansen, and Knochel (2002) observed considerable strain-specific variations in the frequency of *L. monocytogenes* resistance development and a reduced growth of resistants in brain heart infusion (BHI) broth but not in saveloy-type meat model at 5°C. Low temperature, low pH, and presence of NaCl did not increase the sensitivity of mutants to stress nor did it influence the frequency of pediocin resistance development but it reduced the frequency of nisin resistants considerably.

Although resistant and cross-resistant target bacteria may appear when bacteriocins are used at subminimal inhibitory concentrations, the combined use of several appropriate bacteriocins and new preservation technologies could result in better inhibition, thus increasing the safety of the food product (Aymerich et al. 2005; Garriga et al. 2002; Rekhif, Atrih, and Lefebvre 1994; Song and Richard 1997). Moreover, challenge tests with a cocktail of different sensitive strains and different levels of inocula should be recommended to validate the efficiency on food systems, usually contaminated with several strains and lower numbers.

15.5.3 Commercial Availability and Industrial Applications

In the last decade the knowledge on bioprotective cultures, especially concerning bacteriocinogenic cultures and bacteriocins, has been dramatically improved. Nonetheless, the use of LAB that produce bacteriocins is not common in the food industry today. There are already a few cultures on the market introduced as starter or bioprotective cultures with the main objective of contributing to the improvement of microbiological safety. Bactoferm F-LC from Christian Hansen (Hoershom, Denmark) has been patented as a culture capable of preventing growth of *Listeria* in fermented sausages. It is a mixed culture of *Pd. acidilactici* and *Lb. curvatus* producing pediocin and sakacin A, respectively. Christian Hansen also offers other bioprotective cultures for vacuum-packed and MAP meat products containing *Lb. sakei* (B-2) or *Leuc. carnosum* 4010 (B-SF-43). Danisco Cultor has a combined culture composed of *Lb. plantarum* and *S. carnosus* as an antilisterial culture for fermented sausages (either sliceable or spreadable) and for cooked ham (ALCMix1),

and two more culture compositions, COX1 and XPA1, to be applied to minced meat and raw sausages for boiling and frying as bioprotective cultures against undesired bacteria in the cooling chain. Alta™ 2351 and Fargo 23 (Quest International, B.V., The Netherlands) are natural food ingredients produced through a fermentation process of a bacteriocinogenic *Pd. acidilactici* strain with antilisterial activity. The product is approved as an ingredient for foods in the United States and has been introduced on the market as a shelf-life extender in a great variety of meat products such as raw sausages, frankfurters, hamburgers, and MAP cooked ham.

Today nisin is the only bacteriocin commercially available. Cultor (formerly Aplin and Barret, Beaminster, Dorset, UK) produces the product Nisaplin™, and Chr. Hansen A/S (Hoersholm, DK) produces Chrisin™. The nisin products of the two companies have very similar specifications, containing 2.5% of the active component, which corresponds to an antimicrobial activity of 1,000 IU/mg (1 mg pure nisin is equivalent to 40,000 IU; Delves-Broughton 1990). Both Nisaplin™ and Chrisin™ are prepared from a fermentation of selected strains of *Lc. lactis* subsp *lactis*. The use of pediocin PA-1 for food biopreservation has been commercially exploited and is covered by several U.S. and European patents (EP0493779A1). Enterocin A is also covered by a Spanish patent (ES 2 068157). Rhodia Inc. (now owned by Danisco) is developing a casing to be used in hot dog manufacture and other cooked meat products. The films harbor a proprietary combination of bacteriocins, enzymes, and botanicals and the target is *L. monocytogenes*. The added cost is considered economically sound given the large recalls of products contaminated by this pathogen (Chen and Hoover 2003).

15.5.4 Regulation Aspects

Food safety, chemical, and microbiological hazards are among the main focuses of European communities. LAB have been used in food fermentation all over the world for millenia. They have helped to maintain the safety and nutritional quality of many perishable foodstuffs. Consumers have been exposed to an enormous amount of LAB without harmful effects for centuries. Food products with LAB are perceived as being traditional, safe, and even healthy. They are generally recognized as safe based on the experience of common usage by the U.S. Food and Drug Administration (FDA) and the European Commission proposed the term qualified presumption of safety (QPS) for micro-organisms with a history of safe use (European Commission 2002, 2003). In Europe, only Denmark and France have legislation that explicitly regulates the addition of microbial cultures to food and the European Union only stipulates the addition of lactic acid to infant formula (European Commission 1995). Until now, in situ applications of food-grade cultures have been more suitable than the application of purified bacteriocins because special labeling is not necessary if these applications are regarded as processing aid, for example, acidification or flavor compounds (Holzapfel et al. 1995). The only qualification that has to be attached is evidence of the absence of acquired antibiotic resistance and virulence factors mainly regarding enterococci (European Commission 1992).

New purified bacteriocins must fulfill the criteria laid down by the European Commission (1997) concerning novel food ingredients, and safety information

should be provided according to specific guidelines. The Joint Food and Agricultural Organisation/World Health Organisation Expert Committee on Food Additives (1969) recommended acceptance of the use of nisin as a preservative for foods. In 1988, nisin was granted a GRAS status by the U.S. FDA (1988). Today the use of nisin is allowed in more than 50 countries including the European Union, where nisin (E234) is regulated as a preservative with restricted use in some dairy products together with tapioca and semolina puddings, but it is not allowed in meat products (European Commission 1995). Purified nisin has been evaluated for toxicological effects and found harmless, or at least with a very low toxicity, using rats and guinea pig models (Frazer, Sharott, and Hickman 1962; Shtenberg and Ignatev 1970). The Scientific Committee for Food of the European Community (1992) decided to allocate an acceptable daily intake of 0.13 mg/kg body weight based on a nisin product of 40,000 IU/mg.

REFERENCES

Abee, T., L. Krockel, and C. Hill. 1995. Bacteriocins: Modes of action and potentials in food preservation and control of food poisoning. *Int. J. Food Microbiol.* 28:169–185.

Aesen, I. M., S. Markussen, T. Moretro, T. Katla, L. Axelsson, and K. Naterstad. 2003. Interactions of the bacteriocins sakacin P and nisin with food constituents. *Int. J. Food Microbiol.* 87:35–43.

Agence Française de Sécurité Alimentaire des Aliments (AFSSA). 2000. Rapport de la commission d'etudes des risques liés à *Listeria monocytogenes*. Maisons-Alfort, France.

Ananou, S., M. Garriga, M. Hugas, M. Maqueda, M. Martínez-Bueno, A. Gálvez, and E. Valdivia. 2005. Control of *Listeria monocytogenes* in model sausages by enterocin AS-48. *Int. J. Food Microbiol.* 103:179–190.

Augustin, J. C., A. Brouillaud-Delattre, L. Rosso, and V. Carlier. 2000. Significance of inoculum size in the lag time of *Listeria monocytogenes*. *Appl. Environ. Microbiol.* 66:1706–1710.

Aymerich, M. T., M. G. Artigas, M. Garriga, J. M. Monfort, and M. Hugas. 2000. Effect of sausage ingredients and additives on the production of enterocin A and B by *Enterococcus faecium* CTC492: Optimization on *in vitro* production and anti-listerial effect in dry fermented sausages. *J. Appl. Microbiol.* 88:686–694.

Aymerich, M. T., M. Garriga, J. Ylla, J. Vallier, J. M. Monfort, and M. Hugas. 2000. Application of enterocins as biopreservatives against *Listeria innocua* in meat products. *J. Food Prot.* 63:721–726.

Aymerich, M. T., A. Jofré, M. Garriga, and M. Hugas. 2005. Inhibition of *Listeria monocytogenes* and *Salmonella* by natural antimicrobials and high hydrostatic pressure in sliced cooked ham. *J. Food Prot.* 68:173–177.

Aymerich, M. T., B. Martín, M. Garriga, and M. Hugas. 2003. Microbial quality and direct PCR identification of lactic acid bacteria and nonpathogenic staphylococci from artisanal low-acid sausages. *Appl. Environ. Microbiol.* 69:4583–4594.

Baccus-Taylor, G., K. A. Glass, J. B. Luchansky, and A. J. Maurer. 1993. Fate of *Listeria monocytogenes* and pediococcal starter cultures during the manufacture of chicken summer sausages. *Poult. Sci.* 72:1772–1778.

Baron, A., O. Bayer, P. Butz, B. Geisel, B. Gupta, U. Oltersdorf, and B. Tauscher. 1999. Consumer perception of high pressure processing: A three country survey. In *European Conference on Emerging Food Science and Technology, Tampere, Finland. 1999,* 18.

Benkerroum, N., A. Daoudi, T. Hamraoui, H. Ghalfi, C. Thiry, M. Duroy, P. Evrart, D. Roblain, and P. Thonart. 2005. Lyophilized preparations of bacteriocinogenic *Lactobacillus curvatus* and *Lactococcus lactis* subsp. *lactis* as potential protective adjuncts to control *Listeria monocytogenes* in dry-fermented sausages. *J. Appl. Microbiol.* 98:56–63.

Benkerroum, N., and W. E. Sandine. 1988. Inhibitory action of nisin against *Listeria monocytogenes*. *J. Dairy Sci.* 71:3237–3245.

Blom, H., E. Nerbrink, R. Dainty, T. Hagtvedt, E. Borch, H. Nissen, and T. Nesbakken. 1997. Addition of 2.5% lactate and 0.25% acetate controls growth of Listeria monocytogenes in vacuum-packed, sensory-acceptable servelat sausage and cooked ham stored at 4°C. *Int. J. Food Microbiol.* 38:71–76.

Bouttefroy, A., and J. B. Millière. 2000. Nisin-curvaticin 13 combinations for avoiding the regrowth of bacteriocin resistant cells of *Listeria monocytogenes* ATCC 15313. *Int. J. Food Microbiol.* 62:65–75.

Buchanan, R. L., H. G. Stahl, and R. C. Whiting. 1989. Effects and interactions of temperature, pH, atmosphere, sodium chloride, and sodium nitrite on the growth of *Listeria monocytogenes*. *J. Food Prot.* 52:844–851.

Budde, B. B., T. Hornbaek, T. Jacobsen, V. Barkholt, and A. G. Koch. 2003. *Leuconostoc carnosum* 4010 has the potential for use as a protective culture for vacuum-packed meats: Culture isolation, bacteriocin identification, and meat application experiments. *Int. J. Food Microbiol.* 83:171–184.

Buncic, S., S. M. Avery, and S. M. Moorhead. 1997. Insufficient antilisterial capacity of low inoculum *Lactobacillus* cultures on long-term stored meats at 4°C. *Int. J. Food Microbiol.* 34:157–170.

Callewaert, R., M. Hugas, and L. De Vuyst. 2000. Competitiveness and bacteriocin production of enterococci in the production of Spanish-style dry fermented sausages. *Int. J. Food Microbiol.* 57:33–42.

Campanini, M., I. Pedrazzoni, S. Barbuti, and P. Baldini. 1993. Behaviour of *Listeria monocytogenes* during the maturation of naturally and artificially contaminated salami: Effect of lactic-acid bacteria starter cultures. *Int. J. Food Microbiol.* 20:169–175.

Carneiro de Melo, A. M., C. A. Cassar, and R. J. Miles. 1998. Trisodium phosphate increases sensitivity of gram-negative bacteria to lysozyme and nisin. J. Food Prot. 58:977–983.

Center for Science in the Public Interest. 2004. Outbreak alert. Retreived March 2005 from http://www.cspinet.org/new/pdf/outbreakalert2004.pdf

Chasseignaux, E. 1999. Ecologie de *Listeria monocytogenes* dans les ateliers de transformation de viandes des volailles et de porcs. Lyon, France: Université Claude Bernard.

Chen, C.-M., J. G. Sebranek, J. S. Dickson, and A. E. Mendonca. 2004. Combining pediocin with postpackaging irradiation for control of *Listeria monocytogenes* on frankfurters. *J. Food Prot.* 67:1866–1875.

Chen, H., and D. G. Hoover. 2003. Bacteriocins and their food applications. *Compr. Rev. Food Sci. Food Safety.* 2:81–100.

Cleveland, J., T. Montville, I. F. Nes, and M. L. Chikindas. 2001. Bacteriocins: Safe, natural antimicrobials for food preservation: Review article. *Int. J. Food Microbiol.* 71:1–20.

Coma, V., I. Sebti, P. Pardon, A. Deschamps, and F. H. Pichavant. 2001. Antimicrobial edible packaging based on cellulosic ethers, fatty acids, and nisin incorporation to inhibit *Listeria innocua* and *Staphylococcus aureus*. *J. Food Prot.* 94:470–475.

Commission of European Communities. 1992. Reports of the Scientific Committee for Food (26th series, EUR 13913 EN). Brussels, Belgium.

Commission of European Communities. 2000. White paper on food safety. Brussels, Belgium.

Cowden, J. M., P. G. Wall, G. Adak, H. Evans, S. Le Baigue, and D. Ross. 1995. Outbreaks of foodborne infectious intestinal disease in England and Wales: 1992 and 1993. *Commun. Dis. Rep. CDR Rev. 5*(8):109–117.

Cutter, C. N., and G. R. Siragusa. 1994. Decontamination of beef carcass tissue with nisin using a pilot scale model carcass washer. *Food Microbiol.* 11:481–489.

Cutter, C. N., and G. Siragusa. 1995. Population reductions of Gram negative pathogens following treatments with nisin and chelators under various conditions. *J. Food Prot.* 58:977–983.

Dainty, R. H., and B. M. Mackey. 1992. The relationship between the phenotypic properties of bacteria from chill-stored meat and spoilage processes. *J. App. Bacter. Sym. Suppl.* 73:103S–114S.

Degnan, A. J., A. E. Yousef, and J. B. Luchansky. 1992. Use of *Pediococcus acidilactici* to control *Listeria monocytogenes* in temperature-abused vacuum-packaged wieners. *J. Food Prot.* 55:98–103.

Delves-Broughton, J. 1990. Nisin and its uses as a food preservative. *Food Technol.* 44:100–117.

Duffy, L. L., P. B. Vanderlinde, and F. H. Grau. 1994. Growth of *Listeria monocytogenes* on vacuum-packed cooked meats: Effects of pH, a_w, nitrite and ascorbate. *Int. J. Food Microbiol.* 23:377–390.

Dykes, G. A., and J. W. Hastings. 1998. Fitness costs associated with class IIa bacteriocin resistance in *Listeria monocytogenes* B73. *Lett. Appl. Microbiol.* 26:5–8.

Enan, G., S. Alalyan, H. A. Abdel-salam, and J. Debevere. 2002. Inhibition of *Listeria monocytogenes* LMG10470 by plantaricin UG1 *in vitro* and in beef meat. *Nahrung.* 46:411–414.

Ennahar, S., T. Sashihara, K. Sonomoto, and A. Ishizaki. 2000. Class lla bacteriocins: Biosynthesis, structure and activity. *FEMS Microbiol Rev.* 24:85–106.

European Commission. 1995. European Parliament and Council Directive No 95/2/EC of 20 February 1995 on food additives other than colours and sweeteners (Official Journal L 061). Brussels, Belgium.

European Commission. 1997. Regulation (EC) No 258/97 of the European Parliament and of the Council of 27 January 1997 concerning novel foods and novel food ingredients (Official Journal L043). Brussels, Belgium.

European Commission. 2002. Position paper of the Scientific Committee on Animal Nutrition. Brussels, Belgium: Health and Consumer Protection Directorate-General.

European Commission. 2003. On generic approach to the safety assessment of microorganisms used in feed/food and feed/food production. Brussels, Belgium: Health and Consumer Protection Directorate-General.

European Council. 1994. Council Directive 94/65/EC of 14 December 1994 laying down the requirements for the production and placing on the market of minced meat and meat preparations (Official Journal L 368). Brussels, Belgium.

European Council. 2002. Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety (Official Journal L031). Brussels, Belgium.

European Council. 2003. Regulation (EC) No 1642/2003 of the European Parliament and of the Council of 22 July 2003 amending Regulation (EC) No 178/2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety (Official Journal L245). Brussels, Belgium.

Fadda, S., M. T. Aymerich, M. Hugas, and M. Garriga, M. 2005. Use of a GMP/GHP HACCP checklist to evaluate the hygienic status of traditional dry sausages workshops. *Food Prot. Trends*. 25:522–530.

Fang, T. J., and L. W. Lin. 1994a. Growth of *Listeria monocytogenes* and *Pseudomonas fragi* on cooked pork in a modified atmosphere packaging/Nisin combination system. *J. Food Prot*. 57:479–485.

Fang, T. J., and L. W. Lin. 1994b. Inactivation of *Listeria monocytogenes* on raw pork treated with modified atmosphere packaging and nisin. *J. Food Drug Analy.* 2:189–200.

Farber, J. M., Y. Cai, and W. H. Ross. 1996. Predictive modeling of the growth of *Listeria monocytogenes* in CO_2 environments. *Int. J. Food Microbiol*. 32:133–144.

Foegeding, P. M., A. B. Thomas, D. H. Pilkington, and T. R. Klaenhammer. 1992. Enhanced control of *Listeria monocytogenes* by in situ-produced pediocin during dry fermented sausage production. *Appl. Environ. Microbiol*. 58:884–890.

Food and Drug Administration. 1988. Nisin preparation: Affirmation of GRAS status as a direct human food ingredient. *Federal Register.* 53:11247–11250.

Franklin, N. B., K. D. Cooksey, and K. J. K. Getty. 2004. Inhibition of *Listeria monocytogenes* on the surface of individually packaged hot dogs with packaging film coating containing nisin. *J. Food Prot*. 67:480–485.

Frazer, A. C., M. Sharott, and J. R. Hickman. 1962. The biological effects of food additives: I. Nisin. *J. Sci. Food Agric*. 13:32–42.

Gänzle, M. G., C. Hertel, and W. P. Hammes. 1996. Medellhafte Beschreibung des Effektes von Sakacin P auf *Listeria ivanovii* DSM20750 in Abhängigkeit von pH-Wert, NaCl- und Nitritkonzentration. *Fleischwirtschaft*. 76:409–412.

Garriga, M., M. T. Aymerich, S. Costa, J. M. Monfort, and M. Hugas. 2002. Bactericidal synergism through bacteriocins and high pressure in a meat model system during storage. *Food Microbiol*. 19:509–518.

Garriga, M., M. Hugas, P. Gou, M. T. Aymerich, J. Arnau, and J. M. Monfort. 1996. Technological and sensorial evaluation of *Lactobacillus* strains as starter cultures in fermented sausages. *Int. J. Food Microbiol*. 32:173–183.

Garriga, M., B. Marcos, B. Martín, M. T. Veciana-Nogues, S. Bover-Cid, M. Hugas, and M. T. Aymerich. 2005. Starter cultures and high pressure processing to improve the hygiene and safety of slightly fermented sausages. *J. Food Prot*. 68:2341–2349.

Gay, M., O. Cerf, and K. R. Davey. 1996. Significance of pre-incubation temperature and inoculum concentration on subsequent growth of *Listeria monocytogenes* at 14 degrees C. *J. Appl. Bacteriol*. 81:433–438.

Genigeorgis, C. A. 1989. Present state of knowledge on staphylococcal intoxication. *Int. J. Food Microbiol*. 9:327–360.

Gill, A. O., and R. A. Holley. 2002. Interactive inhibition of meat spoilage and pathogenic bacteria by lysozyme, nisin and EDTA in the presence of nitrite and sodium chloride at 24°C. *Int. J. Food Microbiol*. 80:251–259.

Gravesen, A., A. M. Jydegaard-Axelsen, J. Mendes da Silva, T. B. Hansen, and S. Knochel. 2002. Frequency of bacteriocin resistance development and associated fitness costs in *Listeria monocytogenes*. *Appl. Environ. Microbiol*. 68:756–764.

Hagen, B. F., and A. Holck. 1998. Manganese accelerates fermentation of dry fermented sausages. In *44th International Congress of Meat Science and Technology*, Barcelona, Spain, 822–823.

Holzapfel, W. H., R. Geisen, and U. Schillinger. 1995. Biological preservation of foods with reference to protective cultures, bacteriocins and food-grade enzymes. *Int. J. Food Microbiol*. 24:343–362.

Hoover, D. G., C. Metrick, A. M. Papineau, D. F. Farkas, and D. Knorr. 1989. Biological effects of high hydrostatic pressure on food microorganisms. *Food Technol. 43*(3):99–107.

Hugas, M., M. Garriga, M. T. Aymerich, and M. Monfort. 1995. Inhibition of *Listeria* in dry fermented sausages by the bacteriocinogenic *Lactobacillus sake* CTC494. *J. Appl. Bacteriol.* 79:322–330.

Hugas, M., M. Garriga, M. T. Aymerich, and J. M. Monfort. 2002. Bacterial cultures and metabolites for the enhancement of safety and quality in meat products. In *Research advances in the quality of meat products,* ed. F. Toldrà, 225–247. Trivandrum, India: Research Signpost.

Hugas, M., M. Garriga, M. Pascual, M. T. Aymerich, and J. M. Monfort. 2002. Enhancement of sakacin K activity against *Listeria monocytogenes* in fermented sausages with pepper or manganese as ingredients. *Food Microbiol.* 19:519–528.

Hugas, M., B. Neumeyer, F. Pagés, M. Garriga, and W. P. Hammes. 1997. Comparison of bacteriocin-producing lactobacilli on *Listeria* growth in fermented sausages. *Fleischwirtsch Int.* 76:649–652.

Hugas, M., F. Pagés, M. Garriga, and J. M. Monfort. 1998. Application of the bacteriocinogenic *Lactobacillus sakei* CTC494 to prevent growth of *Listeria* in fresh and cooked meat products packed with different atmospheres. *Food Microbiol.* 15:639–650.

Hurst, A. 1981. Nisin. *Adv. Appl. Microbiol.* 27:85–123.

International Commission on Microbiological Specifications for Foods. 1996. Establishment of sampling plans for microbiological safety criteria for foods in international trade, including recommendations for control *Listeria monocytogenes*, *Salmonella enteritidis*, *Campylobacter* and enterohemorragic *E. coli* (Agenda item 11, CX/FH 96/91-16). Rome, Italy: Codex Committee on Food Hygiene.

Jacobsen, T., B. B. Budde, and A. G. Koch. 2003. Application of *Leuconostoc carnosum* for biopreservation of cooked meat products. *J. Appl. Microbiol.* 95:242–249.

Johnson, J. L., M. P. Doyle, R. G. Cassens, and J. L. Schoeni. 1988. Fate of *Listeria monocytogenes* in tissues of experimentally infected cattle and in hard salami. *Appl. Environ. Microbiol.* 54:497–501.

Joint FAO/WHO Expert Committee on Food Additives. 1969. 12th report of the Joint FAO/WHO Expert Committee on Food Additives (WHO Tech. Rep. Series No. 430, FAO Nutrition meetings Rep. Series No. 45). Geneva, Switzerland: FAO/WHO.

Jung, D. S., F. W. Bodyfelt, and M. A. Daeschel. 1992. Influence of fat and emulsifiers on the efficacy of nisin in inhibiting *Listeria monocytogenes* in fluid milk. *J. Dairy Sci.* 75:387–393.

Junttila, J., J. Hirn, P. Hill, and E. Nurmi. 1989. Effect of different levels of nitrite and nitrate on the survival of *Listeria monocytogenes* during the manufacture of fermented sausage. *J. Food Prot.* 52:158–161.

Kalchayanand, N., T. Sikes, C. P. Dunne, and B. Ray. 1994. Hydrostatic pressure and electroporation have increased bactericidal efficiency in combination with bacteriocins. *Appl. Environ. Microbiol.* 60:4174–4177.

Kalchayanand, N., T. Sikes, C. P. Dunne, and B. Ray. 1998. Interaction of hydrostatic pressure, time and temperature of pressurization and pediocin AcH on inactivation of foodborne bacteria. *J. Food Prot.* 61:425–431.

Kathariou, S. 2002. Listeria monocytogenes virulence and patogenicity, a food safety perspective. *J. Food Prot.* 65:1811–1829.

Katla, T., T. Moretro, I. Sveen, I. M. Aesen, L. Axelsson, L. M. Rorvik, and K. Naterstad. 2002. Inhibition of *Listeria monocytogenes* in chicken cold cuts by addiction of sakacin P and sakacin P-producing *Lactobacillus sakei*. *J. Appl. Microbiol.* 93:191–196.

Klaenhammer, T. R. 1993. Genetics of bacteriocins produced by lactic acid bacteria. *FEMS Microbiol. Rev.* 12:39–85.

Kröckel, L. 1997. Use of sakacin P and Lactobacillus sake LB674 to suppress growth of *Listeria monocytogenes* in vacuum-packaged sliced bologna-type sausage. In *Proceedings LACTIC97, Caen (France),* 201.

Kuleasan, H., and M. L. Cakmakci. 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.

Lahti, E., T. Johansson, T. Honkanen-Buzalski, P. Hill, and E. Nurmi. 2001. Survival and detection of *Escherichia coli* O157:H7 and *Listeria monocytogenes* during the manufacture of dry sausage using two different starter cultures. *Food Microbiol*. 18:75–85.

Larsen, A. G., and B. Norrung. 1993. Inhibition of *Listeria monocytogenes* by bavaricin A, a bacteriocin produced by *Lactobacillus bavaricus* MI401. *Lett. Appl. Microbiol.* 17:132–134.

Lauková, A., S. Czikková, S. Laczková, and P. Turek. 1999. Use of enterocin CCM 4231 to control *Listeria monocytogenes* in experimentally contaminated dry fermented Hornád salami. *Int. J. Food Microbiol.* 52:115–119.

Lauková, A., P. Turek, M. Mareková, and J. Nagy. 2003. Use of ent M, new variant of ent P to control *Listeria innocua* in experimentally contaminated Gombasek sausage. *Archiv fur Lebensmittelhygiene. 54*(2):46–48.

Leistner, L. 2000. Basic aspects of food preservation by hurdle technology. *Int. J. Food Microbiol.* 55:181–186.

Leroy, F., and L. De Vuyst. 1999. Temperature and pH conditions that prevail during fermentation of sausages are optimal for production of the antilisterial bacteriocin sakacin K. *Appl. Environ. Microbiol.* 65:974–981.

Liserre, A. M., M. Landgraf, M. T. Destro, and B. D. G. M. Franco. 2002. Inhibition of *Listeria monocytogenes* by a bacteriocinogenic *Lactobacillus sake* strain modified atmosphere-packaged Brazilian sausage. *Meat Sci.* 61:449–455.

Luchansky, J. B., K. A. Glass, K. D. Harsono, A. J. Degnan, N. G. Faith, B. Cauvin, G. Baccus-Taylor, K. Arihara, B. Bater, A. J. Maurer, and R. G. Cassens. 1992. Genomic analyisis of *Pediococcus* starter cultures used to control *Listeria monocytogenes* in turkey summer sausage. *Appl. Environ. Microbiol.* 58:3053–3059.

Mattila, K., P. Saris, and S. Työppönen. 2003. Survival of *Listeria monocytogenes* on sliced cooked sausage after treatment with pediocin AcH. *Int. J. Food Microbiol.* 89:281–286.

Mauriello, G., D. Ercolini, A. La Storia, A. Casaburi, and F. Villani. 2004. Development of polythene films for food packaging activated with an antilisterial bacteriocin from *Lactobacillus curvatus* 32Y. *J. Appl. Microbiol.* 97:314–322.

Mead, P. S., L. Slutsker, V. Dietz, L. F. McCaig, J. S. Bresee, C. Shapiro, P. M. Griffin, and R. V. Tauxe. 1999. Food-related illness and death in the United States. *Emerg. Infect. Dis.* 5:607–625.

Messens, W., J. Verluyten, F. Leroy, and L. De Vuyst. 2003. Modelling growth and bacteriocin production by *Lactobacillus curvatus* LTH 1174 in response to temperature and pH values used for European sausage fermentation processes. *Int. J. Food Microbiol.* 81:41–52.

Ming, X., and M. A. Daeschel. 1993. Nisin resistance of foodborne bacteria and the specific resistance responses of *Listeria monocytogenes* Scott A. *J. Food Prot.* 56:944–948.

Ming, X., G. H. Weber, J. W. Ayres, and W. E. Sandine. 1997. Bacteriocins applied to food packaging materials to inhibit *Listeria monocytogenes* on meats. *J. Food Sci.* 62:413–415.

Moll, G. N., W. N. Konings, and A. J. Driessen. 1999. Bacteriocins: Mechanism of membrane insertion and pore formation. *Antonie Van Leeuwenhoek. 76*(1–4):185–198.

Murray, M., and J. A. Richard. 1997. Comparative study of the antilisterial activity of nisin and pediocin AcH in fresh ground pork stored aerobically at 5°C. *J. Food Prot.* 60:1534–1540.

Natrajan, N., and B. Sheldon. 2000a. Efficacy of nisin-coated polymer films to inactive *Salmonella* THIPHYMURIUM of fresh broiler skin. *J. Food Prot.* 63:1189–1196.

