# READY-TO-EAT FOODS

## Microbial Concerns and Control Measures

Edited by
Andy Hwang
Lihan Huang

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

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

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



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: 978-1-4200-6862-7 (Hardback)



**Library of Congress Cataloging-in-Publication Data**

Ready-to-eat foods : microbial concerns and control measures / editors, Andy Hwang, Lihan Huang.
p. cm.
Includes bibliographical references and index.
ISBN 978-1-4200-6862-7 (hardcover : alk. paper)
1. Food--Microbiology. 2. Food--Safety measures. 3. Ready meals. 4. Convenience foods . 5. Food contamination. I. Hwang, Andy. II. Huang, Lihan. III. Title.

QR115.R43 2010
664.001'579--dc22 2009048853

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**http://www.crcpress.com**

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# *Preface*

Refrigerated ready-to-eat (RTE) foods contaminated with foodborne pathogens such as *Listeria monocytogenes*, *Salmonella* spp., and *Escherichia coli* O157:H7 have been linked to numerous cases of food poisoning worldwide. With the increasing demand for RTE foods that are wholesome and require less handling and preparation, the production and consumption of RTE foods has increased, and the varieties have expanded considerably in recent years. RTE foods now encompass a wide variety of food products, both meat and non-meat, such as cooked or dry meat and poultry products, deli salads, smoked/salted seafood, cheese, fermented dry and semi-dry sausage, and pre-packaged fruits and vegetables. These products are common in the marketplace and are consumed frequently and in large quantities by the general population. The safety of RTE foods has become a growing concern since they can be directly consumed without additional cooking or heating, a step that kills the potentially contaminated pathogenic microorganisms. Several severe and high-profile outbreaks of foodborne illnesses caused by the consumption of RTE foods have prompted the U.S. Department of Agriculture (USDA) and the Food and Drug Administration (FDA) to issue stringent rules and regulations governing the manufacturing of RTE foods. The food industry, academia, and research institutes have conducted extensive research to examine the potential health risk of contaminated RTE foods, to investigate the growth behavior of common contaminating foodborne pathogens, and to develop intervention technologies and control measures. This book provides a comprehensive review of common RTE foods and related food safety issues. It is our desire that this book be a useful tool for scientists and professionals in the areas of food safety and RTE food manufacturing.

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**Cheng-An (Andy) Hwang, Ph.D.** is a research food technologist at the Eastern Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, where he conducted research studying the survival, growth, and inactivation of common foodborne pathogens, such as *Listeria monocytogenes*, *Escherichia coli* O157:H7, and *Salmonella*, in formulated food products. Dr. Hwang has a Ph.D. in food science and technology from the University of Tennessee and has worked in the areas of product development, microbiology, and food safety for more than 15 years. He worked for an international food company for 9 years where he developed and defined processing and formulation parameters for a wide variety of food products to ensure their microbial stability and safety. He also developed protocols for cleaning and sanitizing processing equipments and food dispensing equipments. Andy is a professional member of the Institute of Food Technologists and International Association for Food Protection.

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# chapter 1

# Microbiology of ready-to-eat foods

*Divya Jaroni, Sadhana Ravishankar, and Vijay Juneja*

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## 1.1 Introduction

Ready-to-eat (RTE) foods are those food products that have gone through some kind of processing and can be consumed without undergoing any further bactericidal treatment such as thorough heating. These foods do not require further preparation prior to consumption, except washing, thawing, or moderate reheating (Farber and Harwig, 1996). As the name implies, RTE foods can be readily consumed without further preparation or processing and thus are extremely convenient for present-day, busy

consumers. Surveys of consumer purchase behaviors show an increasing trend in the consumption of RTE foods (Anonymous, 2001, 2003). Hence, the demand for these products from the food industry is huge. Ready-to-eat sandwiches account for 32% of sales from vending machines and include a large share of a multibillion-dollar annual business in the United States (Anonymous, 2001). Supermarkets and convenience stores carry a large selection and variety of RTE food products.

Some examples of RTE foods include the following: meats (deli meats, sausages, hot dogs, corned beef), poultry (buffalo chicken, chicken wraps, deli cuts, chicken salad), dairy products (cheeses, yogurt, sour cream, pasteurized milk), fruits and vegetables (salads and leafy greens, salsa, juices, guacamole), and fish and seafood (cold smoked salmon, sushi, seafood salad, fish soup). Some frozen foods can be included among RTE foods, for example, ice cream, frozen yogurt, frozen fruits, and smoothies. Other non-refrigerated RTE foods include products such as breads, nuts, peanut butter, chocolate, and some snack items.

Due to the increasing demand for and consumption of RTE foods, and owing to the fact that these are not further processed, the microbiological risks to the consumer from these products have also increased. The preparation of RTE sandwiches, salads, and meats involves human handling (cutting or slicing), which can easily contribute to cross-contamination. Ready-to-eat food products have been involved in numerous foodborne illness outbreaks, especially due to cross-contamination of these products during processing and packaging in the food processing plant or handling in consumer households. For example, the *Salmonella enterica* outbreak in late 2008 and early 2009 involving peanut butter was due to heavy rainfall through a leaky roof and a faulty sprinkler system in the processing plant, which provided moisture for the growth of dormant salmonellae that were likely present in raw peanuts or peanut dust.

The most common foodborne pathogenic bacteria found in refrigerated RTE foods include *Listeria monocytogenes*, *Salmonella enterica*, *Escherichia coli* O157:H7, and *Clostridium perfringens*. *Listeria monocytogenes* has caused a number of outbreaks and recalls in dairy and RTE meat products. *Salmonella enterica* and *E. coli* O157:H7 have caused outbreaks due to contamination in fresh produce and salads. *Salmonella enterica* has also caused outbreaks and recalls due to cross-contamination in some non-refrigerated RTE foods such as chocolate, almonds, pistachios, peanut butter–containing products, and infant formula. Improper cooling of prepared RTE foods in large volumes is believed to be the most common cause of *C. perfringens* outbreaks. In this chapter, the microbiology of RTE foods is discussed, with emphasis on four main pathogens: *L. monocytogenes*, *S. enterica, E. coli* O157:H7, and *C. perfringens*.

## *1.2* Listeria monocytogenes

*Listeria monocytogenes* is a facultative, intracellular, Gram-positive bacterium and is ubiquitous in the environment. It is a non-spore-forming and psychrotrophic bacterium that produces flat, dimpled colonies with a black halo on modified Oxford formulation (MOX) agar. *Listeria monocytogenes* is a hardy pathogen and can resist various stresses in the environment. It is well known for forming biofilms, and once *L. monocytogenes* forms biofilms in food processing environments, it is very difficult to eradicate the pathogen from such environments. Hence, *Listeria* biofilms can be a threat to food processors. *Listeria monocytogenes* causes listeriosis in humans, which is accompanied by mild flu-like symptoms such as headache, chills, and fever, along with gastrointestinal symptoms like nausea, vomiting, and diarrhea. Immunocompromised people, infants, pregnant women, and elderly people are more at risk for contracting the disease. Meningitis, abortion, and prenatal septicemia are some of the primary manifestations of listeriosis (FDA, 2001a), which in serious cases can be fatal. In serious cases, especially if untreated, mortality may exceed 25% in predisposed groups (Farber and Harwig, 1996). In healthy individuals, *L. monocytogenes* can cause febrile gastroenteritis characterized by fever, headache, muscle and joint pain, and diarrhea (Sim et al., 2002). In febrile gastroenteritis, usually the organisms are found in high numbers in implicated foods and, when ingested, can cause illness in healthy individuals. Due to differences in virulence of strains and host factors such as age, health, and exposure to certain foods, the minimum infectious dose for *L. monocytogenes* is not known (NACMCF, 1991).

### *1.2.1 Outbreaks of* L. monocytogenes *in RTE foods*

Several foodborne outbreaks since the 1980s have been attributed to *L. monocytogenes* in the United States and many other countries (CDC, 1999). In the United States, listeriosis has been estimated to cause 2,500 cases of foodborne illness annually, resulting in deaths of 500 people and about $200 million in monetary losses (CDC, 2002; FDA, 2001a; Mead et al., 1999). In the 1980s outbreaks of *L. monocytogenes* associated with cheese and milk were of concern to the dairy industry. Soft cheeses made from raw and pasteurized milk such as Mexican-style cheese (Bula et al., 1995; Linnan et al., 1988) have been involved in listeriosis outbreaks. The Mexican-style cheese outbreak of listeriosis that occurred in 1985 resulted in 142 cases, including 48 deaths (Linnan et al., 1988). In 1982, coleslaw made from cabbages contaminated with sheep manure caused an outbreak of listeriosis in 41 people (Schlech et al., 1983).

In 1997, in Italy, a cold corn and tuna salad caused an outbreak of febrile gastroenteritis due to *L. monocytogenes* contamination (Aureli et al., 2000). Other food products that have been involved as vehicles of non-invasive febrile gastroenteritis due to *L. monocytogenes* contamination include smoked mussels (Misrachi et al., 1991), shrimp (Riedo et al., 1994), rice salad (Salamina et al., 1996), chocolate milk (Dalton et al., 1997), cold smoked rainbow trout (Miettenen et al., 1999), and imitation crab meat (Farber et al., 2000). In these outbreaks, a large number of organisms were suspected to have been consumed by patients. For example, in the chocolate milk outbreak, the median numbers consumed were suspected to be as high as $10^{11}$ (Dalton et al., 1997). The issue with some of the RTE foods is that they may get cross-contaminated during handling and may initially contain small numbers of organisms. However, because *L. monocytogenes* is psychrotrophic, a high number of organisms may appear during storage, even at refrigeration temperatures. The shelf life of some RTE foods, especially RTE meats, is as long as 2 to 3 weeks, which gives sufficient time for the organism to multiply and probably cause illness, even in immunocompetent individuals.

There have been many outbreaks of *L. monocytogenes* in RTE meat products. In 1992, there was an outbreak of listeriosis in France due to contaminated jellied pork tongue, in which there were 279 reported cases, 63 deaths, and 22 miscarriages or abortions (Jacquet et al., 1995). The listeriosis outbreak in 1998–1999 in the United States resulted in more than 100 cases of illness, including 21 deaths, in over 14 states and was caused by consumption of post-processing contaminated cured meat products. *Listeria monocytogenes* can contaminate cured meat products and grow to high numbers during refrigerated storage (Beumer et al., 1996; Buncic et al., 1991). This is because the bacterium is able to multiply at temperatures as low as 2°C with curing salts and under low oxygen tension (Lou and Yousef, 1999). Another multistate outbreak of listeriosis in 2000 involved delicatessen sliced processed turkey meat that resulted in 30 cases of illnesses, 4 deaths, and 3 miscarriages or stillbirths (CDC, 2000; Olsen et al., 2005). This outbreak was traced to a single processing plant, and the industry recalled 16 million pounds of processed turkey meat. In New Zealand, in 2000, a series of incidents (30 cases) of non-invasive febrile gastroenteritis caused by *L. monocytogenes* were traced to RTE meats, including luncheon ham and corned beef, and high levels of organisms were isolated from patient fecal samples and from implicated meats (Sim et al., 2002). In 2001, another outbreak of febrile gastroenteritis associated with delicatessen meat (precooked sliced turkey) contaminated with *L. monocytogenes* occurred in the southwestern United States (Frye et al., 2002).

A multistate outbreak of *L. monocytogenes* infections in the northeastern United States was caused by the consumption of sliced turkey

deli meat in 2002 (Gottlieb et al., 2006). This outbreak resulted in 54 confirmed cases, 8 deaths, and 3 stillbirths. The outbreak strain was found in one processing plant and the industry recalled more than 30 million pounds of turkey deli meat. This outbreak prompted the Food Safety and Inspection Service (FSIS), an agency of the U.S. Department of Agriculture (USDA), to issue new regulations, including testing and control programs for *L. monocytogenes* in RTE meat and poultry processing plants.

The previously mentioned outbreaks suggest that the occurrence of *L. monocytogenes* in RTE food products, especially RTE meat products, is a major threat to public health. Outbreaks associated with *L. monocytogenes* therefore have prompted regulatory agencies to impose stringent regulations, especially with regard to RTE foods. Since 1989 there has been a "zero tolerance" policy for *L. monocytogenes* in RTE meat products issued by the USDA. In foods in which *L. monocytogenes* is not capable of growing, Canada and European countries have instituted an action level of 100 CFU/g (Farber and Harwig, 1996; Roberts, 1994; Teufel, 1994). The compliance criteria for *L. monocytogenes* in RTE foods and the sampling scheme for these foods have been discussed (Farber and Harwig, 1996) and are listed in Table 1.1. A quantitative risk assessment for *L. monocytogenes* in RTE foods has been discussed by Rocourt et al. (2003).

### 1.2.2 *Incidence and prevalence of* L. monocytogenes *in RTE foods*

*Listeria monocytogenes* can be prevalent in a variety of RTE foods. Ready-to-eat products like vegetables, meat, poultry, seafood, and dairy have all been found to be contaminated with *L. monocytogenes* (Brackett, 1988). A survey of *L. monocytogenes* in Maryland and northern California showed 577 positive samples out of 31,705 total samples of RTE foods, including luncheon meats, deli salads, soft cheeses, bagged salads, smoked seafood, and seafood salads (Gombas et al., 2003). Seafood salads from markets in Iceland showed a 16% prevalence rate of *L. monocytogenes* (Hartemink and Georgsson, 1991). In a recent survey of salads, *L. monocytogenes* was found in 4.7% of seafood salads and 2.4% of deli salads such as coleslaw, potato salad, tuna salad, and pasta salad (Hwang and Tamplin, 2005). The use of meat, seafood, and cheese in some salads could serve as a source of *L. monocytogenes* in these salads (Smittle, 2000). *Listeria monocytogenes* was isolated from open RTE salad vegetables in retail premises in the United Kingdom (Sagoo et al., 2003). In numerous studies, samples of RTE meat products from retail stores were collected to find the prevalence of *L. monocytogenes*. The contamination level of this pathogen was 72% in corned beef and 33.8% in ham (Grau and Vanderline, 1991) and 15.5%

***Table 1.1*** Compliance Criteria and Sampling for *Listeria monocytogenes* (LM) in Ready-to-Eat (RTE) Foods

| Category | Action Level for LM | GMP Status | Immediate Action | Sampling | Analysis |
|---|---|---|---|---|---|
| 1. RTE foods causally linked to listeriosis (This list presently includes soft cheese, liver pâté, coleslaw mix with shelf-life >10 days, jellied pork tongue, RTE meats) | >0 CFU/50 g | n/a | Class I recall to retail level<br>Consideration of public alert<br>Appropriate follow-up at plant level | 5 sample units (100 g or ml each) taken at random from each lot | 5 × 10 g or 2 × 25 g analytical units are either analyzed separately or composited |
| 2. All other RTE foods supporting growth of LM with refrigerated shelf-life >10 days | >0 CFU/25 g | n/a | Class II recall to retail level<br>Consideration of public alert<br>Appropriate follow-up at plant level | 5 sample units (100 g or ml each) taken at random from each lot | 5 × 5 g analytical units are either analyzed separately or composited |
| 3. RTE foods supporting growth of LM with refrigerated shelf-life ≤10 days and all RTE foods not supporting growth | ≤100 CFU/g | Adequate GMP | Allow sale | 5 sample units (100 g or ml each) taken at random from the lot | 5 × 10 g analytical units are analyzed separately |
| | ≤100 CFU/g | Inadequate or no GMP | Consideration of Class II recall or stop sale | | Where enrichment is necessary 5 × 5 g analytical units are analyzed separately or composited |
| | >100 CFU/g | n/a | Class II recall or stop sale | | |

CFU: Colony Forming Units; GMP: Good Manufacturing Practices
*Source:* Adapted from Farber and Harwig, 1996.

in chicken and turkey products (Rijpens et al., 1996). *Listeria monocytogenes* was isolated from 12% to 18% of precooked RTE chilled foods in England (Gilbert et al., 1989) and about one-third of RTE meat products sampled in Europe and Canada (Johnson et al., 1990).

The survival of *L. monocytogenes* in traditional Greek salads, such as fish roe salad and eggplant salad, stored at 10°C for 15 days was studied (Tassou et al., 2009). In the absence of preservatives, the organism survived well with only one log reduction, and acid adapted cells survived well even in the presence of preservatives. Various RTE foods (3,063 samples) from Florida were tested for *L. monocytogenes,* and 91 samples (2.97%) tested positive (Shen et al., 2006); 71 (78%) of these isolates exhibited multiple antibiotic resistance, and 89 (97.8%) isolates were acid resistant. These results show that RTE food isolates can adapt to various environmental stresses and thus exhibit better stress resistance.

A number of researchers have surveyed RTE foods in various nations throughout the world for incidence of *L. monocytogenes.* Minimally processed RTE fruits, vegetables, sprouts, and salads from retail outlets in Spain were surveyed for the presence of microorganisms; 0.7% of samples tested positive for *L. monocytogenes* (Abadias et al., 2008). Another survey of RTE foods (1,226 samples), including seafood, meat, dairy, and desserts obtained from the retail or food industry in Spain found *L. monocytogenes* in 20% of frozen Atlantic bonito small pies, 7.9% of smoked salmon samples, 11.1% of pork luncheon meat samples, 6.2% of frozen chicken croquettes, 16.9% of cured dried sausage samples, 12.5% of cooked ham samples, 20% of cooked turkey breast samples, 1.3% of fresh salty cheeses, and 15.1% of frozen cannelloni samples (Cabedo et al., 2008). A survey of retail outlet foods, including some RTE foods, in Japan showed the presence of *L. monocytogenes* in 5.4% of 92 smoked salmon, 3.3% of 213 raw seafood, 12.2% of 41 minced beef, 20.6% of 34 minced pork, 37% of 46 minced chicken, 25% of 16 minced pork-beef mixture, and none of the 285 vegetable samples (Inoue et al., 2000). However, another survey of various RTE foods, including raw vegetable salad, cooked salad, cooked rice, boiled noodles, bean curd, and cooked Japanese foods, revealed no presence of *L. monocytogenes* (Kaneko et al., 1999). RTE meat products in Belgian retail outlets were surveyed for *L. monocytogenes* and 13.71% of 824 raw cured meats, 4.9% of 3,405 cooked meats, 21.28% of 874 mayonnaise-based salads, and 11.7% of 786 prepared meals, tested positive for the organism (Uyttendaele et al., 1999). In this study for whole cooked meat products, more samples were positive after slicing than before slicing, indicating cross-contamination during slicing. *Listeria* spp. were recovered from about 15% of RTE food samples in Portugal (Guerra et al., 2001).

Ready-to-eat organic vegetables from retail outlets in the United Kingdom were sampled for the presence of foodborne pathogens, including

*L. monocytogenes,* and none was detected in 3,200 samples (Sagoo et al., 2001). In this survey only 0.5% of samples were of unsatisfactory quality due to presence of non-pathogenic *E. coli* and *Listeria* spp. Another survey of RTE organic vegetables in Northern Ireland found the absence of enteric pathogens, including *L. monocytogenes,* in 86 samples examined (McMahon and Wilson, 2001), indicating good agricultural, production, and harvest practices. Another sampling of 5,228 RTE food samples in the United Kingdom showed an incidence of >0.55% for *L. monocytogenes* and other pathogens (Meldrum et al., 2005). *L. monocytogenes* was present in 4% of samples of RTE baguettes and salads (out of 70 samples) prepared in retail delicatessens in South Africa (Christison et al., 2008). RTE street-vended foods (51 samples) in South Africa were tested for their microbiological quality and safety, and were found to be free of a number of foodborne pathogens, including *L. monocytogenes* (Mosupye and von Holy, 1999).