Natrajan, N., and B. W. Sheldon. 2000b. Inhibition of *Salmonella* on poultry skin using protein- and polysaccharide-based films containing a nisin formulation. *J. Food Prot.* 63:1268–1272.

Nes, I. F., D. B. Diep, L. S. Håvarstein, and M. B. Brurberg. 1996. Biosynthesis of bacteriocins in lactic acid bacteria. In *Lactic acid bacteria: Genetics, metabolism and applications,* eds. G. Venema, J. H. J. Huis in't Veld, and J. Hugenholtz, 113–128. Veldhoven, The Netherlands: Kluwer Academic.

Nes, I. F., and H. Holo. 2000. Class II antimicrobial peptides from lactic acid bacteria. *Biopolymers.* 55:50–61.

Norrung, B., J. K. Andersen, and J. Schlundt. 1999. Incidence and control of *Listeria monocytogenes* in foods in Denmark. *Int. J. Food Microbiol.* 53:195–203.

Ojeniyi, B., H. C. Wegener, N. E. Jensen, and M. Bisgaard. 1996. *Listeria monocytogenes* in poultry and poultry products: Epidemiological investigations in seven Danish abattoirs. *J. Appl. Bacteriol.* 80:395–401.

Pawar, D. D., S. V. S. Malik, K. N. Bhilegaonkar, and S. B. Barbuddhe. 2000. Effect of nisin and its combination with sodium chloride on the survival of *Listeria monocytogenes* added to raw buffalo meat mince. *Meat Sci.* 56:215–219.

Peccio, A., T. Autio, H. Korkeala, R. Rosmini, and M. Trevisani. 2003. *Listeria monocytogenes* occurrence and characterisation in meat-producing plants. *Lett. Appl. Microbiol.* 37:234–238.

Rasch, M., and S. Knochel. 1998. Variations in tolerance of *Listeria monocytogenes* to nisin, pediocin PA-1 and bavaricin A. *Lett. Appl. Microbiol.* 27:275–278.

Rekhif, N., A. Atrih, and G. Lefebvre. 1994. Selection and properties of spontaneous mutants of *Listeria monocytogenes* ATCC 15313 resistant to different bacteriocins produced by lactic acid bacteria strains. *Curr. Microbiol.* 28:237–241.

Sabia, C., S. de Niederhäusern, P. Messi, G. Manicardi, and M. Bondi. 2003. Bacterocin-producing *Enterococcus casseliflavus* IM 416K1, a natural antagonist for control of *Listeria monocytogenes* in Italian sausages ("cacciatore"). *Int. J. Food Microbiol.* 87:173–179.

Salvat, G., M. T. Toquin, Y. Michel, and P. Colin. 1995. Control of *Listeria monocytogenes* in the delicatessen industries: The lessons of a listeriosis outbreak in France. *Int. J. Food Microbiol.* 25:75–81.

Samelis, J., and J. Metaxopoulos. 1999. Incidence and principal sources of *Listeria* spp. and *Listeria monocytogenes* contamination in processed meat and a meat processing plant. *Food Microbiol.* 16:465–477.

Scannell, A. G. M., C. Hill, D. J. Buckley, an E. K. Arendt. 1997. Determination of the influence of organic acids and nisin on shelf-life and microbiological safety aspects of fresh pork sausage. *J. Appl. Microbiol.* 83:407–412.

Scannell, A. G. M., C. Hill, R. P. Ross, S. Marx, W. Hartmeier, and E. K. Arendt. 2000. Development of bioactive food packaging materials using immobilized bacteriocins Lacticin 3147 and Nisaplin. *Int. J. Food Microbiol.* 60:241–249.

Schillinger, U., M. Kaya, and F. K. Lücke. 1991. Behaviour of *Listeria monocytogenes* in meat and its control by bacteriocin-producing strain of *Lactobacillus sake*. *J. Appl. Bacteriol.* 70:473–478.

Schillinger, U., and F. K. Lücke. 1989. Inhibiting salmonella growth in fresh spreadable Mettwurst (dry sausage eaten relatively fresh) made without sugar. *Fleischwirtsch.* 69:879–882.

Schlyter, J. H., K. A. Glass, J. Loeffelholz, A. J. Degnan, and J. B. Luchansky. 1993. The effects of diacetate with nitrite, lactate, or pediocin on the viablity of *Listeria monocytogenes* in turkey slurries. *Int. J. Food Microbiol.* 19:271–281.

Schöbitz, R., T. Zaror, O. León, and M. Costa. 1999. A bacteriocin from *Carnobacterium piscicola* for the control of *Listeria monocytogenes* in vacuum-packaged meat. *Food Microbiol.* 16:249–255.

Sheridan, J. J., G. Duffy, D. A. McDowell, and I. S. Blair. 1994. The occurrence and initial numbers of Listeria in Irish meat and fish products and the recovery of injured cells from frozen products. *Int. J. Food Microbiol.* 22:105–113.

Shtenberg, A. J., and A. D. Ignat'ev. 1970. Toxicological evaluation of some combination of food preservatives. *Food Cosmet. Toxicol.* 8:369–380.

Sobrino, O. J., J. M. Rodríguez, W. L. Moreira, M. F. Fernández, B. Sanz, and P. E. Hernández. 1991. Antibacterial activity of *Lactobacillus sake* isolated from dry sausages. *Int. J. Food Microbiol.* 13:1–10.

Song, H.-J., and J. Richard. 1997. Antilisterial activity of three bacteriocins used at subminimal inhibitory concentrations and cross-resistance of the survivors. *Int. J. Food Microbiol.* 36:155–161.

Stevens, K. A., B. W. Sheldon, N. A. Klapes, and T. R. Klaenhammer. 1991. Nisin treatment for inactivation of *Salmonella* species and other Gram-negative bacteria. *Appl. Environ. Microbiol.* 57:3613–3615.

Stevens, K. A., B. W. Sheldon, N. A. Klapes, and T. R. Klaenhammer. 1992. Effect of treatment conditions on nisin inactivation of Gram-negative bacteria. *J. Food Prot.* 55:763–766.

Stringer, M. 2004. Europe report on food safety objectives: Role in microbiological food safety management Brussels, Belgium: International Life Sciences Institute.

Thomas, L. V., and J. W. T. Wimpenny. 1996. Investigation of the effect of combined variations in temperature, pH, and NaCl concentration on nisin inhibition of *Listeria monocytogenes* and *Staphylococcus aureus*. *Appl. Environ. Microbiol.* 62:2006–2012.

Thorns, C. J. 2000. Bacterial food-borne zoonoses. *Rev. Sci. Tech.* 19:226–239.

Tichaczek, P. S., J. Nissen-Meyer, I. F. Nes, R. F. Vogel, and W. P. Hammes. 1992. Characterization of the bacteriocins curvacin A from *Lactobacillus curvatus* LTH1174 and sakacin P from *L. sake* LTH673. *Sys. Appl. Microbiol.* 15:460–468.

Ukuku, D. O., and L. A. Shelef. 1997. Sensitivity of six strains of *Listeria monocytogenes* to nisin. *J. Food Prot.* 60:867–869.

van den Elzen, A. M., and J. M. Snijders. 1993. Critical points in meat production lines regarding the introduction of *Listeria monocytogenes*. *Vet. Quart.* 15:143–145.

Vázquez-Boland, J. A., Domínguez-Bernal, G., González-Zorn, B., Kreft, J., Goebel, W. 2001. Pathogenicity islands and virulence evolution in *Listeria*. *Microbes Infect.* 3:571–584.

Venema, K., Venema, G., Kok, J. 1995. Lactococcal bacteriocins: mode of action and immunity. *Trends Microbiol.* 3(8):299–304.

Vignolo, G., Fadda, S., de Kairuz, M. N., de Ruiz Holgado, A. A. P., Oliver, G. 1996. Control of *Listeria monocytogenes* in ground beef by lactocin 705, a bacteriocin produced by *Lactobacillus casei* CRL 705. *Int. J. Food Microbiol.* 29:397–402.

Wing, E. J., and S. H. Gregory. 2002. *Listeria monocytogenes*: Clinical and experimental update. *J. Infect. Dis.* 185(Suppl.1):18–24.

Winkowski, K., A. D. Crandall, and T. J. Montville. 1993. Inhibition of *Listeria monocytogenes* by *Lactobacillus bavaricus* MN in beef systems at refrigeration temperatures. *Appl. Environ. Microbiol.* 59:2552–2557.

Yang, R., M. C. Johnson, and B. Ray. 1992. Novel method to extract large amounts of bacteriocins from lactic acid bacteria. *Appl. Environ. Microbiol.* 58:3355–3359.

16 Latest Developments in Meat Bacterial Starters

Régine Talon and Sabine Leroy
INRA, Microbiologie, Centre de Recherches,
Clermont Ferrand Theix

CONTENTS

16.1 Bacterial Meat Starter Cultures 402
16.2 Protective Cultures 403
16.3 Starters and Sensory Quality 404
16.3.1 Texture 404
16.3.2 Color 404
16.3.3 Flavor 404
16.3.3.1 Carbohydrate Catabolism 405
16.3.3.2 Protein Hydrolysis 406
16.3.3.3 Amino Acid Catabolism 407
16.3.3.4 Lipid Hydrolysis 408
16.3.3.5 Fatty Acid Oxidation 408
16.3.3.6 Formation of Esters 409
16.4 Probiotic Cultures 409
16.5 Toward Genomics and Postgenomics to Characterize Starters 411
16.6 Conclusion 413
References 413

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, and thus for fermented meat products, the characterization of micro-organisms responsible for the fermentation, toward the end of the 19th century, led to the development of starter cultures. This development had a major impact on the process and contributed to ensuring consistency of product and reliability of fermentation. Research on starter cultures continued to advance at a very impressive rate through the 20th century and considerable knowledge was developed to manipulate and control these bacteria.

The original and primary purpose of fermentation was to ensure the shelf life and microbiological safety of 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 safeguarding 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 recently developed.

16.1 BACTERIAL MEAT STARTER CULTURES

Most European fermented sausages formulated with nitrates or nitrites are produced with a starter culture, generally consisting of lactic acid bacteria and catalase-positive cocci. In table 16.1, the subspecies of *Lactobacillus curvatus* and *Lactobacillus sakei* are not mentioned because it is not known to which subspecies the commercial starter strains have to be allotted. For *Staphylococcus carnosus*, the two subspecies are used as starter cultures (Hammes and Hertel 1998). *Staphylococcus equorum* is more and more often mentioned in dry-fermented sausages (Mauriello, Casaburi, Blaiotta, and Villani 2004). It is used as commercial starter culture and could be misidentified as *Staphylococcus xylosus* (Morot-Bizot, Talon, and Leroy-Setrin 2003). *S. xylosus* is widely isolated in fermented meat products and usually used as starter culture (Talon, Leroy-Sétrin, and Fadda 2002). Care should be taken in the selection of *Staphylococcus xylosus* starters because some strains exhibit the potential for staphylococcal enterotoxin formation (Rodriguez, Núńez, Córdoba, Bermudez, and Asensio 1996).

The probiotic cultures referred to in the literature as the *Lactobacillus acidophilus* group include *Lactobacillus acidophilus, Lactobacillus amylovorus, Lactobacillus crispatus, Lactobacillus gallinarum, Lactobacillus gasseri,* and *Lactobacillus johnsonii* (Holzapfel, Haberer, Snel, Schillinger, and Huis in't Veld 1998). Most of these

TABLE 16.1
Main Bacteria Species Used in Meat Fermentation

Lactobacillus	*L. acidophilus*[a], *L. alimentarius, L. casei*[a], *L. curvatus, L. farciminis, L. pentosus, L. plantarum, L. rhamnosus*[a], *L. sakei*
Lactococcus	*L. lactis*
Pediococcus	*P. acidilactici, P. pentosaceus*
Bifidobacterium	*Bifidobacterium* spp.[a], *B. lactis*[a], *B. longum*[a]
Staphylococcus	*S. carnosus* subsp. *carnosus, S. carnosus* subsp. *utilis, S. equorum, S. xylosus*
Kocuria	*K. varians*[b]

Note: Data from Hammes and Hertel (1998), Holzapfel, Haberer, Snel, Schillinger, and Huis in't Veld (1998), and Josephsen and Jespersen (2004).

[a] Used in probiotic cultures.

[b] Formerly *Micrococcus varians*.

species have been tested in sausage fermentation (Arihara et al. 1998). Many studies have considered *Lactobacillus rhamnosus* as a probiotic that can be used in meat fermentation (Erkkila, Petäjä, et al. 2001; Erkkila, Suihko, Eerola, Petaja, and Mattila-Sandholm 2001; Sameshima et al. 1998).

16.2 PROTECTIVE CULTURES

The use of bacterial starter cultures with protective effect can enhance the safety of meat products and prevent potential food-borne microbial hazard. Novel strategies using a biopreservation approach are investigated to "naturally" control the growth of pathogenic and spoilage micro-organisms. Rapid growth and production of acids by bacterial meat starter cultures are important for sensory qualities of fermented sausages and for controlling undesirable bacteria. Lactic acid bacteria can inhibit the growth of these micro-organisms by a variety of antimicrobial agents such as organic acids, bacteriocins, and the competition for nutrients in the product (Granly Koch 2004; Klaenhammer 1993). Organic acids including lactic, acetic, and propionic 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 control of *Listeria monocytogenes*, an important food-borne pathogen that can contaminate meat during processing, is of major concern for the meat industry. In vacuum- or modified-atmosphere-packaged samples of sliced, cooked, cured pork meat, the inoculation of bacteriocins-producing *Leuconostoc mesenteroides* and *L. curvatus* strains isolated from dry-fermented sausages resulted in a decrease of the *Listeriae* population by about 1.5 log (Mataragas, Drosinos, and Metaxopoulos 2003). Strains of *L. rhamnosus* and *Lactobacillus plantarum* inoculated in sausage batter experimentally contaminated by *L. monocytogenes* expressed antilisterial activity resulting in *Listeria*-negative fermented sausage at an early stage in the ripening process (Tyopponen, Markkula, Petaja, Suihko, and Mattila-Sandholm 2003). *Pediococcus acidilactici* stimulated by manganese can control the growth of *Escherichia coli* O157:H7 in a laboratory medium but also during beef sausage fermentation (Kang and Fung 1999). The bacteriocinogenesis and the growth of lactic acid bacteria used as protective flora can be inhibited by sodium nitrite and sodium chloride added in the meat batter, but this inhibition is limited by the anaerobic conditions of the fermentation step (Verluyten, Messens, and De Vuyst 2003). Moreover, the lactic acid bacteria strains can be more or less sensitive to this inhibition and their performance can be enhanced by the optimization of preinoculation treatments (Scannell, Schwarz, Hill, Ross, and Arendt 2001).

Most works on bacteriocins concerned lactic acid bacteria and only few data mentioned the production of bacteriocins by catalase-positive cocci (Sahl 1994). However, it has been shown that a strain of *S. xylosus* isolated from Italian sausages produced an inhibitory substance active against *L. monocytogenes* on a solid medium but also in Naples-type sausages in which a 2 log reduction of viable counts of *L. monocytogenes* was measured (Villani et al. 1997). Moreover, the presence of *S. xylosus* in a multispecies biofilm with *L. monocytogenes* and *Pseudomonas fragi* resulted in the decreasing competitiveness of *L. monocytogenes*, compared to its

capacity of adherence in monoculture biofilms (Norwood and Gilmour 2001). *S. equorum* has also been evaluated with respect to its antilisterial potential. 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 *L. monocytogenes* (Carnio et al. 2000).

16.3 STARTERS AND SENSORY QUALITY

Sausages are made with minced meat and fat, mixed with salt and spices, often inoculated by 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.

16.3.1 Texture

During chopping, salt solubilizes muscle proteins (mainly myosin), which on the drop in pH during fermentation coagulate and form gel surrounding 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, Leroy-Sétrin, and Fadda 2004). Acidification by lactic acid bacteria 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).

16.3.2 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 (Ordóñez, Hierro, Bruna, and De la Hoz 1999).

The substrate added to produce NO could be nitrate or nitrite. Use of nitrate involves bacterial reduction to nitrite. It is carried out by cocci Gram-positive bacteria but their nitrate reductases are inhibited by pH below 5.2 (Talon et al. 2004). 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 lactic acid bacteria.

Discoloration of cured meat can be observed by the formation of peroxide. This default can be avoided by the catalase activity of cocci Gram-positive bacteria that protect the color (Nychas and Arkoudelos 1990).

16.3.3 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

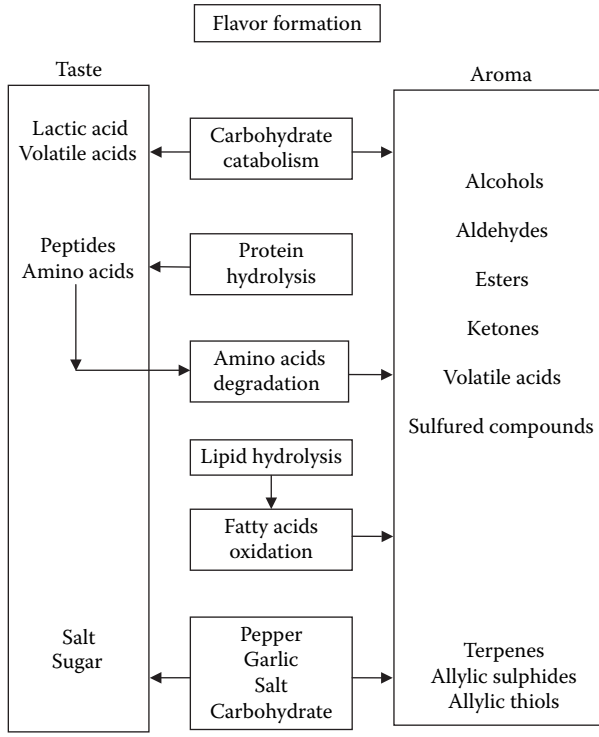


FIGURE 16.1 Formation of important flavor compounds in fermented sausages. Data from Stahnke (2002) and Talon, Leroy-Sétrin, and Fadda (2002).

of spices, and smoke. Others result from the catabolism of carbohydrates, proteins, and lipids from tissue and microbial enzyme reactions and chemical reactions (figure 16.1). Currently, it is still difficult to distinguish between tissue enzymes and microbial enzymes, and also to determine the origin of certain compounds. (For reviews in this field, see Demeyer and Toldrá 2004; Stahnke 2002; Talon et al. 2002; Talon et al. 2004.)

16.3.3.1 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 to 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 lactic acid bacteria (Dainty and Blom 1995).

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 1998). *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, CO_2, and ethanol (Champomier-Vergès, Chaillou, Cornet, and Zagorec 2002) or acetate instead of ethanol (Axelsson 1998).

The role of staphylococci in the carbohydrate catabolism is minor. Yet it has been shown that *Staphylococcus warneri* can produce D- and L-lactate under laboratory conditions, and when inoculated into sausage it can lead to an increase in the D-lactate content. Staphylococci can contribute to the formation of acetic acid (Talon et al. 2002).

Acetic acid 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 (Schmidt and Berger 1998; Stahnke, Sunesen, and De Smedt 1999).

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 the staphylococci (Berdagué, Monteil, Montel, and Talon 1993). Montel, Reitz, Talon, Berdagué, and Rousset-Akrim (1996) have shown the correlation between high diacetyl and acetoin production levels by *Staphylococcus saprophyticus* and *S. warneri* strains under laboratory conditions, and high desorption levels of these compounds in products inoculated with these strains. Stahnke and Sondergaard (2002) have shown that *S. xylsosus* and *S. equorum* produce more diacetyl and acetoin than *S. carnosus*.

16.3.3.2 Protein Hydrolysis

During sausage ripening both the soluble and insoluble proteins are hydrolyzed into smaller proteins and peptides. Myofibrillar proteins are the most degraded proteins with 75% and 56% of the myosin (188 Kda) and actin (42.5 Kda) being degraded into several smaller proteins (13–122 Kda) after 21 days of ripening (Molly et al. 1997). Their breakdown into peptides is due to endogenous enzymes because the use of antibiotics and paucimicrobial meat incubations does not reduce degradation of actin, myosin, or troponin (Molly et al. 1997). Bacterial starters have low proteolytic activity on myofibrillar proteins (Kröckel 1995). Lactic acid bacteria indirectly contribute to proteolysis by reducing the pH, which increases cathepsin D activity (Molly et al. 1997).

Sarcoplasmic proteins are also degraded during sausage maturation. Bacteria are involved in this degradation. Certain *Staphylococcus* and *Kocuria* strains hydrolyze gelatin and sarcoplasmic proteins (Miralles, Flores and Perez-Martinez 1996; Selgas, Garcia, Garcia de Fernando, and Ordóñez 1993). *L. casei*, *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 (CFE), are used as an enzyme source (Fadda et al. 1999a, 1999b; Sanz et al. 1999).

The degradation of peptides into amino acids in sausage results in microbial and tissue activity, but great differences for the content of amino acids were noticed (Beriain, Lizaso, and Chasco 2000; Diaz, Fernández, Garcia de Fernando, de la Hoz,

and Ordóñez 1997; Waade and Stahnke 1997; Zapelena, Astiasarán, and Bello 1999). Verplaeste (1994) and Molly et al. (1997) attribute 40% of the degradation of peptides into amino acids to micro-organisms. Thereby, sausages containing antibiotics have lower amino acid concentrations than those inoculated with *Staphylococcus* and *Kocuria*. Peptidase activity was reported in *L. sakei*, *L. curvatus*, *L. casei,* and *P. pentosaceus* (De Masi, Wardlaw, Dick, and Acton 1990; Montel, Séronie, Talon, and Hébraud 1995; Sanz et al. 1999; Sanz and Toldrá 1997). However, at pH values below 6, such peptidase activity of *L. sakei* was weak (Montel et al. 1995). Fadda et al. (1999a, 1999b) observed high peptidase and aminopeptidase activity on sarcoplasmic or myofibrillar extracts in *L. plantarum*, *L. curvatus,* and *L. sakei* strains from meat.

Peptides and amino acids exhibit five different flavors: sweet, salty, acidic, bitter, and "umami" (Ordóñez et al. 1999). Peptides containing aspartic acid or glutamic acid are described as being acidic, those made up of hydrophobic amino acids are bitter, and others such as aspartame taste sweet (180 times sweeter than sucrose). Although the intrinsic sensory potential of amino acids is disputed, their role as precursors of aroma compounds is becoming more and more evident (Diaz et al. 1997; Toldrá 1998; Zapelena et al. 1999).

16.3.3.3 Amino Acid Catabolism

Amino acids can be transformed into amines, ammonia, or various compounds. Some of them have aromatic properties such as methyl aldehydes, methyl acids, and methyl alcohols.

Volatile amines have been poorly studied in fermented products although biogenic amines are often detected and have to be avoided because of their toxicity (Talon et al. 2002). Among these amines, only putrescine and cadaverine have unpleasant odors with high threshold values, and it is doubtful whether the amounts produced are sufficient to influence taste or smell.

Given its contribution to the increase in pH observed during drying, ammonia could also influence the sensory properties of compounds with ionizable groups (Dainty and Blom 1995). 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, valine), aromatic amino acids (phenylalanine, tyrosine, tryptophan), and sulfured amino acids (methionine), are catabolized into aldehydes, alcohols, and acids, which play an important role in acquiring flavor. With the exception of dimethyldisufide, which confers a putrid odor (Stahnke 2002), 2- or 3-methyl butanol with fermented fruit odors and, above all, 2- or 3-methyl butanal and 2-methyl propanal with malt and cacao odors are important in terms of aroma (Stahnke 2002; Talon et al. 2002). Acids confer an animal and cheese note on products (Stahnke 2002; Talon et al. 2002).

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 (Hinrichsen and Pedersen 1995). Nonetheless, the production of these molecules in sausage is modulated by the inoculated flora and, in particular, by staphylococci. *S. carnosus* is a higher producer of these molecules than *S. xylosus* and *S. equorum*

(Montel et al. 1996; Sondergaard and Stahnke 2002; Stahnke 1995). The catabolism of amino acids by staphylococci is influenced by various parameters such as temperature, salt, nitrate, nitrite, and pH (Olesen, Meyer, and Stahnke 2004; Olesen and Stahnke 2003; Olesen, Stahnke, and Talon 2004; Tjener, Stahnke, Andersen, and Martinussen 2004).

Lactic acid bacteria have restricted aromatic potential. Under laboratory conditions, *L. sakei*, *L. plantarum*, *L. curvatus,* and *P. acidilactici* only weakly degrade leucine, mainly into α–cetoisocaproate, a molecule with no odor (Larrouture, Ardaillon, Pépin, and Montel 2000).

16.3.3.4 Lipid Hydrolysis

Lipolysis in dry sausages releases free fatty acids with chain length between 16 and 18 carbon atoms (Toldrá and Navarro 2002). Lipolysis is of endogenous origin with triglyceride lipases and phospholipases (Molly, Demeyer, Civera, and Verplaeste 1996; Molly et al. 1997; Toldrá 1992). The importance of endogenous lipolysis has been demonstrated either by manufacturing aseptically or in paucimicrobial sausages inoculated by *Kocuria* or staphylococci and comparing the increase in the level of fatty acids (Hierro, De La Hoz, and Ordóñez 1997) and also by manufacturing sausages using antibiotics (Molly et al. 1996). According to Hierro et al. (1997), lipolysis by endogenous enzymes accounted for more than 60% of total free fatty acid release. Similarly, Johansson (1996) considers that about 30% of lipolysis arose from the lipolytic activity of *S. xylosus* inoculated in a sterile mixture of fat and pork lean. Some lipolytic bacteria such as *Staphylococcus* and *Kocuria* are described, nonetheless their activity is very low at a pH of 5.0, which is the pH of northern sausages, whereas at a pH near 6.0, the final pH of some Mediterranean sausages, some lipolytic activities are measured (Kenneally, Leuschner, and Arendt 1998; Talon, Dublet, Montel, and Cantonnet 1995; Talon, Montel, Gandemer, Viau, and Cantonnet 1993). Lipase activity of lactic acid bacteria in meat is very low (Talon et al. 2002).

Short-chain fatty acids have a taste of sour cheese. However, the longer the chain, the weaker the sensory characteristics are. Adding exogenous lipases significantly and rapidly increases the fatty acid concentration without really improving the flavor (Fernández, de la Hoz, Díaz, Cambero, and Ordóñez 1995; Zalacain, Zapelena, Astiasarán, and Bello 1995). In fact, fatty acids are mainly precursors of aroma molecules.

16.3.3.5 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—on the order of ppm—their low sensory threshold, except for alkanes and alkenes which are odorless, means that they have a real effect (Chizzolini, Novelli, and Zanardi 1998). Oxidation of fatty acids can be either chemical (peroxidation) or enzymatic (β-oxidation; Chizzolini et al. 1998).

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 (Chizzolini et al. 1998; Gray, Gomaa, and Buckley 1996). In sausages, micro-organisms play a vital role in the regulation of oxidation. Thereby, model sausages inoculated with *S. carnosus* and *S. xylosus* have lower alkane and aldehyde desorption levels than those inoculated with *S. warneri* and *S. saprophyticus* (Berdagué et al. 1993; Montel et al. 1996). The latter are characterized by a rancid aroma (Montel et al. 1996). Under laboratory conditions, *S. saprophyticus*, *S. warneri,* and especially *S. xylosus* and *S. carnosus* limit the oxidation of linoleic and linolenic unsaturated fatty acids (Talon, Walter, and Montel 2000). The catalase and superoxide dismutase (SOD) activities of *S. carnosus* have been characterized (Barrière, Leroy-Sétrin, and Talon 2001). In *S. xylosus* Barrière, Brückner and Talon (2001) and Barrière, Centeno, et al. (2001) used mutants deficient in SOD or catalase activity to show that these enzymes contribute to limiting the oxidation of unsaturated fatty acids.

The presence of methyl ketones in sausage is associated with the inoculation of *S. carnosus* or *S. xylosus* (Berdagué et al. 1993; Montel et al. 1996). These methyl ketones could arise from incomplete β-oxidation of fatty acids. Usually, β-oxidation degrades saturated fatty acids into acetic acid by successive elimination of acetyl CoA groups. However, 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, Féron, Perrin, Molle, and Talon (2000) have shown that intermediates were freed during the β-oxidation cycle in *S. carnosus*. They also reported thioesterase activity in this strain. Fadda, Lebert, and Talon (2002) and Fadda, Leroy-Sétrin, and Talon (2003) have highlighted the presence of β-decarboxylase activity in *S. carnosus*. These results suggest that *S. carnosus* may produce ketones via this pathway.

16.3.3.6 Formation of Esters

Esters are present in fermented meat products and their aromatic characteristics contribute to the fruity note of the products (Montel et al. 1996; Stahnke 1994). Most of the esters in sausages are ethyl esters. Their production depends on the presence of ethanol and different acids (from 2–8 carbon atoms) as well as on technological factors and micro-organisms.

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 laboratory media, some staphylococci have esterase activities capable of hydrolyzing and forming ethyl esters (Talon and Montel 1997). In sausages, esters are associated with the presence of *S. xylosus* or *S. carnosus* strains (Montel et al. 1996; Stahnke 1994).

16.4 PROBIOTIC CULTURES

The relationship between certain foods and 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 micro-organisms that beneficially affect the health of the host when ingested in adequate amounts (Guarner and Schaafsma 1998).

The success of probiotics in dairy foods, based on the increasing scientific evidence for the beneficial effects of certain well-defined strains (Holzapfel et al. 1998), has led to the development of probiotics for fermented meat products (table 16.1; Hugas and Monfort 1997; Tyopponen, Petaja, and Mattila-Sandholm 2003). The probiotic species originate first from isolates from the intestinal tract. This ecological niche is the natural habitat for *Bifidobacterium* and *L. acidophilus* (Hammes and Hertel 1998) and *L. rhamnosus* (Saxelin 1997). *L. casei* may be also found in the flora of the oral cavity, in various fermented foods, and in spoiled foods (Hammes and Hertel 1998).

Practical criteria have been formulated for selecting probiotic strains. The main requirements are acid and bile tolerances, safety in use, and clinical documentation of health effects (Erkkila and Petaja 2000; Holzapfel et al. 1998). To act as a probiotic, the bacteria must be able to encompass the first challenge: survival in the acidic conditions found in the stomach. The gastric juice has a very low pH of 0.9. However, the presence of food raises the pH value to 3.0 and it takes 2 to 4 hours for the stomach to empty after ingestion of food (Erkkila and Petaja 2000). The second challenge is resistance to the bile salts in the duodenum (Holzapfel et al. 1998). The bile salts have a detergent action on the lipids of micro-organism cell membranes. However, some *Lactobacillus* are able to hydrolyze bile salts via bile salt hydrolase enzyme (Erkkila and Petaja 2000). Resistance to bile salts is variable among the *Lactobacillus* species and strains.

Considering the criteria of acid and bile tolerances, Erkkila and Petaja (2000) screened eight commercial meat starter cultures for their potential probiotic use. It appears that one strain of *L. sakei* and one of *Pediococcus acidilactici* had the best survival capacities under acidic conditions and high concentration of bile salts. Recently, the selection of potentially probiotic lactobacilli from Greek fermented sausages (Papamanoli, Tzanetakis, Litopoulou-Tzanetaki, and Kotzekidou 2003) and Italian fermented sausages (Pennacchia et al. 2004) was realized. Among the Greek sausages, 58% of *L. curvatus* strains (24 isolates) and the seven *L. plantarum* isolates were resistant to 0.3% bile salts. From Italian sausages, strains belonging to the *L. plantarum* and *L. casei* groups were found to be of potential use in the future as probiotic starter cultures for the manufacture of novel fermented sausages.

Some studies describe the behavior of selected probiotic strains for their use in sausage production. Arihara et al. (1998) investigated the potentiality of the probiotic *L. acidophilus* group for their application in meat fermentation. Among the six species, *L. gasseri* was the most appropriate one; it grew in the sausage model and decreased the pH during fermentation. It was also able to inhibit the growth and the production of toxins of *Staphylococcus aureus* inoculated in the model. In parallel, the same group of authors (Sameshima et al. 1998) studied the effect of intestinal *Lactobacillus* starter cultures on the behavior of *S. aureus* in dry-fermented sausages. Of three intestinal *Lactobacillus* strains, *L. rhamnosus* and *L. paracasei* subsp. *paracasei* inhibited the growth and enterotoxin production of *S. aureus*, whereas *L. acidophilus* could not satisfactorily suppress them. The effect of the first two lactobacilli in meat fermentation (fermentation time, acidification) was the same as that of a commercial *L. sakei* starter culture. These results suggest that intestinal *Lactobacillus* could be used for developing probiotic starter cultures in fermented sausages. Pidcock, Heard, and Henriksson (2002) investigated if nontraditional meat starter (NTMS) cultures can be used for

improving the safety of Hungarian salami. When used in conjunction with the commercial meat starter, nine NTMS cultures reduced *Escherichia coli* O111 count by more than 2.5 log units, and ten NTMS reduced *L. monocytogenes* count by more than 2.5 log units. The commercial meat starter alone reduced *E. coli* and *L. monocytogenes* by 1.2 and 1.3 log units, respectively. All NTMS cultures survived in salami throughout fermentation and maturation. It was concluded that NTMS cultures, including one strain of *L. acidophilus*, two of *L. paracasei*, one of *Lactobacillus* spp, and one of *Bifidobacterium lactis* may be used to increase safety of Hungarian salami.

Similarly, three strains of probiotic *L. rhamnosus,* one of *P. pentosaceus* (protective culture for malting procedure), and one of *L. plantarum* (with probiotic properties) were studied for their capacity to act as main fermenting bacteria in sausage manufacturing (Erkkila, Petäjä, et al. 2001). All these bacteria, inoculated in sausage, increased during the fermentation from the level of 6.5 to 7.0 to 8.0 to 9.0 log CFU/g and their presence was confirmed by ribotyping. 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. These probiotic lactic acid bacteria, which did not produced biogenic amines in laboratory tests, did not produce these amines during the ripening of sausages (Erkkila, Suihko, et al. 2001). From all of these studies, it seems possible to successfully use probiotics as starter cultures for dry-fermented sausages, as there are no significant technological and sensorial differences between the sausages fermented by probiotics or nonprobiotics.

The question of usefulness of probiotics in meat products was discussed by Hammes and Hertel (1998). These authors underlined the fact that proof of a beneficial effect should be based on sound studies performed with the probiotics in the meat matrix as it is consumed. Recently, Mahoney and Henriksson (2001) 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 *L. monocytogenes* in mice. Levels up to 6 log CFU/g were detected in feces of mice one day after feeding with salami containing *L. monocytogenes*. Consumption of salami fermented with meat starter or *L. acidophilus* reduced fecal levels by 0.5 to 1.0 and 1.5 log units, respectively. Consumption of salami fermented with meat starter and *L. acidophilus* reduced fecal levels of the pathogen by 2.5 log units. These promising results showed that probiotics in meat products might have the potential to contribute to consumer health.

From all of these studies, it now seems possible to develop meat starter cultures that exhibit probiotic properties and achieve the required technological and sensory tasks in the meat matrix.

16.5 TOWARD GENOMICS AND POSTGENOMICS TO CHARACTERIZE STARTERS

Until recently, classical methods based on biochemical and physiological traits have been used to select the most performant strains for technological use. They were mainly based on acidification and inhibitory tests for lactic acid bacteria. For staphylococci, color and flavor developments were considered.

During the last decade, genetic studies have provided basic knowledge on targeted metabolic activities. Hammes and Hertel (1998) revealed that 14 genetic loci from *L. sakei* had been sequenced and 4 from *L. curvatus*. For *L. sakei*, several chromosomal genes encoding housekeeping, bacteriocin production, stress resistance functions, and carbohydrate and arginine catabolism have been cloned, sequenced, and well characterized (for review see Champomier-Vergès et al. 2002). In particular, sugar metabolism has been extensively investigated in this species, revealing the presence of only L-lactate dehydrogenase encoded by the *ldhL* gene. Different systems for transportation of sugars have been characterized and the genes responsible for ribose catabolism have been cloned and partially characterized (Champomier-Vergès et al. 2002). Several chromosomal genes encoding stress resistance functions such as heat shock genes, oxidative genes, and two-component regulatory systems have been characterized. Three gene clusters involved in the bacteriocin and immunity of *L. sakei* are described (Champomier-Vergès et al. 2002).

Now, for *L. sakei*, a global approach is developed. A strain was selected for a sequencing project, and its physical and genetic chromosome map was established (Dudez et al. 2002). The chromosome size was estimated to be 1,845 kb and a total of 47 clusters were mapped. From these data, 73 new genes were identified with the function of 36 deduced from their similarity to known genes. In parallel, a proteomic approach was developed to study the genes involved in adaptation of *L. sakei* to its environment (Marceau, Mera, Zagorec, and Champomier-Vergès 2001).

The physical and genetic map of one strain of *S. carnosus* was established (Wagner, Doskar, and Götz 1998). The size of the chromosome was estimated to be 2,590 kb. Fifteen genes or gene clusters were positioned on the physical map, including the gene cluster encoding nitrate and nitrite reductases, important traits for color development of fermented sausages. Other genes of *S. carnosus* involved in technological properties such as the branched-chain amino acid aminotransferase producing flavor compounds (Madsen et al. 2002), the superoxide dismutase, and the catalase contributing to the control of lipid oxidation (Barrière, Leroy-Sétrin, and Talon 2001) have been also identified, sequenced, and characterized. The whole genome of one strain of *S. carnosus* has been sequenced by a German team at the University of Tübingen, but the data are not yet available.

For *S. xylosus*, two genes that encoded the superoxide dismutase and the catalase, enzymes involved in antioxidant properties, have been characterized (Barrière, Brückner, and Talon 2001; Barrière, Centeno, et al. 2001). Molecular characterization of sugar utilization systems such as the genes involved in glucose uptake (Fiegler, Bassias, Jankovic, and Bruckner 1999), lactose utilization (Bassias and Bruckner 1998), glucose and fructose utilization (Brückner, Wagner, and Götz 1993), and carbon catabolite repression (Egeter and Bruckner 1996; Huynh, Jankovic, Schnell, and Bruckner 2000) has also been done. The whole genome of one strain of *S. xylosus* is being sequenced in 2005 (Talon and Leroy 2005).

16.6 CONCLUSION

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. The exploitation of the data on bacteria genomes of technological interest will offer new research opportunities by revealing some properties that could explain their adaptation to the meat environment and their interaction with a specific substrate.

REFERENCES

Arihara, K., H. Ota, M. Itoh, Y. Kondo, T. Sameshima, Y. Yamanaka, M. Akimoto, S. Kanai, and T. Miki. 1998. *Lactobacillus acidophilus* group lactic acid bacteria applied to meat fermentation. *J. Food Sci.* 63:544–547.

Axelsson, L. 1998. Lactic acid bacteria: Classification and physiology. In *Lactic acid bacteria: Microbiology and functional aspects* (2nd ed.), eds. S. Salminerand A. Von Wright, 1–72. New York: Marcel Dekker.

Barrière, C., R. Brückner, and R. Talon. 2001. Characterization of the single superoxide dismutase of *Staphylococcus xylosus*. *Appl. Environ. Microbiol.* 67 :4096–4104.

Barrière, C., D. Centeno, A. Lebert, S. Leroy-Sétrin, J. L. Berdagué, and R. Talon. 2001. Roles of superoxide dismutase and catalase of *Staphylococcus xylosus* in the inhibition of linoleic acid oxidation. *FEMS Microbiol. Lett.* 201:181–185.

Barrière, C., S. Leroy-Sétrin, and R. Talon. 2001. Characterization of catalase and superoxide dismutase in *Staphylococcus carnosus* 833 strain. *J. App. Microbiol.* 91:514–519.

Bassias, J., and R. Bruckner. 1998. Regulation of lactose utilization genes in *Staphylococcus xylosus*. *J. Bacteriol.* 180:2273–2279.

Berdagué, J. L., P. Monteil, M. C. Montel, and R. Talon. 1993. Effects of starter cultures on the formation of flavour compounds in dry sausage. *Meat Sci.* 35:275–287.

Beriain, M. J., G. Lizaso, and J. Chasco. 2000. Free amino acids and proteolysis involved in salchichon processing. *Food Control.* 1:41–47.

Brückner, R., E. Wagner, and F. Götz. 1993. Characterization of a sucrase gene from *Staphylococcus xylosus*. *J. Bacteriol.* 175:851–857.

Carnio, M. C., A. Holtzel, M. Rudolf, T. Henle, G. Jung, and S. Scherer. 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. *Appl. Environ. Microbiol.* 66:2378–2384.

Champomier-Vergès, M. C., S. Chaillou, M. Cornet, and M. Zagorec. 2002. *Lactobacillus sakei*: Recent developments and future prospects. *Res. in Microbiol.* 153:115–123.

Chizzolini, R., E. Novelli, and E. Zanardi. 1998. Oxidation in traditional Mediterranean meat products. *Meat Sci.* 49(Suppl. 1):S87–S99.

Dainty, R., and H. Blom. 1995. Flavour chemistry of fermented sausages. In *Fermented meats,* eds. G. Campbell-Platt and P. E. Cook, 176–193. London: Blackie Academic and Professional.

De Masi, T. W., F. B. Wardlaw, R. L. Dick, and J. C. Acton. 1990. Nonprotein nitrogen (NPN) and free amino acid contents of dry, fermented and nonfermented sausages. *Meat Sci.* 27:1–12.

Demeyer, D., M. Raemaekers, A. Rizzo, A. Holck, A. de Smedt, B. Ten Brink, B. F. Hagen, M. C. Montel, E. Zanardi, E. Murbrekk, F. Leroy, F. Vandendriessche, K. Lorentsen, G. Venema, L. Sunesen, L. H. Stahnke, L. De Vuyst, R. Talon, R. Chizzolini, and S. Eerola. 2000. Control of bioflavour and safety in fermented sausages: First results of a European project. *Food Res. Int.* 33:171–180.

Demeyer, D., and F. Toldrá. 2004. Fermentation. In *Encyclopedia of meat sciences,* eds. C. Devine and M. Dikeman, 467–474. Amsterdam: Elsevier.

Diaz, O., M. Fernández, G. D. Garcia de Fernando, L. de la Hoz, and J. A. Ordóñez. 1997. Proteolysis in dry fermented sausages: The effect of selected exogenous proteases. *Meat Sci.* 46:115–128.

Dudez, A. M., S. Chaillou, L. Hissler, R. Stentz, M. Champomier-Vergès, C. A. Alpert, and M. Zagorec. 2002. Physical and genetic map of the *Lactobacillus sakei* 23K chromosome. *Microbiol.* 148(Pt. 2):421–431.

Egeter, O., and R. Bruckner. 1996. Catabolite repression mediated by the catabolite control protein CcpA in *Staphylococcus xylosus*. *Mol. Microbiol.* 21:739–749.

Engelvin, G., G. Féron, C. Perrin, D. Molle, and R. Talon. 2000. Identification of beta-oxidation and thioesterase activities in *Staphylococcus carnosus* 833 strain. *FEMS Microbiol. Lett.* 190:115–120.

Erkkila, S., and E. Petaja. 2000. Screening of commercial meat starter cultures at low pH and in the presence of bile salts for potential probiotic use. *Meat Sci.* 55:297–300.

Erkkila, S., E. Petäjä, S. Eerola, L. Lilleberg, T. Mattila-Sandholm, and M. L. Suihko. 2001. Flavour profiles of dry sausages fermented by selected novel meat starter cultures. *Meat Sci.* 58:111–116.

Erkkila, S., M. L. Suihko, S. Eerola, E. Petaja, and T. Mattila-Sandholm. 2001. Dry sausage fermented by *Lactobacillus rhamnosus* strains. *Int. J. Food Microbiol.* 64:205–210.

Fadda, S., A. Lebert, and R. Talon. 2002. Development of an enzymatic method to quantify methyl ketones from bacterial origin. *J. Agri. Food Chem.* 50:2471–2474.

Fadda, S., S. Leroy-Sétrin, and R. Talon. 2003. Preliminary characterization of β-decarboxylase activities in *Staphylococcus carnosus* 833, a strain used in sausage fermentation. *FEMS Microbiol. Lett.* 228:143–149.

Fadda, S., Y. Sanz, G. Vignolo, M. C. Aristoy, G. Oliver, and F. Toldrá. 1999a. Characterization of muscle sarcoplasmic and myofibrillar protein hydrolysis caused by *Lactobacillus plantarum*. *App. Environ. Microbiol.* 65:3540–3546.

Fadda, S., Y. Sanz, G. Vignolo, M .C. Aristoy, G. Oliver, and F. Toldrá. 1999b. Hydrolysis of pork muscle sarcoplasmic proteins by *Lactobacillus curvatus* and *Lactobacillus sake*. *Appl. and Environ. Microbiol.* 65:578–584.

Fernández, M., L. de la Hoz, O. Díaz, M. I. Cambero, and J. A. Ordóñez. 1995. Effect of the addition of pancreatic lipase on the ripening of dry-fermented sausages: Part 1. Microbial, physico-chemical and lipolytic changes. *Meat Sci.* 40:159–170.

Fiegler, H., J. Bassias, I. Jankovic, and R. Bruckner. 1999. Identification of a gene in *Staphylococcus xylosus* encoding a novel glucose uptake protein. *J. Bacteriol.* 181:4929–4936.

Granly Koch, A. 2004. Biopreservation. In *Encyclopedia of meat sciences,* eds. C. Devine and M. Dikeman, 68–74. Amsterdam: Elsevier.

Gray, J. I., E. A. Gomaa, and D. J. Buckley. 1996. Oxidative quality and shelf life of meats. *Meat Sci.* 43:S111–S123.

Guarner, F., and G. J. Schaafsma. 1998. Probiotics. *Int. J. Food Microbiol.* 39:237–238.

Hammes, W. P., and C. Hertel. 1998. New developments in meat starter cultures. *Meat Sci.* 49(Suppl. 1):S125–S138.

Hierro, E., L. De La Hoz, and J. A. Ordóñez. 1997. Contribution of microbial and meat endogenous enzymes to the lipolysis of dry fermented sausages. *J. Agri. Food Chem.* 45:2989–2995.

Hinrichsen, L. L., and S. B. Pedersen. 1995. Relationship among flavor, volatile compounds, chemical change and microflora in Italian type dry cured ham during processing. *J. Agri. Food Chem.* 43:2932–2940.

Holzapfel, W. H. 2002. Appropriate starter culture technologies for small-scale fermentation in developing countries. *Int. J. Food Microbiol.* 75:197–212.

Holzapfel, W. H., P. Haberer, J. Snel, U. Schillinger, and J. H. Huis in't Veld. 1998. Overview of gut flora and probiotics. *Int. J. Food Microbiol.* 41:85–101.

Hugas, M., and J. M. Monfort. 1997. Bacterial starter cultures for meat fermentation. *Food Chem.* 59:547–554.

Huynh, P. L., I. Jankovic, N. F. Schnell, and R. Bruckner. 2000. Characterization of an HPr kinase mutant of *Staphylococcus xylosus*. *J. Bacteriol.* 182:1895–1902.

Johansson, G. 1996. Bacterial lipolysis by *Staphylococcus xylosus* compared to endogenous lipolysis in meat-fat mixtures of beef or pork. Paper presented at the 42nd International Congress of Meat Science and Technology: Meat for the Consumers. Lillehammer, Norway, Matforsk, Norwegian Food Research Institute.

Josephsen, J., and L. Jespersen. 2004. Starters cultures and fermented products. In *Handbook of food and beverage fermentation technology,* eds. Y. H. Hui, L. Meunier-Goddik, A. S. Hansen, J. Josephsen, W.-K. Nip, P. S. Stanfield, and F. Toldrá, 23–49. New York: Marcel Dekker.

Kang, D. H., and D. Y. C. Fung. 1999. Reduction of *Escherichia coli* O157: H7 by stimulated *Pediococcus* a*cidilactici*. *Lett. in App. Microbiol.* 29:206–210.

Kenneally, P. M., R.-G. Leuschner, and E. K. Arendt. 1998. Evaluation of the lipolytic activity of starter cultures for meat fermentation purposes. *J. App. Microbiol.* 84:839–846.

Klaenhammer, T. R. 1993. Genetics of bacteriocins produced by lactic acid bacteria. *FEMS Microbiol. Rev.* 12:39–85.

Kröckel, L. 1995. Bacterial fermentation of meats. In *Fermented meats,* eds. G. Campbell-Plattad P. E. Cook, 71–109. London: Blackie Academic and Professional.

Larrouture, C., V. Ardaillon, M. Pépin, and M. C. Montel. 2000. Ability of meat starter cultures to catabolize leucine and evaluation of the degradation products by using an HPLC method. *Food Microbiol.* 17:563–570.

Lücke, F. K. 2000. Utilization of microbes to process and preserve meat. *Meat Sci.* 56:105–115.

Madsen, S. M., H. C. Beck, P. Ravn, A. Vrang, A. M. Hansen, and H. Israelsen. 2002. Cloning and inactivation of a branched-chain-amino-acid aminotransferase gene from *Staphylococcus carnosus* and characterization of the enzyme. *Appl. Environ. Microbiol.* 68:4007–4014.

Mahoney, M., and A. Henriksson. 2003. The effect of processed meat and meat starter cultures on gastrointestinal colonization and virulence of *Listeria monocytogenes* in mice. *Int. J. Food Microbiol.* 84:255–261.

Marceau, A., T. Mera, M. Zagorec, and M. C. Champomier-Vergès. 2001. Protein expression under uracil privation in *Lactobacillus sakei*. *FEMS Microbiol. Lett.* 200:49–52.

Mataragas, M., E. H. Drosinos, and J. Metaxopoulos. 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[deg]C. *Food Microbiol.* 20:259–265.

Mauriello, G., A. Casaburi, G. Blaiotta, and F. Villani. 2004. Isolation and technological properties of coagulase negative Staphylococci from fermented sausages of southern Italy. *Meat Sci.* 67:149–158.

Miralles, M. C., J. Flores, and G. Perez-Martinez. 1996. Biochemical tests for the selection of *Staphylococcus* strains as potential meat starter cultures. *Food Microbiol.* 13:1–10.

Molly, K., D. Demeyer, T. Civera, and A. Verplaeste. 1996. Lipolysis in a Belgian sausage: Relative importance of endogenous and bacterial enzymes. *Meat Sci.* 43:235–244.

Molly, K., D. Demeyer, G. Johansson, M. Raemaekers, M. Ghistelinck, and I. Geenen. 1997. The importance of meat enzymes in ripening and flavour generation in dry fermented sausages: First results of a European project. *Food Chemi*. 59:539–545.

Montel, M. C., J. Reitz, R. Talon, J. L. Berdagué, and S. Rousset-Akrim. 1996. Biochemical activities of *Micrococcaceae* and their effects on the aromatic profiles and odours of a dry sausage model. *Food Microbiol*. 13:489–499.

Montel, M. C., M. P. Séronie, R. Talon, and M. Hébraud. 1995. Purification and characterization of a dipeptidase from *Lactobacillus sake*. *App. and Environ. Microbiol*. 61:837–839.

Morot-Bizot, S., R. Talon, and S. Leroy-Setrin. 2003. Development of specific PCR primers for a rapid and accurate identification of *Staphylococcus xylosus*, a species used in food fermentation. *J. Microbiol. Methods*. 55:279–286 .

Norwood, D. E., and A. Gilmour. 2001. The differential adherence capabilities of two *Listeria monocytogenes* strains in monoculture and multispecies biofilms as a function of temperature. *Lett. in App. Microbiol*. 33:320–324.

Nychas, G. J. E., and J. S. Arkoudelos. 1990. Staphylococci: Their role in fermented sausages. *Society for App. Bacteriol. Symp. Ser.* 19:167S–188S.

Olesen, P. T., A. S. Meyer, and L. Stahnke. 2004. Generation of flavour compounds in fermented sausages—The influence of curing ingredients, *Staphylococcus* starter culture and ripening time. *Meat Sci*. 66:675–687.

Olesen, P. T., and L. Stahnke. 2003. The influence of precultivation parameters on the catabolism of branched-chain amino acids by *Staphylococcus xylosus* and *Staphylococcus carnosus*. *Food Microbiol*. 20:621–629.

Olesen, P. T., L. Stahnke, and R. Talon. 2004. Effect of ascorbate, nitrate and nitrite on the amount of flavour compounds produced from leucine by *Staphylococcus xylosus* and *Staphylococcus carnosus*. *Meat Sci*. 68:193–200.

Ordóñez, J. A., E. M. Hierro, J. M. Bruna, and L. De la Hoz. 1999. Changes in the components of dry fermented sausages during ripening. *Crit. Rev. Food Sci. and Nutr.* 39:329–367.

Papamanoli, E., N. Tzanetakis, E. Litopoulou-Tzanetaki, and P. Kotzekidou. 2003. Characterization of lactic acid bacteria isolated from a Greek dry-fermented sausage in respect of their technological and probiotic properties. *Meat Sci*. 65:859–867.

Pennacchia, C., D. Ercolini, G. Blaiotta, O. Pepe, G. Mauriello, and F. Villani. 2004. Selection of *Lactobacillus* strains from fermented sausages for their potential use as probiotics. *Meat Sci*. 67:309–317.

Pidcock, K., G. M. Heard, and A. Henriksson. 2002. Application of nontraditional meat starter cultures in production of Hungarian salami. *Int. J. Food Microbiol*. 76:75–81.

Rodriguez, M., F. Núńez, J. J. Córdoba, E. Bermudez, and M. A. Asensio. 1996. Gram-positive, catalase-positive cocci from dry cured Iberian ham and their enterogenic potential. *App. and Environ.l Microbiol*. 62:1897–1902.

Sahl, H. G. 1994. Staphylococcin 1580 is identical to the lantibiotic epidermin: Implications for the nature of bacteriocins from gram-positive bacteria. *App. and Environ. Microbiol*. 60:752–755.

Sameshima, T., C. Magome, K. Takeshita, K. Arihara, M. Itoh, and Y. Kondo. 1998. Effect of intestinal *Lactobacillus* starter cultures on the behaviour of *Staphylococcus aureus* in fermented sausage. *Int. J. Food Microbiol*. 41:1–7.

Sanz, Y., S. Fadda, G. Vignolo, M. C. Aristoy, G. Oliver, and F. Toldrá. 1999. Hydrolytic action of *Lactobacillus casei* CRL 705 on pork muscle sarcoplasmic and myofibrillar proteins. *J. Agri. Food Chem*. 47:3441–3448.

Sanz, Y., and F. Toldrá. 1997. Purification and characterization of an aminopeptidase from *Lactobacillus sake*. *J. Agri. Food Chem*. 45:1552–1558.

Saxelin, M. 1997. *Lactobacillus* GG: A human probiotic strain with thorough clinical documentation. *Food Rev. Int.* 13:293–313.

Scannell, A. G. M., G. Schwarz, C. Hill, R. P. Ross, and E. K. Arendt. 2001. Pre-inoculation enrichment procedure enhances the performance of bacteriocinogenic *Lactococcus lactis* meat starter culture. *Int. J. Food Microbiol.* 64:151–159.

Schmidt, S., and R. G. Berger. 1998. Microbially formed aroma compounds during the maturation of dry fermented sausage. *Adv. in Food Sci.* 20:144–152.

Selgas, M. D., J. M. Garcia, G. D. Garcia de Fernando, and J. A. Ordóñez. 1993. Lipolytic and proteolytic activity of micrococci isolated from dried fermented sausages. *Fleischwirtschaft.* 73:1164–1166.

Sondergaard, A. K., and L. Stahnke. 2002. Growth and aroma production by *Staphylococcus xylosus, S. carnosus* and *S. equorum:* A comparative study in model systems. *Int. J. Food Microbio.* 75:99–109.

Stahnke, L. H. 1994. Aroma components from dried sausages fermented with *Staphylococcus xylosus*. *Meat Sci.* 38:39–53.

Stahnke, L. H. 1995. Dried sausages fermented with *Staphylococcus xylosus* at different temperatures and with different ingredient levels: Part II. Volatile components. *Meat Sci.* 41:193–209.

Stahnke, L. H. 2002. Flavour formation in fermented sausage. In *Research advances in quality of meat and meat products,* ed. F. Toldrá, 193–223. Trivandrum, India: Research Signpost.

Stahnke, L. H., L. O. Sunesen, and A. De Smedt. 1999. Sensory characteristics of European dried fermented sausages and the correlation to volatile profile. *Thirteenth Forum for App. Biotech.* 64/5b:559–566.

Talon, R., N. Dublet, M. C. Montel, and M. Cantonnet. 1995. Purification and characterization of extracellular *Staphylococcus warneri* lipase. *Current Microbiol.* 30:11–16.

Talon, R., and S. Leroy. 2005. *Staphylococcus xylosus C2a: Whole genome shotgun*. Available at: www.genoscope.cns.fr/externe/Francais/Projets/Projet_NN/organisme_NN.html.

Talon, R., S. Leroy-Sétrin, and S. Fadda. 2002. Bacterial starters involved in the quality of fermented meat products. In *Research advances in quality of meat and meat products,* ed. F. Toldrá, 175–191. Trivandrum, India: Research Signpost.

Talon, R., S. Leroy-Sétrin, and S. Fadda. 2004. Dry fermented sausages. In *Handbook of food and beverage fermentation technology,* eds. Y. H. Hui, L. Meunier-Goddik, A. S. Hansen, J. Josephsen, W.-K. Nip, P. S. Stanfield, and F. Toldrá, 397–416. New York: Marcel Dekker.

Talon, R., and M. C. Montel. 1997. Hydrolysis of esters by staphylococci. *Int. J. Food Microbiol.* 36:207–214.

Talon, R., M. C. Montel, G. Gandemer, M. Viau, and M. Cantonnet. 1993. Lipolysis of pork fat by *Staphylococcus warneri*, *Staphylococcus saprophyticus* and *Micrococcus varians*. *App. Microbiol. and Biotech.* 38:606–609.