Sources other than foods can aid in promoting the transfer, survival, and growth of foodborne pathogens to RTE foods. Pesticides used in vegetable crop cultivation promoted the survival and growth of foodborne pathogens, including *L. monocytogenes* in some studies (Coghlan, 2000; Guan et al., 2001), while in some other studies *L. monocytogenes* did not survive in pesticides (Ng et al., 2005). Those pesticides promoting survival or growth are a concern for vegetable growers, since they can be a source of contamination of vegetables in the field. Cleaning and handling tools such as floor mops, cleaning cloths, and disposable gloves used in retail delicatessens in South Africa were sampled for the presence of bacteria, including *L. monocytogenes* (Christison et al., 2007). The organism was detected in five cleaning tool samples as well as other bacteria detected in several samples. These tools, therefore, can play a role as reservoirs for transferring contamination to RTE meats prepared in delicatessens.

Contamination of RTE products can occur from many different sources. Raw meat used for manufacturing of meat products, the processing environment, processing equipment, and the workers engaged in the manufacturing can cause contamination. RTE meat products are cooked in casings but are re-exposed to the processing environment to be packed in a final retail-packaging wrap. This procedure can lead to contamination of RTE meat products with pathogens on conveyors, meat surfaces, in condensation drippage, contaminated air filters, splashed standing water, or the workers themselves. Many RTE products are often consumed without further thorough cooking, which can lead to illness. Current efforts in meat and meat product safety have included reduction of pathogens in the raw meat during slaughtering, using treatments like carcass pasteurization, vacuum steaming, acid or water rinse, and other methods. Strategies for controlling or reducing *L. monocytogenes* in RTE foods are discussed later in this chapter.

Due to several large outbreaks that occurred in the 1980s and 1990s, the U.S. Food and Drug Administration (FDA) and the USDA-FSIS established a policy for RTE foods which mandates that the presence of *L. monocytogenes* at 0.04 CFU/g in a RTE food renders the product adulterated (Smoot and Pierson, 1997). Since then the RTE food industry has taken stringent measures to control *L. monocytogenes* in their products. However, *L. monocytogenes* contamination continues to occur, probably attributable to its ubiquitous nature. Surveillance and monitoring of RTE foods by the USDA and FDA have indicated that as many as 5% of some of the RTE foods such as sliced luncheon meats and prepared deli style salads show *L. monocytogenes* contamination (Hitchins, 1996; Levine et al., 2001). The incidence of *L. monocytogenes* in RTE foods can range from 1% to 10% (Farber and Harwig, 1996).

### 1.2.3 *Strategies for controlling* L. monocytogenes *in RTE foods*

In May 1999, the FSIS announced strategies for controlling *L. monocytogenes* in RTE food products (FSIS, 1999). These include mandatory implementation of Hazard Analysis and Critical Control Points (HACCP) at various processing steps to reduce the number of pathogens in the manufacturing environment, end-product testing, and educating consumers on the risk of listeriosis and prevention of *L. monocytogenes* growth. For long-term goals, the FSIS conducted studies of *L. monocytogenes* post-production growth and risk assessment in RTE meat products (FSIS, 1999). Based on the results of risk assessment conducted by FSIS with FDA, the agency acknowledged three other approaches for controlling *Listeria monocytogenes* in RTE meat products. The first alternative was for the establishments to check for HACCP plans, go through a lethality treatment, and incorporate growth-inhibiting agents and processes for *Listeria* in their products. The second alternative was that the establishment would employ a lethality treatment and incorporate a growth-inhibiting agent or a process to address *Listeria monocytogenes*. The third alternative was for the establishment to rely on sanitation Standard Operating Procedures (SOPs) for processing environments, particularly food contact surfaces, to be free of *Listeria*.

*Listeria monocytogenes* is a difficult organism to control because of its hardy characteristics. It has a relatively high tolerance to heat compared to other non-spore-forming organisms, can survive and multiply at refrigeration temperatures in the presence or absence of oxygen, withstand a wide pH range (pH 4.1–9.6) and concentrations of salt up to 12% to 13% (Lou and Yousef, 1999). Some strains can grow at a water activity ($a_w$) as low as 0.9 and at pH value as low as 4.4 (Farber and Peterkin, 1991; Miller, 1992; Walker et al., 1990). Also, *Listeria* can form biofilms on various food and food contact surfaces. Since *L. monocytogenes* is able to grow

at refrigeration temperatures, if RTE foods are contaminated, it can grow to dangerous levels during storage and transportation from production facility to retail distributors, even at refrigeration temperatures.

On RTE fresh fruits and vegetables, *L. monocytogenes* can be removed using washing treatments with or without sanitizers. Washing fresh produce using cold chlorinated water is a common practice used by the industry. Washing, using solutions of natural antimicrobials such as plant extracts, could prove useful and is an area that needs further investigation. Delaquis et al. (2002) utilized warm (47°C) and cold (4°C) chlorinated water (100 mg/l chlorine) for washing cut iceberg lettuce which was stored at 1°C or 10°C. Cold chlorinated water was more effective against *L. monocytogenes* than was warm chlorinated water, which permitted growth of the organism on lettuce stored at 10°C. Washing treatments on whole cantaloupes using 1000 ppm chlorine or 5% hydrogen peroxide for 2 minutes and storing at 4°C for 15 days reduced the pathogen population by 3.5 logs (Ukuku and Fett, 2002). On fresh cut pieces from the sanitized cantaloupes, *L. moncytogenes* survived but did not grow during 15 days storage at 4°C.

The growth of *L. monocytogenes* in RTE meat products can be controlled by changing internal parameters of food products, such as reduction of $a_w$ and pH with direct acidification, incorporation of antimicrobials in meat products, and modified atmospheric packaging (Nilsson et al., 1997; Pothuri, 1995; Zeitoun and Debevere, 1991). In the processed meats, reduction procedures include incorporating antimicrobials and post-packaging treatments of RTE meat products with microwave (Schalch et al., 1995), steam (Cygnarowicz-Provost, 1994), or hot water (Cooksey et al., 1993a, 1993b). Antimicrobials of natural origin or chemical preservatives can be used and can be added as direct ingredients in the formulation. Bacteriocins are compounds produced by microorganisms that have antimicrobial activity against closely related species. Pediocin and nisin are examples of commonly used bacteriocins. Compounds like organic acids and their salts have shown antimicrobial activity against common foodborne pathogens. These compounds are classified as Generally Recognized As Safe (GRAS) when added to RTE meat products.

Research undertaken within the past decade has indicated that bactericidal compounds such as salts of organic acids and bacteriocins can control *L. monocytogenes* in meat products. In many instances combination treatments work better than individual treatments. Sodium diacetate used as a dipping treatment inhibited growth of *L. monocytogenes* on sliced pork bologna stored at 4°C for 120 days (Samelis et al., 2001). Sodium lactate used alone or in combination with sodium acetate, sodium diacetate, or glucono-δ-lactone inhibited growth of *L. monocytogenes* on frankfurters during 120 days of storage (Samelis et al., 2002). Heating the

frankfurters further reduced the organism population. A combination of sodium lactate and sodium diacetate was bacteriocidal against *L. monocytogenes* strain Scott A and bacteriostatic against a cocktail of six strains of *L. monocytogenes* (Mbandi and Shelef, 2002). Chen et al. (2002) showed that 3000 and 6000 AU of pediocin reduced the population of *L. monocytogenes* by 1.5 and 2.0 logs, respectively, on frankfurters stored at 4°C for up to 12 weeks. On beef franks, a combination of sodium lactate, sodium diacetate, and pediocin used as a dipping treatment reduced the populations of *L. monocytogenes* single strains as well as cocktail by 1 to 1.5 logs and 1.5 to 2.5 logs, respectively, after 2 and 3 weeks of storage at 4°C (Uhart et al., 2004).

Sodium diacetate as individual treatment or in combination with potassium benzoate and/or sodium lactate as dip treatments inhibited *L. monocytogenes* on frankfurters (Lu et al., 2005). Acetic acid, sodium diacetate, and potassium benzoate inhibited *L. monocytogenes* growth on pork bologna for 120 days of storage (Samelis et al., 2001). Seman et al. (2008) assessed the effects and interactions of sodium benzoate, sodium diacetate, and sodium chloride for inhibiting *L. monocytogenes* in RTE meat products, and sodium benzoate was more effective when combined with sodium diacetate and sodium chloride. These authors concluded that low moisture products such as bologna or wieners could have a shelf life longer than 18 weeks, if they were formulated with 0.1% sodium benzoate and 0.1% sodium diacetate.

A number of studies have investigated the effectiveness of natural compounds derived from plants or microorganisms against *L. monocytogenes* in various non-meat RTE foods. Citron essential oil was effective against *L. monocytogenes* in fruit-based salads, reducing the population by >4 logs in 3 days, and below detection in about 7 days (Belletti et al., 2008). The antimicrobial activity of some plant extracts and essential oils used as flavor ingredients in confectionary products stored at 7°C and 20°C for 9 days were studied; strawberry flavor in chocolate inhibited *L. monocytogenes* (Kotzekidou et al., 2008). A combination of nisin and ALTA™2341 (pediocin-like product) reduced *L. monocytogenes* on smoked salmon packaged under vacuum or 100% $CO_2$ (Szabo and Cahill, 1999). Lactic acid bacteria were inhibitory to *L. monocytogenes* on vacuum-packed cold smoked salmon stored at 6°C or 8°C for 35 days (Mejlholm and Dalgaard, 2007). Green tea powder added to Oriental-style rice cakes stored at 22°C ± 2°C was more effective than rosemary leaf powder against *L. monocytogenes* (Lee et al., 2009).

Since RTE meat and poultry products have been involved in a number of outbreaks with *L. monocytogenes*, antimicrobial interventions on the surface of these products have been researched extensively. Nisin incorporated at 2500 IU/ml into methyl cellulose or hydroxypropyl methyl

cellulose solutions made into films inactivated *L. monocytogenes* on the surface of hot dogs (Franklin et al., 2004). Alginate films incorporated with essential oils of Spanish oregano, Chinese cinnamon, and winter savory and pretreated with 20% $CaCl_2$ were effective against *L. monocytogenes* on bologna stored at 4°C; however, these films were not effective on ham (Oussalah et al., 2007). A combination of nisin with grapeseed extract inactivated *L. monocytogenes* on turkey frankfurters at 4°C and 10°C; however, neither antimicrobial treated alone was effective (Sivarooban et al., 2007). Edible zein film coatings containing nisin and calcium propionate reduced the population of *L. monocytogenes* on RTE chicken by 5 logs at 8°C and to undetectable levels at 4°C in 24 days (Janes et al., 2002).

Ready-to-eat meats can also be treated using physical means for inactivating *L. monocytogenes.* The physical methods employed include heat, high pressure, drying, smoking, and irradiation. High hydrostatic pressure treatment (400 MPa, 17°C, 10 min) of sliced cooked ham, in combination with 1.8% potassium lactate followed by storage at 1°C was effective in inactivating *L. monocytogenes* (Aymerich et al., 2005). Ionizing radiation alone and in combination with sodium diacetate and potassium lactate was effective against RTE frankfurter on a roll product (Sommers and Boyd, 2006). Foong et al. (2004) treated RTE meats using irradiation against *L. monocytogenes.* To obtain 3- and 5-log reductions, respectively, dosages of 1.5 and 2.5 kGy were needed for bologna, roast beef, and smoked turkey, and 2.0 and 3.0 kGy were needed for frankfurters and ham. Combining irradiation with antimicrobials may help reduce the high dose and also prevent the growth of *L. monocytogenes* during storage. Combinations of irradiation at 1- or 2-kGy doses with potassium benzoate and sodium lactate, or sodium lactate and sodium diacetate, or a combination of all three salts inhibited growth of *L. monocytogenes* on RTE turkey breast rolls stored at 4°C for 42 days (Zhu et al., 2009). Combination treatments help in maintaining sensory attributes of the product by permitting use of lower levels of antimicrobials or lower intensity of the physical treatment than needed, when these antimicrobials or physical treatments are used alone as a single treatment.

Using post-package pasteurization of RTE deli meats including turkey, ham, and roast beef by submersion heating for 2 to 10 minutes at 195°F to 205°F, 2- to 4-log reductions in *L. monocytogenes* populations were achieved (Muriana et al., 2002). An integrated pasteurization-packaging system using 121°C for 1.5 seconds, followed by vacuum sealing of the top films of food packages, rendered a 3-log reduction in *L. monocytogenes* population on cooked franks (Murphy et al., 2005). Processing bologna using a cooking cooling cycle commonly used by the industry resulted in a 5-log reduction of *L. monocytogenes* (Sallami et al., 2006). Ozone was found to be of limited effectiveness against

*L. monocytogenes* on RTE cured ham (Julson et al., 2001). RTE meat products such as summer sausage, smoked cured beef, beef jerky, and snack stick pork rind were made using drying, fermentation, and/or smoking, inoculated with *L. monocytogenes* cocktail, repackaged under vacuum or air and stored at 21°C or 5°C for 11 weeks (Ingham et al., 2004). There was a decrease in the population of the pathogen from 0.8 to 3.3 logs in various products under different conditions, indicating that these processes combined with storage for at least 1 week would be an effective post-lethality strategy for controlling *L. monocytogenes* in some of these products.

Al-Holy et al. (2004) employed radio frequency heating along with nisin to inactivate *L. innocua* (a surrogate for *L. monocytogenes*) on sturgeon caviar, and the combination treatment at 65°C was effective; no survivors were detected. Pulsed electric field treatment (30–50 kV/cm) followed by exposure to nisin (10–100 IU) reduced *L. innocua* (a surrogate for *L. monocytogenes*) population in skim milk by 2 to 3.8 logs (Calderón-Miranda et al., 1999). Pulsed electric field treatment alone, however, was not effective against *L. monocytogenes* in skim milk (Fleischman et al., 2004). Nisin in combination with moderate heat (60°C for 5 min or 65°C for 2 min) reduced *L. monocytogenes* population by 3 to 5 logs in cold pack lobster meat; either treatment alone had only 1- to 3-log reductions (Budu-Amoako et al., 1999). Mild heating (60°C for 3 min) with nisin reduced *L. monocytogenes* on sturgeon caviar stored for 28 days at 4°C to below detection; no synergy was observed between nisin, lactic acid, or chlorous acid (Al-Holy et al., 2005).

## *1.3* Salmonella enterica

The *Salmonella* genus includes rod-shaped, Gram-negative, non-spore-forming, predominantly motile enteric (intestinal) bacteria. The bacteria range in diameter from 0.7 to 1.5 µm, and in lengths from 2 to 5 µm, with peritrichous flagella. These bacteria obtain their energy from oxidation-reduction reactions using organic substrates and are facultative anaerobes. Most *Salmonella* species produce hydrogen sulfide, are unable to ferment lactose, and can readily be detected by growing on media containing ferrous sulfate (Giannella, 1996).

*Salmonella* has been known to cause illness for over 100 years. The genus *Salmonella* was named after Daniel Elmer Salmon, an American veterinary pathologist. Although Theobald Smith was the actual discoverer of the type bacterium (*Salmonella enterica* var. choleraesuis) in 1885, Dr. Salmon was the administrator of the research program; hence the organism was named after him (USFDA, 2007). Smith and Salmon had been investigating the cause of common hog cholera and suggested

*Salmonella* as the causal agent. However, it was later found out that this organism (now known as *Salmonella enterica*) rarely caused enteric symptoms in pigs (Todar, 2008). *Salmonella* are closely related to *Escherichia coli* (~ 90% homology at DNA level) and are found in warm- and cold-blooded animals, in humans, and in nonliving habitats. They cause illnesses in humans such as typhoid fever, paratyphoid fever, and the foodborne illness salmonellosis (Ryan and Ray, 2004).

There are more than 2,500 serotypes of *Salmonella*, based upon the somatic or cell wall antigens (O-antigen), flagellar antigens (H-antigen), and surface or envelope antigens (Todar, 2008). Two of these serotypes, namely, *Salmonella enterica* serotype Enteritidis and *Salmonella enterica* serotype Typhimurium are the most common in the United States and account for 50% of all human infections (CDC 2005a; Vugia et al., 2004). The infectious dose of *Salmonella* is small, probably ranging from 15 to 20 cells, and most strains cause gastroenteritis with an incubation period of 6 to 72 hours (CDC, 2001). The initial stage of infection includes symptoms such as nausea, cramping, and vomiting, followed by a second stage typified by severe diarrhea and cramping lasting for 2 to 7 days.

*Salmonella* causes one of the most common enteric infections in the United States, salmonellosis, which is the second most common bacterial foodborne illness reported (second to *Campylobacter* infection) (CDC, 2005b). However, the actual number of infections may be 30 or more times greater since many cases with mild symptoms are not diagnosed or reported (Mead et al., 1999). According to the CDC estimates, 1.4 million cases occur annually in the United States, amounting to about 30,000 confirmed cases of salmonellosis each year. *Salmonella* illnesses result in approximately 600 deaths annually, which accounts for 31% of all food-related deaths (CDC, 2005b). The incidence of salmonellosis appears to be rising both in the United States and in other industrialized nations. In 2005, more than 36,000 cases were reported from public health laboratories across the nation, which represented a 12% decrease compared to the previous decade, but a 1.5% increase compared to those reported in 2004 (CDC, 2007b). Isolation of *S.* Enteritidis from humans has shown that *Salmonella* infections have increased dramatically (six-fold or more) in the past decade, particularly in the northeast United States and is spreading south and west, with sporadic outbreaks in other regions (U.S. Food and Drug Administration, 2009).

### *1.3.1 Outbreaks associated with* Salmonella *in RTE foods*

*Salmonella* has been linked to several foodborne outbreaks associated with RTE foods in the past two decades. RTE foods most commonly associated

with *Salmonella* include poultry products, egg products, milk and dairy products, seafood, yeast, coconut, sauces and salad dressings, cake mixes, cream-filled desserts and toppings, dried gelatin, peanut butter, cocoa, chocolate, and fresh produce (USFDA, 2009).

Foodborne outbreaks of *Salmonella* have been a major concern in RTE meat and poultry products. In June 2007, a multistate outbreak of *Salmonella* infections associated with frozen pot pies occurred in the United States (CDC, 2007a). The outbreak involved 401 cases of salmonellosis in 41 states; 32% of affected people were hospitalized. The outbreak strain was isolated from 13 samples of unopened Banquet pot pies collected from the homes of patients. Further investigations revealed that 77% of patients failed to cook the product properly due to confusion regarding microwaving instructions. In February 1995, 93 cases of salmonellosis associated with beef jerky were identified by the New Mexico Department of Health (NMDOH) (CDC, 1995b). The *Salmonella* serotypes isolated from patients were *Salmonella* Typhimurium (31 persons), *Salmonella* Montevideo (12 persons), and *Salmonella* Kentucky (11 persons).