Talon, R., D. Walter, and M. C. Montel. 2000. Growth and effect of staphylococci and lactic acid bacteria on unsatured free fatty acid. *Meat Sci.* 54:41–47.

Tjener, K., L. H. Stahnke, L. Andersen, and J. Martinussen. 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. *Int. J. Food Microbiol.* 97:31–42.

Toldrá, F. 1992. The enzymology of dry-curing of meat products. In *New technologies for meat and meat products: Part II. Muscle enzymology and meat ageing,* eds. F. J. M. Smulders, F. Toldrá, J. Flores, and M. Prieto, 209–231. ECCEAMST.

Toldrá, F. 1998. Proteolysis and lipolysis in flavour development of dry-cured meat products. *Meat Sci*. 49:S101–S110.

Toldrá, F., and J. L. Navarro. 2002. Action of muscle lipases during the processing of dry-cured ham. In *Research advances in quality of meat and meat products,* ed. F. Toldrá, 249–254. Trivandrum, India: Research Signpost.

Tyopponen, S., A. Markkula, E. Petaja, M. L. Suihko, and T. Mattila-Sandholm. 2003. Survival of *Listeria monocytogenes* in north European type dry sausages fermented by bio-protective meat starter cultures. *Food Control*. 14:181–185.

Tyopponen, S., E. Petaja, and T. Mattila-Sandholm. 2003. Bioprotectives and probiotics for dry sausages. *Int. J. Food Microbiol*. 83:233–244.

Verluyten, J., W. Messens, and L. De Vuyst. 2003. The curing agent sodium nitrite, used in the production of fermented sausages, is less inhibiting to the bacteriocin-producing meat starter culture *Lactobacillus curvatus* lth 1174 under anaerobic conditions. *App. and Environ. Microbiol*. 69:3833–3839.

Verplaeste, A. 1994. Influence of raw meat properties and processing technology on aroma quality of raw fermented meat products. Paper presented at the 40th International Congress of Meat Science and Technology, The Hague, Netherlands.

Villani, F., L. Sannino, G. Moschetti, G. Mauriello, O. Pepe, R. Amodio-Cocchieri, and S. Coppola. 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 Microbiol*. 14:555–566.

Waade, C., and L. H. Stahnke. 1997. Dried sausages fermented with *Staphylococcus xylosus* at different temperatures and with different ingredient levels: Part IV. Amino acid profile. *Meat Sci*. 46:101–104.

Wagner, E., J. Doskar, and F. Götz. 1998. Physical and genetic map of the genome of *Staphylococcus carnosus* TM300. *Microbiol*. 144:509–517.

Zalacain, I., M. J. Zapelena, I. Astiasarán, and J. Bello. 1995. Dry fermented sausages elaborated with lipase from *Candida cylindracea*: Comparison with traditional formulations. *Meat Sci*. 40:55–61.

Zapelena, M. J., I. Astiasarán, and J. Bello. 1999. Dry fermented sausages made with a protease from *Aspergillus oryzae* and/or a starter culture. *Meat Sci*. 52:403–409.

17 Modified Atmosphere Packaging

Joseph G. Sebranek
Iowa State University

Terry A. Houser
University of Florida

CONTENTS

17.1 Definitions 421
17.2 Historical 422
17.3 Purposes 422
17.4 Applications 423
17.5 Gases Used in MAP Systems 424
17.5.1 Carbon Dioxide 424
17.5.2 Nitrogen 426
17.5.3 Oxygen 427
17.5.4 Carbon Monoxide 428
17.5.5 Dynamic Headspace Changes in MAP Systems 429
17.6 Product Characteristics Following MAP Applications 430
17.6.1 Fresh Meat 430
17.6.1.1 High-Oxygen MAP 430
17.6.1.2 High-Carbon-Dioxide MAP 431
17.6.1.3 MAP with Carbon Monoxide 432
17.6.2 Cooked and Cured-and-Cooked Meat Products 432
17.7 Comparisons of MAP with Vacuum Packaging for Meat and Poultry 433
17.7.1 Vacuum Versus MAP Packaging of Fresh Meat 433
17.7.2 Vacuum Versus MAP Packaging of Cooked and Cured-and-Cooked Meat Products 434
17.7.3 Effects of High-Carbon-Dioxide MAP 434
17.8 Packaging Films and Film Properties 435
17.8.1 Nylon 435
17.8.2 Polyester 436
17.8.3 Polypropylene 436
17.8.4 Ethylene Vinyl Alcohol 436
17.8.5 Polyethylenes 436

17.8.6 Polyvinyl Chloride 436
17.8.7 Polyvinylidene Chloride 436
17.9 Effects of Map on Pathogens in Meat and Poultry 437
17.9.1 *Salmonella* spp. 437
17.9.2 *Yersinia enterocolitica* 437
17.9.3 *Escherichia coli* O157:H7 437
17.9.4 *Campylobacter jejuni* 438
17.9.5 *Staphylococcus aureus* 438
17.9.6 *Clostridium botulinum* 438
17.9.7 *Clostridium perfringens* 438
17.9.8 *Bacillus cereus* 439
17.9.9 *Listeria monocytogenes* 439
17.10 MAP as Component in the Hurdle Concept 440
17.11 Active Packaging Components 440
17.12 Leakers and Package Integrity 441
17.13 Regulatory Issues 442
17.14 Conclusions 443
References 443

Modified atmosphere packaging (MAP) for meat and poultry products is a food preservation concept that is far from new. The development of plastic films many years ago 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. Carbon dioxide (CO_2) gas has been 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 Jones 1996; Gill and Tan 1980; Kraft and Ayres 1952; Livingston, Breuer, Killifer, Bidner, and McKeith 2004; Sebranek 1985). Various other gases including nitrogen, oxygen, and carbon monoxide have been studied more recently as MAP technology for meat and poultry products has continued to develop (Kropf 2004). The interest in commercial applications for MAP systems has increased tremendously in the past 20 years or so 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 to consistently deliver wholesome, high-quality products to consumers. Consumers demand, and have come to expect, fresh, 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 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.

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 micro-organisms, 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 because there are less throwaways from 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, with improved product appearance 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, although a longer product shelf life and less waste can more than pay the difference. Specialized equipment and worker training may be necessary and, if so, these factors represent increased time and monetary commitments. Appropriate gas mixtures may need to be determined for specific products, because necessary information may not available. Finally, in some cases, safety concerns can be an issue for some of the gases used and for some of the products packaged in anaerobic atmospheres.

17.1 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; Rao and Sachindra 2002). 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 are dynamic and 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 diffusion 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 100% carbon dioxide atmosphere. Active packaging (AP) is 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 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. 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.

17.2 HISTORICAL

The use of carbon dioxide as a preservative for meat was suggested as early as 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 (Kraft and Ayres 1952). 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% to 25% were common for red meat species (beef, pork, lamb; Ledward 1970). Nitrogen was typically added to exclude oxygen in atmospheres that included increased carbon dioxide. Poultry, because of less obvious color change than red meat, may permit the use of greater concentrations of carbon dioxide without color losses (Rao and Sachindra 2002). 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, Dryden, and Marchello 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). Most recently, interest in use of carbon monoxide for MAP packaging has been growing. 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 very limited around the world, with only Australia and New Zealand in addition to the United States allowing its use. Carbon monoxide was used in Norway for retail meat packaging from 1985 until 2004 (Sørheim, Aune, and Nesbakken 1997), but because of trade agreement changes with the European Union, the approval of carbon monoxide for fresh meat packaging in Norway was discontinued in 2004.

17.3 PURPOSES

The primary purpose of MAP systems for meat and poultry products is to achieve a longer shelf life 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 that 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 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 MAP systems 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 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 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 micro-organisms 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. Research in this area is continuing and the role of MAP in product safety will be better understood in the near future.

17.4 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% to 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 balance. The purpose of oxygen is color development. Carbon monoxide at 0.3% to 0.5% may also be included, where permitted, for color development. In this case, because color is more stable, carbon dioxide can be included at greater concentrations that will contribute to improved 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 individual packages) or stand-alone small units

(typically retail-ready packages). 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 individual units. The case-ready pack approach uses high-barrier films for the individual packages to retain the MAP gas mixture that is placed inside the package.

17.5 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% to 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. However, limited regulatory approval for carbon monoxide use has resulted in much less use of this gas in MAP systems than of carbon dioxide, nitrogen, and oxygen. 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). 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.

17.5.1 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 micro-organisms. 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 micro-organisms, 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 (Daniels, Krishnamurthi, and Rizvi 1985). 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. This 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). Although the mechanism of action of carbon dioxide that results in bacterial inhibition is not entirely clear, 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, Debevere, and Van Impe 1998; Devlieghere, Geeraerd, Versyck, Vandewaetere, Van Impe, and Debevere 2001; Jakobsen and Bertelsen 2004; Löwenadler and Rönner 1994; Mitz 1979). 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 amount 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 consequently, it is usually recommended that the amount of carbon dioxide used in MAP for fresh meat, if used alone, be applied in excess of the amount needed to saturate the product. Research has suggested that an optimum level of carbon dioxide ranges from 1 to 2 L/kg meat (Gill 1988) with some recommendations as high as 3L/kg (Kropf 2004). However, several factors can influence carbon dioxide solubility in meat tissue. For example, fat content, water content, pH, and temperature are product-related factors that affect carbon dioxide absorption (Gill 1988; Jakobsen and Bertelsen 2002; Mullan and McDowell 2003; Zhao, Wells, and McMillin 1995). Package-related factors that are important include gas partial pressure and the ratio of headspace volume to meat volume are important package factors (Jakobsen and Bertelsen 2002; Zhao, Wells, and McMillin 1994, 1995).

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. 1998; 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 among 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 recently 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 2L of gas per kg of meat was necessary to prevent package collapse. For volume ratios greater than 2L/kg, the partial pressure of carbon dioxide was most predictive of the amount of absorbed gas (Jakobsen and Bertelsen 2002). The product factors of fat and 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 (Daniels et al. 1985). 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, Sebranek, Rust, and Honeyman 2003; Kropf 2004). This may be the result of carbon dioxide extending the lag phase of bacterial growth for a bacteriostatic effect rather than a bacteriocidal effect.

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 (Bruce, Wolfe, Jones, and Price 1996). In our laboratory, we observed increased height of ground beef patties after cooking and increased circumference of frankfurters after heating when these products had been stored in high (99.5%) carbon dioxide atmospheres. Both products were softer in texture than products stored under vacuum. Sørheim, Ofstad, and Lea (2004) reported greater cooking losses from ground beef stored in 100% carbon dioxide, and observed fissures in the product following cooking.

17.5.2 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 micro-organisms. 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, Jensen, Olsen, Skibsted, and Bertelsen 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, typically have low microbial numbers. For these products, 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.

17.5.3 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 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% to 80% in MAP helps to form a deeper layer of oxymyoglobin pigment that will extend the time during which the color appears attractive (Kropf 2004). However, because oxygen also promotes growth of rapidly proliferating, aerobic micro-organisms, oxygen in MAP systems for fresh meat is usually combined with 20% to 25% carbon dioxide to achieve improved microbial control (Kropf 2004). 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 mm to 10 mm of mercury (normal atmospheric partial pressure of oxygen is 159.2 mm Hg) 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 decreased, 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 be no more than 0.01% (100 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 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 not been subjected to adequate 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. 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. Some of these modifiers can be incorporated into the packaging film (Kropf 2004). Packaging with AP systems is discussed more thoroughly in Section 17.11.

17.5.4 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. Carbon monoxide binds very strongly to blood hemoglobin and muscle myoglobin. The binding to hemoglobin is the basis for concerns about human toxicity because the carbon monoxide displaces oxygen and prevents oxygen transport. Although exposure to high concentrations of carbon monoxide can be fatal, low-level exposure is not harmful (Krause et al. 2003; Sørheim et al. 1997). Concern about toxicity and human exposure, however, is one reason why carbon monoxide has not been widely 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 single gas or as a component of "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. A beneficial result of this reaction is a stable, bright red meat color that is visibly indistinguishable from the cherry-red meat color produced by oxygen. The color induced by carbon monoxide, however, 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 has been growing interest in the use of low levels of carbon monoxide for MAP packaging. Research has demonstrated that concentrations of 0.3% to 0.5% carbon monoxide are adequate to result in stable, attractive meat color and that at these concentrations, no human hazard exists (Sørheim et al. 1997). The U.S. 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 (Sørheim, Nissen, and Nesbakken 1999). 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).

There are several ways to achieve improved fresh meat color stability using carbon monoxide and MAP systems. The system used in Norway from 1985 to 2004 consisted of retail packages containing a gas atmosphere of approximately 0.4% CO, 60% CO_2, and 40% N_2 (Sørheim et al. 1999). In this case, the carbon-monoxide-containing atmosphere remains in contact with the product throughout distribution and retail display. This concept is similar to that recently approved by the U.S. FDA (2004).

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% to 100% carbon monoxide for up to 24 hours, followed by VP, resulted in bright red color for several weeks (Jayasingh, Cornforth, Carpenter, and Whittier 2001). This approach would allow exposure of meat cuts to carbon monoxide in a central facility followed by VP for distribution and retail display.

A third approach that has been utilized for commercial applications 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 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, Mancini, Hachmeister, Kropf, Merriman, Del Duca, and Milliken 2004).

17.5.5 Dynamic Headspace Changes in MAP Systems

Because MAP systems, by definition, are a one-time 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 (Daniels et al. 1985; 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 when muscle enzyme and metabolic systems are most active (Daniels et al. 1985). Clearly, the amount of carbon dioxide emitted by respiration will be dependent on 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 micro-organisms becomes more extensive. Absorption of gases by the product generally reaches equilibrium early, during the first 12 to 72 hours after packaging (Gill 1988; Jakobsen and Bertelsen 2004; Zhao et al. 1994). 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 (Daniels et al. 1985).

17.6 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 has 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.

17.6.1 Fresh 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 also incorporate carbon monoxide.

17.6.1.1 High-Oxygen MAP

Case-ready MAP packages that utilize high oxygen levels for color development of red meat most often utilize a gas blend of about 70% to 80% oxygen and 20% to 30% carbon dioxide. This combination results in a deeper-than-usual layer of red oxymyoglobin for improved color life and improved shelf life, both of which are increased about threefold over that of conventional overwrapped aerobic packages (Bartkowski et al. 1982; Kropf 2004). 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, Hunt, Mancini, Hachmeister, Kropf, and Unruh 2004). Lamb loins packaged in high-oxygen atmospheres showed improved color stability and limited oxidative change during storage (Kennedy, Buckley, and Kerry 2004). High-oxygen packaging of pork has also been demonstrated to achieve increased color life and consumer acceptability (Buys 2004). Enhanced fresh pork (injection of low-salt brine) has become a very widely used means of improving palatability and consistency of fresh pork. Research with high-oxygen MAP for enhanced pork has shown color advantages similar to conventional pork cuts (Livingston et al. 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 (Jackson, Acuff, Vanderzant, Sharp, and Savell 1992; Jensen, Fiensted-Jensen, Skibsted, and Bertelsen 1998). A study of beef loin muscles stored in MAP with 20% to 80% oxygen showed that 55% oxygen or more was necessary for maintaining good color (Jakobsen and Bertelsen 2000). In this study, oxygen concentration had a relatively small effect on lipid oxidation compared to storage temperature, which was found to have a much larger impact on lipid oxidation.

Exposure of beef to high-oxygen atmospheres has been observed to result in "premature browning" when the beef is cooked (Killinger, Hunt, Campbell, and Kropf 2000; Seyfert, Hunt, Mancini, Kropf, and Stroda 2004; Seyfert, Mancini, and Hunt 2004). 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 a 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, Cornforth, Carpenter, Sorheim, Pettee, and Whittier 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. This 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, Hunt, Hachmeister, Kropf, and Johnson 2005).

17.6.1.2 High-Carbon-Dioxide MAP

Packaging with elevated carbon dioxide is sometimes referred to as a low- or ultra-low-oxygen system (Kropf 2004). This terminology is used 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% to 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 reduced (Kropf 2004). 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). The microbial shelf life of fresh meat products is typically best with high-carbon-dioxide atmospheres. A comparison of pork loins, for example, packaged in

vacuum, carbon dioxide alone, nitrogen alone, or oxygen and carbon dioxide (2:1) resulted in 12 days without off-odors for nitrogen and oxygen and carbon dioxide atmospheres, but 21 days for carbon dioxide alone and vacuum (Gill and Jones 1996).

17.6.1.3 MAP with Carbon Monoxide

Fresh meat products packaged with carbon monoxide demonstrate dramatically improved color stability. For example, beef steaks and ground beef packaged with 1% carbon monoxide resulted in stable red color for at least 5 to 7 days longer (over 29 days in total) than a high-oxygen (70%) package (Luño, Beltrán, and Roncalés 1998). In another study, comparison of ground beef, beef steaks, and pork chops packaged in 0.4% CO, 60% CO_2 , and 40% N_2 or 70% O_2, and 30% CO_2 showed that the carbon monoxide atmosphere extended the color life 3 to 7 days longer than that of the high-oxygen atmosphere (Sørheim et al. 1999). Pork chops packaged in 0.5% CO, 70% CO_2 and 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 7 days of storage (Krause et al. 2003). Including CO 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.

17.6.2 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 and 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, Jakobsen, W, Martinussen, Skibsted, and Bertelsen 2003). However, headspace volume relative to product volume was found to be among the most important factors 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). A comparison of packaging for dry-cured ham slices in vacuum, 100% nitrogen, and 20% carbon dioxide and 80% nitrogen for effects on product color, texture, and microbial growth showed no differences among the three packaging systems (García-Esteban, Ansorena, and Astiasarán 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 very recent study investigated the stability of cured meat pigment to both autoxidation and photo-oxidation in the presence of 0%, 20%, and 90% carbon dioxide atmospheres (Møller, Nannerup, and Skibsted 2005). Autoxidation was decreased by carbon dioxide, which might have implications for reducing rancidity and off-flavor development in MAP systems. Other researchers have reported reduced lipid oxidation in the presence of carbon dioxide atmospheres (Sørheim et al. 1997). Photo-oxidation, 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 and nitrogen blends can be used for MAP applications involving cooked or cured-and-cooked meat and poultry products.

17.7 COMPARISONS OF MAP WITH VACUUM PACKAGING FOR MEAT AND POULTRY

17.7.1 Vacuum Versus MAP Packaging of 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% to 20% of the gases present (Tewari, Jayas, and Holley 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 (Rao and Sachindra 2002). 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 shelf life of products by 50% to 400% or more over that of aerobic packaging (Rao and

Sachindra 2002). 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 (Parrish, Boles, Rust, and Olson 1991), although MAP has been shown to be equally effective for this application as well (Lee, Sebranek, and Parrish 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. The effects of physical pressure on the product surface can be avoided by the use of MAP systems.

17.7.2 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 (Rao and Sachindra 2002). The principal 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.

17.7.3 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, 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). Consequently, it has been suggested that very high carbon dioxide concentrations could be used for both fresh and cooked and cured-and-cooked products to further improve shelf life.

Because high-carbon-dioxide atmospheres can affect meat pH, 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% to 100% carbon dioxide, reduced cooking yield of 1% to 3% resulted (Sørheim et al. 2004). There were no effects on product texture in this case. However, it has been observed that a softer cooked texture resulted for both ground beef and frankfurters following packaging in 99.5% carbon dioxide (Sebranek 2005). These products, when cooked, also demonstrated visible changes including an increase in height of cooked ground beef patties and an increase in the circumference of cooked frankfurters following storage in 99.5% to 100% carbon dioxide atmospheres.

The introduction of physical changes in meat product structure as a result of cooking after packaging in high-carbon-dioxide MAP appears to be due to evolution of dissolved carbon dioxide during heating. These physical changes are likely to affect fat and water binding, cooking yields, texture, and product appearance. Further research on the concentrations of carbon dioxide and other product variables that affect these product changes will be important for determining the best way to maximize the antimicrobial effect of carbon dioxide in MAP.

17.8 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. Added features such as the closable zipper have made flexible film packaging more attractive to consumers (Lazar 2004). Currently available films offer a range of oxygen and moisture barriers, shrinking properties, sealing characteristics, smoke and 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. 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. There are no single films that provide all the properties needed for effective MAP systems. The following discussion 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.

17.8.1 Nylon

Nylon or polyamides are widely used for strength and are resistant to puncture, abrasion, and tearing (Mullan and McDowell 2003). The toughness of nylon means that it is often used as a component of fresh meat pouches where puncture-resistance is important. Nylon is generally hard to heat-seal and is combined with other films for sealing ease. Because of the strength and rigidity, this film is often part of thermoformed base packs for MAP applications (Kirwan and Strawbridge 2003).

17.8.2 Polyester

Polyesters are condensation polymers, polyethylene terephthalate, with a wide range of properties. These films are generally good gas and water barriers and are heat resistant (Mullan and McDowell 2003). Polyester is often used for barrier pouches and lids for tray packs. 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 (Kirwan and Strawbridge 2003).

17.8.3 Polypropylene

Polypropylene applications for MAP systems are most often found in rigid base trays. This film is a good water-vapor barrier but a poor gas barrier so combinations with high gas-barrier films are likely (Mullan and McDowell 2003). This film has a high heat tolerance but can be heat-sealed (Kirwan and Strawbridge 2003).

17.8.4 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). The film is often coextruded with nylon and other films for fresh meat applications.

17.8.5 Polyethylenes

Polyethylenes are commonly used in multiple-layer package films 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 (Kirwan and Strawbridge 2003).

17.8.6 Polyvinyl Chloride

Polyvinyl chloride is a film 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). The film is not a good gas barrier so combinations with barrier films are usually necessary for MAP applications.

17.8.7 Polyvinylidene Chloride

Polyvinylidene chloride is a flexible film that is a superior barrier for water vapor, gases, and odors (Mullan and McDowell 2003). 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 are often monolayer films of polyvinylidene chloride but the film is most often used in coextrusions with multiple layers of other flexible film materials (Kirwan and Strawbridge 2003).

17.9 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 micro-organisms. 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, psychotrophic 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*, *C. perfringens*, *Bacillus cereus*, and *Listeria monocytogenes.*

17.9.1 *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). A review of several studies (Farber 1991) suggests that high carbon dioxide (60%–100%) MAP can slow the growth of *Salmonella* compared to aerobic packaging but the organism will survive in the carbon dioxide environment. Other researchers have reported that *Salmonella* strains grew faster in high carbon dioxide than in oxygen atmospheres (Nissen, Alvseike, Bredholt, Holck, and Nesbakken 2000). 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.

17.9.2 *Yersinia enterocolitica*

Y. enterocolitica is a facultative anaerobe and can grow well in the absence of oxygen (Farber 1991; Knabel 1995). Yersiniosis is an infection frequently resulting from undercooked meat, most often pork (Farber 1991; Gill and Reichel 1989). Several studies have reported that high-carbon-dioxide atmospheres will slow the growth of *Y. enterocolitica* (Gill and Reichel 1989) but there are reports that this pathogen can grow on beef even under a 100% carbon dioxide atmosphere (Gill and Reichel 1989). Adequate cooking, rather than MAP, provides assurance of safety from this pathogen.

17.9.3 *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. However, at warm temperatures (20°C), carbon dioxide is reported to have little effect on growth of this organism (Rao and Sachindra 2002).

17.9.4 *Campylobacter jejuni*

C. jejuni is classified as a microaerophilic organism (Farber 1991) meaning that it prefers a small amount of oxygen. *C. jejuni* causes illnesses as a food-borne infection and seems to be largely unaffected by carbon dioxide atmospheres. 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 (Farber 1991). *C. jejuni* is frequently associated with poultry and adequate cooking is the preferred method for assuring product safety (Knabel 1995).

17.9.5 *Staphylococcus aureus*

S. aureus is a pathogen that causes illness by producing a toxin during growth, resulting in what is considered 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 (Farber 1991; Rao and Sachindra 2002). 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.

17.9.6 *Clostridium botulinum*

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 (Farber 1991). Further, refrigeration temperatures are normally sufficient to prevent toxin production. Although the probability of *C. botulinum* toxin in meat and poultry is relatively low, it has been reported that storage of hamburger sandwiches in a nitrogen-packed atmosphere at 12°C permitted toxin production by *C. botulinum,* though toxin was not produced at 8°C (Kautler, Lynt, Lilly, and Solomon 1981). In fish products, where *C. botulinum* contamination is high, anaerobic modified atmospheres are not considered safe for refrigerated products (Farber 1991).

17.9.7 *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 (Hintlian and Hotchkiss 1987). 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.

17.9.8 *Bacillus cereus*

B. cereus is a toxin-producing pathogen that induces a food poisoning illness, but the illness is usually not fatal. Although this pathogen is among the less common causes of food-borne 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 (Farber 1991). Carbon dioxide has been reported to be much more effective against *B. cereus* than nitrogen or anaerobic atmospheres (Molin 1983).

17.9.9 *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, is widespread, very hardy, and very difficult to eradicate from food processing plants. Further, listeriosis is a food-borne infection that can result in a 20% to 30% fatality rate in an outbreak (Devlieghere et al. 2001; Farber 1991). 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.

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). Others have reported carbon dioxide inhibition of this organism even at abusive temperatures (Avery, Rogers, and Bell 1995). In a study of irradiation and MAP for control of *L. monocytogenes,* it was reported that irradiation in air was more lethal to the organism than in MAP with either carbon dioxide or nitrogen (Thayer and Boyd 1999). However, lethality of irradiation was greater in a 100% carbon dioxide atmosphere compared to nitrogen. Further, recovery and growth of *L. monocytogenes* following irradiation was inhibited by MAP with 40% or more carbon dioxide.

Much of the research on MAP systems and *L. monocytogenes* has been done with fresh meat and poultry. However, there is major concern for this pathogen in cooked, RTE products. Most of these products are packaged in vacuum or 100% nitrogen. There is a need for more research on the effects of carbon dioxide in MAP systems for inhibition of *L. monocytogenes* on RTE meat and poultry products.

17.10 MAP AS COMPONENT IN THE HURDLE CONCEPT

Most micro-organisms 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 micro-organisms 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 micro-organisms 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 micro-organisms respectively (Gray, Elliott, and Tomlins 1984; Sawaya et al. 1995). Combining MAP with irradiation for fresh meat has been shown to be effective for reducing initial microbial counts and suppressing growth of survivors during storage (Zhao, Sebranek, Dickson, and Lee 1996). Irradiation combined with MAP has been studied extensively, though in the case of irradiation, there are trade-offs between increased microbial lethality of irradiation in the presence of oxygen (Avery et al. 1995) and potential losses of quality when products are irradiated in air. However, irradiation combined with MAP has been found to be effective for accelerated aging of beef at elevated temperature (Lee et al. 1996). Packaging in MAP with CO has also been reported to be a very effective way to overcome the discoloration of beef that occurs as a result of irradiation treatment (Kusmider, Sebranek, Lonergan, and Honeyman 2002).

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.

17.11 ACTIVE PACKAGING COMPONENTS

Although 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.

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 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. In the United States, these packets must be labeled "Do Not Eat" to prevent accidental consumption. 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 high-carbon-dioxide-containing package is sealed. However, this concept does not appear to be used a great deal for meat and poultry.

Finally, packaging films that include antimicrobial compounds would be a powerful complement for MAP systems to help control micro-organisms and pathogens that tolerate MAP atmospheres. There has been a considerable amount of research on antimicrobial films and coatings (Ozdemir and Floros 2004) but most of these systems are still in various stages of development.

17.12 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 or the seal is not intact, the gas atmosphere is likely to be lost very quickly and product safety and quality will be compromised. 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, among others (Mullan and McDowell 2003). Most of these methods are destructive

and will 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 (Smolander, Hurme, and Ahvenainen 1997). 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, Papkovskaia, Papkovsky, and Kerry 2002).

17.13 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, although safety concerns for fish in anaerobic MAP systems has been expressed (Farber 1991). Carbon monoxide, on the other hand, has not been permitted for very many applications. The most substantial use was in Norway where 0.5% carbon monoxide was approved for use in 1985 and has been utilized for over half of the retail packaging of fresh meat (Sørheim et al. 1997). However, because Norway is moving toward conformity with the European Union for commodity trade, use of carbon monoxide for red meat packaging was discontinued in 2004 (Sørheim, Nissen, Aune, and Nesbakken 2001).

Because of a considerable amount of research demonstrating the advantages of low-carbon-monoxide MAP, in February 2002, the U.S. FDA approved use of 0.4% carbon monoxide for MAP used as part of a master pack system (ActiveTech by Pactiv Corp.; 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 (U.S. FDA 2002). 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. At the time of this writing, the Pactiv system is being used for some commercial applications in the United States but the Precept system has not yet been put into commercial use.

Whereas 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. 1997; Sørheim et al. 2001; U.S. FDA 2002, 2004), the perception of danger continues to limit approval of potential applications of this gas.

17.14 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 (Tewari et al. 1999). 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

Avery, S. M., A. R. Rogers, and R. G. Bell. 1995. Continued inhibitory effect of carbon dioxide packaging on *Listeria monocytogenes* and other microorganisms on normal pH beef during abusive retail display. *Int. J. Food Sci. Tech.* 30:725–735.

Bartkowski, L., F. D. Dryden, and J. A. Marchello. 1982. Quality changes of beef steaks stored in controlled gas atmospheres containing high or low levels of oxygen. *J. Food Protect.* 45:41–45.

Brooks, J. 1933. The effects of carbon dioxide on the color changes or bloom of lean meat. *J. Soc. Chem. Ind.* 52:17–19.

Bruce, H. L., F. H. Wolfe, S. D. M. Jones, and M. A. Price. 1996. Porosity in cooked beef from controlled atmosphere packaging is caused by rapid CO_2 evolution. *Food Res. Int.* 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 Sci.* 68:641–647.

Clark, D. S., and C. P. Lentz. 1969. Use of carbon dioxide for extending shelf-life of prepackaged beef. *Canadian Food Sci. Tech.* J. 5:175–178.

Daniels, J. A., R. Krishnamurthi, and S. S. H. Rizvi. 1985. A review of effects of carbon dioxide on microbial growth and food quality. *J. Food Protect.* 48:532–537.

Devlieghere, F., J. Debevere, and J. Van Impe. 1998. Effect of dissolved carbon dioxide and temperature on the growth of *Lactobacillus sake* in modified atmospheres. *Interact. J. Food Microbiol.* 41:231–238.

Devlieghere, F., A. H. Geeraerd, K. J. Versyck, B. Vandewaetere, J. Van Impe, and J. Debevere. 2001. Growth of *Listeria monocytogenes* in modified atmosphere-packed, cooked meat products: A predictive model. *Food Microbiol.* 18:53–66.

Dixon, N. M., and D. B. Kell. 1989. The inhibition by CO_2 of the growth and metabolism of micro-organisms. *J. Appl. Bacteriol.* 67:109–136.

Farber, J. M. 1991. Microbiological aspects of modified-atmosphere packaging: A review. *J. Food Protec.* 54:58–70.

García-Esteban, M., D. Ansorena, and I. Astiasarán. 2004. Comparison of modified atmosphere packaging and vacuum packaging for long period storage of dry-cured ham: Effect on color, texture and microbiological quality. *Meat Sci.* 67:57–63.

Gill, C. O. 1988. The solubility of carbon dioxide in meat. *Meat Sci.* 22:65–71.

Gill, C. O., and T. Jones. 1996. The display life of retail packaged pork chops after their storage in master packs under atmosphere of N_2, CO_2 or O_2+ CO_2. *Meat Sci.* 42:203–213.

Gill, C. O., and M. D. Reichel. 1989. Growth of the cold-tolerant pathogens *Yersinia enterocolitica, Aeromonas hydrophila* and *Listeria monocytogenes* on high-pH beef packaged under vacuum or carbon dioxide. *Food Microbiol.* 6:223–230.

Gill, C. O., and K. H. Tan. 1980. Effect of carbon dioxide on growth of meat spoilage bacteria. *Appl. Environ. Microbiol.* 39:317–319.

Gray, R. J. H., P. H. Elliott, and R. I. Tomlins. 1984. Control of two major pathogens on fresh poultry using a combination of potassium sorbate/carbon dioxide packaging treatment. *J. Food Sci.* 49:142–145, 179.

Hintlian, C. B., and J. Hotchkiss. 1987. Comparative growth of spoilage and pathogenic organisms on modified atmosphere-packaged cooked beef. *J. Food Protect.* 50:218–223.

Hunt, M. C., R. A. Mancini, K. A. Hachmeister, D. H. Kropf, M. Merriman, G. Del Duca, and G. Milliken. 2004. Carbon monoxide in modified atmosphere packaging affects color, shelf life and microorganisms of beef steaks and ground beef. *J. Food Sci.* 45:45–52.

Jackson, T. C., G. R. Acuff, C. Vanderzant, T. R. Sharp, and J. W. Savell. 1992. Identification and evaluation of volatile compounds of vacuum and modified atmosphere packaged beef strip loins. *Meat Sci.* 31:175–190.

Jakobsen, M., and G. Bertelsen. 2000. Colour stability and lipid oxidation of fresh beef: Development of a response surface model for predicting the effects of temperature, storage time, and modified atmosphere composition. *Meat Sci.* 54:49–57.

Jakobsen, M., and G. Bertelsen. 2002. The use of CO_2 in packaging of fresh meats and its effect on chemical quality changes in the meat: A review. *J. Muscle Foods.* 13:143–168.

Jakobsen, M., and G. Bertelsen. 2004. Predicting the amount of carbon dioxide absorbed in meat. *Meat Sci.* 68:603–610.

Jay, J. M. 2000. *Modern food microbiology* (6th ed.). Gaithersburg, MD: Aspen Publishers.

Jayasingh, P., D. P. Cornforth, C. E. Carpenter, and D. Whittier. 2001. Evaluation of carbon monoxide treatment in modified atmosphere packaging or vacuum packaging to increase color stability of fresh beef. *Meat Sci.* 59:317–324.

Jensen, C., M. Fiensted-Jensen, L. H. Skibsted, and G. Bertelsen. 1998. Effects of rape seed oil, copper (B) sulphate and vitamin E on drip loss, color, and lipid oxidation of chilled pork chops packed in atmosphere. *Meat Sci.* 50:211–221.

John, L., D. Cornforth, D. E. Carpenter, O. Sørheim, B. C. Pettee, and D. R. Whittier. 2004. Comparison of color and thiobarbituric acid values of cooked hamburger patties after storage of fresh beef chubs in modified atmospheres. *J. Food Sci.* 69:C608–C614.

Kautler, D. A., R. K. Lynt, T. Lilly, Jr., and H. M. Solomon. 1981. Evaluation of the botulism hazard from nitrogen-packed sandwiches. J. Food Protect. 44:59–61.

Kennedy, C., D. J. Buckley, and J. P. Kerry. 2004. Display life of sheep meats retail packaged under atmospheres of various volumes and compositions. *Meat Sci.* 68:649–658.

Killinger, K. M., M. C. Hunt, R. E. Campbell, and D. H. Kropf. 2000. Factors affecting premature browning during cooking of store-purchased ground beef. *J. Food Sci.* 65:585–587.

Kirwan, M. J., and J. W. Strawbridge. 2003. Plastics in food packaging. In *Food packaging technology,* eds. R. Coles, D. McDowell, and M. J. Kirwan, 174–240. Boca Raton, FL: CRC Press.

Knabel, S. J. 1995. Fordborne illness: Role of home food handling practices. *Food Tech. 49*(4):119–131.

Kraft, A. A., and J. C. Ayres. 1952. Post-mortem changes in stored meats: IV. Effect of packaging materials on keeping quality of self-service meats. *Food Technol. 6*(1):8–12.

Krause, T. R., J. G. Sebranek, R. E. Rust, and M. S. Honeyman. 2003. Use of carbon monoxide packaging for improving the shelf life of pork. *J. Food Sci.* 68:2596–2603.

Kropf, D. H. 2004. Packaging/modified- and controlled- atmosphere. In *Encyclopedia of meat sciences,* eds. W. Jensen, C. Devine, and M, Dikeman, 962–969. Oxford, UK: Elsevier.

Kusmider, E. A., J. G. Sebranek, S. M. Lonergan, and M. S. Honeyman. 2002. Effects of carbon monoxide packaging on color and lipid stability of irradiated ground beef. *J. Food Sci.* 67:3463–3468.

Lazar, V. 2004. Packaging sells. *Meat Processing. 43*(4):28–33.

Ledward, P. A. 1970. Metmyoglobin formation in beef stored in carbon dioxide-enriched and oxygen-depleted atmosphere. *J. Food Sci.* 35:33–37.

Lee, M., J. G. Sebranek, and F. C. Parrish, Jr. 1996. Accelerated postmortem aging of beef utilizing electron-beam irradiation and modified atmosphere packaging. *J. Food Sci.* 61:133–136, 141.

Livingston, M., M. S. Breuer, J. Killifer, B. Bidner, and F. McKeith. 2004. Shelf life characteristics of enhanced modified atmosphere packaged pork. *Meat Sci.* 68:115–122.

Löwenadler, J., and U. Rönner. 1994. Determination of dissolved carbon dioxide by coulometric titration in modified atmospheres systems. *Lett. in Appl. Microbiol.* 18:285–288.

Luño, M., J. A. Beltrán, and P. Roncalés. 1998. Shelf life extension and colour stabilization of beef packaged in a low O_2 atmosphere containing CO: Loin steaks and ground meat. *Meat Sci.* 48:75–84.

Mancini, R. A., M. C. Hunt, K. A. Hachmeister, D. H. Kropf, and D. E. Johnson. 2005. Exclusion of oxygen from modified atmosphere packages limits beef rib and lumber vertebrae marrow discoloration during display and storage. *Meat Sci.* 69:493–500.

Mitz, M. A. 1979. CO_2 biodynamics: A new concept of cellular control. *J. Theor. Biol.* 80:537–551.

Molin, G. 1983. The resistance to CO_2 of some food-related bacteria. *Europ. J Appl. Microbiol. Biotech.* 18:214–217.

Møller, J. K. S., M. Jakobsen, C. J. Weber, T. Mortinussen, L. H. Skibsted, and G. Bertelsen. 2003. Optimization of colour stability of cured ham during packaging and retail display by a multifactorial design. *Meat Sci.* 63:169–175.

Møller, J. K. S., J. S. Jensen, M. B. Olsen, L. H. Skibsted, and G. Bertelsen. 2000. Effect of residual oxygen on color stability during chill storage of sliced pasteurized ham packaged in modified atmosphere. *Meat Sci*. 54:399–405.

Møller, J. K. S., L. Nannerup, and L. H. Skibsted. 2005. Effect of carbon dioxide on autoxidation and photooxidation of nitrosylmyoglobin. *Meat Sci*. 69:71–78.

Mullan, M., and D. McDowell. 2003. Modified atmosphere packaging. In *Food packaging technology,* eds. R. Coles, D. McDowell, and M. J. Kirwan, 303–331. Boca Raton, FL: CRC Press.

Nannerup, L. D., M. Jakobsen, F. Van den Berg, J. S. Jensen, J. K. S. Møller, and G. Bertelsen. 2004. Optimizing colour quality of modified atmosphere packed sliced meat products by control of critical packaging parameters. *Meat Sci*. 68:577–585.

Nissen, H., O. Alvseike, S. Bredholt, A. Holck, and T. Nesbakken. 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. *Int. J. Food Microbiol*. 59:211–220.

Ozdemir, M., and J. D. Floros. 2004. Active food packaging technologies. *Crit. Rev. Food Sci. Nutr.* 44:185–193.

Parrish, F. C., Jr., J. A. Boles, R. E. Rust, and D. G. Olson. 1991. Dry and wet aging effects on palatability attributes of beef loin and rib steaks from three quality grades. *J. Food Sci*. 56:601–603.

Rao, D. N., and N. M. Sachindra. 2002. Modified atmosphere and vacuum packaging of meat and poultry products. *Food Rev. Int*. 18:263–293.

Sawaya, W. N., A. S. Elnawawy, S. Al-Zenki, J. Al-Otaibi, H. Al-Omirah, and H. Al-Amiri, H. 1995. Storage stability of chicken as affected by MAP and lactic acid treatment. *J. Food Sci*. 60:611–614.

Sebranek, J .G. 1985. Stabilizing the properties of meat products with packaging systems. In *Proceedings of the Meat Industry Research Conference,* 150–167. Savoy, IL: American Meat Science Association.

Sebranek, J. G. 2005. Unpublished data.

Seyfert, M., M. C. Hunt, R. Mancini, K. A. Hachmeister, D. H. Kropf, and J. A. Unruh. 2004. Accelerated chilling and modified atmosphere packaging affect colour and colour stability of injection-enhanced beef round muscles. *Meat Sci*. 68:209–219.

Seyfert, M., M. C. Hunt, R. A. Mancini, D. H. Kropf, and S. I. Stroda. 2004. Internal premature browning in cooked steaks from enhanced beef round muscles packaged in high- or ultra-low oxygen modified atmosphere. *J. Food Sci*. 69:143–146.

Seyfert, M., R. A. Mancini, and M. C. Hunt. 2004. Internal premature browning in cooked ground beef patties from high-oxygen-modified-atmosphere packaging. *J. Food Sci*. 69:C721–C725.

Smiddy, M., N. Papkovskaia, D. B. Papkovsky, and J. D. Kerry. 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 Res. Int*. 35:577–584.

Smolander, M., E. Hurme, and R. Ahvenainen. 1997. Leak indicators for modified atmosphere packages. *Trends in Food Sci. Technol*. 8:101–106.

Solomon, J. 2004. Eliminating oxygen. *Meat Poult*. *50*(9):38–41.

Sørheim, O., T. Aune, and T. Nesbakken. 1997. Technological, hygienic and toxicological aspects of carbon monoxide used in modified atmosphere packaging. *Trends Food Sci. Technol*. 8:307–312.

Sørheim, O., H. Nissen, T. Aune, and T. Nesbakken. 2001. Use of carbon monoxide in retail meat packaging. In *Proceedings of the Reciprocal Meat Conference,* 47–51. Savoy, IL: American Meat Science Association.

Sørheim, O., H. Nissen, and T. Nesbakken. 1999. The storage life of beef and pork packaged in an atmosphere with low carbon monoxide and high carbon dioxide. *Meat Sci.* 52:157–164.

Sørheim, O., R. Ofstad, and P. Lea. 2004. Effects of carbon dioxide on yield, texture and microstructure of cooked ground beef. *Meat Sci.* 67:231–236.

Tan, K. H., and C. O. Gill. 1982. Physiological basis of CO_2 inhibition of a meat spoilage bacterium, *Pseudomonas flourescens*. *Meat Sci.* 7:9–17.

Tewari, G., D. S. Jayas, and R. A. Holley. 1999. Centralized packaging of retail meat cuts: A review. *J. Food Protect.* 62:418–425.

Thayer, D. W., and G. Boyd. 1999. Irradiation and modified atmosphere packaging for the control of *Listeria monocytogenes* on turkey meat. *J. Food Protect.* 62:1136–1142.

U.S. Food and Drug Administration. 2002. Agency Response Letter GRAS Notice No. GRN 000083. Retrieved November 2004, from http://www.cfsan.fda.gov/~rdb/opa-gO83.html.

U. S. Food and Drug Administration. 2004. Agency Response Letter GRAS Notice No. GRN 000143, 2004. Retrieved November 2004, from http://www.cfsan.fda.gov/~rdb/opa-g143 .html.

Zhao, Y., J. G. Sebranek, J. Dickson, and M. Lee. 1996. Bacteriological, physiochemical, and sensory quality of fresh pork chops with low-dose irradiation and modified-atmosphere packaging. *J. Food Protect.* 59:493–501.

Zhao, Y., J. H. Wells, and K. W. McMillin. 1994. Applications of dynamic modified atmosphere packaging systems for fresh red meats. *J. Muscle Foods.* 5:299–328.

Zhao, Y., J. H. Wells, and K. W. McMillin. 1995. Dynamic changes of headspace gases in CO_2 and N_2 packaged fresh beef. *J. Food Sci.* 60:571–575, 591.

18 Perspectives for the Active Packaging of Meat Products

Véronique Coma
Laboratoire de Chimie des Substances Végétales
Centre de Recherche en Chimie Moléculaire,
Université Bordeaux

CONTENTS

18.1 General Developments in Active Packaging for Meat Preservation 452
18.1.1 O_2-Scavenging Technology 452
18.1.2 CO_2 Scavenging and Emitting Technology 454
18.1.3 Moisture Regulators 455
18.1.4 Release or Absorption of Flavors and Odors and Antioxidant Release 455
18.1.5 Commercial Generators of Antimicrobial Substances 456
18.2 Developments and Perspectives in Antimicrobial Packaging for Meat Preservation 456
18.2.1 Antimicrobial Packaging Produced by Adding a Sachet into the Package 456
18.2.1.1 CO_2 Generators 456
18.2.1.2 Chlorine Dioxide Generators 457
18.2.2 Bioactive Agents Merely Dispersed in the Packaging 457
18.2.2.1 Bacteriocins 459
18.2.2.2 Spices and Essential Oils 460
18.2.2.3 Enzymes 461
18.2.2.4 Preservatives and Additives 461
18.2.3 Bioactive Agents Coated on the Surface of the Packaging Material 462
18.2.3.1 Migration Process 464
18.2.3.2 Released Through Evaporation in the Headspace 464
18.2.4 Antimicrobial Macromolecules With Film-Forming Properties 465
18.2.4.1 Naturally Antimicrobial Macromolecules Exhibiting Film-Forming Properties 465
18.2.4.2 Surface-Bounded Bioactive Agents 466

18.2.5 Bioactive Edible Coating 466
18.3 Conclusion 467
References 469

Food-borne disease has always topped the list of food safety concerns for most governments around the world. Recent highly publicized outbreaks of *Listeria*, *Salmonella*, and *Escherichia coli* 0157:H7 have placed food-borne diseases at the top of consumers' list of food safety concerns (Jones 2002). Therefore, the food industry is currently subjected to increasingly strict rules and controls at the request of the consumer. In particular, recent outbreaks of contaminations associated with meat products have heightened consumer concerns. Providing physical protection as well as moisture and oxygen barriers is a given. With today's quality demands, that might not be enough! Growing concerns regarding the safety of intermediate-moisture foods warrant a greater emphasis on the development of active packaging, defined as "a type of packaging that changes the condition of the packaging to extend shelf-life or improve safety or sensory properties while maintaining the quality of the food" (EC Proposes Regulations 2003; Quintavalla and Vicini 2002). Principal active packaging systems include those that involve oxygen scavenging, moisture absorption and control, carbon dioxide and ethanol generation, and antimicrobial systems. As related by Strathmann, Pastoreli, and Simoneau (in press), the most common examples of active packaging materials are oxygen scavengers to prevent oxidation of food (removing the residual oxygen from the headspace and absorbing oxygen diffused through the packaging during storage) and moisture removers or desiccants for preventing spoilage by micro-organisms or a change in sensory properties. Furthermore, some materials change the carbon dioxide content of the atmosphere in the packaging. However, only limited applications are actually identified in Europe. This phenomenon is related to the lack of a specific regulation for the use of these types of packaging. Preliminary investigations have been performed with a multipartner research project financed by the European Commission on Evaluating Safety, on effectiveness, economic and environmental impact, and consumer acceptance of active and intelligent packaging, but more data are needed. Although little developed in Europe, in the past decade, active packaging has become one of the major areas of research in food packaging. Of these active packaging systems, the antimicrobial version is of great importance. As reported by Oussalah, Caillet, Salmieri, Saucier, and Lacroix (2004), carved beef has a short shelf life that varies between three and five days when kept at +4°C. *Pseudomonas, Enterobacteriacea,* and lactic acid bacteria are responsible for meat deterioration. Meat and meat products may be also contaminated by *L. monocytogenes, S. typhimurium, E. coli* 0157:H7 and *Yersinia enterolitica,* responsible for food-borne illnesses and deaths. Thus, additional measures should be used to ensure the safety of such products. In addition to the development of pathogens, microbial growth commonly induces undesirable organoleptic changes during the storage of meat. If bacterial growth at the meat surface by a packaging film could be delayed or halted, large gains in product shelf life would be possible (Ouattara, Simard, Holley, Piette, and Begin 1997). Depending on the region, some of the traditional methods of preserving foods

from the effect of microbial growth (thermal processing, drying, freezing, irradiation, etc.) cannot be applied to some food products, such as fresh meats and ready-to-eat products. Moreover, as discussed by Skandamis and Nychas (2002), despite the extended shelf life of refrigerated products stored under vacuum pack or modified atmosphere packaging (MAP) conditions, there is an increasing concern about the growth and survival of microaerophilic psychotrophic pathogens.

For all these reasons, the red meat industry has increased the use of preservative packaging systems to satisfy the demand for extended product storage life and reduction of spoilage losses. To prevent the development and spread of spoilage and pathogenic micro-organisms via meat foodstuffs, antimicrobial packaging materials could be a potential alternative solution. Indeed, combining biocide agents directly into a packaging material could provide several advantages. First, only the necessary amount of biocide would be used. This system could be more efficient by maintaining high concentrations on food surfaces with a low migration of active substances. As opposed to mixing antimicrobial compounds directly with food, their incorporation into film would localize the functional effect at the food surface. This approach can be used to impart a strong, localized, functional effect, without excessively elevating the concentration of the additives in foods (figure 18.1). Second, the agent would not be a direct additive to the food product. Third, the incorporation of bactericidal agents or growth inhibitors into meat formulations may result in partial inactivation of the active substances by product constituents and is therefore expected to have only a limited effect on the surface flora. In addition, if the packaging materials were made of a biopolymer, there would be environmental advantages (Gennadios, Hanna, and Kurth 1997). Due to the strong development of antimicrobial packagins in the active packaging area, this chapter, starting with a short section related to developments in active packaging for meat preservation, surveys the range of use of antimicrobial packaging processes to extend shelf life and to assure the innocuity and preservation of meat products. According to Cooksey (2001), there are three basic categories of antimicrobial films:

1. Incorporation of the antimicrobial substances into a sachet connected to the package from which the bioactive substance is released during further storage.
2. Direct incorporation of the antimicrobial additive into the packaging film.
3. Coating of the packaging with a material that acts as a carrier for the additive.

These categories of materials can release the antimicrobial agents onto the surface of the food (Appendini and Hotchkiss 2002; Buonocore, Del Nobile, Panizza, Corbo, and Nicolais 2003; Halek and Garg 1989; Sebti, Pichavant, and Coma 2002; Weng, Chen, and Chen 1999; Weng and Hotchkiss 1992). The antimicrobial agents may either migrate into the food through diffusion and partitioning or be released through evaporation in the headspace. This system is more efficient compared to a direct application of antimicrobial agent onto meat surfaces, because it slows the migration of the agents away from the surface, thus helping to maintain high concentrations where they are needed.

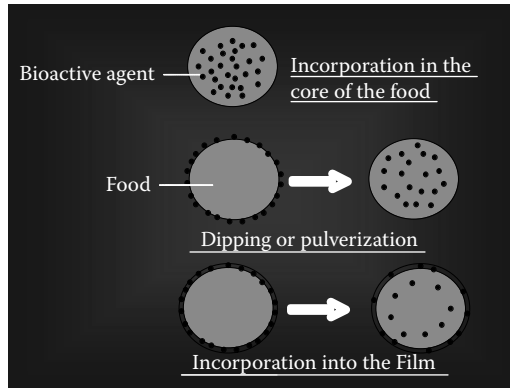


FIGURE 18.1 Different incorporation modes of additives in food products (incorporation into the foodstuff, dipping or pulverization, and incorporation into a film) and consequences. The black points correspond to the antimicrobial compound.

Moreover, antimicrobial macromolecules with film-forming properties, such as cationic amino-polysaccharides, can produce antimicrobial films (Begin and Van Calsteren 1999; Coma et al. 2002; Ouattra, Simard, Piette, Bégin, and Holley 2000; Pen and Jiang 2003). Packaging materials with bioactive agents chemically bonded to the polymer can be included in this category. The release of the biocide agent would not be required for this system. Therefore, legal issues and standards concerning the rate of migration of substances in packaging into food products do not limit the development of such bioactive materials. However, the limitation is the necessity of a direct contact between the packaging and the food. The last category might be related with bioactive edible coating, directly applied onto the food.

18.1 GENERAL DEVELOPMENTS IN ACTIVE PACKAGING FOR MEAT PRESERVATION

Major active packaging techniques are concerned with substances absorbing oxygen, carbon dioxide, ethylene, moisture, and flavors and odors and releasing carbon dioxide, antioxidants, flavors, and antimicrobial agents. Examples of current and future applications of active packaging in meat food preservation are shown in table 18.1. A good presentation of the different systems of active and intelligent packaging materials was proposed by Gontard (2000).

18.1.1 O_2-Scavenging Technology

In many cases, food deterioration is caused by the oxidation of food constituents or spoilage by molds in the presence of O_2. O_2 absorbers applied to meat product packaging can prevent the growth of molds, aerobic bacteria such as *Pseudomonas,* and oxidative damage of muscle pigments and flavors to avoid discoloration (Vermeiren, Devlieghere, Van Beest, De Kruijf, and Debevere 1999).

As specified in EC Proposes Regulations (2003), among the packaging technologies developed by and for meat and meat products, MAP has led to the evolution

TABLE 18.1
Examples of Current and Future Applications of Active Packaging for Meat Food Preservation

Concept	Meat Foods
O_2 scavenger	Salami, smoked meat
CO_2 scavenger	Beef jerky, poultry products
CO_2 emitter	Fresh meat
Moisture scavenger	Meat
Biocide systems	Meat and foods in general

Adapted from Vermeiren et al. 1999

of fresh and minimally processed food preservation. In such packaging, an initial atmosphere can be generated by injecting a desired initial gas mixture that potentially changes as a result of multiple variables including gas permeability, water vapor transfer through the package material, relative humidity, temperature and so on. Residual oxygen can, however, be responsible for various degradation phenomena. O_2-scavenging technology may be used appropriately to remove residual O_2 after MAP or vacuum packaging. Moreover, this system can absorb the O_2 that permeates through the packaging film. O_2 absorbers can also be a complement to vacuum packaging to avoid photo-oxidation phenomena, in particular for sliced delicatessen products. Indeed, presentations in small packages with a transparent cover to show the food product are more and more appreciated; unfortunately, if oxygen traces are still present when the package is put on the shelf, the photo-oxidation phenomena start to take effect, leading to a rapid discoloration of the meat. The majority of currently commercially available O_2 scavengers (table 18.2) are based on iron oxidation and are incorporated into a sachet (Ageless-Mitsubishi Gas Chemical Co, Japan/ATCO O_2-absorber-Standa industry, France):

$$FeOH_2 + \tfrac{1}{4}O_2 + \tfrac{1}{2}H_2O \rightarrow Fe(OH)_3$$

Multisorb Technologies (United States, Fresh pax and Fresh max) and Bioka Ltd. (Finland) commercially produce other O_2 absorber sachets, which can be found in the packages of many foods such as meat products (e.g., smoked ham and salami). This type of sachet is also used for the conservation of large pieces of meat to limit the growth of spoilage micro-organisms such as *Pseudomonas*, before cutting up the meat.

An alternative to sachets is the incorporation of the scavenger into the packaging structure itself (e.g., *zero 2*, Southcorp Australia Ltd., Australia; Amoco Chemical, United States, etc.). It should be noted that the speed and capacity of O_2-scavenging films are considerably low compared with iron-based O_2-scavenger sachets. The O_2-scavenging film of Ageless-OMAC (Mitsubishi Gas Chemical Co, Japan) is activated by heat. Today, it is used in particular for the preservation of pasteurized cooked rice and could potentially be used for meat preservation.

TABLE 18.2
Some Commercial Active Packaging Materials for Food Applications

Concept	Trade Name	Company	Forms
CO_2 emitting/O_2 scavenging	Ageless G	Mitsubishi Gas Chemical (Japan)	Sachets
CO_2 scavenging/O_2 scavenging	Ageless G	Mitsubishi Gas Chemical (Japan)	Sachets
CO_2 emitting	Verifrais	SARL Codimer (France)	Sachets
CO_2 emitting/O_2 scavenging	Freshpax M	Multisorb Technologies (United States)	Sachets
CO_2 scavenging	Freshlock	Multisorb Technologies (United States)	Sachets
CO2 emitting		Standa (France)	Gel into sachets in contact with the food
Absorption of liquid water		Standa (France)	
	Antimicrobial Substances		
Ethanol vapor emitting	Ethicap Negamold	Freund	Sachets
	Oiteck	Nippon Kayalan (Japan)	Sachets
Silver Zeolite	AgIon™	AgIon Technologies	Paper, plastic packaging
Triclosan	Microban	Microban Prod. (United Kingdom)	Plastic packaging
Allylisothiocyanate	WasaOuro	Lintec Corp. (Japan)	Sheets
Glucose oxydase (H_2O_2)	Bioka	Bioka Ltd. (Finland)	Sachets
Chlorine dioxide	Microsphere	Bernard Technologies (United States)	Sachets, film, wraps, plastics

Close-fitting packages such as the vacuum pack for meats are an example where the headspace is very small and oxygen permeation is the cause of quality loss. It is in such circumstances that oxygen-scavenging plastic films are particularly needed (Rooney 2000).

In parallel to O_2 scavengers, due to a potential microbial development CO_2 generators are increasingly envisaged in packaging materials used to extend the shelf life of meat products.