While meat and poultry products top the list of RTE foods associated with *Salmonella* outbreaks, other RTE foods have also been implicated in *Salmonella* outbreaks. Toasted oats cereal was implicated in a multistate outbreak of *Salmonella* serotype Agona in April and May 1998 (Martin and Bean, 1995). A total of 209 cases with at least 47 hospitalizations were reported. *Salmonella* Agona is an uncommon serotype, accounting for approximately 1.5% of human isolates reported to the Public Health Laboratory Information System (PHLIS) (Martin and Bean, 1995). Like most other *Salmonella* serotypes, *S.* Agona is found in a variety of animal reservoirs, including poultry, cattle, pigs, and animal feed. This outbreak represented the first commercial cereal product to have been implicated in a *Salmonella* outbreak, although an infant cereal product was implicated in an outbreak of *Salmonella* Senftenberg in the United Kingdom (Rushdy et al., 1988). *Salmonella* spp. are relatively resistant to desiccation and can survive for long periods in dry environments such as cereal (Mitscherlich and Marth, 1984). Other outbreaks of this serotype have been attributed to dried milk and to a commercial peanut-flavored snack (Sramova et al., 1991). Another outbreak of *Salmonella* Enteritidis occurred in 1994 in ice cream which had 80 confirmed cases (CDC, 1994).

In recent years, fresh produce has been repeatedly implicated as a vehicle for foodborne illnesses due to *Salmonella*. Together with *E. coli* O157:H7, *S. enterica* is responsible for approximately 61% of all produce-associated illnesses (Olsen et al., 2000). Green onions, lettuce, spinach, cantaloupes, tomatoes, cabbage, strawberries, raspberries (Beuchat, 1996), alfalfa sprouts, parsley (Harris et al., 2001), and tree nuts (CDC, 2009b; USFDA,

2004) have been implicated as vehicles of bacterial infections. According to the Center for Science in the Public Interest (CSPI, 2009), alfalfa sprouts have been implicated in more than 20 *Salmonella* outbreaks since 1995. An outbreak of *S. enterica* was associated with irrigation water used to produce alfalfa sprouts in California (Winthrop et al., 2003). *Salmonella* Saintpaul was identified as the causative agent for 228 cases of foodborne illnesses that recently occurred in Nebraska and 13 other states, due to consumption of alfalfa sprouts between January and May 2009. The outbreak was traced to alfalfa sprouts grown at multiple facilities that used seeds possibly originating from a common seed producer, identified as Caudill (CDC, 2009b). In April 2008, another large outbreak of *Salmonella* Saintpaul was initially linked to raw tomatoes in New Mexico and Texas, when CDC issued warnings against consumption of raw tomatoes, including round red and Roma tomatoes (CDC, 2008). Over the summer, the outbreak spread to 43 states, the District of Columbia, and Canada. By August, more than 1,400 people were confirmed ill. However, as the outbreak continued to spread, the advisory on tomatoes was lifted and issued for Mexican-grown raw jalapeño and serrano peppers. On July 30, the FDA confirmed the presence of *Salmonella* Saintpaul at a farm in Mexico, both in irrigation water and on produce.

Between October 2006 and January 2007, an outbreak of 26 cases of *Salmonella* Litchfield infection in the states of Western Australia and Queensland was linked to consumption of papaya (Gibbs et al., 2009). Inspection of two of the three farms in Western Australia that supplied the contaminated papaya revealed that *Salmonella* Litchfield was not detected in papaya samples, fungal sprays, or water samples from the farms; however, other serotypes of *Salmonella* were detected in untreated river water used for washing papaya at one of the farms.

The most recent peanut butter outbreak of *S. enterica* spanned 47 states and involved more than 500 cases and 8 fatalities (CDC, 2009c). Inadvertent moisture in the production facility possibly allowed the growth of dormant *Salmonella* organisms that were likely present in raw peanuts or peanut dust. A leaky roof in the plant during a rainstorm and a faulty sprinkler system were suspected to have contributed to the moisture. An earlier outbreak in 2006–2007 involved peanut butter and *S. enterica* (CDC 2007b). Yet another *Salmonella* outbreak was definitively linked to raw almonds, leading to the recall of roughly 18 million pounds of raw almonds produced by Paramount Farms (USFDA, 2004). Environmental investigations into the outbreak revealed that *Salmonella* was present at the farm when three samples from two huller-shellers that supplied the farm during the period of interest tested positive for *Salmonella* contamination.

### *1.3.2 Incidence and prevalence of* Salmonella *in RTE foods*

Ready-to-eat products such as salad vegetables, leafy greens, meat, poultry, seafood, dairy, and tree nuts, along with herbs, spices, and dried seeds, have all been found to be contaminated with *Salmonella*. The organism is more prevalent in RTE products of animal origin, possibly due to its enteric nature and the ability to survive extreme environmental conditions. Prevalence of *Salmonella* was estimated in different types of RTE food products of animal origin in Catalonia, Spain (Cabedo et al., 2008). A total of 1,379 samples consisting of 187 RTE fish products and 569 RTE meat products, 484 RTE dairy products, and 139 RTE dishes and desserts were collected and analyzed for the presence of *Salmonella*. The organism was isolated from 1.2% of smoked salmon samples, 1.5% of frozen chicken croquettes, 2% of cooked ham samples, and 11.1% of cured dried sausage samples. Between 1990 and 1999, FSIS conducted microbiological testing for nine different categories of RTE meat and poultry products that were produced at approximately 1,800 federally inspected production facilities (Levine et al., 2001). The cumulative *Salmonella* prevalence over the 10-year period was found to be 0.31% in jerky; 0.10% in cooked, uncured poultry products; 0.07% in large-diameter cooked sausages; 0.20% in small-diameter cooked sausages; 0.22% in cooked beef, roast beef, and cooked corned beef; 0.05% in salads, spreads, and pâtés; and 0.22% in sliced ham and luncheon meat. For dry and semidry fermented sausages, the cumulative 3-year *Salmonella* prevalence was 1.43%.

Recent outbreaks of salmonellosis due to consumption of products from plant origin have highlighted the relevance of sources of infections other than animal origin (e.g., egg, poultry, meat). An international outbreak of multi-drug-resistant *S.* Typhimurium DT 104 was correlated to the consumption of halvah, an Asian sweet confection made from sesame seeds. In a follow-up study conducted by Brockmann et al. (2004), several sesame seed products were examined for the occurrence of *Salmonella*. Of the 117 RTE food products made from sesame seeds, salmonellae were isolated from 11 (9.4%) samples (Brockmann et al., 2004). In addition to finding *S.* Typhimurium DT 104 in the halvah involved in the outbreak, *S.* Offa, *S.* Tennessee, and *S.* Poona were also isolated from sesame paste (tahini) and sesame seed sold for raw consumption in cereals. Due to the high desiccation tolerance of *Salmonella*, dried herbs and spices have also been found to be contaminated with this organism. The FDA has noted an increase in the number of recalls of dried spices due to bacterial contamination (Viji et al., 2006). Accordingly, Viji et al. (2006) reviewed spice recalls in the United States that took place between 1970 and 2003. Of the 21 recalls involving 12 spice types contaminated with bacterial pathogens,

20 recalled spices contained *Salmonella*, with paprika being the most common spice involved in the recalls.

A more recent study carried out by the Health Protection Agency and the Local Authorities Co-ordinators of Regulatory Services (LACORS) (2009) revealed the presence of *Salmonella* in a small number of RTE dried seed samples. Between October 2007 and March 2008, 3,735 samples of RTE seeds of different varieties (alfalfa, hemp, linseed, melon, poppy, pumpkin, sesame, sunflower, other [watermelon, celery] and mixed seeds) were collected from retail premises including supermarkets, health food shops, convenience shops, and market stalls. *Salmonella* was detected in 0.6% of samples collected; samples included sesame seeds, linseed, sunflower seeds, alfalfa seeds, melon seeds, and mixed seeds. The most frequently contaminated seed type was melon seeds (8.5% contained *Salmonella*).

Consumer demand for fresh fruits and vegetables and for convenience foods has increased in the past decade, causing an expansion of the minimally processed vegetables (MPVs) market. *Salmonella* is one of the more common pathogenic microorganisms that can be transmitted to humans by these products. Fröder et al. (2007) evaluated the microbial quality of a variety of MPVs. A total of 133 samples of minimally processed leafy salads were tested for the presence of *Salmonella* from retailers in the city of São Paulo, Brazil. Salmonella was detected in 4 (3%) of the 133 samples. Similarly, a study of retail, bagged, prepared RTE salad vegetables was undertaken by Sagoo et al. (2003) to determine the microbiological quality of these vegetables. Examination of 3,852 samples of the salad vegetables revealed that 6 (0.2%) samples were of unacceptable microbiological quality because of the presence of *Salmonella* (*Salmonella* Newport PT33 [1 sample], *Salmonella* Umbilo [3 samples], and *Salmonella* Durban [1 sample]). Prevalence of *Salmonella* on fresh produce collected from the southern United States was evaluated by Johnston et al. (2005). A total of 398 produce samples (leafy greens, herbs, and cantaloupe) were collected. The prevalence of *Salmonella* for all samples was 0.7% (3 of 398).

### 1.3.3 *Strategies for controlling* Salmonella *in RTE foods*

*Salmonella* is associated with a number of foods. Due to its enteric nature, meat (cattle, pigs, goats, chickens, etc.) may be contaminated from the intestinal contents during evisceration of animals, during washing, and during post-harvest processing. However, proper cooking of the meat and meat products will generally eliminate the contamination. Therefore, control of *Salmonella* focuses on adequate cooking of potentially contaminated foods. The multistate outbreak of *Salmonella* infections associated with inadequately cooked frozen pot pies emphasizes the need to thoroughly cook non-RTE frozen foods. Additionally, these products need to

be clearly labeled, with cooking instructions validated to account for variability in microwave wattage and common misconceptions among consumers regarding the nature of non-RTE foods.

Ready-to-eat foods such as cream, mayonnaise, and creamed foods can support multiplication of *Salmonella.* Additionally, many RTE foods are either consumed raw or are minimally processed. Vegetables and fruits may carry *Salmonella* when contaminated with fecal matter or when washed with polluted water. Minimally processed cut and packaged salad is exposed to a range of conditions during growth, harvest, preparation, and distribution, and these conditions may increase the potential for microbial contamination. It is therefore essential to prevent cross-contamination by implementation of good agricultural practices and good hygiene practices from farm to fork.

Additionally, *Salmonella* is extremely tolerant to various growth conditions. It can grow over a temperature range of 7°C to 46°C, survive in water activity as low as 0.94, and in pH ranging from 4.4 to 9.4 (FSIS, 2008). The desiccation resistance of *Salmonella,* along with its ability to survive varying temperature and pH conditions, plays a role in the survival of this organism in RTE products. Therefore, conditions recommended for the prevention of bacterial growth during production of certain RTE meat products such as jerky include rapid drying at high temperatures (i.e., initial drying temperature greater than 155°F [68.3°C] for 4 hours, then greater than 140°F [60°C] for an additional 4 hours) and decreased water activity (i.e., $a_w = 0.86$) (Holley, 1985a, 1985b). *Salmonella* infection can thus also be prevented by avoiding multiplication of the organism in food (constant storage at 4°C), and by using pasteurized and sterilized products (fruit juices, milk, and milk products).

FSIS is the public health regulatory agency in the USDA that is responsible for the safety of the United States' commercial supply of meat, poultry, and egg products. As part of its responsibility, the FSIS issued the "Pathogen Reduction; Hazard Analysis and Critical Control Point (PR/HACCP) Systems, Final Rule" in 1996. This rule sets performance standards for establishments that slaughter a selective class of food animals, or those that produce selected classes of raw ground products, to verify that industry systems are effective in controlling the contamination of raw meat and poultry products with disease-causing bacteria, such as *Salmonella*. FSIS requires all plants to reduce bacteria by means of the PR/HACCP system. FSIS has also taken steps at addressing the problems of *Salmonella* contamination on meat and poultry products. Since 1983, FSIS has been conducting regulatory microbiological testing programs on RTE meat and poultry products. These programs are designed to evolve continuously in response to public health concerns and provide an overall indication of trends.

## *1.4* Escherichia coli *O157:H7*

*Escherichia coli*, considered to be part of the normal micro-flora of the intestinal tract of humans and other warm-blooded animals (Drasar and Hill, 1974), was first described by Theodor Escherich in 1885 (Escherich, 1988). Generally, *E. coli* strains that colonize the human intestine are harmless. However, they may become pathogenic and have the potential to cause infection among immunocompromised individuals or when the integrity of the defense barrier system of the intestinal mucosa is compromised (Nataro and Kaper, 1998). Over the years some *E. coli* strains have developed the ability to cause disease of the gastrointestinal, urinary, or central nervous system, even in healthy individuals (Nataro and Kaper, 1998). The diarrheagenic *E. coli* that have been associated with foodborne illnesses are grouped into five categories: enteropathogenic *E. coli* (EPEC), enteroinvasive *E. coli* (EIEC), enterotoxigenic *E. coli* (ETEC), enteroaggregative *E. coli* (EAggEC), and enterohemorrhagic *E. coli* (EHEC). *Escherichia coli* O157:H7 belongs to the EHEC group of *E. coli* that has been associated with foodborne illnesses. It is one of the most important strains belonging to the EHEC group and has been commonly associated with hemorrhagic colitis and hemolytic uremic syndrome around the world.

*Escherichia coli* O157:H7 was first isolated in 1975 from a woman in California diagnosed with severe bloody diarrhea (Riley et al., 1983). It was first recognized as an important pathogen in 1982 following two foodborne outbreaks of hemorrhagic colitis (Riley et al., 1983; Wells et al., 1983). In 1983, Karmali et al. (1983) reported an association between infection with Shiga toxin–producing *E. coli* and hemolytic uremic syndrome. In recognition of its distinct clinical manifestations, *E. coli* O157:H7 became the first of several strains to be referred to as EHEC, which are now believed to account for more than 90% of all cases of hemolytic uremic syndrome in industrial countries (Scotland et al., 1985). *Escherichia coli* O157:H7 is genetically very closely related to *E. coli* O55:H7, an EPEC strain associated with infantile diarrhea (Whittman et al., 1993). It can adhere to epithelial cells and produce the characteristic "attaching and effacing" lesions (Nataro and Kaper, 1998) similar to the EPEC strain. According to LeClerc et al. (1996), *E. coli* O157:H7 may be particularly adept at incorporating foreign DNA material due to its intrinsically high rate of defects in the DNA repair mechanisms.

Several factors have been associated with the virulence of *E. coli* O157:H7; one factor is its ability to produce one or more Shiga toxins. It was demonstrated in 1983 that the genes controlling the production of the two toxins are bacteriophage encoded (O'Brien et al., 1984; Scotland et al., 1983; Smith et al., 1983). It is believed that the bacteriophage was acquired by the *E. coli* O157:H7 strains directly or indirectly from *Shigella*

(Buchanan and Doyle, 1997). Production of Shiga toxins in itself is not sufficient to cause disease. *E. coli* O157:H7 has other characteristics that help make it virulent and deadly. Adhesion to the epithelial cells lining the intestinal tract may be one important aspect of the organism's pathogenic potential (Griffin, 1995). Other factors thought to contribute to the virulence of *E. coli* O157:H7 include a 60MDa virulence plasmid (pO157) and the locus of enterocyte effacement (LEE) (Mead and Griffin, 1998). The involvement of the 60MDa plasmid in adherence has been suggested, but the reports on its exact role are conflicting (Karch et al., 1987; Toth et al., 1991; Tzipori et al., 1987). It has also been suggested that the pO157 encodes a hemolysin that, in concert with specialized transport systems, may allow *E. coli* O157:H7 to use iron from blood released into the intestine (Law and Kelly, 1995).

### 1.4.1 *Outbreaks associated with* E. coli *O157:H7 in RTE foods*

In the 10 years following the 1982 outbreak, approximately 30 *E. coli* O157:H7 outbreaks were recorded in the United States (Griffin and Tauxe, 1991). The actual number that occurred is probably much higher because *E. coli* O157:H7 infections did not become a reportable disease (required to be reported to public health authorities) until 1987 (Keene et al., 1991). As a result, only the most geographically concentrated outbreaks would have garnered enough attention to prompt further investigation (Keene et al., 1991). It is important to note that only about 10% of infections occur in outbreaks; the rest are sporadic. It is estimated that *E. coli* O157:H7 causes approximately 73,000 illnesses in the United States each year. The Centers for Disease Control and Prevention (CDC) has estimated that 85% of *E. coli* O157:H7 infections are foodborne in origin (Mead et al., 1999). Between 1982 and 2002, of the 350 outbreaks caused by *E. coli* O157:H7, 52% were caused by foodborne sources (Rangel et al., 2005). Although undercooked or raw hamburger (ground beef) has been implicated in many of the documented outbreaks, RTE foods and produce such as sausages, dried (uncooked) salami (Alexander et al., 1994), non-pasteurized milk and cheese, yogurt, non-pasteurized apple juice and cider (Cody et al., 1999), orange juice, alfalfa and radish sprouts (Breuer et al., 2001), lettuce, and spinach (Friedman et al., 1999) have also been implicated in several *E. coli* O157:H7 outbreaks.

Due to the association of *E. coli* O157:H7 with cattle, milk and milk products have been implicated in several foodborne disease outbreaks associated with this organism. Seven outbreaks of *E. coli* O157:H7 have been associated with dairy products, including four from consuming raw milk. The others involved cheese curds and butter made from raw milk,

and commercial ice cream bars (possibly due to cross-contamination) (Rangel et al., 2005).

Beef jerky and other processed meat products, including fermented salami and sausages which are considered to be RTE and, therefore, are expected to be pathogen-free, have also been implicated in foodborne outbreaks of *E. coli* O157:H7 (Alexander et al., 1994; Williams et al., 2000). In the 1994 foodborne illness outbreak in Washington and California, 20 laboratory confirmed cases of *E. coli* O157:H7 were linked to the consumption of dry cured sausage. An outbreak of *E. coli* O157:H7 infection due to consumption of Genoa salami was identified in the spring of 1998 in southern Ontario. These products are made from raw ground meat, usually beef and pork, and are preserved through fermentation and drying, with the addition of salt and spices. It was a common belief among the manufacturers that pathogens like *E. coli* were unable to grow in foods processed under low pH, low water activity, and high salinity conditions and that fermented sausages, therefore, did not require cooking and were RTE with no further preparation.

Foodborne outbreaks of *E. coli* O157:H7 associated with fresh produce were first reported in 1991 (Rangel et al., 2005) and have become increasingly common over the past two decades. The increased consumption of fresh fruits and vegetables in the United States appears to correlate with increased produce-associated outbreaks (Rangel et al., 2005). Since 1995, there have been 22 outbreaks of *E. coli* O157:H7 associated with fresh lettuce or spinach (USFDA, 2001). According to surveillance results for *E. coli* O157 outbreaks reported to CDC between 1982 and 2002, fresh produce accounted for 38 of 183 (21%) foodborne outbreaks and 34% of 5,269 cases related to foodborne outbreaks (Rangel et al., 2005). The median number of cases in produce-associated outbreaks was significantly greater than that of ground beef-associated outbreaks (20 vs. 8 at $p < 0.001$) (Rangel et al., 2005). Fresh produce types most commonly associated with these outbreaks included lettuce (34%), apple cider or apple juice (18%), salad (16%), coleslaw (11%), melons (11%), sprouts (8%), and grapes (3%). These outbreaks most commonly occurred in restaurants (39%), out of which 47% were reported to be due to cross-contamination during food preparation. However, more than half (53%) of the produce-associated outbreaks did not involve cross-contamination in the kitchen. The outbreaks were due to produce already contaminated with *E. coli* O157 before purchase and included 7 outbreaks associated with apple cider or apple juice, 7 of 10 lettuce-associated outbreaks, 3 of 4 coleslaw-associated outbreaks, and 3 alfalfa sprout or clover sprout associated outbreaks (Ackers et al., 1998; Besser et al., 1993; Cody et al., 1999; Hilborn et al., 1999; Mahon et al., 1997). These produce items may have become contaminated in the field from manure or contaminated irrigation water; during processing due

to contaminated wash water or ice, contaminated equipment, or poor handling practices; during transport; or through contaminated storage equipment.