18.1.2 CO_2 Scavenging and Emitting Technology

As for O_2 scavengers, this type of active packaging is frequently associated with MAP systems. For meat preservation, CO_2 generators are mainly used because of their inhibitory activity against aerobic bacteria and fungi. Among oxygen, CO_2, and N_2, which are most often used in MAP systems, CO_2 is the only one with a direct antimicrobial effect, resulting in an increased lag phase and generation time during the logarithmic phase of growth. These systems are studied in more detail later in this chapter (18.2.1.1). The association of active systems, CO_2 generators, and O_2 absorbers, are often envisaged. When a package has been flushed with a mixture of

gases including CO_2, the CO_2 dissolves partly in the product and creates a partial vacuum. In such cases, the simultaneous release of CO_2 from inserted sachets, which consume O_2, is desirable. Such systems are based on either ferrous carbonate or a mixture of ascorbic acid and sodium bicarbonate (Ageless G, Mitsubishi Gas Chemical, Japan; Freshpax M., Multisorb Technologies, United States; Verifrais, SARL Codimer, France).

18.1.3 Moisture Regulators

Foods that are susceptible to moisture damage need to be packaged in a high-humidity-barrier material. A certain amount of moisture, however, can be trapped in the packaging or develop during distribution. According to Rooney (2000), the circumstances in which water needs to be controlled in food packs include the accumulation of liquid water as in tissue fluids from cut and fresh meats, and the condensation of water vapor from high water activity in foods undergoing temperature cycling, for example.

For example, to prevent the first phenomenon, the Verifrais process (Codimer, France) is based on a lower compartment in polystyrene trays connected by holes to the upper one containing the meat. Juice of the meat is absorbed and, at the same time, carbon dioxide is released, which potentially inhibits microbial growth. Standa Industrie (France) has also developed a system based on a gel incorporated into a sachet, Atco Pad, which progressively absorbs the tissue fluid from fresh meat products.

Meat-based products may be sensitive to the dehydration process. Excessive water evaporation through the packaging material may result in the desiccation of the packed foodstuff or may favor lipid oxidation. To prevent this and establish the desired relative humidity in the package headspace, a film with the appropriate water vapor permeability or a moisture-controlling sachet can be used (Standa, France). On the other hand, desiccants (drip-absorbent sheets: Thermarite, Australia; Toppan, Japan; Peaksorb, Peakfresh Products, Australia; sachets with silica gel or others: Desipak, Sorb-it, Tri-sorb, and 2-in-1, Multisorb Technologies, United States) are successfully being used for some meat-based foods to lower water activity, thereby reducing the growth of molds, yeast, and spoilage bacteria on foods with high water content, such as ready-to-eat meals. Examples of applications are in the removal of melting ice from frozen meat or frozen blood or tissue fluid from meat to make the package more attractive to the consumer (Vermeiren et al. 1999). In conclusion, interception of the excess water vapor in a food package has been approached previously by use of desiccant sachets. A thin layer of one or more humectants placed between two layers of polyvinyl alcohol (PVA) film (highly permeable to water vapor) is a process in development.

18.1.4 Release or Absorption of Flavors and Odors and Antioxidant Release

The absorption of food flavors by packaging materials may result in loss of flavor, taste intensities, and changes in the organoleptic profile of foods (Vermeiren et al. 1999). Flavor incorporation in packaging material might be used to minimize flavor

scalping. Flavor release might also provide a means of masking off-odors coming from the food or the packaging. Further applications of flavor-enriched packaging materials include the possibility of improving the organoleptic quality of the product by emitting desirable flavors into the food and encapsulating pleasant aromas. This type of system is not extensively used in meat packaging.

Antioxidants are widely used as food additives to improve the oxidation stability of lipids and to prolong shelf life, mainly for dried products and O_2-sensitive foods. This type of active packaging is still in the experimental stage and few commercial applications are known. Today, research is mainly based on cereal products.

18.1.5 Commercial Generators of Antimicrobial Substances

This type of system, based on the release of antimicrobial substances, is included in the next section.

18.2 DEVELOPMENTS AND PERSPECTIVES IN ANTIMICROBIAL PACKAGING FOR MEAT PRESERVATION

Among all the literature developed on the subject, only the studies related to meat product preservation are presented here, in a nonexhaustive way. Several approaches to the application of antimicrobial agents in food packages exist. As the majority of commercially developed systems have shown, the bioactive substances may be included in a sachet added before closing the package. Antimicrobial substances might also be incorporated in the packaging or coated on the surface of the material. Some film-forming molecules exhibit antimicrobial activity and may be used as directly bioactive packaging materials. Finally, inhibitory activity may come from a bioactive edible coating or film, associated with lighter packaging.

Commercial generators of antimicrobial substances (second part of table 18.2) may be based on CO_2, ethanol, or chlorine dioxide generators. An Ethicap sachet (Freund, Japan) could be used as a source of ethanol vapor. Other systems, such as Silver zeolite, are also proposed. However, ethanol generators are more developed in bakery packaging (MAP) due to their antifungal activity.

18.2.1 Antimicrobial Packaging Produced by Adding a Sachet into the Package

Only a few research studies deal with new antimicrobial systems incorporated into sachets.

18.2.1.1 CO_2 Generators

The inhibitory action of CO_2 has differential effects on micro-organisms. Thus, whereas aerobic bacteria such as *Pseudomonas* are inhibited by moderate to high levels of CO_2 (10%–20%), micro-organisms such as lactic acid bacteria can be stimulated by CO_2. Furthermore, pathogens such as *Clostridium perfringens*,

C. botulinum, and *L. monocytogenes* are minimally affected by CO_2 levels lower than 50%. However there is concern that by inhibiting spoilage micro-organisms, a food product may be made to appear edible while containing a high charge of pathogens that have multiplied due to a lack of indigenous competition (U.S. Food and Drug Administration 2001). For most applications in meat and poultry preservation, high CO_2 levels (10%–80%) are desirable because these high levels inhibit surface microbial growth and thereby extend shelf life (Vermeiren et al. 1999).

18.2.1.2 Chlorine Dioxide Generators

Chlorine dioxide can exist in a gaseous, liquid, or solid state. Its efficiency against bacteria, fungi, and viruses can be delivered from a solid state, called Microspheres (Bernard Technologies, United States), through the interaction of moisture to produce a controlled and sustained release of chlorine dioxide in gaseous form. According to the company, no residue is left, nor is the food product contained in the packaging tainted in any way. Sustained and controlled release of chlorine dioxide is related to exposure to humidity greater than 80% and light. The result is high activity against a broad spectrum of micro-organisms, including actively growing vegetative cells and spores. Microsphere powder can be delivered from sachet previously incorporated into the packaging. The sustained and controlled release of chlorine dioxide from the Microspheres can be varied from peak delivery of 1 ppm to 100 ppm for periods of days to six weeks. Applications for this technology are just beginning to unfold in the food industry to reduce food safety risks for meat, poultry, fish, dairy, confectionery, and baked goods.

18.2.2 Bioactive Agents Merely Dispersed in the Packaging

Some commercial systems based on bioactive compounds directly incorporated into the packaging are mentioned in the second part of table 18.2. A good example is the AgION antimicrobials which are based on silver, a powerful antimicrobial metal ion, as the active ingredient. AgION compounds are compatible with a wide range of polymers and they will not volatilize during processing. AgION antimicrobial products can be compounded into bulk polymers, incorporated into fibers during spinning, and mixed into a variety of coatings. Another example is the Triclosan (2,4,4'-Trichloro-2'-hydroxydiphenyl-ether), the antimicrobial substance used by Microban (United Kingdom). For more than 20 years this chemical compound has been used effectively in personal hygiene products such as toothpaste, mouthwash, deodorant, and soap, as well as an antibacterial agent in the hospital environment. This protection is achieved by combining Triclosan with any of the major polymers (e.g., PE, PP, PVC). The Triclosan fits into the empty spaces of the polymer and migrates to the surface to start its work against any developing bacteria. During washing, the molecules closest to the surface are cleaned away but are immediately replaced by other protective molecules. In the mid-1990s, Freund (Japan) combined an oxygen absorber with an inhibitive agent to produce Negamold, used for fresh fish, meat, delicatessen products, milk products, and cheeses.

Such systems are mainly elaborated by adding the antimicrobial compound to the extruder when the film or coextrusion is produced. A disadvantage is that the high temperatures and shearing associated with the extrusion process can deteriorate the antimicrobial additives. Edible film, used as the primary packaging, could be an alternative. Different antimicrobial molecules, such as bacteriocins, enzymes, chelatants, and organic acids (i.e., propionic and sorbic acids), included in different films, have been tested to suppress pathogenic or saprophyte bacteria growth in meat product preservation (table 18.3).

TABLE 18.3
Selected Bioactive Compounds Directly Incorporated Into the Packaging for Potential Applications of Meat Preservation

Active Component	Polymer Carrier	Example of Tested Substrate and Target Strain	References
Bacteriocin			
Nisin	Cellulose films, PE	Ham	Scannell et al. (2000)
	PE	Beef carcass tissue	Siragusa, Cutter, and Willett (1999)
	HPMC	Culture media; *Listeria monocytogenes*	Sebti and Coma (2002)
Lacticin	Cellulose film, PE	Ham	
Pediocin	Cellulose	Cooked meats	Ming, Weber, Ayres, and Sandine (1997)
Enzymes			
Glucose oxidase	Alginate	Fish	Field, Pivarnick, Barnett, and Rand (1986)
Bioactive polymer			
Chitosan	Chitosan	Cooked ham	Ouattara, Simard, Piette, Bégin, and Holley (2000)
Chitosan	Chitosan	Culture media; *Listeria monocytogenes*	Coma et al. (2002)
Organic acids			
Lactic acid	Alginate	Beef muscle	Siragusa and Dixon (1992)
Propionic acid	Chitosan	Cooked ham	Ouattara et al. (2000)
Others			
Tocopherol	LDPE	Beef	Moore et al. (2000)
Triclosan	Plastic matrix	Food-borne pathogenic bacteria and bacteria associated with meat surface	Cutter (1999)

For the systems described here, a contact between the food and the package is obviously necessary and a migration process from the packaging materials is expected. Therefore, potential food applications include especially vacuum-packaged products (Devliedhere, Vermeiren, and Debevere 2004).

18.2.2.1 Bacteriocins

Bacteriocins are antibacterial peptides produced by lactic bacteria. These agents are generally heat stable, apparently hypoallergenic, and readily degraded by proteolytic enzymes in the human intestinal tract. Numerous bacteriocins have been characterized. Although some bacteriocins, such as pediocin PA-1 and lacticin 3147, have been developed for possible approval and use, nisin remains the most commercially important bacteriocin because of its relatively long history of safe use and documented effectiveness against important Gram-positive food-borne pathogens and spoilage agents. Indeed, as mentioned by Siragusa, Cutter, and Willett (1999), nisin, produced by *Lactococcus lactis* (generally recognized as safe [GRAS] status) and pediocin, produced by the meat fermentation starter culture bacterium *Pediococcus acidilactici*, have been demonstrated to be active against *L. monocytogenes* and other Gram-positive bacterial pathogens on meat surfaces when applied. Siragusa et al. (1999) have also incorporated nisin in a liquid form, into a polyethylene-based plastic film that was used to vacuum-pack beef carcasses. They demonstrated the retention of nisin activity when incorporated into the plastic formulation and also that the conditions used to produce the film did not eliminate the antimicrobial activity of nisin. As mentioned, nisin retained activity against *Lactobacillus helveticus* and *Brochothrix thermosphacta* inoculated in carcass surface tissue sections. An initial reduction of 2-log10 cycles of *B. thermosphacta* was observed with nisin-impregnated packaged beef within the first 2 days of storage at 4°C. After 20 days of refrigerated storage at 4°C or 12°C (to simulate temperature abuse), *B. thermosphacta* populations from nisin-impregnated plastic-wrapped samples were significantly less than controls (without nisin). Growth inhibition of *L. monocytogenes* and other pathogen strains potentially found in meat products was often studied (Coma, Sebti, Deschamps, and Pichavant 2001; Degnan, Buyong, and Luchansky 1993; Ming and Daeschel 1993; Stevens, Sheldon, Klapes, and Klaenhammer 1991). Examples are the use of nisin, pediocin AcH, and enterocins A and B in meat and meat products (Aymerich, et al. 2000; Gill and Holley 1998; Murray and Richard 1997), and pediocin AcH and sakacin P in chicken (Goff, Bhunia, and Johnson 1996; Katla et al. 2002). Large variations in the degree of inhibition were observed in these studies.

The activity of bacteriocins in foods has been the subject of only a few studies. In these cases the activity was considerably reduced, or not detectable, after some days of storage (Goff et al. 1996; Katla et al. 2002; Murray and Richard 1997). Bacteriocins are amphiphilic peptides susceptible to adsorption to food macromolecules and proteolytic degradation. These properties may limit their use as preservation agents. Aasen et al. (2003) have demonstrated the influence of different crucial factors on the efficiency of bacteriocins (i.e., nisin and sakarin) and the required dosage in foods. More than 80% of the added bacteriocin is adsorbed to the muscle protein, but the activity of the protein-bound bacteriocin still remains to be assessed. Proteolytic activity causes degradation of sakacin P, and probably other bacteriocins in non-heat-treated foods, but the losses can be compensated by increased dosages. Fat may inactivate the bacteriocin in liquid food and forcemeat. Factors that may influence the recovery and efficiency of bacteriocins are binding to meat components, partitioning into polar or nonpolar food components, and conditions that destabilize

the biological activity, like proteolytic degradation or oxidation (Daeschel McGuire, and Al-Makhlafi 1992; Murray and Richard 1997). Reduced activity or recovery of bacteriocins in foods with high fat content were shown for nisin (Bell and Lacy 1986; Davies and Adams 1994) and acidocin CH5 (Chumchalová, Josephsen, and Plocková 1998). Bacteriocins may also adsorb to proteins in the food matrix by ionic or hydrophobic bonds. These kinds of interactions and their effect on the inhibition efficiency have been studied less than the influence of fat, but, for example, Murray and Richard (1997) demonstrated that protein binding might cause a significant reduction in free bacteriocin in foods. Addition of casein reduced the activity of sakacin P, curvacin, and nisin in synthetic media. Degradation attributed to proteolytic activity was demonstrated for pediocin AcH and nisin in raw pork meat (Murray and Richard 1997).

According to Roller et al. (2002), although bacteriocins have the potential to protect some foods from spoilage, their application in raw or processed meat products is limited because binding with meat particles and fat may cause loss of activity. However, as reported by Helander, Nurmiaho-Lassila, Ahvenainen, Rhoades, and Roller (2001) or Carneiro de Melo, Cassar, and Miles (1998), substances lacking inherent toxicity yet causing increases in bacterial outer membrane permeability could find applications in food protection, as they would sensitize harmful Gram-negative bacteria to other potentially inhibitory substances by faciliting their entry into the bacterial cells. For example, the resistance of Gram-negative bacteria to the biocidal action of lysozyme diminished when the outer membrane was perturbed with ethylene diamine tetraacetate (EDTA). Apart from direct bactericidal or bacteriostatic activity, chitosan's ability to disrupt the permeability barrier of the outer membrane in Gram-negative bacteria expands the applicability of chitosan as an antimicrobial substance in foods.

18.2.2.2 Spices and Essential Oils

Spices are rich in phenolic compounds, such as flavonoids and phenolic acids, which exhibit a wide range of biological effects, including antioxidant and antimicrobial properties (Oussalah et al. 2004; Suppalku, Miltz, Sonneveld, and Bigger 2003). Basil (*Ocimum basilicum*) is a popular culinary herb and its essential oils have been used extensively for many years in the flavoring of confectionary and baked goods, condiments, sausages and meats, and so on (Suppalku et al. 2003). According to these authors, basil essential oil has a potential use in food preservation, especially in conjunction with technologies of antimicrobial packages for food products. Further research on the antimicrobial activity of the main components is required to evaluate the usefulness in the shelf life extension of packaged foods such as meat and poultry. Lacroix, Chiasson, Borsa, and Ouattara (2004) and Ouattara, Giroux, Smoragiewicz, Saucier, and Lacroix (2002) evaluated the combined effect of gamma irradiation and application of cross-linked film coatings containing spice powders on microbial growth related to ground beef contamination. Cross-linked films based on caseinate and whey protein isolate were combined with thyme, rosemary, and sage spice.

Oussalah et al. (2004) evaluated the ability of a milk-protein-based edible film containing 1% essential oils of oregano, pimento, or a mixture of both spices to

control *Pseudomonas spp.* and *E. coli* H0157:H7 growth on surface-inoculated beef muscle. The use of film containing essential oils reduced significantly the micro-organism level in meat as compared to meat samples coated with free-essential oil film and meat without film during seven days of storage. These authors also showed that both bacteria seem to use milk-protein-based film in the absence of essential oils as a substrate to sustain their growth. Moreover, films containing oregano essential oil were the most effective against the growth of both bacteria, whereas pimento-based films presented the highest antioxidant activity. Finally, the films allowed a progressive release of phenolic compounds during storage. After seven days, the film remained effective. Thus, the use of edible films containing essential oils as a preservation method for meat is promising. However, further research must be conducted to control the diffusion rate of the bioactive compounds to the meat surface during storage.

Research is needed to determine whether natural plant extracts could act as an antimicrobial agent, as an odor or flavor enhancer in packaged foods, and as a component in antimicrobial packages.

18.2.2.3 Enzymes

Materials may be the support for enzyme systems. Gill and Holley (2000) used lysozyme, a 14,6 kDa single peptide protein produced by many animals including man, which exhibits enzymatic activity against the peptidoglycan of the cell wall of both Gram-positive and Gram-negative bacteria, to limit bacterial growth in meat. Previously, these authors showed that a combination treatment of lysozyme, nisin, and EDTA may be effective in controlling the growth of spoilage and safety bacteria of cured meat products (Gill and Holley 1998). These biocides were directly incorporated into meat products (e.g., ham or bologna-based sausages), but may be combined into a packaging material.

18.2.2.4 Preservatives and Additives

Numerous studies concerning the antimicrobial activity of packaging materials have been reported and different methods of antimicrobial activity determination have been used. It is therefore difficult to compare the results of these studies because of substantial variations in the bioactive compounds included in the matrix, packaging matrix, test micro-organisms, and test methods. There is a need for the development and validation of standard methods to accurately determine the efficiency of bioactive packaging on the preservation of meat products.

Sorbic acid and its more soluble salts are widely used as preservatives in various food products. Good solubility, stability, and ease of manufacture make potassium sorbate the most widely used form in food systems. Ouattara et al. (2000) evaluated the feasibility of using acetic or propionic acids included in a chitosan matrix that was designed to slowly release the bacterial inhibitor. This system was studied to improve the preservation of vacuum-packaged processed meats during refrigerated storage. They observed that the growth of *Enterobacteriaceae* and *Serratia liquefaciens* was delayed or completely inhibited as a result of film application.

Using again the example of the Triclosan, Cutter (1999) showed that material containing 1,500 ppm of this ether inhibited the growth of bacterial strains associated with meat alterations, such as *B. thermosphacta*, *S. aureus*, *Shigella*, *S. typhimurium*, and so on. As specified by Quintavalla and Vicini (2002), the use of Triclosan for food contact applications has been recently allowed in EU countries by the Scientific Committee for Food, in the tenth additional list of monomers and additives for food contact materials, with a quantitative restriction on migration of 5 mg/Kg of foods.

Because the activity of antimicrobial film is based on the diffusion of antimicrobial entities, knowledge of diffusivity of these entities is the most important factor in developing these packaging systems. Choi et al. (2005) determined the diffusivity of potassium sorbate incorporated in a K-carrageenan film and investigated the effects of pH and temperature on this diffusivity to produce an antimicrobial food packaging system.

A lot of information is available on the diffusion of various molecules in food, such as water in drying operations or NaCl in cheese (Simal, Sanchez, Bon, Femenia, and Rossello 2001; Tütüncü and Labuza 1996). Comparatively few data could be found on the diffusivity of molecules such as bioactive substances in foods or model gel systems, for example, sorbic acid diffusivity in agar gels (Giannakopoulos and Guilbert 1986a, 1986b) or diffusion of sucrose in gellan gels (Bayarri, Rivas, Costell, and Duran 2001). Mattisson, Roger, Jönsson, Axelsson, and Zacchi (2000) studied the influence of the gel matrix on lysozyme and myoglobine diffusion. Modeling bioactive substances diffusion is crucial to understand and to modulate film activity and to investigate which type of food could be protected efficiently using active films. Several methods have already been used by several authors who worked on different solute diffusion in food (Giannakopoulos and Guilbert 1986a, 1986b). Sebti, Carnet-Ripoche, Blanc, Saurel, and Coma (2003) studied the diffusion of nisin in model gel, and a simple model using Fick's second law was developed to determine the apparent diffusion coefficient of nisin in agarose gel. The diffusive process of nisin in agarose model gel was verified, taking into account possible factors influencing the diffusion rates such as nisin concentration, temperature, and time of diffusion.

For these bioactive packages based on migration phenomenon, further studies are necessary to adapt antimicrobial activity for various meat-based food applications.

18.2.3 Bioactive Agents Coated on the Surface of the Packaging Material

An alternative to the incorporation of antimicrobial compounds during extrusion is to apply the antimicrobial additives as a coating. This has the advantage of placing the specific antimicrobial additive in a controlled manner without subjecting it to high temperature or shearing forces. In addition, the coating can be applied at a later step, minimizing the exposure of the product to contamination.

Coating can serve as a carrier for antimicrobial compounds to maintain high concentrations of preservatives on the surface of foods. Bioactive activity may be based on migration or release by evaporation in the headspace (table 18.4).

TABLE 18.4
Selected Bioactive Compounds Coated on the Surface of Packaging Materials

Active Component	Polymer Carrier	Example of Tested Substrate and Target Strain	Process to Release the Biocide	References
Bacteriocin				
Nisin	Silicon coating	Beef tissue	Migration	Daeschel, McGuire, and Al-Makhlafi (1992)
Nisin/pediocin	Plastic vacuum package	*L. monocytogenes* on ham and turkey	Migration	Ming, Weber, Ayres, and Sandine (1997)
Spice powders				
Thyme, rosemary, sage	Cross-linked caseinate and whey protein film	Ground beef	Migration	Lacroix, Chiasson, Borsa, and Ouattara (2004)
Oregano, pimentos	Milk protein films	*Pseudomonas*, *E. coli* 0157:H7 on beef muscle	Migration	Oussalah, Caillet, Salmieri, Saucier, and Lacroix (2004)
Essential oil				
Volatile compounds associated with modified atmosphere packaging	Paper immersed into essential oil extracts	Meat samples	Evaporation in the headspace	Skandamis and Nychas (2002)

18.2.3.1 Migration Process

In Japan, silver-substituted zeolite has been developed as the most common antimicrobial agent associated with plastics. Zeolite, which has some of its surface atoms replaced by silver, is laminated as a thin layer (3–6 μm) on the surface of the food contact polymer and appears to release silver ions. Silver ions, which inhibit a wide range of metabolic enzymes, have strong antimicrobial activity with a broad spectrum (Quintavalla and Vicini 2002).

Bacteriocins could be coated or adsorbed to polymer surfaces. Examples include nisin and methylcellulose coatings for polyethylene films and nisin coatings for poultry based on an adsorption of nisin on polyethylene, ethylene vinyl acetate, polypropylene, polyamide, polyester, acrylics, and polyvinyl chloride (Chen and Williams 2005). Ming, Weber, Ayres, and Sandine (1997) applied bacteriocins to the inner surface of plastic vacuum-packaging bags. They reported, using the coated materials with nisin and pediocin, inhibition of *L. monocytogenes* growth on ham, turkey breast meat, and beef under refrigerated conditions.

18.2.3.2 Released Through Evaporation in the Headspace

Because most food packaging systems consist of (1) the packaging material, (2) the food, and (3) the headspace in the package, antimicrobial agents may be either initially coated on the packaging materials to migrate into the food through diffusion and partitioning, or be released through evaporation in the headspace. The latter can be achieved with essential oils that are volatile and are regarded as "natural" alternatives to chemical preservatives. In addition, their use in foods meets the current demands of consumers for mildly processed or natural products. According to Skandamis and Nychas (2002), their application in active packaging can be of great importance, even if their practical application is limited due to flavor considerations, as well as their effectiveness, which is moderated due, especially, to interaction with food ingredients. Skandamis and Nychas evaluated the efficiency of volatile compounds of essential oils in combination with the use of MAP conditions. From paper immersed in pure essential oil extract and placed within the packaging but not in contact with the meat, these authors showed longer shelf life of meat samples supplemented with volatile compounds of oregano essential oil (*Origanum vulgare*). Volatile compounds of this essential oil affected both growth and metabolic activity of micro-organisms of meat stored at modified atmosphere. However, such inhibition is not as strong as that due to the contact of pure essential oil with micro-organisms when it is added directly onto the surface of meat.

Further research is required to establish the parameters for optimal antimicrobial efficiency, adhesion on packaging support, or the desorption procedure from the materials. Such parameters as plastics formulation, levels of antimicrobial agents, biocide purity, and varying plastic composition will be the focus of further studies. Food composition, as previously mentioned, will necessarily have consequences on the biocide efficiency. Preservation by combined methods generally results in enhanced antibacterial effects and therefore extended shelf life.

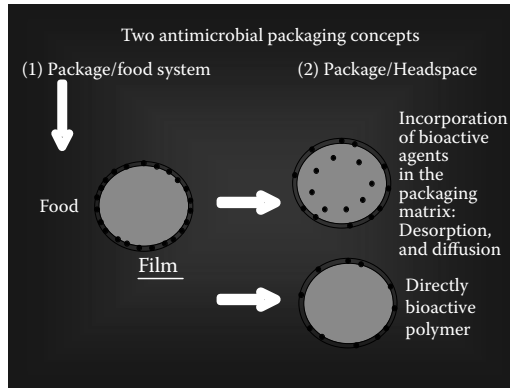


FIGURE 18.2 Both antimicrobial packaging concepts: package-food and package-headspace systems.

18.2.4 Antimicrobial Macromolecules With Film-Forming Properties

18.2.4.1 Naturally Antimicrobial Macromolecules Exhibiting Film-Forming Properties

Macromolecules such as chitosan, which exhibit an antifungal and an antibacterial activity, also show abilities to retain included antimicrobial substances (Begin and Van Calsteren 1999; Coma et al. 2002; Jeon, Kamil, and Shahidi 2002; Muzzarelli et al., 1990; Ouattara et al. 2000; Shahidi, Arackchi, and Jeon 1999). Chitosan consists of polymeric of especially 1,4 linked 2-amino-2-deoxy-β-D-glucose and, although more active against spoilage yeasts, it also inhibits some Gram-negative and Gram-positive bacteria. As reported by Helander et al. (2001), the polycationic structure of chitosan can be expected to interact with the predominantly anionic components of the bacteria surface (lipopolysaccharides, proteins). This binding leads to a disruption of the integrity of the outer membrane, resulting in loss of the barrier function. Chitosan has recently been affirmed as GRAS by the U.S. FDA (2001), thus removing some of the regulatory restrictions on its use in foods. Chitosan coating can serve, at the same time, as a carrier. Ouattara et al. (1997) evaluated the feasibility of using an incorporation of acetic and propionic acid into a chitosan matrix with or without the addition of lauric acid or cinnamaldehyde. The various films were tested against indigenous lactic acid bacteria and *Enterobacteriaceae*, and against *Lactobacillus sakei* or *Serratia liquefaciens*, surface-inoculated onto meat products. These authors showed that whereas lactic acid bacteria were not affected by the antimicrobial films, the growth of *Enterobacteriaceae* and *S. liquefaciens* was delayed or completely inhibited as a result of film application. However, improvements are necessary before the concept can be developed into a successful technology. It appears that better antimicrobial agents have to be found, which would be active against a broader range of bacteria, including the lactic acid bacteria responsible for spoilage of refrigerated vacuum-packaged processed meats. Moreover, a better carrier than chitosan has to be found to slow the release of antimicrobial agents.

Antimicrobial efficiency demonstrated in the laboratory is not always realized in foods, due to the highly reactive nature of the polycationic chitosan, which interacts with proteins, fats, and other anionic substances in foods. As reported by Sagoo, Board, and Roller (2002), the growth of Gram-negative bacteria, particularly *Pseudomonas* and *Enterobacteriaceae*, is repressed in sulphited sausages. Sulphites have a long history of safe use in meat products. However, exposure to sulphites has been linked to the aggravation of asthmatic and other respiratory problems in some sensitive individuals. Sagoo et al. showed that treatment with chitosan increases the shelf life of raw sausages stored at chilled temperatures from 7 to 15 days. In general, spoilage yeasts were more sensitive than Gram-positive bacteria, which were in turn more sensitive than Gram-negative bacteria to the biocidal action of chitosan. These authors concluded that the antilisterial action of chitosan was particularly notable and could potentially be exploited in chilled foods.

The effect of chitosan in meat preservation was also studied by Darmadji and Izumimoto (1994), including microbiological, chemical, sensory, and color qualities. These authors showed that 0.01% of chitosan inhibited the growth of some spoilage bacteria such as *Bacillus subtilis*, *E. coli*, *Pseudomonas fragi,* and *S. aureus*. In meat, during incubation at 30°C for 48 hours or storage at 4°C for 10 days, 0.5% to 1% chitosan inhibited the growth of spoilage bacteria, reduced lipid oxidation, and resulted in better sensory attributes. These authors reported that chitosan at a concentration of 1% reduced bacterial counts by an average of 1-2 log $CFU.g^{-1}$ in minced beef patties stored at 4°C for 10 days. Both latter studies used chitosan as a bioactive preservative and not as a bioactive packaging.

18.2.4.2 Surface-Bounded Bioactive Agents

In contrast to naturally antimicrobial polymers, some bioactive materials have been produced by modifying the surface composition of the polymer, for example, by electron irradiation (amine groups on the surface). This type of antimicrobial packaging consists of binding an agent to the surface of the package and this requires a molecular structure large enough to retain activity on the microbial cell wall even though bound to the plastic. Such agents are likely to be limited to enzymes or other antimicrobial proteins (Quintavalla and Vicini 2002). These functional groups were immobilized on the surface, which prevents the migration of biocides in the food. Cohen et al. (1995) showed a conversion of amide to amine groups of nylon by irradiation. Not only electron irradiation or ultraviolet treatments can be used to modify film surfaces; plasma treatments are under development as well.

18.2.5 Bioactive Edible Coating

Another possibility is to incorporate the antimicrobial compound into an edible coating, applied by dipping or spraying directly onto the food. Much effort has been made in recent years to develop biodegradable or edible materials to produce environmentally friendly packaging. Selection of the incorporated active agents is limited to edible compounds. Because they have to be consumed together with the edible coating layers and foods, their edibility and safety are essential. Chitosan-based

films, mentioned earlier, whether associated or not with other bioactive compounds (bacteriocins, organic acids, etc.) may be included in these packaging concepts. According to Gennadios et al. (1997), edible coatings can improve the quality of fresh, frozen, and processed meat and poultry products by, for instance:

- Delaying moisture loss.
- Reducing lipid oxidation and discoloration.
- Enhancing product appearance.
- Functioning as a carrier of food additives.

For instance, cellulose derivatives such as hydroxy propyl methyl cellulose (HPMC) are a promising raw material for edible coatings associated with antimicrobial entities. HPMC-based coatings associated with nisin, where the bioactivity was based on the release of nisin previously incorporated into the film-forming solution, was studied. The effectiveness of bacteriocin protective cultures has mainly been studied in meats (Jacobsen, Koch, Gravesen, and Knochel 2002; Katla et al. 2002). Agar coatings associated with nisin on fresh poultry have been studied by Natrajan and Sheldon(1995). Substantial reductions in *S. typhimurium* growth were recorded after storage at 4°C for 96 hours. Ming et al. (1997) observed a total inhibition of the *L. monocytogenes* growth on ham, turkey meat, and beef when bacteriocins were associated with cellulose-based matrix. As reported by Quintavalla and Vicini (2002), commercial application of this technology is described in U.S. Patent 5,573,797 assigned to Viskase Co. The package is a film, such as regenerated cellulose, containing a heat resistant *Pediococcus*-derived bacteriocin in a synergistic combination with a chelating agent to inhibit listerial strains.

The potential association of a chitosan-based packaging with bacteriocin could be interesting. Although this system was only studied as preservative agent by Roller et al. (2002), using a chitosan-carnocin based combination and a chitosan-sulphite combination, results have shown potential applications for edible films.

Siragusa et al. (1999) investigated the potential for decontamination of raw beef meat by application of organic acids immobilized in calcium alginate gels. Immobilized lactic or acetic acid resulted in a significantly greater reduction of *Listeria monocytogenes* growth, compared to acid treatment without an edible polymer.

18.3 CONCLUSION

Within the available arsenal of preservation techniques, the food industry is increasingly investigating the replacement of traditional food preservation techniques (intense heat treatments, salting, acidification, drying, and chemical preservation) by new preservation technologies. The most investigated among the latter are non-thermal inactivation processes in which active packaging holds a considerable place. In spite of the intensive research efforts and investments, very few of these new preservation methods have until now been implemented by the food industry.

One approach to extend the storage and shelf life of fresh meats is to introduce antimicrobials, preferably those that are naturally occurring, to the surface of the meat (Aymerich et al. 2000; Cutter and Siragusa 1997; Gill and Holley 1998; Nattress, Yost,

and Baker 2001). Often antimicrobials are evaluated in an *in vitro* system especially against bacteria associated with meat spoilage (i.e., *B. thermosphacta*, *Carnobacterium,* etc.) or pathogen strains (*L. monocytogenes, Salmonella,* etc.).

Because microbial contamination of meat products occurs primarily at the surface due to postprocessing handling, the use of packaging films containing antimicrobial agents could be more efficient, by slow migration to the food surface, thus helping maintain high concentrations where they are needed.

According to Devlieghere et al. (2004), two aspects that are crucial for the practical application of some biocides, are often overlooked: the changes in the organoleptic and texture properties of the food when added, and the interaction of these compounds with food ingredients and the influence of this interaction on its efficiency. For instance, these authors have shown that moderate concentrations of NaCl, present in many food products, are often already responsive for the inhibition of the antimicrobial activity of chitosan (Devliedhere, Vermeulen, and Debevere 2002). The pH of food influences the ionization (dissociation–association) of most active chemicals, and could change the antimicrobial activity of organic acids and their salts. Moreover, each food has its own characteristic microflora. As reported by Quintavalla and Vicini (2002), the release kinetics of antimicrobial agents have to be designed to maintain the concentration above the critical inhibitory concentration with respect to the contaminating micro-organisms that are likely to be present.

Another type of smart film, developed with food safety in mind, is currently undergoing testing. This novel system, when incorporated into a packaging film, uses an antibody detection system to detect pathogens. It expresses a positive finding as a symbol on the surface of the package, thereby alerting food handlers to the presence of pathogens. Although this technology is promising, it is still in its infancy and comprehensive assessments have yet to be performed. Several limitations have been suggested with this technology; for example, it would not be able to detect pathogens at concentrations below 10^4 CFU/g or cm^2 and would not detect pathogens within the product (U.S. Food and Drug Administration 2001).

Concerning EC legislation aspects, the European Commission is planning to propose a directive allowing new forms of food packaging to be introduced in the European market and a new directive would allow the introduction of active and intelligent packaging. The proposed directive would set basic safety and labeling guidelines for active and intelligent packaging, but more detailed rules would be needed for more sophisticated systems. The directive would also include traceability requirements (EC Proposes Regulations 2003).

In conclusion, despite the extended shelf life of refrigerated products stored under vacuum-packed or MAP conditions, there is an increasing concern about the growth and survival of microaerophilic psychotrophic pathogens. Thus, additional methods should be used to ensure the safety of such products. Smart, interactive, and active packaging are terms that have been used to describe the innovative concept of package structures.

REFERENCES

Aasen, I. M., S. Markussen, T. Møretrø, T. Katla, L. Axelsson, and K. Naterstad. 2003. Interactions of the bacteriocins sakacin P and nisin with food constituents. *Int. J. Food Microbiol.* 87:35–43.

Appendini, P., and H J. Hotchkiss. 2002. Review of antimicrobial food packaging. *Inno. Food Sci. Emerg. Tech.* 3:113–126.

Aymerich, M., J. Garriga, J. Ylla, J. M. Vallier, J. M. Monfort, and M. Hugas. 2000. Application of enterocins as biopreservatives against *Listeria innocua* in meat products. *J. Food Prot.* 63:721–726.

Bayarri, S., I. Rivas, E. Costell, and L. Duran. 2001. Diffusion of sucrose and aspartame in kappa-carrageenan and gellan gum gels. *Food Hydrocolloids.* 15:67–73.

Begin, A., and M. R. Van Calsteren. 1999. Antimicrobial films produced from chitosan. *Int. J. Biol. Macromol.* 26:63–67.

Bell, R. G., and K. M. Lacy. 1986. Factors influencing the determination of nisin in meat products. *J. Food Technol.* 21:1–7.

Buonocore, G. G., M. A. Del Nobile, A. Panizza, M. R. Corbo, and L. Nicolais. 2003. A general approach to describe the antimicrobial agent release from highly swellable films intended for food packaging applications. *J. Controlled Release.* 90:97–107.

Carneiro de Melo, A. M. S., C. A. Cassar, and R. J. Miles. 1998. Trisodium phosphate increases sensitivity of gram-negative bacteria to lysozyme and nisin, *J. Food Prot.* 61:839–844

Chen, H., and J. Williams. 2005. Bacteriocins offer enormous promise in food packaging safety advances. Retrieved from http://www.silliker.com/html/eResearch/vol1issue2.php.

Choi, J. H., W. Y. Choi, D. S. Cha, M. J. Chinnan, H. J. Park, D. S. Lee, and J. M. Park. 2005. Diffusivity of potassium sorbate in K-carrageenan based antimicrobial film. *LWT: Food Sci. and Tech.* 38:417–423.

Chumchalová, J., J. Josephsen, and M. Plocková. 1998. The antimicrobial activity of acidocin CH5 in MRS broth and milk with added NaCl, NaNO3 and lysozyme. *Int. J. Food Microbiol.* 43:33–38.

Cohen, J. D., C. W. Erkenbrecher, S. L. Haynie, M. J. Kelley, H. Kobsa, A. N. Roe, and M. H. Scholla. 1995. Process for preparing antimicrobial polymeric materials using irradiation. U.S. Patent 5,428, 078.

Coma, V., A. Martial-Gros, S. Garreau, A. Copinet, F. Salin, and A. Deschamps. 2002**.** Edible anti-microbial films based on chitosan matrix. *J. Food Sci.* 67:1162–1169.

Coma, V., I. Sebti, A. Deschamps, and H. F. Pichavant. 2001. Anti-microbial edible packaging based on cellulosic ethers, fatty acids and nisin incorporation to inhibit *Listeria monocytogenes* and *Staphylococcus aureus*. *J. Food Prot.* 64:470–475.

Cooksey, K. 2001. Antimicrobial food packaging materials**.** Additives for Polymers. 8:6–10.

Cutter, C. N. 1999. The effectiveness of Triclosan-incorporated-plastic against bacteria on beef surfaces. *J. Food Prot.* 62:474–479.

Cutter, C. N., and G. R. Siragusa. 1997. Growth of Brochothrix thermosphactain ground beef following treatments with nisin in calcium alginate gels. *Food Microbiol.* 14:425–430.

Daeschel M. A., J. McGuire, and H. Al-Makhlafi. 1992. Antimicrobial activity of nisin absorbed to hydrophilic and hydrophobic silicon surfaces. *J. Food Prot.* 55:731–735.

Darmadji, P., and M. Izumimoto. 1994. Effect of chitosan in meat preservation. *Meat Sci.* 38:243–254.

Davies, E. A., and M. R. Adams. 1994. Resistance of *Listeria monocytogenes* to the bacteriocin nisin. *Int. J. Food Microbiol.* 21:341–347.

Degnan, A. J., N. Buyong, and J. B. Luchansky. 1993. Anti-listerial activity of pediocin AcH in model food systems in the presence of an emulsifier or encapsulated within liposomes. *Int. J. Food Microbiol.* 18:127–138.

Devliedhere, F., L. Vermeiren, and J. Debevere. 2004. New preservation technologies: Possibility and limitations. *Int. Dairy J.* 14:273–285.

Devliedhere, F., A. Vermeulen, and J. Debevere. 2002. Chitosan as a food preservative: Possibility and limitations. Paper presented at the 18th International ICFMH Symposium FOOD MICRO, Lillehammer, Norway, August 18–23.

EC proposes regulations to encourage active packaging. 2003. *Additives for Polymers*. 12:10.

Field, C. E., L. F. Pivarnick, S. M. Barnett, and A. Rand. 1986. Utilization of glucose oxidase for extending the shelf-life of fish. *J. Food Sci.* 51:66–70.

Gennadios, A., M. A. Hanna, and L. B. Kurth. 1997. Application of edible coatings on meats, poultry and seafoods: A review. *Lebensmittel-Wissenschaft und-Technologie*. 30:337–350.

Giannakopoulos, A., and Guilbert, S. 1986a. Determination of sorbic acid diffusivity in model food gels. *J. Food Tech.* 21:339–353.

Giannakopoulos, A., and Guilbert, S. 1986b. Sorbic acid diffusivity in relation to the composition of high and intermediate moisture model gels and foods. *J. Food Tech.* 21:477–485.

Gill, A. O., and R. A. Holley. 1998. *In vitro evaluation of the effects of nitrite and NaCl on the antimicrobial activity of lysozyme, nisin and EDTA combination treatments* (Internal report). Abbotsford, BC, Canada: Canadian Inovatech.

Gill, A. O., and R. A. Holley. 2000. Inhibition of bacterial growth on ham and bologna by lysozyme, nisin and EDTA. *Food Res. Int.* 33:83–90.

Goff, J. H., A K. Bhunia, and M. G. Johnson. 1996. Complete inhibition of low levels of *Listeria monocytogenes* on refrigerated chicken meat with pediocin AcH bound to heat-killed *Pediococcus acidilactici* cells. *J. Food Prot.* 59:1187–1192.

Gontard, N. 2000. *Les emballages actifs*. Paris: Ed Tec & Doc.

Halek, W., and A. Garg. 1989. Fungal inhibition by a fungicide coupled to an ionomeric film. *J. Food Safety.* 9:215–222.

Helander, I. M., E.-L. Nurmiaho-Lassila, R. Ahvenainen, J. Rhoades, and S. Roller. 2001. Chitosan disrupts the barrier properties of the outer membrane of Gram-negative bacteria. *Int. J. Food Microbiol.* 71:235–244.

Jacobsen, T., A. G. Koch, A. Gravesen, and S. Knochel. 2002. Biocontrol of class IIa bacteriocin sensitive and resistant *Listeria monocytogenes* on sliced meat products. Paper presented at the 18th International ICFMH Symposium FOOD MICRO, Lillehammer, Norway, August 18–23.

Jeon, Y. J., J. Y. V. A. Kamil, and F. Shahidi. 2002. Chitosan as an edible film for quality preservation of herring and Atlantic cod. *J. Agr. Food Chem.* 50:5167–5178.

Jones, J. M. 2002. Food safety. In *Chemical and functional properties of food components,* ed. Z. E. Sikorski, 291–305. Boca Raton: CRC Press.

Katla, T., T. Moretto, I. Sveen, I. M. Aasen, L. Axelsson, L. M. Rorvik, and K. Naterstad. 2002. Inhibition of *Listeria monocytogenes* in chicken cold cuts by addition of sakacin P and sakacin P-producing *Lactobacillus sakei*. *J. App. Microbiol.* 93:191–196.

Lacroix, M., F. Chiasson, J. Borsa, and B. Ouattara. 2004. Radiosensitization of *Escherichia coli* and *Salmonella typhi* in presence of active compounds. *Rad. Phys. Chem.* 71:65–68.

Mattisson, C., P. Roger, B. Jönsson, A. Axelsson, and G. Zacchi. Diffusion of lysozyme in gels and liquids, a general approach for the determination of the diffusion coefficients using holographic laser interferometry. *J. Chromatogr.* 743:151–167.

Ming, X., and M. Daeschel. 1993. Nisin resistance of foodborne bacteria and the specific resistance responses of *Listeria monocytogenes*. *J. Food Prot*. 56:944–948.

Ming, X., G. Weber, J. Ayres, and W. Sandine. 1997. Bacteriocins applied to food packaging materials to inhibit *Listeria monocytogenes* on meats. *J. Food Sci*. 62:413–415.

Moore, M. E., I. Y. Han, J. C. Acton, A. A. Ogale, C. R. Barmore, and P. L. Dawson. 2000. Effects of antioxidants in polyethylene film on fresh beef color. *J. Food Sci*. 68:99–104.

Murray, M., and J. A. Richard. 1997. Comparative study of the antilisterial activity of nisin A and pediocin AcH in fresh ground pork stored aerobically at 5°C. *J. Food Prot*. 60:1534–1540.

Muzzarelli, R., R. Tarsi, O. Filippini, E. Giovanetti, G. Biagini, and P. R. Varaldo. 1990. Antimicrobial properties of N-carboxybutyl chitosan. *Antimicro. Agents Chemo*. 34:2019–2023.

Natrajan, N., and B. W. Sheldon. 1995. Evaluation of bacteriocin-based packaging and edible film delivery systems to reduce Salmonella in fresh poultry. *Poult. Sci*. 74:31.

Nattress, F. M., C. K. Yost, and L. P. Baker, 2001. Evaluation of the ability of lysozyme and nisin to control meat spoilage bacteria. *Food Microbiol*. 70:111–119.

Ouattara, B., M. Giroux, W. Smoragiewicz, L. Saucier, and M. Lacroix. 2002. Combined effect of irradiation, ascorbic acid and edible coating on the improvement of microbiological and biochemical characteristics of ground beef. *J. Food Prot*. 65:981–987.

Ouattara, B., R. E. Simard, R. A. Holley, G. J. P. Piette, and A. Begin. 1997. Inhibitory effect of organic acids upon meat spoilage bacteria. *J. Food Prot*. 230:246–253.

Ouattara. B., R. E. Simard, G. Piette, A. Bégin, and R. A. Holley. 2000. Inhibition of surface spoilage bacteria in processed meats by application of antimicrobial films prepared with chitosan. *Int. J. Food Microbiol*. 62:139–148.

Oussalah, M., S. Caillet, S. Salmieri, L. Saucier, and M. Lacroix. 2004. Antimicrobial and antioxidant effects of milk protein-based film containing essential oils for the preservation of whole beef muscle. *J. Agric. Food Chem*. 52:5598–5605.

Pen, L. T., and Y. M. Jiang. 2003. Effects of chitosan coating on shelf life and quality of fresh-cut Chinese water chestnut. *Lebensm.-Wiss. U.-Tech*. 36:359–364.

Quintavalla, S., and L. Vicini. 2002. Antimicrobial food packaging in meat industry. *Meat Sci*. 62:373–380.

Roller, S., S. Sagoo, R. Board, T. O'Mahony, E. Caplice, G. Fitzgerald, M. Fogden, M. Owen, and H. Fletcher. 2002. Novel combinations of chitosan, carnocin and sulphite for the preservation of chilled pork sausages. *Meat Sci*. 62:165–177.

Rooney, M. 2000. Active packaging in charged films. In *Les emballages actifs,* ed. N. Gontard, 229–239. Paris: TEC & DOC.

Sagoo, S., R. Board., and S. Roller. 2002. Chitosan inhibits growth of spoilage microorganisms in chilled pork products. *Food Microbiol*. 19:175–182.

Scannell, A. G. M., C. Hill, R. P. Ross, S. Marx, W. Hartmeier, and E. K. Arendt. 2000. Development of bioactive food packaging materials using immobilised bacteriocins Lacticin 3147 and Nisaplin®. *Int. J. Food Microbiol*. 60:241–249.

Sebti, I., A. Carnet-Ripoche, D. Blanc, R. Saurel, and V. Coma. 2003. Controlled diffusion of an antimicrobial peptide from a biopolymer film. *J. Chem. Eng. Res. Des. Trans. IchemE*. 81:1099–1104.

Sebti, I., and V. Coma. 2002. Active edible polysaccharide coating and interactions between solution coating compounds. *Carbohydrate Polym*. 49:139–144.

Sebti, I., F. Pichavant, and V. Coma. 2002. Edible bioactive fatty acid-cellulosic derivatives composites used to food packaging applications. *J. Agric. Food Chem*. 50:4290–4294.

Shahidi, F., U. J. K. Arackchi, and Y. J. Jeon. 1999. Food applications of chitin and chitosans. *Trends Food Sci. Tech.* 10:37–51.

Simal, S., E. S. Sanchez, J. Bon, A. Femenia, and C. Rossello. 2001. Water and salt diffusion during cheese ripening: Effect of the external and internal resistances to mass transfer. *J. Food Eng.* 48:269–275.

Siragusa, G. R., C. N. Cutter, and J. L. Willett. 1999. Incorporation of bacteriocin in plastic retains activity and inhibits surface growth of bacteria on meat. *Food Microbiol.* 16:229–235.

Siragusa, G. R., and J. S. Dickson. 1992. Inhibition of *Listeria monocytogenes* on beef tissue by application of organic acids immobilized in a calcium alginate gel. *J. Food Sci.* 57:293–296.

Skandamis, P. N., and G. J. E. Nychas. 2002. Preservation of fresh meat with active and modified atmosphere packaging conditions. *Int. J. Food Microbiol.* 79:35–45.

Stevens, K. L., B. W. Sheldon, N. A. Klapes, and T. R. Klaenhammer. 1991. Nisin treatment for inactivation of Salmonella species and other gram-negative bacteria. *Appl. Environ. Microbiol.* 57:3613–3615.

Strathmann, S., S. Pastorelli, and C. Simoneau. in press. *Investigation of the interaction of active packaging material with food aroma compounds: Sensors and actuators.*

Suppalku, P., J. Miltz, K. Sonneveld, and. S. W Bigger. 2003. Antimicrobial properties of basil and its possible application in food packaging. *J. Agric. Food Chem.* 51:3197–3207.

Tütüncü, M. A., and T. P. Labuza. 1996. Effect of geometry on the effective moisture transfer diffusion coefficient. *J. Food Eng.* 30:433–447.

U.S. Food and Drug Administration. 2001. Center for Food Safety and Applied Nutrition. Office of Premarket Approval, GRAS Notices Received in 2001. Retrieved from http:/vm.cfsan.fda.gov.

Vermeiren, L., F. Devlieghere, M. Van Beest, N. De Kruijf, and J. Debevere. 1999. Developments in the active packaging of foods. *Trends Food Sci. Tech.* 10:77–86.

Weng, Y. M., M. J. Chen, and W. Chen. 1999. Antimicrobial food packaging materials from poly(ethylene-co-methacrylic acid). *Lebensmittel-Wissenschaft und-Technologie.* 32:191–195.

Weng, Y. M., and J. H. Hotchkiss. 1992. Inhibition of surface molds on cheese by polyethylene containing the antimycotic imazalil. *J. Food Prot.* 55:367–369.

Index

α-ketoglutarate 174
α-connectin 199–201
β-connectin 199–201
1-heptene 169
1-hexene 169
1-nonene 169
1-octene 169
2-alkylcyclobutanones 156
2-butanone 180
2-methyl butanal 169–170
^{31}P NMR 118
3-methyl butanal 169–170

AB *See* tenderstretched
ABB 68
Absorption spectroscopy 88
Accelerated processing 353–367
ACE 246–249, 256–259
Acetic acid 166
Acidulants 166
Actin 199, 206
Actin-myosin interaction 196
Actomyosin 199, 206–208
Actomyosin toughness 201–202
Additives 176–178
Adductor 4, 6
Adenosine triphosphate 74, 118, 206
Adept 68
Advanced Technology for Efficient Pork Production 55–60, 68
AE *See* amplification efficiency
Aeromonas hydrophilia 163
Aging of meat 194, 253–254, 262
AgResearch 65
AI *See* artificial insemination
Aitch-bone hung carcasses 82
Alcohols 174
Aldehydes 168, 174, 177, 180
Allergen 277, 289, 291
Alpha particles 157
Alternative carcass positioning 219
American Meat Institution Foundation 176
Amides 174
Amines 287, 338, 407, 411
Amino acids 3, 203, 260–262, 293–294, 297, 331–337, 339–342, 373, 405–408
Aminopeptidases *See* proteases
Ammonia 338
Amplification 133
Amplification efficiency 134
Amplification facilitators 138
Ampl*Taq* Gold 139
Angiotensin See ACE
Animals bioengineering 1–14
Anserine *See* dipeptides
Antihypertensive 246–249, 256–260. *See also* peptides
Antimicrobial 321–322, 324, 456
Antioxidant 156, 163, 175–176, 180, 246, 253, 261–262, 280, 285, 287, 295–297, 318, 320–321
Apollo 17 158
Apollo-Soyuz Test Project 158
Aroma *See* flavor
ARTEPP *See* Advanced Technology for Efficient Pork Production
Artificial insemination 29
As yet uncultured 143
Ascorbate 177
Ascorbic acid 166, 177–178, 180
Ash 3
Asparagine 174
Aspergillus 343
ASTP *See* Apollo-Soyuz Test Project
ATP *See* adenosine triphosphate
ATPase 196, 238
ATPase activity 208
Attec 54
Audio/Visual Cueing Device 229
Autofluorescence 107, 110, 139
AUTO-FOM 54
Automated chill rooms 50
Automated grading 50
Automation 44
  and hygiene 50
  for beef carcass production 60–63
  for lamb carcass production 63–67
  for pork carcass production 50–60
  in modern slaughterhouse 43–69

in modern slaughterhouse industrial drivers 45–46
risks and pitfalls 46–48
system components 48–49
Automeat 53
AYU *See* as yet uncultured

BAC *See* bacterial artificial chromosome
Bacillus cereus 166, 439
Background flora 146
Background toughness 201–202
Bacon 311, 322
Bacterial artificial chromosome 27
Bacterial enteric pathogens 131
Bacteriocins 373–390, 403, 411, 458–460, 462–464, 467
BANSS 55–56
Beef
boning automation 63
carcass break-up 62–63
carcass splitting 62
dehiding 61
evisceration 62
killing 60–61
primalization automation 62–63
quality QTL 29–31
Benzene 156
Benzoate 178
BF *See Biceps femoris*
BGF *See* background flora
Biceps femoris 4, 6, 195, 232–233
BIMA *See* Biochemical Index of Miofibrillar Aging
Bioactive
components 277–279, 286, 457–464, 466–467
peptides 245–264
Biochemical Index of Miofibrillar Aging 196
Bioengineering
bovine 3–4
caprine 8
food safety implications 13
ovine 5–8
porcine 8–13
Biotechnology 1–14, 279
Bis(methylthio-)methane 169
Blade tenderization 228, 230, 233
Blending *See* chopping
Bovine 257, 283–284
serum albumin 140
spongiform encephalopathy 37
BPW *See* buffered peptone water
Breed improvement 33–34
Brine 313–314
composition 119
curing 313, 354–356
impregnation 356–366
Brisance 222
BSA *See* bovine serum albumin
BSE *See* bovine spongiform encephalopathy
BT *See* blade tenderization
BTH 233
Buffered peptone water 139, 144
Buoyant density centrifugation 141, 146

Calcium chloride injection 7
Callipyge phenotype 39
Callipyge sheep 6, 7
Calpain 7–10, 201, 231
Calpains See proteases
Calpastatin 7–11, 231
Calpastatin polymorphism FF genotype 13
Campylobacter 132, 141–142, 373, 438
Campylobacter jejuni 146, 164
Cancer 277–278, 280, 287
Canonical correlation analysis 106–107
Carbohydrate 405–406
Carbon
dioxide 420–426, 429–431, 433, 434–435, 437–442, 451, 454–456
monoxide 422–423, 428–429, 432, 440, 442
Carbon monoxide-myoglobin 173–175, 179
Carbonyl compounds 163
Carboxylic acids 174
Carboxypeptidases *See* proteases
Carcass compisition gene 33
Cardiovascular 277–278, 280, 287, 294–295
Carnitine 293–294
Carnosine 163 *See also* dipeptides
CAST *See* calpastatin
CAST/Rsal *See* Rsal restriction enzyme
Catalase 296, 404
Cathepsins *See* proteases
CCA *See* canonical correlation analysis
CCD detector 101–102, 110
cDNA 34–35
Cellulose 11
Center for Veterinary Medicine 3
Ceroids 101, 103
Chelators 176
Chicken *See* poultry
Chitosan 458–459, 465–468
Chlorine dioxide 457
Cholesterol 3, 253, 280, 287–289, 294, 298
Chopping 314, 404
Chromatography 255
CHU *See* commercial prototype steel vessel
cis-3-nonenals 169
Citrate 177
Citric acid 177
CLA *See* conjugated linolenic acid
Cloning 1

Clostridium 310, 315, 322–324, 372, 437–438, 456–457
Clostridium botulinum 141, 164
CO_2 stunning 51
Collagen 101, 105, 258
Collagen proportion of protein 90
Color 279–280, 310, 312, 315, 404, 422–423, 427–433, 467
Colorants 315
CO-Mb *See* carbon monoxide-myoglobin
Commercial prototype steel vessel 222–229
Compton effect 160
Compton electron 160
Conditioning 194
Conjugated linoleic acid 246, 277, 283, 291–292
Conjugated linolenic acid 12–13
Connectin 196
Connective fat 102–104
Connective tissue 102–104, 117, 196, 201–203
Conversion of α-connectin to β-connectin 199–201
Cooperative Research and Development Agreement 220, 229
C_p *See* crossing point
CRADA *See* Cooperative Research and Development Agreement
Crossing point 133
CSIRO 60
CT *See* connective tissue
Ct *See* crossing point
Cured meat 297, 309–324, 355
Curie 157
Curing *See* cured meat
CVM *See* Center for Veterinary Medicine
Cysteine 170