In 1996, a large *E. coli* O157 outbreak, attributed to the consumption of commercial unpasteurized apple juice, occurred in three western states and British Columbia, involving 70 illnesses, mostly children. More than 30% of patients were hospitalized and hemolytic uremic syndrome developed in 20% resulting in death of one child (Cody et al., 1999). Since 1998, only two outbreaks due to unpasteurized apple cider have been reported, one at a local fair and one from locally produced cider. In September 2006, there was an outbreak of foodborne illness caused by *E. coli* O157:H7 in 26 U.S. states which was traced to organic bagged fresh spinach (CDC, 2006; FDA, 2007). The outbreak resulted in 204 cases of illness, including 31 cases involving hemolytic uremic syndrome, 104 hospitalizations, and 3 deaths. Investigations by the CDC and a joint report by the California Department of Health Services (CDHS, 2007) and the FDA concluded that the probable source of the outbreak was a cattle ranch that had leased land to a spinach grower. Although it could not be definitively decided how the spinach became contaminated, the presence of wild pigs on the ranch and the close proximity of surface waterways to irrigation wells were considered "potential environmental risk factors." The report also noted that flaws in the spinach producer's transportation and processing systems could have further spread contamination. A few months later, following this outbreak, another outbreak implicated bagged lettuce in fast-food restaurants (FDA, 2006a, 2006b, 2006c), thus raising concerns about the microbiological quality of RTE produce.

### 1.4.2 *Incidence and prevalence of* E. coli *O157:H7 in RTE foods*

Although *E. coli* O157:H7 is most commonly associated with undercooked or raw hamburger (ground beef), RTE foods and produce including leafy greens, alfalfa sprouts, unpasteurized fruit juices, dry-cured salami, raw or unpasteurized milk, and cheese curds have also been associated with the organism. A survey of the prevalence of *E. coli* O157 in raw meats, raw cow's milk, and raw-milk cheeses was conducted in southeast Scotland over a 2-year period starting in April 1997. *Escherichia coli* O157 was isolated from beef sausage and beef burger produced by the same retail butcher shop. Cooley et al. (2007) described the incidence of *E. coli* O157 in a major produce production region in California following the produce-associated outbreaks between 2002 and 2006. Approximately 1,000 *E. coli* O157 isolates obtained from cultures of more than 100 individual samples were typed using Multi-Locus Variable-number-tandem-repeat Analysis

(MLVA) to identify the potential fate and transport of *E. coli* O157 in this region. The organism was isolated at least once from 15 of 22 different watershed sites over a 19-month period. The incidence of *E. coli* O157 increased significantly when heavy rain caused an increased flow rate in the rivers.

In a study by Dingman (1999), cider samples were obtained from 11 cider mills operating in Connecticut during the 1997 to 1998 production season to test for the presence of *E. coli.* Three hundred fourteen cider samples were tested, out of which 11 (4%) were found to contain *E. coli.* Of the 11 mills, 6 (55%) tested positive for *E. coli* in the cider at least once during the production year. The microbiological quality of RTE food products sold in Taiwan was determined in a total of 164 samples of RTE food products, purchased in 1999–2000 from convenience stores and supermarkets in central Taiwan (Fang et al., 2003). The incidence of *E. coli* in these RTE food products was found to be 7.9%. Among the five types of food products tested, the highest incidence of *E. coli* (16%) was detected in hand-rolled sushi and food products made from ham.

More recently, a study was carried out by the Health Protection Agency and LACORS (2009) in 3,735 samples of RTE dried seeds, where 1.5% seed samples were found to contain unsatisfactory levels of *E. coli*, an indicator of poor hygiene. Retail samples of seeds of different varieties (alfalfa, hemp, linseed, melon, poppy, pumpkin, sesame, sunflower, other [watermelon, celery] and mixed seeds) were collected from supermarkets, health food shops, convenience shops, and market stalls. *Escherichia coli* was detected in melon, pumpkin, sesame, hemp, poppy, linseed, sunflower, and mixed seeds. The most frequently contaminated seed type was melon seeds.

### *1.4.3 Strategies for controlling* E. coli *O157:H7 in RTE foods*

Although outbreaks from *E. coli* O157:H7 are often associated with ground beef products, other food products have also been implicated, and these include salami, sprouts, lettuce, unpasteurized apple juice, unpasteurized milk, yogurt, and cheese. Since most of these foods are considered to be RTE with no final cooking step, control of the disease from these products is difficult. Since the infectious dose of *E. coli* O157:H7 is very low (Tilden et al., 1996), any survival in an RTE product has the potential to cause illness. *Escherichia coli* O157:H7 may be present in raw ground beef that is used in the manufacturing of other RTE meat products (Alexander et al., 1994). Appropriate measures must therefore be taken to control *E. coli* O157:H7 in the final product. Following good farm and manufacturing practices could be the first step to reducing the risk of contamination with *E. coli* O157:H7. In

a recent FDA report, surface waterways exposed to cattle feces were identified as potential environmental risk factors for *E. coli* O157:H7 contamination of spinach associated with the 2006 outbreak (USFDA, 2007). Several reported outbreaks of *E. coli* O157:H7 in the United States between 1986 and 1996 associated with fruits and vegetables were also due to contamination of produce with animal manure, contamination of the water supply used to wash produce, or by cross-contamination of the fresh produce with raw meat products during meal preparation (Beuchat, 1996). The prevention of cross-contamination from raw beef to RTE products is therefore also critical. In addition, proper personal hygiene practices should be followed by workers at the pre- and post-harvest levels during handling of RTE products since even low doses can cause severe illness.

Several intervention strategies to reduce the prevalence of *E. coli* O157:H7 in meat are currently practiced by the food industry, including the HACCP program. According to the USDA regulation established in 1994, any raw ground beef and non-intact cuts of beef contaminated with *E. coli* O157:H7 are considered to be adulterated. To minimize contamination of food products that generally enter the food chain in the raw state, prevention of fecal contamination during slaughter, milking procedure, and pre- and post-harvest processing of fresh produce is important. Washing of hides prior to removal has been shown to significantly reduce levels of contamination in beef products (Ahmadia et al., 2006; Bosilevac et al., 2004; Sheridan, 2007). Decontamination strategies, which include the use of FDA- and USDA-approved antimicrobial treatments to reduce *E. coli* O157:H7 counts on carcass surfaces, hot water or steam treatments, and steam vacuuming, are some of the procedures that can eliminate or significantly reduce the numbers of the organism on meat surfaces.

*Escherichia coli* O157:H7 is also known to be acid tolerant and survives well in fermented and acidic foods. Studies have shown that *E. coli* O157:H7 can survive many of the typical dry fermentation processing conditions (Faith et al., 1998; Glass et al., 1992; Tilden et al., 1996). The organism's tolerance to acidic conditions has also been reported in the processing of other foods such as apple cider (Miller and Kaspar, 1994) and mayonnaise (Zhao and Doyle, 1994). These findings have led to significant changes in the industry and in the manufacturing of dry fermented sausage in the United States (Tilden et al., 1996). In 1996, the USDA-FSIS recommended that deli meat manufacturers adopt one of five options for the control of *E. coli* when they make fermented sausages (Blue Ribbon Task Force, 1996).

Pasteurization or other risk-reducing strategies, such as labeling, irradiation, disinfection, and sprays, for final product or ingredients are currently being considered by several countries. For fresh produce that is

consumed raw, good agricultural practices that significantly reduce the potential for fecal contamination of the crops growing in the fields is critical. In 2005 and 2006, produce-specific guidelines to minimize the potential for product contamination with *E. coli* O157:H7 were published by the FDA for fresh produce, including tomatoes, melons, lettuce, and leafy greens. For example, pasteurization of fruit juices was suggested as an effective control measure for these products, since *E. coli* O157:H7 is more sensitive to heat than is *Salmonella*. The range of temperatures that support growth of *E. coli* O157:H7 is more limited than that for generic *E. coli*, with a minimum of 46°F (8°C) and a maximum of 113°F (45°C). Interventions currently used by the fresh produce industry to reduce foodborne pathogens include several physical and chemical treatments (Gutierrez et al., 2008; Periago and Moezelaar, 2001). Fresh-cut lettuce and leafy greens go through one or more vigorous washing processes before they are packaged and sold to consumers. A wide range of sanitizers including ozone, peroxyacetic acid, chlorine dioxide, chlorinated trisodium phosphate, oxidized water, and acidified sodium chlorite have been evaluated for their ability to reduce *E. coli* O157:H7 on fresh produce (Stopforth et al., 2008). A number of studies have investigated efficacies of various antimicrobials on fresh produce such as tomatoes, lettuce, broccoli, and apples (Beuchat et al., 2001; Kilonzo-Nthenge et al., 2006) against *E. coli* O157:H7. However, these treatments do not always eliminate pathogenic microorganisms. For example, efforts by the industry to decrease contamination of sprouts have had limited success (Brooks et al., 2001; Taormina et al., 1999). Scientific studies have demonstrated that washing produce with water or a chlorine-based solution reduces *E. coli* O157 counts only modestly (Beuchat, 1999; Beuchat et al., 2004; Beuchat and Ryu, 1997). Water used in post-harvest operations to wash fresh produce may itself become a source of contamination if it contains pathogenic microorganisms and if there is insufficient wash water disinfectant present (Solomon et al., 2003). Therefore, once consumers obtain contaminated produce intended for raw consumption, little can be done to prevent illness. Until effective measures for preventing *E. coli* O157 contamination of produce items such as lettuce, cabbage, and sprouts can be implemented, consumers should be educated about potential risks associated with consuming these items raw. Further regulatory and educational efforts are needed to improve the safety of produce items.

Government regulatory agencies in the United States such as the USDA and the FDA have also taken steps to reduce the incidences of foodborne illnesses associated with bacterial pathogens, including *E. coli* O157:H7. Following the large multistate outbreak of *E. coli* O157 infections in 1993 in the western United States, a surveillance network called PulseNet was developed, in order to prevent future severe outbreaks.

PulseNet is the national network for molecular sub-typing of foodborne bacteria, coordinated by CDC. The participating laboratories include state health departments, some local health departments, USDA, and FDA. Another surveillance network established by the CDC is the Foodborne Diseases Active Surveillance Network (FoodNet), which is a joint effort by 10 state health departments, the USDA, and FDA. FoodNet conducts active surveillance for foodborne diseases and also conducts related epidemiologic studies to look at both sporadic cases and outbreaks to help better understand the epidemiology of foodborne illnesses and target effective prevention strategies.

## *1.5* Clostridium perfringens

*Clostridium perfringens* is an anaerobic (microaerophilic), Gram-positive, non-motile, spore-forming rod. The organism is widely distributed in the environment and is found in soil, dust, and raw ingredients such as spices, used in food processing, and in the intestinal tract of humans and animals (ICMSF, 1996; Juneja, Thippareddi, and Friedman, 2006). According to Smith and Williams (1984), it may be one of the most widely occurring bacterial pathogens. Raw protein foods of animal origin are frequently contaminated with *C. perfringens*. While the organism has the ability to produce enterotoxin (CPE), a large proportion of *C. perfringens* found in raw foods is CPE-negative (Saito, 1990). The CPE gene associated with food poisoning is commonly located on the chromosome while a non-food poisoning CPE gene is commonly located on a plasmid (Collie and McClane, 1998).

*Clostridium perfringens* food poisoning typically occurs from the ingestion of $>10^6$ viable vegetative cells of the organism in temperature-abused RTE meat products and is one of the most common types of foodborne illnesses (Labbe, 1989; Labbe and Juneja, 2002). Acidic conditions encountered in the stomach may actually trigger the initial stages of sporulation of the vegetative cells of *C. perfringens*. Once in the small intestine, the vegetative cells sporulate, releasing an enterotoxin upon sporangial autolysis. The enterotoxin is responsible for the pathological effects in humans as well as the typical symptoms of acute diarrhea with severe abdominal cramps and pain (Duncan and Strong, 1969; Duncan et al., 1972). Pyrexia and vomiting are usually not encountered in affected individuals. The typical incubation period before onset of symptoms is 8 to 24 hours, and acute symptoms usually last less than 24 hours. Full recovery within 24 to 48 hours is normal. Fatalities are rare in healthy individuals. Foodborne outbreaks of *C. perfringens* can be confirmed if $>10^5$ CFU/g of the organism or $>10^6$ spores/g are detected in the implicated food or feces, respectively (Labbe and Juneja, 2002).

Intrinsic and extrinsic factors that affect survival and growth of *C. perfringens* in food products and contribute to outbreaks include temperature, oxygen, water activity ($a_w$), pH, and curing salts. *Clostridium perfringens* strains that carry the CPE gene on the chromosome have previously been reported to be 60 times more heat resistant than the non-food poisoning strains (Sarker et al., 2000).

Heat treatment applied to raw product during preparation of RTE meat or poultry products is usually sufficient to kill vegetative cells of *C. perfringens* but not the spores present in the raw product. Likewise, slow cooking associated with low-temperature, long-time cooking will not eliminate *C. perfringens* spores. Cooking temperatures, if designed to inactivate *C. perfringens* spores, may negatively affect the product quality and desirable organoleptic attributes of foods. Therefore, spores are likely to survive the normal pasteurization/cooking temperatures applied to RTE foods. The surviving, heat-activated *C. perfringens* spores may germinate, outgrow, and multiply into large number of vegetative cells in cooked RTE foods if the rate and extent of cooling are not sufficient or if the processed foods are temperature abused. The abuse may occur during transportation, distribution, storage, or handling in supermarkets or during preparation of foods by consumers, which includes low-temperature, long-time cooking of foods as well as the scenarios when the foods are kept on warming trays before final heating or reheating. Thus, meat-containing RTE foods can be contaminated by vegetative cells that start out as spores originating in the raw meat or spices used in their production. In addition, cooking usually increases the anaerobic environment in food and reduces the number of competing spoilage organisms, which is ecologically important because *C. perfringens* competes poorly with the spoilage flora of many foods. Mean generation times in autoclaved ground beef during slow heating from 35°C to 52°C ranged from 13 to 30 minutes with temperature increases of 6°C to 12.5°C per hour (Willardsen et al., 1978). Another study also demonstrated growth of the organism in autoclaved ground beef during linear temperature increases (4.1°C–7.5°C per hour) from 25°C to 50°C (Roy et al., 1981).

Studies have described growth of *C. perfringens* during cooling of cooked RTE, uncured meat products. In a study by Tuomi et al. (1974), when cooked ground beef gravy inoculated with a mixture of vegetative cells and spores of *C. perfringens* NCTC 8239 was cooled in a refrigerator, rapid growth of the organism was reported to occur during the first 6 hours of cooling when the gravy temperature was in an ideal growth temperature range. Shigehisa et al. (1985) reported on germination and growth characteristics of *C. perfringens* spores inoculated into ground beef at 60°C and cooled to 15°C at a linear cooling rate of 5°C to 25°C per hour. They observed that the organism did not grow during exposure to falling

temperature rates of 25°C to 15°C per hour. However, multiplication of the organism was observed when the rate was less than 15°C per hour. Interestingly, the total population did not change for the first 150 minutes, regardless of the cooling rate. This study is not totally applicable to typical retail food operations because cooling is not linear; it is exponential. Steele and Wright (2001) evaluated growth of *C. perfringens* spores in turkey roasts cooked to an internal temperature of 72°C, followed by cooling in a walk-in cooler from 48.9°C to 12.8°C in 6, 8, or 10 hours. Results of that study indicated that an 8.9-hour cooling period was adequate to prevent growth of *C. perfringens* with a 95% tolerance interval. To simulate commercial chili cooling procedures, Blankenship et al. (1988) conducted exponential cooling experiments in which the cooling time was 4 hours and 6 hours for a temperature decline from 50°C to 25°C. This is approximately equivalent to a cooling rate of 12 hours and 18 hours for temperatures of 54.4°C to 7.2°C. They observed a declining growth rate in the case of 4 hours and 6 hours cooling time. Juneja et al. (1994) reported that no appreciable growth (<1.0 $\log_{10}$ CFU/g) occurred if cooling took 15 hours or less when cooked ground beef, inoculated with heat activated *C. perfringens* spores, was cooled from 54.4°C to 7.2°C at an exponential rate. However, *C. perfringens* grew by 4 to 5 $\log_{10}$ CFU/g if the cooling time was greater than 18 hours. This implies that *C. perfringens* is capable of rapid growth in meat systems, making this organism a particular concern to meat processors, as well as to the food service industry.

A limited amount of published research is available regarding growth of the pathogen in cooked cured meats during cooling. Taormina et al. (2003) inoculated bologna and ham batter with *C. perfringens* spores, followed by cooking and either cooling procedures typically used in industry or extended chilling. In that study, growth of the organism was not detected in any of the products tested during chilling from 54.4°C to 7.2°C. Zaika (2003) reported complete inhibition of *C. perfringens* germination and growth in cured ham with NaCl concentrations of 3.1% when cooled exponentially from 54.4°C to 7.2°C within 15, 18, or 21 hours. Cooked cured turkey cooled from 48.9°C to 12.8°C did not support *C. perfringens* growth in 6 hours; however, a 3.07-log increase was observed following 24 hours cooling time (Kalinowski et al., 2003).

### *1.5.1 Outbreaks of* C. perfringens *in RTE foods*

The incidence of *Clostridium perfringens* gastrointestinal illnesses in the United States has been estimated by the CDC to be 248,000 cases per year leading to 41 hospitalizations and 7 deaths each year, with 100% of these cases being due to foodborne transmission of the pathogen (Mead et al., 1999) and with an estimated cost of $200 per case (Todd, 1989). It is currently

estimated that the incidence of foodborne illness from *C. perfringens* is a factor of 10 to 350 times the number of cases actually reported (Mead et al., 1999). In another report from 1993 to 1997, *C. perfringens* accounted for 2.1% of the outbreaks and 3.2% of the cases of foodborne illnesses and was the third most common cause of confirmed outbreaks and cases of foodborne illness (Olsen, 2000). There is no evidence to suggest that the contrary exists today. Most cases of *C. perfringens* food poisoning are mild and are not reported. In 1994, the total cost of illnesses due to *C. perfringens* was estimated at $123 million in the United States (Anonymous, 1995). The estimated large number of illnesses due to *C. perfringens* clearly stresses the importance of cooling foods quickly after cooking with proper refrigeration during shelf-life storage.

*Clostridium perfringens* outbreaks usually result from improper handling and preparation of foods, such as inadequate cooling, at the home, retail, or food service level, rarely involving commercial meat processors (Bean et al., 1997; Bean and Griffin, 1990; Bryan, 1988; CDC, 2000; Taormina et al., 2003). Major contributing factors leading to food poisoning associated with *C. perfringens* include its ability to form heat-resistant spores that can survive commercial cooking operations, as well as the ability to germinate, outgrow, and multiply at a very rapid rate during post-cook handling, primarily under conditions conducive to germination. Such conditions occur when the cooling of large batches of cooked foods is not fast enough to inhibit bacterial growth or the foods are held at room temperature for an extended period or are temperature abused. Germination and outgrowth of *C. perfringens* spores during cooling of thermally processed meat products has been reported extensively (Juneja et al., 1994, 1999). Accordingly, improper cooling (40.9%) of food products has been cited as the most common cause of *C. perfringens* outbreaks (Angulo et al., 1998).

Meat and poultry products were associated with the vast majority of *C. perfringens* outbreaks in the United States, probably due to the fastidious requirement for more than a dozen amino acids and several vitamins for the organism to grow in these products (Brynestad and Granum, 2002; Labbe and Juneja, 2002). Roast beef, turkey, meat-containing Mexican foods, and other meat dishes have been associated with *C. perfringens* food-poisoning outbreaks (Bryan, 1969, 1988). Roast beef and other types of cooked beef were primarily implicated as vehicles of transmission for 26.8% of 190 *C. perfringens* enteritis outbreaks in the United States from 1973 to 1987 and 33.9% of 115 outbreaks from 1977 to 1984, although poultry products were also commonly implicated (Bean and Griffin, 1990; Bryan, 1988). In retrospect, most outbreaks of *C. perfringens* food poisoning can be avoided by adequate cooking of meat products followed by holding at hot temperatures or rapid cooling.

### *1.5.2 Incidence and prevalence of* C. perfringens *in RTE foods*

*Clostridium perfringens* is commonly found on vegetable products and in other raw and processed foods. The organism is frequently found in meats, generally through fecal contamination of carcasses, contamination from other ingredients such as spices, or post-processing contamination. *Clostridium perfringens* was detected in 36%, 80%, and 2% of fecal samples from cattle, poultry, and pigs, respectively (Tschirdewahn et al., 1991). The organism was found on 29%, 66%, and 35% of beef, pork, and lamb carcasses, respectively (Smart et al., 1979). *Clostridium perfringens* was isolated from 43.1% of processed and unprocessed meat samples tested in one study, including beef, veal, lamb, pork, and chicken products (Hall and Angelotti, 1965). Many areas within broiler chicken processing plants are contaminated with the organism (Craven, 2001), and the incidence of *C. perfringens* on raw poultry ranges from 10% to 80% (Waldroup, 1996). *Clostridium perfringens* was detected in 47.4% of raw ground beef samples (Ladiges et al., 1974), and a mean level of 45.1 *C. perfringens* per $cm^2$ was detected on raw beef carcass surface samples (Sheridan et al., 1996). *Clostridium perfringens* was detected on 38.9% of commercial pork sausage samples (Bauer et al., 1981), and on raw beef, equipment, and cooked beef in food service establishments (Bryan and McKinley, 1979). In a survey conducted in the United Kingdom by Hobbs et al. (1965), 67% of the vacuum-packaged fish products samples were positive for clostridia, predominantly *C. perfringens.* About 50% of raw or frozen meat and poultry contains *C. perfringens* (Labbe, 1989). *Clostridium perfringens* spores were isolated from 80% of 54 different spices and herbs (Deboer et al., 1985). This presents a public health hazard since spices and herbs are commonly used in meat and meat products. However, early surveys of foods did not determine the enterotoxin-producing ability of isolates. In recent surveys, on the incidence of *C. perfringens* in raw and processed foods, an incidence level of 30% to 80% has been found (Lin and Labbe, 2003).

To determine the level of contamination, Kalinowski et al. (2003) reported that out of 197 raw comminuted meat samples analyzed, all but two samples had undetectable levels (<3 spores per g) and two ground pork samples contained 3.3 and 66 spores per gram. In another survey, Taormina et al. (2003) examined a total of 445 whole muscle and ground or emulsified raw pork, beef, and chicken product mixtures acquired from industry sources for *C. perfringens* vegetative cells and spores. Out of 194 cured whole-muscle samples examined, 1.6% were positive for vegetative cells and spores were not detected. Out of 152 cured ground or emulsified samples, 48.7% and 5.3% were positive for vegetative cells and spores, respectively. Populations of vegetative cells and spores did not exceed 2.72 and 2.00 $\log_{10}$ CFU/g. These studies suggest a low incidence of spores in

raw, cured, whole-muscle ham as well as low levels of spores in cured, ground, emulsified meats, and in raw comminuted meat samples.

Li et al. (2007) surveyed soils and home kitchen surfaces in the Pittsburgh, Pennsylvania, area to determine the prevalence of *cpe*-positive *C. perfringens* isolates in these two environments and reported that neither soil nor home kitchen surfaces represent major reservoirs for type A isolates with chromosomal *cpe* that cause food poisoning, although soil does appear to be a reservoir for *cpe*-positive isolates causing non-foodborne gastrointestinal diseases. Rahmati and Labbe (2008) reported 17 samples positive for *C. perfringens*, one possessed enterotoxin gene, out of 347 fresh and processed seafood samples examined. In another study (Wen and McClane, 2004), a survey of American retail foods reported that approximately 1.7% of raw meat, fish, and poultry items sold in retail foods stores contained type A isolates carrying a chromosomal *cpe* gene, and no plasmid *cpe* gene was found in any of those surveyed retail foods. In a national survey of the retail meats conducted in Australia, *C. perfringens* was not recovered from any of the 94 ground beef samples and was isolated from 1 of 92 samples of diced lamb (Phillips et al., 2008). These surveys indicate a low incidence of *C. perfringens* in retail meats.

### 1.5.3 *Strategies for controlling* C. perfringens *in RTE foods*

Due to its ubiquitous nature and rapid growth in meat products, *C. perfringens* can be a potential hazard in processed meat and poultry products. The FDA (2001b) Food Code dictates that cooked, potentially hazardous foods such as meats should be cooled from 60°C to 21°C within 2 hours and from 60°C to 5°C within 6 hours. In the United Kingdom, it is recommended that uncured cooked meats be cooled from 50°C to 12°C within 6 hours and from 12°C to 5°C within 1 hour (Gaze et al., 1998). Safe cooling times for cured meats may be up to 25% longer (Gaze et al., 1998). The USDA-FSIS compliance guidelines (USDA-FSIS, 2001) for chilling of thermally processed meat and poultry products state that these products should be chilled following the prescribed chilling rates, or require that process authorities validate the safety of customized chilling rates to control spore-forming bacteria. Specifically, the guidelines state that cooling from 54.4°C to 26.7°C should take no longer than 1.5 hours, and cooling from 26.7°C to 4.4°C should take no longer than 5 hours (USDA-FSIS 2001). Additional guidelines allow for the cooling of certain cured cooked meats from 54.4°C to 26.7°C in 5 hours and from 26.7°C to 7.2°C in 10 hours (USDA-FSIS 2001). If meat processors are unable to meet the prescribed time-temperature cooling schedule, they must be able to document that the customized or alternative cooling regimen used will result in a less than 1-$\log_{10}$ CFU increase in *C. perfringens* in the finished product. If the cooling

guidelines cannot be achieved, computer modeling, product sampling, or both, can be used to evaluate the severity and microbiological risk of the process deviation, and additional challenge studies may be necessary to determine if performance standards have been met.

The presence of inhibitory agents in the products can affect germination of *C. perfringens* spores and may also affect the minimum growth temperatures for the germinated spores. Recent studies have shown the efficacy of certain antimicrobial agents against the growth of *C. perfringens* during cooling of meat products. For instance, Sabah, Thippareddi, et al. (2003) found that 0.5% to 4.8% sodium citrate inhibited growth of *C. perfringens* in cooked, vacuum-packaged, restructured beef cooled from 54.4°C to 7.2°C within 18 hours. The same researchers also demonstrated growth inhibition of the microorganism by oregano in combination with organic acids during cooling of *sous-vide* cooked ground beef products (Sabah, Juneja, et al., 2003). Organic acid salts such as 1% to 1.5% sodium lactate, 1% sodium acetate, 0.75% to 1.3% buffered sodium citrate (with or without sodium diacetate), 1.5% sodium lactate supplemented with sodium diacetate inhibited germination and outgrowth of *C. perfringens* spores during the chilling of marinated ground turkey breast (Juneja and Thippareddi, 2004a, 2004b). In another study, Thippareddi et al. (2003) reported complete inhibition of *C. perfringens* spore germination and outgrowth by sodium salts of lactic and citric acids (2.5% and 1.3%, respectively) in roast beef, pork ham, and injected turkey products. Incorporation of plant-derived natural antimicrobials, such as thymol (1%–2%), cinnamaldehyde (0.5%–2%), oregano oil (2%), and carvacrol (1%–2%), as well as the biopolymer chitosan (0.5%–3%) derived from shellfish, and green tea catechins (0.5%–3%) individually inhibited *C. perfringens* germination and outgrowth during exponential cooling of ground beef and turkey (Juneja, Thippareddi, Bari, et al., 2006; Juneja, Thippareddi, and Friedman, 2006; Juneja and Friedman, 2007; Juneja et al., 2007). Therefore, natural compounds can be used as ingredients in processed meat products to provide an additional measure of safety to address the *C. perfringens* hazard during chilling and subsequent refrigeration of meat products, thus further minimizing risk to the consumer.

Numerous studies have examined the heat resistance of *C. perfringens* spores, vegetative cells, or both. Heat resistance varies among strains of *C. perfringens*, although both heat-resistant and heat-sensitive strains can cause food poisoning (Labbe and Juneja, 2006). Environmental stresses, such as storage and holding temperatures, and low-temperature, long-time cooking expose the contaminating vegetative and spore-forming foodborne pathogens to conditions similar to heat shock, thereby rendering the heat-shocked organisms more resistant to subsequent lethal heat treatments. Researchers have reported on the quantitative assessment of

heat resistance, the heat shock response, and the induced thermotolerance to assist food processors in designing thermal processes for the inactivation of *C. perfringens*, thereby ensuring the microbiological safety of cooked foods (Juneja, Novak, Eblen, et al., 2001; Juneja et al., 2003). Heat shocking vegetative cells suspended in beef gravy at 48°C for 10 minutes allowed the microorganisms to survive longer and increased the heat resistance to as high as 1.5-fold (Juneja, Novak, Eblen, et al., 2001). The thermal resistance (D-values in minutes) of *C. perfringens* cells heated in beef gravy at 58°C ranged from 1.21 minutes (C 1841 isolate) to 1.60 minutes (F 4969 isolate). Compared to the control (no heat shock), the increase in heat resistance after heat shocking at 58°C ranged from 1.2-fold (B 40 isolate) to 1.5-fold (NCTC 8239 isolate). The D-values of *C. perfringens* spores heated in beef gravy at 100°C ranged from 15.50 minutes (NCTC 8239 isolate) to 21.40 minutes (NB 16 isolate) (Juneja et al., 2003). Compared to the control (no heat shock), the increase in heat resistance of *C. perfringens* spores at 100°C after heat shocking ranged from 1.1-fold (NCTC 8238 isolate) to 1.5-fold (F 4969 isolate). Similar results were obtained by Heredia et al. (1997), who found that a sublethal heat shock at 55°C for 30 minutes increased tolerance of both spores and vegetative cells to a subsequent heat treatment. In their study, when the heat resistance of *C. perfringens* vegetative cells, grown at 43°C in fluid thioglycollate broth (FTG) to an $A_{600}$ of 0.4 to 0.6, was determined, the D-values at 55°C of 9 and 5 minutes for FD-1 and FD1041 strains, respectively, were reported. The D-values of the heat-shocked cells were 85 and 10 minutes, respectively. Heredia et al. (1997) heat shocked sporulating cells of *C. perfringens* at 50°C for 30 minutes and then determined the D-values at 85°C or 90°C. The authors reported that a sublethal heat shock increased the thermotolerance of *C. perfringens* spores by at least 1.7- to 1.9-fold; the D-values at 85°C increased from 24 to 46 minutes, and at 95°C, from 46 to 92 minutes, respectively. Bradshaw et al. (1982) reported D-values at 99°C for *C. perfringens* spores suspended in commercial beef gravy ranging from 26 to 31.4 minutes. Miwa et al. (2002) found that spores of enterotoxin-positive *C. perfringens* strains were more heat-resistant than were enterotoxin-negative strains. Similarly, food poisoning isolates of the organism are generally more heat-resistant than *C. perfringens* spores from other sources (Labbe, 1989). Sarker et al. (2000) reported D-values at 100°C for 12 isolates of *C. perfringens* spores, carrying either chromosomal *cpe* gene or plasmid *cpe* gene, in DS medium ranged from 0.5 minutes to 124 minutes. Sarker et al. (2000) reported D-values at 55°C for *C. perfringens* cells grown at 37°C in FTG of 12.1 minutes and 5.6 minutes for E13 and F5603 strains, respectively. These authors also reported a strong association of the food poisoning isolates and increased heat resistance; the D-values at 55°C or 57°C for the *C. perfringens* chromosomal *cpe* isolates were significantly higher

($p < 0.05$) than the D-values of the *C. perfringens* isolates carrying a plasmid *cpe* gene; however, differences in heat resistance were not observed at higher temperatures. Nevertheless, understanding these variations in heat resistance is certainly necessary in order to design adequate cooking regimes to eliminate *C. perfringens* vegetative cells in RTE foods.

Studies have been conducted to assess the effects and interactions of multiple food formulation factors on the heat resistance of spores and vegetative cells of *C. perfringens*. In a study by Juneja and Marmer (1996), when the thermal resistance of *C. perfringens* spores (expressed as D-values in minutes) in turkey slurries that included 0.3% sodium pyrophosphate at pH 6.0 and salt levels of 0%, 1%, 2%, or 3% was assessed, the D-values at 99°C decreased from 23.2 minutes (no salt) to 17.7 minutes (3% salt). In a beef slurry, the D-values significantly decreased ($p < 0.05$) from 23.3 minutes (pH 7.0, 3% salt) to 14.0 minutes (pH 5.5, 3% salt) at 99°C (Juneja and Majka, 1995). While addition of increasing levels (1%–3%) of salt in turkey (Juneja and Marmer, 1996) or a combination of 3% salt and pH 5.5 in beef (Juneja and Majka, 1995) can result in a parallel increase in sensitivity of *C. perfringens* spores at 99°C, mild heat treatments given to minimally processed foods will not eliminate *C. perfringens* spores. Juneja and Marmer (1998) examined the heat resistance of vegetative *C. perfringens* cells in ground beef and turkey containing sodium pyrophosphate (SPP). The D-values in beef that included no SPP were 21.6, 10.2, 5.3, and 1.6 minutes at 55°C, 57.5°C, 60°C, and 62.5°C, respectively; the values in turkey ranged from 17.5 minutes at 55°C to 1.3 minutes at 62.5°C. Addition of 0.15% SPP resulted in a concomitant decrease in heat resistance as evidenced by reduced bacterial D-values. The D-values in beef that included 0.15% SPP were 17.9, 9.4, 3.5, and 1.2 minutes at 55°C, 57.5°C, 60°C, and 62.5°C, respectively; the values in turkey ranged from 16.2 minutes at 55°C to 1.1 minutes at 62.5°C. The heat resistance was further decreased when the SPP level in beef and turkey was increased to 0.3%. Heating such products to an internal temperature of 65°C for 1 minute killed >8 $\log_{10}$ CFU/g. The z-values in beef and turkey for all treatments were similar, ranging from 6.22°C to 6.77°C. Thermal death time values from this study should assist institutional food service settings in designing thermal processes that ensure safety against *C. perfringens* in cooked beef and turkey.

Researchers have assessed the efficacy of added preservatives on inhibiting or delaying the growth of *C. perfringens* in extended shelf-life, refrigerated, processed meat products. When ground turkey containing 0.3% sodium pyrophosphate and 0%, 1%, 2%, or 3% salt was *sous-vide* processed (71.1°C) and held at 28°C, lag times of 7.3, 10.6, 11.6, and 8.0 hours were observed for salt level of 0%, 1%, 2%, and 3%, respectively (Juneja and Marmer 1996). Growth of *C. perfringens* spores in cooked ground turkey with added 0.3% sodium pyrophosphate was inhibited for 12 hours at 3%

salt, pH 6.0, and 28°C. After 16 hours, spores germinated and grew at 28°C from 2.25 to >5 $\log_{10}$ CFU/g in *sous-vide* processed (71.1°C) turkey samples regardless of the presence or absence of salt (Juneja and Marmer, 1996). While *C. perfringens* spores germinated and grew at 15°C to >5 $\log_{10}$ CFU/g in turkey with no salt by day 4, the presence of 3% salt in samples at 15°C completely inhibited germination and subsequent multiplication of vegetative cells even after 7 days of storage (Juneja and Marmer 1996). Thus, the addition of 3% salt in *sous-vide* processed ground turkey containing 0.3% SPP delayed growth for 12 hours at 28°C and completely inhibited the outgrowth of spores at 15°C (Juneja and Marmer 1996). However, 3% salt in RTE products will not inhibit germination and growth of *C. perfringens* spores if refrigerated products are temperature abused to 28°C for an extended period. In another study (Juneja and Majka, 1995) the combination of 3% salt and pH 5.5 inhibited *C. perfringens* growth from spores in *sous-vide* processed ground beef supplemented with 0.3% SPP at 15°C and 28°C. Growth from *C. perfringens* spores occurred within 6 days in *sous-vide* processed (71.1°C) pH 7.0 ground beef samples, but was delayed until day 8 in the presence of 3% salt at pH 5.5 at 15°C (Juneja and Majka, 1995). *C. perfringens* growth from a spore inoculum at 4°C was not observed in *sous-vide* cooked turkey or beef samples (Juneja and Majka, 1995; Juneja and Marmer, 1996). In a related study, Juneja et al. (1996) showed that *C. perfringens* growth in cooked turkey can be effectively inhibited in an atmosphere containing 25% to 75% $CO_2$, 20% $O_2$, and balance $N_2$ in conjunction with good refrigeration; however, the atmosphere cannot be relied upon to eliminate the risk of *C. perfringens* food poisoning in the absence of proper refrigeration (Juneja et al., 1996). Kalinowski et al. (2003) investigated the fate of *C. perfringens* in cooked-cured and uncured turkey at refrigeration temperatures. In their study, *C. perfringens* decreased by 2.52, 2.54, and 2.75 $\log_{10}$ CFU/g in cured turkey held at 0.6°C, 4.4°C, and 10°C, respectively, and the reductions in levels were similar in uncured turkey.

The efficacy of sodium lactate (NaL) in inhibiting the growth from spores of *C. perfringens* in a *sous-vide* processed food has been assessed. Inclusion of 3% NaL in *sous-vide* beef goulash inhibited *C. perfringens* growth at 15°C, delayed growth for a week at 20°C, and had little inhibitory effect at 25°C (Aran 2001). While addition of 4.8% NaL restricted *C. perfringens* growth from spores for 480 hours at 25°C in *sous-vide* processed (71.1°C) marinated chicken breast, it delayed growth for 648 hours at 19°C. *Clostridium perfringens* growth was not observed at 4°C regardless of NaL concentration (Juneja, 2006). These studies suggest that NaL can have significant bacteriostatic activity against *C. perfringens* and may provide *sous-vide* processed foods with a degree of protection against this microorganism, particularly if employed in conjunction with adequate refrigeration.