D *See* diffusivity coefficient
Danfotech 53
Danish Carcass Classification Centre 54
Danish Meat Research Institute 51–54, 60, 68
Deamination 338
Debaryomices *See* yeasts
Decarboxylation 338
Delta-like 1 7
Deoxymyoglobin 171, 178
DEXA *See* dual X-ray absorptiometry
DHA *See* fatty acids
Diacetate 178
Differential scanning calorimetry 202
Diffusivity coefficient 119
Dimethyl disulfide 169, 177–178
Dimethyl sulfide 169, 178
Dimethyl trisulfide 178
Diode array 92
Diode array detectors 93
Dipeptides 246, 261, 285, 293, 297, 342
Discoloration *See* color
DLK1 *See* Delta-like 1
DMRI *See* Danish Meat Research Institute
DNA 37, 156, 205
DNA analogs 136
DNA markers 26
DNA polymerase 132, 136–138, 140
DNAase I inhibition capacity 208
dNTP 138
Dolly 2
Double muscling 32
Double packaging 179–180
Drip loss 9
Dry curing 312–313, 355
Drying 119, 330, 341, 356, 451
DSC *See* differential scanning calorimetry
DTI 118
Dual X-ray absorptiometry 120–121
Durand 53

E-beam 162
Edible coating 452, 458, 460, 466–467
Elastin 101, 261
Electrical conductivity 91
Electrical stimulation 76, 219
Electrical stunning 51
Electrically based pulsed plasma technology 230, 237
Electron beams 161, 236
Electron microscopy 231
Electronic wavelength switching 92
Electrons 157, 159
Electrophoresis 28, 38
Embryo splitting 2
Emission spectroscopy 88
Endomysium 202
Endopeptidases *See* proteases
Energy metabolism 117–118
Enterobacteriaceae 166, 461, 465–466
Enterocins *See* bacteriocins
Environmental effects 36
Enviro™ pig 11
Enzymes 254–255
EPA *See* fatty acids
Escherichia coli 142, 164–165, 205, 372, 385–386, 403, 437–438, 450, 463, 466
Escherichia coli O157:H7 12–13, 164, 205, 236–237, 239
Esterases *See* lipases
Esters 174
ESTs *See* expressed sequence tags
ESW *See* Electrically based pulsed plasma technology
Ethylene vinyl alcohol 436

Eurofins 37
Exopeptidases *See* proteases
Explosion Simulation Technology 229
Expressed sequence tags 28
Extended aging 7

F-actin 199, 202, 208
FAD 101
Fanuc 68
Fanuc M710 60
Fast-twitch glycolytic fibers 10
Fat *See* lipids
Fat content 116
Fatty acids 3, 7, 246, 277–285, 291–293, 296
FDA 8, 157–158, *See also* U.S. Food and Drug Administration
Feed 279–286
Fermentation 262, 341, 374, 390, 401–402, 404, 410, *See* fermented
Fermented
  meats 254, 263, 401–402, 409–410
  sausages 254, 345, 376–379, 383–384, 389, 404
Ferricytochrome 174
Ferrocytochrome 174
Ferrylmyoglobin 173
FG *See* fast-twitch glycolytic fibers
Fibers 262, 278, 293, 295, 314
Fish 256–257
  oil 281–283, 292
Flavor 280, 310, 312–313, 320–321, 342, 343–345, 355, 404–408, 411, 423, 432, 441, 452, 455
Flavor-related components 203
Floatation 141,146–147
Fluorescence 28, 90
  analysis 106–107
  applications 102–104
  emission spectroscopy 105
  NIR 122
  online analysis 109
  spectroscopy 90, 100–109
  techniques 101–102
Fluorescent compounds 135–136
Food and Agriculture Organization 9
Food irradiation 156–162
Food safety 46
Food Science Australia 60–63
Food Technology and Safety Laboratory 220, 229
Food-borne pathogens 156
Fourier-transform instruments 92
Fourier-transform Raman system 110
Fragmentation of myofibrils 197–199
Frankfurter 291–293, 296–297, 316, 320, 376–378, 380–382, 387, 390, 426, 435
Free fatty acids 163
Free radicals 109, 156, 161, 163–164, 169, 177
Freezing-thawing 118–119
Front face fluorescence spectroscopy 101
Frozen ham 360–366
FTSL *See* Food Technology and Safety Laboratory
Functional
  meat 277–285
  meat products 262–263, 276–300
  peptides 246–264
Fututech 60–62
Fututech system 61

G-actin 208
Gallate 180
Gallic esters 177
Gamma irradiation 166
Gamma rays 160, 162
Gas chromatography-mass spectroscopy 108
Gas sensors 107
Gaseous composition 164–165
GC-MS *See* gas chromatography-mass spectroscopy
GDF-8 32
Gel electrophoresis 38
Gene 279, 411
Gene expression patterns 34–36
Gene technology 21–39
Generic meat automation systems 50
Genes controlling variations in traits locating 25–26
Genetic control of meat quality traits 24–25
Genetic manipulation 1–14
Genetic markers 26–28
Genetic selection 23–24
Genetic Solutions 37
Genetic variations 35–36
Genetics 279, 411
Gene-trap locus 2 7
Genome mapping 23
Genome maps 28
Genomics 23, 411–412
GF–1 7
GH *See* growth hormone
Gluco-delta-lactone 166
Glucose 203
GLUT4 11. *See also* insulin-sensitive glucose transporter
Glutamine 174
Gluten 288–289
Gluteus medius 6, 104
Gluthathione 173
Glyceraldehydes 174
Glycerophosphoryl choline 117

Glycine 174
GMS *See* guided microwave spectrometry
GPC *See* glycerophosphoryl choline
Grading carcasses methods 121
GRF *See* growth-hormone releasing factor
Growth hormone 1, 5–6
Growth-hormone releasing factor 5
GTL2 *See* gene-trap locus 2
Guided microwave spectrometry 114–115, 122

HACCP *See* Hazard Analysis and Critical Control Point
Hafnia 238
Ham 254
  dry-cured 254, 313, 339–341, 345, 355–367, 381, 409, 433
  cooked 291–293, 296, 385, 389–390, 458
  smoked 453
Hamburger 377, 390
Hazard Analysis and Critical Control Point 73
HDP *See* hydrodynamic pressure processing
  and food safety enhancements 235–239
  and further processed meat products 233–235
  and muscle proteins 230–233
HDP technology and meat tenderness enhancement 222–229
Health 246–253, 256–263, 276–278, 286, 294–295, 411
  claim 299
Heme pigments 171
Hemoglobin 101, 142, 261, 428
Hexanal 180
HHP *See* high hydrostatic pressure
High hydrostatic pressure 193–212, 220, 236
High intensity pulsed light 236
High performance liquid chromatography 203
High pressure
  on prerigor muscle 195
  on postrigor muscle 195
High pressure assisted freezing and thawing 211
High pressure heat treatments 219
High pressure treatment
  and color 204
  and fat stability 204
  and flavor-related components 203
  and micro-organisms 205–206
High voltage electric discharges 236–237
High voltage electrical stimulation 81, 83
Hormones 9
Hot dog 377, 385, 390
Hot-boning 77–78
  benefits 78
  disadvantages 78
  of meat 73–83
HPLC 255, *See also* high performance liquid chromatography
Huge memory 46
Hugoniot compression 221
HVES *See* high voltage electrical stimulation
HVSW *See* Electrically based pulsed plasma technology
Hybridization probes 136
Hydrodynamic pressure 7
Hydrodynamic Pressure Processing 219–239
Hydrodyne process 220
Hydrogen peroxide 163
Hydrogen sulfide 169
Hydrolysis 250–255, 258–260, 262, 405–408
Hydrolyzate *See* hydrolysis
Hydroperoxides 109, 163
Hydroperoxyl radicals 174
Hydroxyl radicals 167
Hydroxylycyl pyridoline 102
Hydroxyproline 104
Hypertension 277–278

IAEA *See* Internal Atomic Energy Agency
IBD *See* identical by descent
Identical by descent 31
IdentiGEN 37
IGF-1 transgene 12–13
IGF2 *See* insulin-like growth factor 2
IM *See* intermediate moisture
Immune stimulants 156
IMP *See* inosinic acid
Impedance 91
Increased strength 46
Industrial Research Ltd. 61
Industry 263, 300, 310, 319, 389, 443
Infrared detection 46
Infraspinatus 4, 6, 105
Inorganic phosphates 117
Inosinic acid 203
Insulin 7
Insulin-like growth factor 2 33, 39
Insulin-sensitive glucose transporter 10
Intermediate moisture 167
Internal Atomic Energy Agency 158
IR *See* midinfrared
IRL 62
Iron *See* minerals
Irradiated meat
  consumer attitude 175–176
Irradiation 155–181, 386–439, 451, 459, 466
  color changes in meat 171–175
  dose 163
  and other intervention 166–167
  process 159–162
  quality changes in meat 167–180

IS *See Infraspinatus*
Isoleucine 170
Isotonic principle 238
Itec 54

Japan Meat Grading Association 4
Japanese Meat Grading Standard 4

Ketones 168, 174, 234
Kocuria 334, 402, 406, 408
KUKA 62, 68
KUKA KR125 56
KUKA robot 61, 67

Laboratory model steel vessel 222–229
Lactate 178
Lactic acid 166, 177, 383, 403, 405–406, 440, 458
Lactic acid bacteria 238, 263, 278, 334–337, 342, 345, 372–380, 383–384, 388–390, 402–405, 408–410, 459, 465
Lactobacillus See lactic acid bacteria
Lactobacillus alimentarius 164
Lactobacillus curvatus 164
Lactobacillus sake 164
Lactobacillus spp. 237
Lactococcus See lactic acid bacteria
Lactose 288–291
Lambs 283–284
Latissimus dorsi 4
LD 105
Le Chatelier principle 237
Legislation 46
Leucine 170
Leupeptin 201
LHU *See* laboratory model steel vessel
LightCycler™ 139
Linkage disequilibrium 28, 31
Linoleic acid 11
Linolenic acid 11
Lipases 279, 406, 408
Lipid oxidation 108–109, 167–169, 180
Lipids 3, 278–285, 287–289, 291–293, 296, 405, 408–409
Lipofuscin 101, 103
Lipolysis *See* lipases
Lipolytic *See* lipases
Listeria 144
Listeria innocua 12–13
Listeria monocytogenes 12, 142, 144, 163–166, 205–206,209–210, 237, 372–375, 379–390, 403–404, 439, 450, 457, 459, 463–464, 467–468
Lithotripsy 229
LNA *See* locked nucleic acids
Locked nucleic acids 136
Longissimus 6, 231–233
Longissimus dorsi 80, 103, 203–204, 211
Longissimus lumborum 6, 9
Longissimus thoracis 4, 6, 105–106
Low-voltage electrical stimulation 81, 83
LT *See Longissimus thoracis*
LTL *See Longissimus thoracis et lumborum*
LVES *See* low-voltage electrical stimulation
Lysostaphin 4
Lysyl pyridinoline 102

M. longissimus 79
M. longissimus dorsi 81
M. semimembranosus 79, 81
M. semitendinosus 81
Macpro 67
Macro-arrays 35
Mahalanobis distances classification 95
Maillard 427
Malignant hyperthermia syndrome 117
Manipulation of beef postmortem 75–77
MAP *See* modified atmosphere packaging
Mapping populations 28–29
Marbling score 4
Marker-assisted selection 23, 26, 38
MARS LANDING *See* Medical and Related Links to Agricultural Network for Devlopment and Innovation with Guelph 11
MAS *See* marker-assisted selection
MAS programs 37
Mass spectrometry 28, 38
Mass transport 356–360
Massaging 314, 324, 354
Masseter 104
Mastitis 4
Maternal expressed gene 8 7
Mb 175
MDC *See* Mahalanobis distances classification
Meat
  cooked 321, 375–380, 403, 423, 432, 434
  cured 297, 309–324, 375–378, 386, 403, 423, 432, 434
  dry-cured 341–345, 375–378, 386
  fresh 430, 434, 467
  frozen 467
Meat & Livestock Australia 62
Meat composition 163
Meat cutting 49
Meat decontamination 155–181
Meat Industry Research Institute of New-Zealand 63–67
Meat processing 89–91
Meat quality 1–14, 21–39, 73, 219–239
Meat quality parameters 117–118
Meat Research Corporation of Australia 67

Meat safety 1–14, 219–239
Meat tenderization 194–203
Mechanization 44
Medical and Related Links to Agricultural Network for Devlopment and Innovation with Guelph 11
MEG8 *See* maternal expressed gene 8
Mercaptan 169
Mercaptomethane 169
Metallothionein 5–6
Methanethiol 178
Methionine 170
Methylbenzene 156
Methyl thioacetate 169
Metmyoglobin 173, 204
MEXA *See* multiple X-ray absorptiometry
Micro-arrays 35
Microbial factors 165–166
Microbial growth 355
Microbial inactivation 236–238
Micrococaceae 342–343, 402
Microorganisms 156, 251–252, 263, 310
Microsatellite markers 37–38
Microwave 90, 160
Microwave spectrometry 112–115
Midinfrared 91
Minerals 252, 277–278
MIRINZ *See* Meat Industry Research Institute of New-Zealand
MLA project 61
Modified atmosphere packaging 164, 179
Moisture determination 99
Molecular beacons 136
Mono X-ray absorptiometry 120
MRF *See* muscle regulatory factor
MRI 116, 118
MRNA 142
MRS *See* NMR spectroscopy
MSTN *See* porcine loci myostain
Multiple X-ray absorptiometry 120
Muscle 256–250, 279, 285–286,296, 322, 330–331, 339–340
Muscle regulatory factor 9
Muscle tenderness 75–76
Muscle types 105–106
Myofibril units 175
Myofibrils 196–199
Myofribrillar fragmentation index 220
Myoglobin 104, 142, 171–173, 177–178, 204, 280, 316, 404, 428
Myosin 199, 206–208
Myosin binding protein H 35

NADH 101
NADPH 101
NAS *See* National Academy of Sciences
NASA *See* National Aeronautics and Space Administration
National Academy of Sciences 2
National Aeronautics and Space Administration 158
National Research Council 2
Near infrared spectroscopy 90, 111, 113, 122
Neutrons 157, 159
Nicotinamide 176–177
NIR *See* near infrared
  analysis of fresh meat 93–97
  analysis of semifrozen meat 97
  instrumentation 92–93
  online applications 93
  spectroscopy 91–10
  transflectance analysis of whole muscle meat 97–100
Nisin *See* bacteriocins
Nitrate 310, 330, 355, 404
Nitrite 296, 309–324, 330, 355, 375, 404, 408
Nitrogen 420, 424–427, 429, 442
Nitrosamines 287, 311, 315, 324
Nitrosyl hemochrome 173
NMR *See* nuclear magnetic resonance
NMR relaxometry 116
Noncontact reflectance mode microwave 113–114
Nonpyrotechnic shock wave meat processing tenderization scheme 229–230
NRC *See* National Research Council
Nuclear Magnetic resonance 115–120
Nuclear transfer cloning 8
Nutrient *See* nutrition
Nutrition 276–277, 279, 299, 318
Nylon 435

Obesity 277–278
Obturator foramen 76
oct-1-one 169
oct-3-one 169
Off-flavor 157
Off-odor 177
Off-odor production 169–171
Oligosaccharides 295
Online monitoring of meat quality 87–123
Online spectroscopic analysis
  sampling problems 121–122
Opioids 249–251
ORP *See* oxidation-reduction potential
Oscillating magnetic fields 236
Oxidation 253, 295, 315
  lipids 253, 280, 318–320, 324, 406, 408, 427
Oxidation-reduction potential 174, 177–179
Oxygen 422–428, 430–431, 440, 442, 452–454
Oxymyoglobin 171–173

Packaging 178–179
active 384–386, 449–468
modified atmosphere 379, 385, 389, 403, 421–443, 451–456, 464, 468
vacuum 354, 379, 385, 403, 422, 425, 431, 433–435, 439, 451, 458
Packaging and additive combinations 180
Pale, soft, exudative 117
Partial least squares 96
Pascal principle 238
Paté 291, 381
Paternal expressed gene 11 7
Pathogenic microorganisms 162, 236
Pathogens 131–147, 156, 373–390
Patties 296–297
PCA *See* principal component analysis
PCr *See* phosphocreatine
PCR *See* polymerase chain reaction
avoiding false negative and false positive results 142–143
conversion of hetrigeneous samples into homogenuous samples 141–142
inhibition 138–142
inhibitors 138–141
purification 145–146
quantification 143
PCR-RFLPs 27
PEC *See* plastic explosive containers
Pediococcus See lactic acid bacteria
Pediocin 167
PEG11 *See* paternal expressed gene 11
Pentosidine 102
Peptide nucleic acid 136
Peptides 203, 245–264, 293–294, 331–334, 339–342, 373, 405–407. *See also* proteolysis
ACE inhibitory 247–249, 256–259
antihypertensive 247–249
antimicrobial 251
antioxidant 253, 261–262
antithrombotic 252
bioactive 245–264
hypocholesterolemic 252
immunomodulating 250
mineral binding 252
opiod 249–250, 260–261
prebiotic 251
savory 262, 407
Perimysium 202
Peroxisome proliferator-activated receptor-gamma coactivator-1 10
PGC-1% *See* Peroxisome proliferator-activated receptor-gamma coactivator-1
Phenol reagent positive materials 203
Phenolic compounds 177
Phenylalanine 101
Phosphate 289–290
Phosphocreatine 117
Phospholipids 101, 174 *See also* lipids
Phytase 11
Phytate 297
Phytochemicals 277–278, 285, 291, 298
Pi *See* inorganic phosphates
Pickle 313–314, 316, 324
Pi-Vac Elasto-Pack 79–81
Plastic explosive containers 222–229
PLS *See* partial least squares
PLSR 104
PNA *See* peptide nucleic acid
Polyamide 464
Polyester 436
Polyethylenes 436, 464
Polymerase chain reaction 27, 132–147
Polymorphisms 23, 25, 36–37
Polyphenols 177
Polyphosphates 177
Polypropylene 436, 464
Polyunsaturated fatty acids 11, 171
Polyvinyl chloride 436, 464
Polyvinylidene chloride 436
Porcine halothane sensitivity 117
Porcine loci myostatin 9–10
Pork 257–259, 279–283
boning 60
carcass break-up 54–59
dehairing 51–52
evisceration 52–53
grading 54
killing 51
primalization 54–55
splitting 53–54
Porphyrins 101
Postmortem electrical stimulation 75
Potassium benzoate 178
Poultry 256–259, 297, 379, 385, 420–421, 432–434, 440, 467
PPARGC1 *See* Peroxisome proliferator-activated receptor-gamma coactivator-1
PPCS 67
PPM *See* phenol reagent positive materials
Prebiotics 251–252, 262, 277–278, 294–295
Prediction errors 97
Pre-PCR processing 136–138
Prerigor freezing 7
Preservation 456–467
Preservatives *See* preservation
Pressure
high hydrostatic 386–387
Pressure-induced meat tenderization 194–203
Pressure-processed cooked ham 209

Pressure-processed dry-cured ham 209–211
Pressure-processed pork ham 208–209
Pressure-processing of meat products 206–211
Primer extension 38
Primers 132, 138
Principal component analysis 96
Probiotics 262–263, 278, 287, 294.295, 403, 409–411
Process control 46
Product consistency 46
Product shelf life 238–239
Production quality 46
Pronuclear microinjection 2
Propanal 180
Propionic acid 166
Proteases 246, 254–255, 259–260, 279–280, 329–345, 404, 406
Proteasome *See* proteases
Protein content 9
Proteinases *See* proteases
Proteins 3, 245–264, 288
  meat 253–264, 278, 293–294, 389
  myofibrillar 257–258, 331–332, 335, 339–342, 404–407
  plant 294
  sarcoplasmic 258, 342, 406–407
  whey 294
Proteolysis 330, 337–345, 406
Proteolytic *See* proteases
Protons 159
PSE *See* pale, soft, exudative
Pseudomonas 164, 238, 453, 456, 463, 466
Psoas major 204
PSW *See* pulsed shock wave
PUFA *See* polyunsaturated fatty acids
Pulsed shock wave 237
P-Vac packaging 79–82
Pyridoxal 101
Pyruvate 174

QPCR 142–143
QTL *See* quantitative test loci
QTN *See* quantitative trait nucleotide
Quadriceps femoris 6
Quality changes control 176–180
Quantitative trait loci 9, 26–31, 33, 38
Quantitative trait nucleotide 33
Quantitative traits 26

Radiation absorbed dose 162
Radiation resistance 166
Radionuclides 160
RAMAN spectroscopy 109–112
Rancidity 318
Ranodyne receptor 9
Ready-to-eat meats 163, 180
Real-time detection in meats 143–147
Real-time PCR 131–147
  principle 132–135
Reflectance diode array NIR instruments 96–97
Reflectance filter instruments 93–95
Reflectance NIR 122
Regulations 390–391, 452, 468
Remote reflectance 99
Restriction fragment length polymorphism 27
RFLP *See* restriction fragment length polymorphism
Rheology 106
Riboflavin 101
Ripening 339–341
RMSECV *See* root mean square error of cross-validation
RMSEP 104, 115, 121
Roast beef 296
Root mean square error of cross-validation 94, 97
Rosemary oleoresin 177
Rsal restriction enzyme 9
RTE *See* ready-to-eat
R*Tth* 139–141
Ruminants 283–284
RyR *See* ranodyne receptor

Saccharomyces cerevisiae 237
Safety 264, 298, 373, 389–390, 411, 443
Sakacin *See* bacteriocins
Salami 316, 411, 453
Salmonella 141–142, 144–146, 164, 205–206, 373–375, 385–387, 437, 468
Salmonella enteridis 205
Salmonella typhimurium 12–13, 164, 167, 236–237
Salt 288–290, 309–310, 320, 330, 339–340, 345, 355–366, 375, 388–389, 404–406, 408, 430, 462, 468
Salting 355
Sarcomere structure 231
Sarcomeres 175,197–198
Sarcoplasmatic reticulum 231
Sausages 291, 298, 375, 390, 406, 408–409
  cooked 292
  dry 262, 293, 296, 341–342, 345, 375–378, 402–403, 410
  semidry 341, 345
Scanning electron microscopy 202
Scavenging 452–455
Scorpion primers 136
Scott Automation 67
SDS-PAGE 199, 201, 232
Seasoning 312
Selective breeding 23

Semimembranosus 6, 232
Semitendinosus 4, 6, 103, 105–106, 228, 234
Sensory 279–281, 356, 374, 380, 404–409, 411
Sensory analysis 95, 106
Sequence-specific fluorescent probes 135
Serratia 238
Serratia liquefaciens 461, 465
Sesamol 177–178, 180
SFK 53
SFK-Danfotech 52
Sheep
  boning automation 66–67
  carcass break-up 65
  evisceration 65
  killing 64
  pelting 64–65
  primalization automation 65
Shigella 462
Shock waves
  alternative producing methods 229–230
Silent polymorphisms 27
SIMCA *See* soft independent modeling of class analogy
Single nucleotide polymorphisms 27–28, 33, 38
SL *See* sodium lactate
SLC2A4 *See* insulin-sensitive glucose transporter
Slow-twitch oxidative 10
Smart Cycler® 139
Smoke 310, 313, 330, 405
SNPs *See* single nucleotide polymorphisms
SO *See* slow-twitch oxidative
Sodium chloride *See* salt
Sodium lactate 178
Sodium nitrite 177
Sodium tripolyphosphate 177
Soft independent modeling of class analogy 96
Somatic cell nuclear transfer 2
Sorbate 178
SP *See* sugar phosphates
Spectra Research 229
Spectroscopic techniques 87–123
Spectroscopy 88–89
Spice 312, 320, 388, 404, 460–461, 463
Spinach desaturase 11
Spoilage 313, 373
Spoilage microorganisms 236
SR *See* sarcoplasmatic reticulum
SS *See Supraspinatus*
ST *See Semitendinosus*
Stanols 298
Staphylococcus
  aureus 2, 204, 237, 334, 342, 372, 374, 385, 438, 462, 466
  carnosus 402, 406–407, 409, 411
  saprophiticus 409
  typhimurium 450, 462, 467
  warneri 409
  xylosus 402, 406–409, 411
Starter cultures 374–375, 401–413
Sterols 298
Storage modulus 208
Stork 53
Strecker 337, 407
Streptococcus spp. 238
Sugar phosphates 117
Sulfur compounds 179
Sulfur-containing amino acids 169
Sulfur dioxide 169
Sulfur volatiles 170, 180
Summer sausage 379
Sunrise primers 136
Superoxide radicals 174
Supraspinatus 6
S-volatiles 178
SYBR Green I 135, 139
Synthesis 255

Taq DNA polymerase 138
TaqMan probes 136
Taste 262, 322, 338–339, 342, 355, 388, 404, 407, 422
Taurine *See* amino acids
TB *See* tenderbound
TB *See Triceps brachii*
TBA *See* thiobarbituric acid
TBARS *See* thiobarbituric acid reactive substances
Tenderbound 82
Tendercut 77
Tenderization 255
Tenderizing meat 219
Tenderness 77, 105–106 *See also* texture
Tenderness increasing of hot-boned meat 79–83
Tenderstretch hanging 76
Tenderstretched 82
Tensor fasciae latae 105
Texture 175, 290, 313, 355, 404, 423, 435
TFL *See Tensor fasciae latae*
TG *See* transgene
TGFβ *See* transforming growth factor
Thawing 363
Thiobarbituric acid 109, 204
Thiobarbituric acid reactive substances 108, 168, 180
Titin 196, 199
TOBEC See total body electrical conductivity
Tocopherol 177–178, 180
Total body electrical conductivity 121
Total volatiles 168
Traceability 23, 37–38, 46

Trait genes finding 31–33
Transamination 338
Transflectance 98–99
Transforming growth factor 32
Transgenesis 1
Transgenic cloning 2
Transmission instruments 93
Transmittance measurements 98
trans-6-nonenals 169
Triacylglycerol *See* lipids
Triceps brachii 6, 105–106
Trichina 157
Trichinella spiralis 158, 236
Trimethyl sulfide 169
Tripolyphosphate 234
Tropinin T 8, 35
Tryptophan 101, 105–106
Tth 141
Tth DNA 141
Tth polymerase 140
Tumbling 314, 324, 354
Turkey products 289, 293, 296, 359, 379, 386
Tyrosine 101

U.S. Army Medical Department 157–158
U.S. Department of Agriculture 3, 158
U.S. Food and Drug Administration 2
U.S. Patent and Trademark Office 2
Ultrafiltration 256
Ultrasonics 91
Ultraviolet-absorbance spectroscopy 107
Ultraviolet light 157
Ultraviolet rays 236
Unami taste 203
Unspecific double-stranded DNA 135
USDA *See* U.S. Department of Agriculture
USPTO *See* U.S. Patent and Trademark Office

Vacuum
  impregnation 356–367
  packaging 164, 178
Vacuum-steam-vacuum technology 167
Variability 74
Variability of tenderness 74–75
Viable but nonculturable 132
Viable culturable cells 143
Vibrio 132
Vibrio vulnificus 142
Video image analysis 91
Viruses 165
Vitamin A 101, 103
Vitamin D 101, 103
Vitamin D_3 7
Vitamin E 163
Vitamin K 101, 103
Vitamins 278, 281, 293, 296
VNC *See* viable but nonculturable
VNC cells 143–144
von Roentgen 157
VSV *See* vacuum-steam-vacuum

Warner Bratzler shear force 80, 82, 95
Water activity 313, 366, 375, 407
Water binding 290, 295, 435
Water holding capacity 9, 118, 175
Water loss 359
WBSF *See* Warner Bratzler shear force
WHC *See* water holding capacity
Whey protein 176
Wiener 379
Wrapping technique 79–80

Xenotransplantation 8
X-ray spectroscopic techniques 120–121
X-ray vision 46
X-rays 90, 122, 157, 160, 162
Xylan 11

Yeast 335–338, 343–344
Yersinia 238, 373, 437, 450
Yersinia enterocolitica 12–13, 141–146, 205

Z-line disintegration 196

Food Science and Technology

In recent years the meat industry has incorporated important technological advances that, to this point, have not been addressed in a single source. Comprehensive and authoritative, **Advanced Technologies for Meat Processing** presents the latest developments concerning the quality, analysis, and processing of meat and meat products. Featuring contributions from a panel of international experts, the book details technologies used in the meat processing chain. It describes important processing methodologies such as gene technology, automation, irradiation, hot boning, high pressure, vacuum-salting, enzymes, starters, and bacteriocins.

The book begins by exploring various production systems that include the use of modern biotechnology, automation in slaughterhouses, and rapid nondestructive online detection systems. It proceeds to describe new technologies such as decontamination, high pressure processing, and fat reduction. The book then examines functional meat compounds such as peptides and antioxidants and the processing of nitrate-free products and dry-cured meat products. It also discusses bacteriocins that fight against meat-borne pathogens and the latest developments in bacterial starters for improved flavor in fermented meats. It concludes with a discussion of recent packaging systems of the final products.

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- Details information on advanced processing technology such as gene technology for production
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DK3813
ISBN 1-57444-587-1
90034
9 781574 445879

Taylor & Francis
Taylor & Francis Group
A CRC PRESS BOOK
www.taylorandfrancisgroup.com

6000 Broken Sound Parkway, NW
Suite 300, Boca Raton, FL 33487
270 Madison Avenue
New York, NY 10016
2 Park Square, Milton Park
Abingdon, Oxon OX14 4RN, UK