Predictive bacterial growth models that describe *C. perfringens* spore germination and outgrowth during cooling of food systems have been generated by researchers using constant temperature data. Juneja et al. (1999) presented a model for predicting the relative growth of *C. perfringens* from spores, through lag, exponential, and stationary phases of growth, at temperatures spanning the entire growth temperature range of about 10°C to 50°C. Huang (2003a, 2003b, 2003c) used different mathematical methods to estimate the growth kinetics of *C. perfringens* in ground beef during isothermal, square-waved, linear, exponential, and fluctuating cooling temperature profiles. Juneja, Novak, Marks, et al. (2001) developed a predictive cooling model for cooked, cured beef based on growth rates of the organism at different temperatures. This model estimated that exponential cooling from 51°C to 11°C in 6, 8, or 10 hours would result in an increase of 1.43, 3.17, and 11.8 $\log_{10}$ CFU/g, respectively, assuming that the ratio of the mathematical lag time to the generation time for cells in exponential phase of growth was equal to 8.068, the estimated geometric mean. A similar model was later developed for cooked, cured chicken (Juneja and Marks, 2002). Juneja, Huang, et al. (2006) also developed a model for predicting growth of *C. perfringens* from spore inocula in cured pork ham. In their study, isothermal growth of *C. perfringens* at various temperatures from 10°C to 48.9°C were evaluated using a methodology that employed a numerical technique to solve a set of differential equations, simulating the dynamics of bacterial growth; the authors described the effect of temperature on the kinetic parameter $k_D$ by the modified Ratkowski model. According to the coefficient of the model, the estimated theoretical minimum and maximum growth temperatures of *C. perfringens* in cooked cured pork were 13.5°C and 50.6°C, respectively. The kinetic and growth parameters obtained from these studies can be used in evaluating growth of *C. perfringens* from spore populations during dynamically changing temperature conditions such as those encountered in meat processing.

In a model for growth of *C. perfringens* during cooling of cooked uncured beef (Juneja et al., 2008), for a temperature decline from 54.4°C to 27°C in 1.5 hours, a $\log_{10}$ relative growth of about 1.1 was predicted, with a standard error of about 0.08 $\log_{10}$. While the observed results for two replicates were 0.43 and 0.90 $\log_{10}$, for the same temperature decline in 3 hours, the predicted $\log_{10}$ relative growth was about 3.6 $\log_{10}$ (with a standard error of about 0.07), and the observed $\log_{10}$ relative growths were 2.4 $\log_{10}$ and 2.5 $\log_{10}$. When the cooling scenarios extended to lower temperature, the predictions were somewhat better, taking into account the larger relative growth: for a cooling scenario of 54.4°C to 27°C in 1.5 hours and 27°C to 4°C in 12.5 hours, the average observed and predicted

$\log_{10}$ relative growths were 2.7 $\log_{10}$ and 3.2 $\log_{10}$, respectively; when cooling was extended from 27°C to 4°C in 15 hours, the average observed and predicted $\log_{10}$ relative growths were 3.6 $\log_{10}$ and 3.7 $\log_{10}$, respectively. For the latter cooling scenario the levels were greater than 6 $\log_{10}$, still less than stationary levels of about 7 $\log_{10}$ or 8 $\log_{10}$. The differences of the estimates obtained for the models were insignificant. Smith-Simpson and Schaffner (2005) collected data under changing temperature conditions and developed a model to predict growth of *C. perfringens* in cooked beef during cooling. It was suggested that the accuracy of the germination, outgrowth, and lag (GOL) time model has a profound influence upon the overall prediction, with small differences in GOL time prediction (~1 hour) having a very large effect on the predicted final concentration of *C. perfringens*. Amezquita et al. (2004) developed an integrated model for heat transfer and dynamic growth of *C. perfringens* during cooling of cured ham and demonstrated that the effective integration of engineering and microbial modeling is a useful quantitative tool for ensuring microbiological safety. The previously mentioned models can be successfully used to design microbiologically "safe" cooling regimes for cooked meat and poultry products.

Recent research has focused on combining traditional inactivation, survival, and growth-limiting factors at sub-inhibitory levels with emerging novel non-thermal intervention food preservation techniques using ionizing radiation, high hydrostatic pressure, or exposure to ozone. For example, the efficacy of high pressure is considerably enhanced when combined with heat, antimicrobials, or ionizing radiation. The effect of the combined intervention strategies is either additive or synergistic in which the interaction leads to a combined effect of greater magnitude than the sum of the constraints applied individually. For example, the lethal effect of heat on spores can be enhanced after exposure to ozone. Novak and Yuan (2004) reported that the spores were more sensitive to heat at 55°C or 75°C following 5 ppm of aqueous ozone for 5 minutes. Shelf-life extension of meat processed with 5 ppm $O_3$ for 5 minutes and containing *C. perfringens* spores combined with modified atmosphere packaging as a "hurdle" technology was proven to be effective in inhibiting spore germination and outgrowth over 10 days storage at $CO_2$ concentrations above 30% and 4°C (Novak and Yuan, 2004). Likewise, *C. perfringens* cells exposed to 3 ppm $O_3$ for 5 minutes following mild heat exposure (55°C for 30 min) were more susceptible to ozone treatment.

When *C. perfringens* spores were suspended in peptone solution and exposed to combination treatments of hydrostatic pressure (138–483 MPa), time (5 min), temperature (25°C–50°C), inactivation of spores ranged between 0.1 and 0.2 log cycles (Kalchayanand et al., 2004). When suspended

spores were pressurized at 50°C for 5 minutes and stored at 4°C or 25°C for 24 hours, 12% to 52% spores germinated, indicating that germination increased both at 4°C and 25°C during 24 hours. $Log_{10}$ reductions of spores were 6.1 log/ml when bacteriocins were supplemented in the recovery medium. These results show that germinated spores at high levels could be killed by using a bacteriocin-based preservative in foods.

## *1.6 Conclusion*

Ready-to-eat foods are becoming increasingly popular among the present-day consumers due to their convenience value. Since these foods are either minimally processed or are consumed raw, the microbiological risks associated with these products has also increased. The four major pathogens that have been repeatedly implicated in foodborne illnesses associated with these foods are *L. monocytogenes*, *Salmonella* spp., *E. coli* O157:H7, and *C. perfringens*.

Among the four pathogens discussed in this chapter, *L. monocytogenes* has been historically the most common foodborne pathogen linked to RTE foods due to its hardy and ubiquitous nature and the difficulty in eradicating it from the food-processing environment. The high prevalence of *L. monocytogenes* in RTE products is a major threat to public health. Outbreaks associated with *L. monocytogenes* have prompted regulatory agencies to impose stringent regulations, with a USDA "zero tolerance" policy, in place since 1989, for *L. monocytogenes* in RTE meat products. The FSIS-USDA has also mandated three alternative approaches for controlling *L. monocytogenes* in RTE meats.

*Salmonella* and *E. coli* O157:H7 infections due to contamination of RTE foods are becoming increasingly common, especially in fresh produce. Since these microorganisms are part of the natural intestinal microflora of food animals, contamination of RTE food products from these organisms is easy. Additionally, both *Salmonella* and *E. coli* O157:H7 can survive extreme temperature, moisture, and pH conditions, with *Salmonella* being slightly more temperature and desiccation tolerant than *E. coli* O157:H7. These traits make these pathogens adept at surviving and proliferating in food-processing environments, making their control or elimination in RTE foods challenging.

Meat and poultry are the most common foods associated with *C. perfringens* food poisoning. Foodborne illness by this organism is usually a result of inadequate refrigeration and inadequate reheating of RTE meat and poultry products. Ingestion of large number of organisms in a contaminated food is necessary for the pathogen to survive passage through the stomach and for it to sporulate and elaborate enterotoxin in

the intestines. The preferred method for controlling the growth of this pathogen is not necessarily initial heating, but rather adequate cooling and adequate reheating following cooling to inactivate any cells produced due to bacterial multiplication during cooling. Control measures are in place that take advantage of the microorganism's limitations to growth with respect to oxygen, water activity, pH, curing salts, organic acids, and natural inhibitors. Recently, many predictive growth models have been developed to accurately estimate *C. perfringens* survival following various types of food-processing scenarios. The best strategy for controlling *C. perfringens* appears to be a hurdle approach combined with careful handling of foods to avoid temperature abuse. Regulatory requirements for *C. perfringens* in foods in the United States follow the USDA-FSIS compliance guidelines.

However, foodborne infections from these pathogens can be reduced by following a comprehensive farm-to-table approach to food safety. The critical links in this food safety chain are the farmers, industry, food inspectors, retailers, food service workers, and consumers, who can play a significant role in improving the microbiological safety of RTE foods.

## 1.7 Future outlook

The popularity of RTE foods and the resulting increased consumption calls for enhanced and food-specific measures to control or eliminate pathogens in these foods. Mitigation strategies are needed to reduce or prevent contamination during growing, harvesting, and processing of raw ingredients used in preparation of RTE foods. In addition, there is a critical need for continued education of food industry personnel at pre- and post-harvest processing levels and at the retail level, and that of consumers about risks and prevention measures. In particular, continued efforts are needed to understand factors contributing to contamination of fresh produce and processed foods. Evaluation of the safety of processing methods can ensure more reliable and consequently safer processes. Efforts also need to be directed toward development and implementation of effective intervention strategies. Control measures must be applied along the entire production chain, from farm, via processing, to the table, and applied as early as possible in the production chain. The epidemiology of several of the existing and potential foodborne pathogens is also poorly understood. New DNA-based tools for the specific detection/enumeration and identification of pathogenic bacteria can be useful in clarifying which species and sub-groups are of real concern.

Microorganisms can successfully adapt to changes in food production, processing, and preservation techniques, which can result in new and emerging foodborne pathogens and the re-emergence of pathogens with a

known history of causing foodborne illnesses. It is therefore important to meet the challenges, resulting from the extraordinary capability of foodborne pathogens to adapt to stressful conditions, through science-based research. However, not all of the challenges of preventing foodborne illness can be met by scientific research alone. New food safety policies in response to scientific research, which increase understanding of food safety hazards and a constantly changing food-processing industry, must also be put in place to reduce future foodborne diseases. Regulatory agencies such as the FDA need to be provided with adequate funding to conduct sufficient inspections, and the authority to mandate recalls. The laws, regulations, agencies, and organizations that are part of the food safety system are frequently not up to date with the current scientific knowledge of the risks associated with foodborne pathogens. It is therefore crucial to better understand these risk factors along the farm-to-fork continuum, along with the associated costs and benefits of implementing mitigation strategies that would help implement future systemic changes to enhance food safety.

## *References*

Abadias, M., J. Usall, M. Anguera, C. Solsona, and I. Viñas. 2008. Microbiological quality of fresh, minimally-processed fruit and vegetables, and sprouts from retail establishments. *Int. J. Food Microbiol.* 123:121–129.

Ackers, M. L., B. E. Mahon, E. Leahy, B. Goode, T. Damrow, P. S. Hayes, W. F. Bibb, D. H. Rice, T. J. Barrett, L. Hutwagner, P. M. Griffin, and L. Slutsker. 1998. An outbreak of *Escherichia coli* O157:H7 infections associated with leaf lettuce consumption. *J. Infect. Dis.* 177:1588–1593.

Ahmadia, B. V., A. G. J. Velthuisa, H. Hogeveena, and R. B. M. Huirnea. 2006. Simulating *Escherichia coli* O157:H7 transmission to assess effectiveness of interventions in Dutch dairy-beef slaughterhouses. *Prevent. Vet. Med.* 77:15–30.

Alexander, E. R., J. Boase, M. Davis, L. Kirchner, C. Osaki, T. Tanino, M. Samadpour, P. Tarr, M. Goldoft, P. Bradley, B. Hinton, P. Tighe, B. Pearson, and G. R. Flores. 1994. *Escherichia coli* O157:H7 outbreak linked to commercially distributed dry-cured salami—Washington and California. *Morb. Mortal. Wkly Rep.* 44:157–160.

Al-Holy, M., M. Lin, and B. Rasco. 2005. Destruction of *Listeria monocytogenes* in sturgeon (*Acipenser transmontanus*) caviar by a combination of nisin with chemical antimicrobials or moderate heat. *J. Food Prot.* 68:512–520.

Al-Holy, M., J. Ruiter, M. Lin, D.-H. Kang, and B. Rasco. 2004. Inactivation of *Listeria innocua* in nisin-treated salmon (*Oncorhynchus keta*) and sturgeon (*Acipenser transmontanus*) caviar heated by radio frequency. *J. Food Prot.* 67:1848–1854.

Amezquita, A., C. L. Weller, L. Wang, H. Thippareddi, and D. E. Burson. 2004. Development of an integrated model for heat transfer and dynamic growth of *Clostridium perfringens* during the cooling of cooked boneless ham. *Int. J. Food Microbiol*. 101:123–144.

Angulo, F. L., A. C. Voetsch, D. Vugia, J. L. Hadler, M. Farley, C. Hedberg, P. Cieslak, D. Morse, D. Dwyer, and D. L. Swerdlow. 1998. Determining the burden of human illness from foodborne diseases—CDC's emerging infectious disease program foodborne diseases active surveillance network (FoodNet). *Vet. Clin. N. Amer. Food Anim. Pr*. 14:165–172.

Anonymous. 1995. Food poisoning—an overview. *Int. Poultry Prod*. 4:20–21.

Anonymous. 2001. *Hot stuff. Food service sampler—from rich products*. TCP Publishing, Buffalo, NY.

Anonymous. 2003. It's what's for dinner. *Prep. Foods* 172:24.

Aran, N. 2001. The effect of calcium and sodium lactates on growth from spores of *Bacillus cereus* and *Clostridium perfringens* in a '*sous-vide*' beef goulash under temperature abuse. *Int. J. Food Microbiol*. 63:117–123.

Aureli, P., G. C. Fiorucci, D. Caroli, G. Marchiaro, O. Novara, L. Leone, and S. Salmaso. 2000. An outbreak of febrile gastroenteritis associated with corn contaminated by *Listeria monocytogenes. N. Engl. J. Med.* 342:1236–1241.

Aymerich, T., A. Jofre, 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.

Bauer, F. T., J. A. Carpenter, and J. O. Reagan. 1981. Prevalence of *Clostridium perfringens* in pork during processing. *J. Food Prot*. 44:279–283.

Bean, N. H., J. S. Goulding, M. T. Daniels, and F.J. Angulo. 1997. Surveillance for foodborne disease outbreaks—United States, 1988–1992. *J. Food Prot.* 60:1265–1286.

Bean, N. H. and P. M. Griffin. 1990. Foodborne disease outbreaks in the United States, 1973-1987: pathogens, vehicles, and trends. *J. Food Prot.* 9:804–817.

Belletti, N., R. Lanciotti, F. Patrignani, and F. Gardini. 2008. Antimicrobial efficacy of citron essential oil on spoilage and pathogenic microorganisms in fruit-based salads. *J. Food Sci.* 73:M331–338.

Besser, R. E., S. M. Lett, J. T. Weber, M. P. Doyle, T. J. Barrett, J. G. Wells, and P. M. Griffin. 1993. An outbreak of diarrhea and hemolytic uremic syndrome from *Escherichia coli* O157:H7 in fresh-pressed apple cider. *J. Am. Med. Assoc*. 269:2217–2220.

Beuchat, L. R. 1996. Pathogenic microorganisms associated with fresh produce. *J. Food Prot.* 59:204–216.

Beuchat, L. R. 1999. Survival of enterohemorrhagic *Escherichia coli* O157:H7 in bovine feces applied to lettuce and the effectiveness of chlorinated water as a disinfectant. *J. Food Prot.* 62:845–849.

Beuchat, L. R., B. B. Adler, and M. M. Lang. 2004. Efficacy of chlorine and a peroxyacetic acid sanitizer in killing *Listeria monocytogenes* on iceberg and romaine lettuce using simulated commercial processing conditions. *J. Food Prot.* 67:1238–1242.

Beuchat, L. R. and J. H. Ryu. 1997. Produce handling and processing practices. *Emerg Infect Dis.* 3:459–465.

Beuchat, L. R., T. E. Ward, and C. A. Pettigrew. 2001. Comparison of chlorine and a prototype produce wash product for effectiveness in killing *Salmonella* and *Escherichia coli* O157:H7 on alfalfa seeds. *J. Food Prot.* 64:152–158.

Beumer, R. R., M. C. te Giffel, E. de Boer, and F. M. Rombouts. 1996. Growth of *Listeria monocytogenes* on sliced cooked meat products. *Food Microbiol*. 13:333–340.

Blankenship, L. C., S. E. Craven, R. G. Leffler, and C. Custer. 1988. Growth of *Clostridium perfringens* in cooked chili during cooling. *Appl. Environ. Microbiol*. 53:1104–1108.

Blue Ribbon Task Force. 1996. Dry fermented sausage and *E. coli* O157:H7. Chicago National Cattlemen's Beef Association. Report no. 11–316.

Bosilevac, J. M., T. L. Wheeler, M. Rivera-Betancourt, X. Nou, T. M. Arthur, S. D. Shackelford, M. P. Kent, D. Jaroni, M. S. Osborn, M. Rossman, J. O. Reagan, M. Koohmaraie. 2004. Protocol for evaluating the efficacy of cetylpyridinium chloride as a beef hide intervention. *J. Food Prot*. 67:303–309.

Brackett, R. E. 1988. Presence and persistence of *Listeria monocytogenes* in food and water. *Food Technol.* 42(4):163–164, 178.

Bradshaw, J., G. Stelma, V. Jones, J. Peeler, J. Wimsatt, J. Corwin, and R. Twedt. 1982. Thermal inactivation of *Clostridium perfringens* enterotoxin in buffer and in chicken gravy. *J. Food Sci.* 47:914–916.

Breuer, T., D. H. Benkel, R. L. Shapiro, W. N. Hall, M. M. Winnett, M. J. Linn, J. Neimann, T. J. Barrett, S. Dietrich, F. P. Downes, D. M. Toney, J. L. Pearson, H. Rolka, L. Slutsker, and P. M. Griffin. 2001. A multistate outbreak of *Escherichia coli* O157:H7 infections linked to alfalfa sprouts grown from contaminated seeds. *Emerg. Infect. Dis.* 7:977–982.

Brockmann, S. O., I. Piechotowski, and P. Kimmig. 2004. *Salmonella* in sesame seed products. *J. Food Prot.* 67:178–180.

Brooks, J. T., S. Y. Rowe, P. Shillam, D. M. Heltzel, S. B. Hunter, L. Slutsker, R. M. Hoekstra, and S. P. Luby. 2001. *Salmonella* Typhimurium infections transmitted by chlorine-pretreated clover sprout seeds. *Am. J. Epidemiol*. 154:1020–1028.

Bryan, F. L. 1969. What the sanitarian should know about *Clostridium perfringens* foodborne illness. *J. Milk Food Technol*. 32:381–389.

Bryan, F. L. 1988. Risks associated with vehicles of foodborne pathogens and toxins. *J. Food Prot*. 51:498–508.

Bryan, F. L. and T. W. McKinley. 1979. Hazard analysis and control of roast beef preparation in foodservice establishments. *J. Food Prot*. 42:4–18.

Brynestad, S. and P. E. Granum. 2002. *Clostridium perfringens* and foodborne infections. *Int. J. Food Microbiol.* 74:195–202.

Buchanan, R. L. and M. P. Doyle. 1997. Foodborne disease significance of *Escherichia coli* O157:H7 and other enterohemorrhagic *E. coli*. *Food Technol*. 51(10): 69–76.

Budu-Amoako, E., R. F. Ablett, J. Harris, and J. Delves-Broughton. 1999. Combined effect of nisin and moderate heat on destruction of *Listeria monocytogenes* in cold-pack lobster meat. *J. Food Prot.* 62:46–50.

Bula, C., J. Billie, and M. P. Galuser. 1995. An epidemic of food-borne listeriosis in western Switzerland: description of 57 cases involving adults. *Clin. Infect. Dis.* 20:66–72.

Buncic, S. L., L. Paunovic, and D. Radisic. 1991. The fate of *Listeria monocytogenes* in fermented sausages and in vacuum-packaged frankfurters. *J. Food Prot.* 54:413–417.

Cabedo, L., L. P. Barrot, and A. T. Canelles. 2008. Prevalence of *Listeria monocytogenes* and Salmonella in ready-to-eat food in Catalonia, Spain. *J. Food Prot.* 71:855–859.

Calderón-Miranda, M. L., G. V. Barbosa-Cánovas, and B. G. Swanson. 1999. Inactivation of *Listeria innocua* in skim milk by pulsed electric fields and nisin. *Int. J. Food Microbiol.* 51:19–30.

California Department of Health Services. 2007. Investigation of an *Escherichia coli* O157:H7 outbreak associated with Dole pre-packaged spinach. March 21, 2007. http://www.dhs.ca.gov/fdb/local/PDF/2006%20Spinach%20Report%20Final%20redacted.PDF. Accessed May 2009.

Center for Science in the Public Interest (CSPI). 2009. Outbreak Alert! Database. http://www.cspinet.org/foodsafety/outbreak/pathogen.php. Accessed April 2009.

Centers for Disease Control and Prevention (CDC). 1994. Outbreak of *Salmonella enteritidis* associated with nationally distributed ice cream products—Minnesota, South Dakota, and Wisconsin, 1994. *Morb. Mortal. Wkly Rep.* 43:740–741.

Centers for Disease Control and Prevention (CDC). 1995. Outbreak of salmonellosis associated with beef jerky—New Mexico, 1995. *Morb. Mortal. Wkly Rep.* 44:785–788.

Centers for Disease Control and Prevention (CDC). 1999. Update: multistate outbreak of listeriosis—United States, 1998-1999. *Morb. Mortal. Wkly Rep.* 47:1117–1118.

Centers for Disease Control and Prevention (CDC). 2000. Multistate outbreaks of listeriosis—United States, 2000. *Morb. Mortal. Wkly Rep.* 49:1129–1130.

Centers for Disease Control and Prevention (CDC). 2001. Diagnosis and management of foodborne illnesses: a primer for physicians. *Morb. Mortal. Wkly Rep.* 50:1–69

Centers for Disease Control and Prevention (CDC). 2002. Multistate outbreak of listeriosis—United States, 2002. *Morb. Mortal. Wkly Rep.* 51:950–951.

Centers for Disease Control and Prevention (CDC). 2005a. *Salmonella* surveillance: annual summary, 2004. Atlanta, GA: U.S. Department of Health and Human Services, CDC.

Centers for Disease Control and Prevention (CDC). 2005b. *Salmonella*: technical fact sheet. http://www.cdc.gov/ncidod/dbmd/diseaseinfo/salmonellosis_t.htm. Accessed April 2009.

Centers for Disease Control and Prevention (CDC). 2006. Ongoing multistate outbreak of *Escherichia coli* serotype O157:H7 infections associated with consumption of fresh spinach—United States, September 2006. *Morb. Mortal. Wkly. Rep.* 55:1045–1046.

Centers for Disease Control and Prevention (CDC). 2007a. Investigation of outbreak of human infections caused by *Salmonella enterica*. http://www.cdc.gov/Salmonella/4512eyeminus.html. Accessed May 2009.

Centers for Disease Control and Prevention (CDC). 2007b. *Salmonella* surveillance: annual summary, 2005. Atlanta, Georgia: U.S. Department of Health and Human Services.

Centers for Disease Control and Prevention (CDC). 2008. Outbreak of *Salmonella* serotype Saintpaul infections associated with multiple raw produce items—United States, 2008. *Morb. Mortal. Wkly. Rep*. 57:929–934.

Centers for Disease Control and Prevention (CDC). 2009a. Preliminary FoodNet data on the incidence of infection with pathogens transmitted commonly through food—10 states, 2008. *Morb. Mortal. Wkly. Rep.* 58:333–337.

Centers for Disease Control and Prevention (CDC). 2009b. Outbreak of *Salmonella* serotype Saintpaul infections associated with eating alfalfa sprouts—United States, 2009. *Morb. Mortal. Wkly. Rep.* 58:1–3.

Centers for Disease Control and Prevention (CDC). 2009c. Multistate outbreak of *Salmonella* infections associated with peanut butter and peanut butter–containing products—United States, 2008–2009. *Morb. Mortal. Wkly. Rep*. 58:1–6.

Chen, C. M., J. G. Sebranek, J. S. Dickson, and A. Mendonca. 2002. Use of pediocin with other barriers for control of *Listeria monocytogenes* on ready-to-eat (RTE) processed meats. Project Report to American Meat Institute Foundation. Available at: http://www.amif.org/ht/a/GetDocumentAction/i/7410. Accessed May 2009.

Christison, C. A., D. Lindsay, and A. Von Holy. 2007. Cleaning and handling implements as potential reservoirs for bacterial contamination of some ready-to-eat foods in retail delicatessen environments. *J. Food Prot.* 70:2878–2883.

Christison, C. A., D. Lindsay, and A. Von Holy. 2008. Microbiological survey of ready-to-eat foods and associated preparation surfaces in retail delicatessens, Johannesburg, South Africa. *Food Control* 19:727–733.

Cody, S. H., M. K. Glynn, J. A. Farrar, K. L. Cairns, P. M. Griffin, J. Kobayashi, M. Fyfe, R. Hoffman, A. S. King, J. H. Lewis, B. Swaminathan, R. G. Bryant, and D. J, Vugia. 1999. An outbreak of *Escherichia coli* O157:H7 infection from unpasteurized commercial apple juice. *Ann. Intern. Med.* 130:202–209.

Coghlan, A. C. 2000. Food poisoning bugs thrive in crop sprays. *New Sci.* 2259:20.

Collie, R. E., and B. A. McClane. 1998. Evidence that the enterotoxin gene can be episomal in *Clostridium perfringens* isolates associated with non-food-borne human gastrointestinal diseases. *J. Clin. Microbiol*. 36:30–36.

Cooksey, D. K., B. P. Klein, F. K. McKeith, and H. K. Blaschek. 1993a. Reduction of *Listeria monocytogenes* in the precooked vacuum packaged beef using post packaging pasteurization. *J. Food Prot.* 56:1034–1038.

Cooksey, D. K., B. P. Klein, F. K. McKeith, and H. K. Blaschek. 1993b. Post packaging pasteurization reduces *Clostridium perfringens* and other bacteria in prepackaged vacuum-packaged beef loin chunks. *J. Food Sci.* 58:239–241.

Cooley, M., D. Carychao, L. Crawford-Miksza, M. T. Jay, C. Myers, C. Rose, C. Keys, J. Farrar, and R. E. Mandrell1. 2007. Incidence and tracking of *Escherichia coli* O157:H7 in a major produce production region in California. *PLoS ONE*. 11:1–16.

Craven, S. E. 2001. Occurrence of *Clostridium perfringens* in the broiler chicken processing plant as determined by recovery in iron milk medium. *J. Food Prot*. 64:1956–1960.

Cygnarowicz-Provost, M., R. C. Whiting, and J. C. Craig. 1994. Steam surface pasteurization of beef frankfurters. *J. Food Sci.* 59:1–5.

Dalton, C. B., C. C. Austin, J. Sobel, P. S. Hayes, W. F., Bibb, L. M. Graves, B. Swaminathan, M. E. Proctor, and P. M. Griffin. 1997. An outbreak of gastroenteritis and fever due to *Listeria monocytogenes* in milk. *N. Engl. J. Med.* 336:100–105.

Deboer, E., W. Spiegelenberg, and F. Jenssen. 1985. Microbiology of spices and herbs. *Ant. Van Leeuw*. 51:435–438.

Delaquis, P., S. Stewart, S. Cazaux, and P. Toivonen. 2002. Survival and growth of *Listeria* monocytogenes and *Escherichia coli* O157:H7 in ready-to-eat iceberg lettuce washed in warm chlorinated water. *J. Food Prot.* 65:459–464.

Dingman, D. W. 1999. Prevalence of *Escherichia coli* in apple cider manufactured in Connecticut. *J. Food Prot.* 62:567–573.

Drasar, B. S., and M. J. Hill. 1974. The distribution of bacterial flora in the intestine. 36–43. In B. S. Drasar and M. J. Hill (eds.), *Human intestinal flora*. Academic Press, London, UK.

Duncan, C. L., and D. H. Strong. 1969. Ileal loop fluid accumulation and production of diarrhea in rabbits by cell-free products of *Clostridium perfringens*. *J. Bacteriol.* 100:86–94.

Duncan, C. L., D. H. Strong, and M. Sebald. 1972. Sporulation and enterotoxin production by mutants of *Clostridium perfringens*. *J. Bacteriol.* 110:378–391.

Escherich, T. 1988. The intestinal bacteria of the neonate and breast-fed infant. 1885. *Rev. Infect. Dis.* 10(6):1220–1225.

Faith, N. G., N. Parniere, T. Larson, T. D. Lorang, C. W. Kaspar, J. B. Luchansky. 1998. Viability of *Escherichia coli* O157:H7 in salami following conditioning of batter, fermentation and drying of sticks, and storage of slices. *J. Food Prot.* 61:377–382.

Fang, T. J., Q.-K. Wei, C.-W. Liao, M.-J. Hung, and T.-H. Wang. 2003. Microbiological quality of 18 °C ready-to-eat food products sold in Taiwan. *Int. J. Food Microbiol.* 80:241–250.

Farber, J. M., E. M. Daley, M. T. Mackie, and B. Limerick. 2000. A small outbreak of listeriosis potentially linked to the consumption of imitation crab meat. *Lett. Appl. Microbiol.* 31:100–104.

Farber, J. M., and J. Harwig. 1996. The Canadian position on *Listeria monocytogenes* in ready-to-eat foods. *Fd. Cont.* 7:253–258.

Farber, J. M., and P. I. Peterkin. 1991. *Listeria monocytogenes*, a foodborne pathogen. *Microbiol. Rev.* 55:476–511.

Fleishman, G., S. Ravishankar, K. Ghiron, and V. M. Balasubramaniam. 2004. The lethality of pulsed electric field (PEF) treatment on *Listeria monocytogenes* in a static chamber. *Food Microbiol.* 21:91–95.

Food and Drug Administration (FDA). 2001a. Background provided by FDA to IFT. *J. Food Prot.* 66:1059–1060.

Food and Drug Administration (FDA). 2001b. Food Code 1999. Part 3.5: Limitation of growth of organisms of public health concern. http://www.fda.gov/Food/FoodSafety/RetailFoodProtection/FoodCode1999. Accessed May 2009.

Food and Drug Administration (FDA). 2006a. Nationwide *E. coli* O157:H7 outbreak: questions & answers. September 16, 2006; Updated October 20, 2006. http://www.cfsan.fda.gov/~dms/spinacqa.html. Accessed May 2009.

Food and Drug Administration (FDA). 2006b. Taco Bell *E. Coli* O157:H7 lettuce outbreak. December 14, 2006. http://www.cfsan.fda.gov/~dms/tacobqa.html. Accessed May 2009.

Food and Drug Administration (FDA) and Center for Food Safety and Applied Nutrition (CFSAN). 2006c. Commodity specific food safety guidelines for the lettuce and leafy greens supply chain. April 25, 2006. http://www.cfsan.fda.gov/~dms/lettsup.html. Accessed March 2009.

Food Safety and Inspection Services (FSIS). 1999. FSIS announces strategy to control *Listeria monocytogenes* in ready-to-eat meat and poultry products. http://www.fsis.usda.gov/oa/haccp/small10.htms. Accessed May 2009.

Food Safety and Inspection Service (FSIS). 2008. Disposition/food safety: overview of food microbiology. http://www.fsis.usda.gov/PDF/PHVt-Food_Microbiology.pdf. Accessed May 2009.

Foong, S. C. C., G. L. Gonzalez, and J. S. Dickson. 2004. Reduction and survival of *Listeria monocytogenes* in ready-to-eat meats after irradiation. *J. Food Prot.* 67:77–82.

Franklin, N., K. Cooksey, and K. Getty. 2004. Inhibition of *Listeria monocytogenes* on the surface of individually packaged hot dogs with a packaging film coating nisin. *J. Food Prot.* 67:480–485.

Friedman, M. S., T. Roels, J. E. Koehler, L. Feldman, W. F. Bibb, and P. Blake. 1999. *Escherichia coli* O157:H7 outbreak associated with an improperly chlorinated swimming pool. *Clin. Infect. Dis.* 29:298–303.

Fröder, H., C. G. Martins, K. L. O. De Souza, M. Landgraf, B. D. G. M. Franco, and M. T. Destro. 2007. Minimally processed vegetable salads: microbial quality evaluation. *J. Food Prot.* 70:1277–1280.

Frye, D. M., R. Zweig, J. Sturgeon, M. Tormey, M. LeCavalier, I. Lee, L. Lawani, and L. Pascola. 2002. An outbreak of febrile gastroenteritis associated with delicatessen meat contaminated with *Listeria monocytogenes. Clin. Infect. Dis.* 35:943–949.

Gaze, J. E., R. Shaw, and J. Archer. 1998. Identification and prevention of hazards associated with slow cooling of hams and other large cooked meats and meat products. Review No. 8. Campden and Chorleywood Food Research Association, Gloucestershire, UK.

Giannella, R. A. 1996. Salmonella. *Baron's medical microbiology* (4th ed.). University of Texas Medical Branch, Galveston, TX.

Gibbs, R., N. Pingault, T. Mazzucchelli, L. O'Reilly, B. MacKenzie, J. Green, R. Mogyorosy, R. Stafford, R. Bell, L. Hiley, K. Fullerton, and P. Van Buynder. 2009. An outbreak of *Salmonella enterica* serotype Litchfield infection in Australia linked to consumption of contaminated papaya. *J. Food Prot.* 72:1094–1098.

Gilbert, R. J., K. L. Miller, and D. Roberts. 1989. *Listeria monocytogenes* and chilled foods. *Lancet* 1:383–384.

Glass, K. A., J. M. Loeffelholz, P. J. Ford, and M. P. Doyle. 1992. Fate of *Escherichia coli* O157:H7 as affected by pH or sodium chloride and in fermented, dry sausage. *Appl. Environ. Microbiol.* 58:2513–2516.

Gombas, D. E., Y. Chen, R. S. Clavero, and V. N. Scott. 2003. Survey of *Listeria monocytogenes* in ready-to-eat food. *J. Food Prot.* 66:559–569.

Gottlieb, S. L., E. C. Newbern, P. M. Griffin, L. M. Graves, R. M. Hoekstra, N. L. Baker, S. B. Hunter, K. G. Holt, F. Ramsey, M. Head, P. Levine, G. Johnson, D. Schoonmaker-Bopp, V. Reddy, L. Kornstein, M. Gerwel, J. Nsubuga, L. Edwards, S. Stonecipher, S. Hurd, D. Austin, M. A. Jefferson, S. D. Young, K. Hise, E. D. Chernak, J. Sobel, and the Listeriosis Outbreak Working Group. 2006. Multistate outbreak of listeriosis linked to turkey deli meat and subsequent changes in U.S. regulatory policy. *Clin. Infect. Dis.* 42:29–36.

Grau, F. H., and P. B. Vanderline. 1991. Occurrence, numbers, and growth of *Listeria monocytogenes* on some vacuum packaged processed meats. *J. Food Prot.* 55:4–7.

Griffin, P. M. 1995. *Escherichia coli* O157:H7 and other enterohemorrhagic *Escherichia coli*. 739–761. In M. J. Blaser, P. D. Smith, J. I. Ravdin, H. B. Greenberg, and R. L. Guerrant (eds.), *Infections of the gastrointestinal tract*. Raven Press, Ltd., New York.

Griffin, P. M., and R. V. Tauxe. 1991. The epidemiology of infections caused by *Escherichia coli* O157:H7, other enterohemorrhagic *E. coli*, and the associated hemolytic uremic syndrome. *Epidemiol. Rev.* 13: 60–98.

Guan, T. Y., G. Blank, A. Ismond, and R. Van Acker. 2001. Fate of foodborne bacterial pathogens in pesticide products. *J. Sci. Food Agri.* 81:503–512.

Guerra, M. M., J. McLaughlin, and F. A. Bernardo. 2001. *Listeria* in ready-to-eat and unprocessed foods produced in Portugal. *Food Microbiol*. 18:423–429.

Gutierrez, J., G. Rodriguez, C. Barry-Ryan, and P. Bourke. 2008. Efficacy of plant essential oils against foodborne pathogens associated with ready-to-eat vegetables: antimicrobial and sensory screening. *J. Food Prot.* 71:1846–1854.

Hall, H.E., and R. Angelotti. 1965. *Clostridium perfringens* in meat and meat products. *Appl. Microbiol*. 13:352–357.

Harris, L. J., L. R. Beuchat, T. M. Kajs, T. E. Ward, and C. H. Taylor. 2001. Efficacy and reproducibility of a produce wash in killing *Salmonella* on the surface of tomatoes assessed with a proposed standard method for produce sanitizers. *J. Food Prot*. 64:1477–1482.

Hartemink, R., and G. Georgsson. 1991. Incidence of *Listeria* species in seafood and seafood salads. *Int. J. Food Microbiol.* 12:189–195.

Health Protection Agency and LACORS. 2009. *Salmonella* and *E. coli* bacteria found in "ready-to-eat" seeds. http://www.hpa.org.uk/webw/HPAweb&HPAwebStandard/HPAweb_C/1235464508651?p=1231252394302. Accessed May 2009.

Heredia, N. L., G. A. Garcia, R. Luevanos, R. G. Labbe, and J. S. Garcia-Alvarado. 1997. Elevation of the heat resistance of vegetative cells and spores of *Clostridium perfringens* type A by sublethal heat shock. *J. Food Prot*. 60:998–1000.

Hobbs, G., D. Cann, B. Wilson, and J. Shewan. 1965. The incidence of organisms of the genus *Clostridium* in vacuum-packed fish in the United Kingdom. *J. Appl. Bacteriol*. 28:265–270.

Hilborn, E. D., J. H. Mermin, P. A. Mshar, J. L. Hadler, A. Voetsch, C. Wojtkunski, M. Swartz, R. Mshar, M.-A. Lambert-Fair, J. A. Farrar, M. K. Glynn, and L. Slutsker. 1999. A multistate outbreak of *Escherichia coli* O157:H7 infections associated with consumption of mesclun lettuce. *Arch. Intern. Med.* 159:1758–1764.

Hitchins, A. D. 1996. Assessment of alimentary exposure of *Listeria monocytogenes*. *Int. J. Food Microbiol.* 30:71–85.

Holley, R. A. 1985a. Beef jerky: viability of food-poisoning microorganisms on jerky during its manufacture and storage. *J. Food Prot.* 48:100–106.

Holley, R. A. 1985b. Beef jerky: fate of *Staphylococcus aureus* in marinated and corned beef during jerky manufacture and 2.5 C storage. *J. Food Prot.* 48:107–171.

Huang, L. 2003a. Dynamic computer simulation of *Clostridium perfringens* growth in cooked ground beef. *Int. J. Food Microbiol*. 87:217–227.

Huang, L. 2003b. Estimation of growth of *Clostridium perfringens* in cooked beef under fluctuating temperature conditions. *Food Microbiol*. 20:549–559.

Huang, L. 2003c. Growth kinetics of *Clostridium perfringens* in cooked beef. *J. Food Safety* 23:91–105.

Hwang, C. A., and M. L. Tamplin. 2005. The influence of mayonnaise pH and storage temperature on the growth of *Listeria monocytogenes* in seafood salad. *Int. J. Food Microbiol.* 102:277–285.

Ingham, S. C., D. R. Beuge, B. K. Dropp, and J. A. Losinski. 2004. Survival of *Listeria monocytogenes* during storage of ready-to-eat products processed by drying, fermentation, and/or smoking. *J. Food Prot.* 67:2698–2702.

Inoue, S., A. Nakama, Y. Arai, Y. Kokubo, T. Maruyama, A. Saito, T. Yoshida, M. Terao, S. Yamamoto, and S. Kumagai. 2000. Prevalence and contamination levels of *Listeria monocytogenes* in retail foods in Japan. *Int. J. Food Microbiol.* 59:73–77.

International Commission on Microbiological Specifications for Foods (ICMSF). 1996. Microorganisms in foods, pp. 112–125, vol. 5. In *Characteristics of microbial pathogens*. Blackie Academic and Professional, London.

Jacquet, C., B. Catimel, R. Broscb, C. Bucbrieser, I. Dehaumont, V. Goulet, A. Lepoutre, P. Veit, and J. Rocourt. 1995. Investigations related to the epidemic strain involved in the French listeriosis outbreak in 1992. *Appl. Environ. Microbial.* 61:2242–2246.

Janes, M. E., S. Kooshesh, and M. G. Johnson. 2002. Control of *Listeria monocytogenes* on the surface of refrigerated, ready-to-eat chicken coated with edible zein film coatings containing nisin and/or calcium propionate. *J. Food Sci.* 67:2754–2757.

Johnson, J. L., M. P. Doyle, and R. G. Cassens. 1990. *Listeria monocytogenes* and other *Listeria* spp. in meat and meat products. A review. *J. Food Prot*. 53:81–91.

Johnston, L. M., L.-A. Jaykus, D. Moll, M. C. Martinez, J. Anciso, B. Mora, C. L. Moe. 2005. A field study of the microbiological quality of fresh produce. *J. Food Prot.* 68:1840–1847.

Julson, J. L., K. Muthukumarappan, and D. Henning. 2001. Effectiveness of ozone for controlling *Listeria monocytogenes* in ready to eat cured ham. Research Report: Pork Safety. 1–6. http://www.pork.org/PorkScience/Research/Documents/99-221-JULSON-SDSU.pdf. Accessed May 2009.

Juneja, V. K. 2006. Delayed *Clostridium perfringens* growth from a spore inocula by sodium lactate in *sous-vide* chicken products. *Food Microbiol*. 23:105–111.

Juneja, V. K., M. L. Bari, Y. Inatsu, S. Kawamoto, and M. Friedman. 2007. Control of *Clostridium perfringens* by green tea leaf extracts during cooling of cooked ground beef, chicken, and pork. *J. Food Prot.* 70:1429–1433.

Juneja, V. K., and M. Friedman. 2007. Carvacrol, cinnamaldehyde, oregano oil, and thymol inhibit *Clostridium perfringens* spore germination and outgrowth in ground turkey during chilling. *J. Food Prot.* 218–222.

Juneja, V. K., L. Huang, and H. Thippareddi. 2006. Predictive model for growth of *Clostridium perfringens* in cooked cured pork. *Intern. J. Food Microbiol*. 110:85–92.

Juneja, V. K., and W. M. Majka. 1995. Outgrowth of *Clostridium perfringens* spores in cook-in-bag beef products. *J. Food Safety* 15:21–34.

Juneja, V. K., and H. M. Marks. 2002. Predictive model for growth of *Clostridium perfringens* during cooling of cooked cured chicken. *Food Microbiol*. 19:313–327.

Juneja, V. K., H. Marks, and H. Thippareddi. 2008. Predictive model for growth of *Clostridium perfringens* during cooling of cooked uncured beef. *Food Microbiol*. 25:42–55.

Juneja, V.K., and B. S. Marmer. 1996. Growth of *Clostridium perfringens* from spore inocula in *sous-vide* turkey products. *Int. J. Food Microbiol*. 21:115–123.

Juneja, V. K., and B. S. Marmer. 1998. Thermal inactivation of *Clostridium perfringens* vegetative cells in ground beef and turkey as affected by sodium pyrophosphate. *Food Microbiol*. 15:281–287.

Juneja, V. K., B. S. Marmer, and J. E. Call. 1996. Influence of modified atmosphere packaging on growth of *Clostridium perfringens* in cooked turkey. *J. Food Safety* 16:141–150.

Juneja, V. K., J. S. Novak, B. S. Eblen, and B. A. McClane. 2001. Heat resistance of *Clostridium perfringens* vegetative cells as affected by prior heat shock. *J. Food Safety* 21:127–139.

Juneja, V. K., J. S. Novak, L. Huang, and B. S. Eblen. 2003. Increased thermotolerance of *Clostridium perfringens* spores following sublethal heat shock. *Food Control* 14:163–168.

Juneja, V. K., J. S. Novak, H. M. Marks, D. E. Gombas. 2001. Growth of *Clostridium perfringens* from spore inocula in cooked cured beef: development of a predictive model. *Innov. Food Sci. Emer. Technol*. 2:289–301.

Juneja, V. K., O. P. Snyder, and M. Cygnarowicz-Provost. 1994. Influence of cooling rate on outgrowth of *Clostridium perfringens* spores and cooked ground beef. *J. Food Prot.* 57:1063–1067.

Juneja, V. K., and H. Thippareddi. 2004a. Control of *Clostridium perfringens* in a model roast beef by salts of organic acids during chilling. *J. Food Safety* 24:95–108.

Juneja, V. K., and H. Thippareddi. 2004b. Inhibitory effects of organic acid salts on growth of *Clostridium perfringens* from spore inocula during chilling of marinated ground turkey breast. *Intern. J. Food Microbiol.* 93:155–163.

Juneja, V. K., H. Thippareddi, M. L. Bari, Y. Inatsu, S. Kawamoto, and M. Friedman. 2006. Chitosan protects cooked ground beef and turkey against *Clostridium perfringens* spores during chilling. *J. Food Sci.* 71:M236-M240.

Juneja, V. K., H. Thippareddi, and M. Friedman. 2006. Control of *Clostridium perfringens* in cooked ground beef by carvacrol, cinnamaldehyde, thymol or oregano oil during chilling. *J. Food Prot*. 69:1546–1551.

Juneja, V. K., R. C. Whiting, H. M. Marks, and O. P. Snyder. 1999. Predictive model for growth of *Clostridium perfringens* at temperatures applicable to cooling of cooked meat. *Food Microbiol*. 16:335–349.

Kalchayanand, N., C. P. Dunne, A. Sikes, and B. Ray. 2004. Germination induced and inactivation of *Clostridium* spores at medium-range hydrostatic pressure treatment. *Innov. Fd. Sci. Emerg. Tech.* 5:277–283.

Kalinowski, R. M., R. B. Tompkin, P. W. Bodnaruk, and W. P. Pruett. 2003. Impact of cooking, cooling, and subsequent refrigeration on the growth or survival of *Clostridium perfringens* in cooked meat and poultry products. *J. Food Prot.* 66:1227–1232.

Kaneko, K.-I., H. Hayashidani, Y. Ohtomo, J. Kosuge, M. Kato, K. Takahashi, Y. Shiraki, and M. Ogawa. 1999. Bacterial contamination of ready-to-eat foods and fresh products in retail shops and food factories. *J. Food Prot.* 62:644–649.

Karch, H., J. Heesemann, R. Laufs, A. D. O'Brien, C. O. Tacket, and M. M. Levine. 1987. A plasmid of enterohemorrhagic *Escherichia coli* O157:H7 is required for expression of a new fimbrial antigen and for adhesion to epithelial cells. *Infect. Immun*. 55:455–461.

Karmali, M. A., B. T. Steele, M. Petric, and C. Lim. 1983. Sporadic cases of hemolytic-uremic syndrome associated with faecal cytotoxin and cytotocin-producing *Escherichia coli* in stools. *Lancet* 1:619–620.

Keene, W., J. M. McAnulty, F. C. Hoesly, L. P. Williams, K. Hedberg, G. L. Oxman, T. J. Barrett, M. A. Pfaller, and D. A. Fleming. 1991. A swimming-associated outbreak of hemorrhagic colitis caused by *Escherichia coli* O157:H7 and Shigella Sonnei. *N. Engl. J. Med.* 331:579–584.

Kilonzo-Nthenge, A., C. Fur-Chi, and S. L. Godwin. 2006. Efficacy of home washing methods in controlling surface microbial contamination on fresh produce. *J. Food Prot.* 69:330–334.

Kotzekidou, P., P. Giannakidis, and A. Boulamatsis. 2008. Antimicrobial activity of some plant extracts and essential oils against foodborne pathogens in vitro and on the fate of inoculated pathogens in chocolate. *Lebensm-Wiss. Technol.* 41:119–127.

Labbe, R. G. 1989. *Clostridium perfringens,* pp. 192–234. In M. P. Doyle (ed.), *Foodborne bacterial pathogens.* Marcel Dekker, New York.

Labbe, R. J., and V. K. Juneja. 2002. *Clostridium perfringens,* pp. 192–126. In D. O. Cliver and H. Riemann (eds.), *Foodborne diseases*. Academic Press, Inc., San Diego, CA.

Labbe, R. J., and V. K. Juneja. 2006. *Clostridium perfringens* Gastroenteritis. In H. Riemann and D. O. Cliver (eds.), *Foodborne infections and intoxications*. Academic Press, Inc., San Diego, CA.

Ladiges, W.C., J.F. Foster, and W.M. Ganz. 1974. Incidence and viability of *Clostridium perfringens* in ground beef. *J. Milk Food Technol*. 37:622–626.

Law, D., and J. Kelly. 1995. Use of heme and hemoglobin by *Escherichia coli* O157:H7 and other Shiga-like-toxin-producing *E. coli* serogroups. *Infect. Immun*. 63:700–702.

LeClerc, J. E., B. Li, W. L. Payne, and T. A. Cebula. 1996. High mutation frequencies among *Escherichia coli* and *Salmonella* pathogens. *Sci.* 274:1208–1211.

Lee, S.-Y., S.-Y. Gwon, S.-J. Kim, and B. K. Moon. 2009. Inhibitory effect of commercial green tea and rosemary leaf powders on the growth of foodborne pathogens in laboratory media and oriental-style rice cakes. *J. Food Prot.* 72:1107–1111.

Levine, P., B. Rose, S. Green, G. Ransom, and W. Hill. 2001. Pathogen testing of ready-to-eat meat and poultry products collected at federally inspected establishments in the United States, 1990 to 1999. *J. Food Prot.* 64:1188–1193.

Linnan, M. J., L. Mascola, X. Dong Lou, V. Goulet, S. May, C. Salminen, D. W. Hird, M. L. Yonekura, I. Hayes, R. Weaver, A. Audurier, B. D. Plikaytis, X. L. Fannin, A. Kleks, and C. V. Broome. 1988. Epidemic listeriosis associated with Mexican-style cheese. *N. Eng. J. Med.* 319:823–828.

Li, J., S. Sayeed, and B. A. McClane. 2007. Prevalence of enterotoxigenic *Clostridium perfringens* isolates in Pittsburgh (Pennsylvania) area soils and home kitchens. *Appl. Environ. Microbiol.* 73:7218–7224.

Lin, Y. T., and R. Labbe. 2003. Enterotoxigenicity and genetic relatededness of *Clostridium perfringens* isolates from retail food. *Appl. Environ. Microbiol.* 69:1642–1646.

Lou, Y., and A. E. Yousef. 1999. Characteristics of *Listeria monocytogenes* important to food processors, pp. 131–224. In E. T. Ryser and E. H. Marth (eds.), *Listeria, listeriosis and food safety.* Marcel Dekker Inc., New York.

Lu, Z., J. G. Sebranek, J. S. Dickson, A. F. Mendonca, and T. B. Bailey. 2005. Inhibitory effects of organic acid salts for control of *Listeria monocytogenes* on frankfurters. *J. Food Prot.* 68:499–506.

Mahon, B. E., A. Ponka, W. N. Hall, K. Komatsu, S. E. Dietrich, A. Siitonen, G. Cage, P. S. Hayes, M.-A. Lambert-Fair, N. H. Bean, P. M. Griffin, and L. Slutsker. 1997. An international outbreak of *Salmonella* infections caused by alfalfa sprouts grown from contaminated seeds. *J. Infect. Dis.* 175:876–882.

Martin, S. M., and N. H. Bean. 1995. Data management issues for emerging diseases and new tools for managing surveillance and laboratory data. *Emerg. Infect. Dis.* 1:124–128.

Mbandi, E., and L. A. Shelef. 2002. Enhanced antimicrobial effects of combination of lactate and diacetate on *Listeria monocytogenes* and *Salmonella* spp. in beef bologna. *Int. J. Food Microbiol.* 76:191–198.

McMahon, M. A. S., and I. G. Wilson. 2001. The occurrence of enteric pathogens and *Aeromonas* species in organic vegetables. *Int. J. Food Microbiol.* 70:155–162.

Mead, P. S., and M. Griffin. 1998. *Escherichia coli* O157:H7. *Lancet* 352:1207–1212

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. Inf. Dis.* 5:607–625.

Mejlholm, O., and P. Dalgaard. 2007. Modeling and predicting the growth of lactic acid bacteria in lightly preserved seafood and their inhibiting effect on *Listeria monocytogenes*. *J. Food Prot*. 70:2485–2497.

Meldrum, R. J., C. D. Ribeiro, R. M. M. Smith, A. M. Walker, M. Simmons, D. Worthington, and C. Edwards. 2005. Microbiological quality of ready-to-eat foods: results from a long-term surveillance program (1995 through 2003). *J. Food Prot.* 68:1654–1658.

Miettinen, M. K., A. Siitonen, P. Heiskanen, H. Haajanen, K. J. Bjorkroth, and H. J. Korkeala. 1999. Molecular epidemiology of an outbreak of febrile gastroenteritis caused by *Listeria monocytogenes* in cold-smoked rainbow trout. *J. Clin. Microbiol.* 37:2358–2360.

Miller, A. J. 1992. Combined water activity and solute effects on growth and survival of *L. monocytogenes* Scott A. *J. Food Prot.* 55:414–418.

Miller, L. G., and C. W. Kaspar. 1994. *Escherichia coli* O157:H7 acid tolerance and survival in apple cider. *J. Food Prot.* 57:460–464.

Misrachi, A., A. J. Watson, and D. Coleman. 1991. *Listeria* in smoked mussels in Tasmania. *Comm. Dis. Intel.* 15:427.

Mitscherlich, E., and E. H. Marth. 1984. *Microbial survival in the environment*. Springer-Verlag, New York.

Miwa, N., T. Masuda, A. Kwamura, K. Terai, and M. Akiyama. 2002. Survival and growth of enterotoxin-positive and enterotoxin-negative *Clostridium perfringens* in laboratory media. *Int. J. Food Microbiol*. 72:233–238.

Mosupye, F. M., and A. Von Holy. 1999. Microbiological quality and safety of ready-to-eat street-vended foods in Johannesburg, South Africa. *J. Food Prot.* 62:1278–1284.

Muriana, P. M., W. Quimby, C. A. Davidson, and J. Grooms. 2002. Postpackage pasteurization of ready-to-eat deli meats by submersion heating for reduction of *Listeria monocytogenes*. *J. Food Prot.* 65:963–969.

Murphy, R. Y., R. E. Hanson, N. Feze, N. R. Johnson, L. L. Scott, and L. K. Duncan. 2005. Eradicating *Listeria monocytogenes* from fully cooked franks by using an integrated pasteurization-packaging system. *J. Food Prot.* 68:507–511.

Nataro, J. P., and J. B. Kaper. 1998. Diarrheagenic *Escherichia coli. Clin. Microbiol. Rev.* 11:1–60.

National Advisory Committee on Microbiological Criteria for Foods (NACMCF). 1991. Foodborne listeriosis. *Int. J. Food. Microbiol.* 14:185–246.

Ng, P. J., G. H. Fleet, and G. M. Heard. 2005. Pesticides as a source of microbial contamination of salad vegetables. *Int. J. Food. Microbiol.* 101:237– 250.

Nilsson, L., H. H. Huss, and L. Gram. 1997. Inhibition of *Listeria monocytogenes* on cold smoked salmon by nisin and carbon dioxide atmosphere. *Int. J. Food Microbiol.* 38:217–227.

Novak, J. S., and Y. T. C. Yuan. 2004. The fate of *Clostridium perfringens* spores exposed to ozone and/or mild heat pretreatment on beef surfaces followed by modified atmosphere packaging. *Food Microbiol*. 21(6):667–673.

O'Brien, A. D., J. W. Newland, S. F. Miller, R. K. Holmes, H. W. Smith, and S. B. Formal. 1984. Shiga-like toxin-converting phages from *Escherichia coli* strains that cause hemorrhagic colitis or infantile diarrhea. *Sci*. 226:694–696.

Olsen, S. J., L. C. MacKinnon, J. S. Goulding, N. H. Bean, and L. Slutsker. 2000. Surveillance for foodborne disease outbreaks—United States, 1993-1997. *Morb. Mortal. Wkly Rep.* 49:1–62.

Olsen, S. J., M. Patrick, S. B. Hunter, V. Reddy, L. Kornstein, W. R. MacKenzie, K. Lane, S. Bidol, G. A. Stoltman, D. M. Frye, I. Lee, S. Hurd, T. F. Jones, T. N. LaPorte, W. Dewitt, L. Graves, M. Wiedmann, D. J. Schoonmaker-Bopp, A. J. Huang, C. Vincent, A. Bugenhagen, J. Corby, E. R. Carloni, M. E. Holcomb, R. F. Woron, S. M. Zansky, G. Dowdle, F. Smith, S. Ahrabi-Fard, A. R. Ong, N. Tucker, N. A. Hynes, and P. Meada. 2005. Multistate outbreak of *Listeria monocytogenes* infection linked to delicatessen turkey meat. *Clin. Infect. Dis.* 40:962–967.

Oussalah, M., S. Caillet, S. Salmieri, L. Saucier, and M. Lacroix. 2007. Antimicrobial effects of alginate-based films containing essential oils on *Listeria monocytogenes* and *Salmonella* Typhimurium present in bologna and ham. *J. Food Prot.* 70:901–908.

Periago, P. M., and R. Moezelaar. 2001. Combined effect of nisin and carvacrol at different pH and temperature levels on the viability of different strains of *Bacillus cereus*. *Int. J. Microbiol.* 68:141–148.

Petit, L., M. Gilbert, and M.R. Popoff. 1999. *Clostridium perfringens*: toxinotype and genotype. *Trends Microbiol*. 7:104–110.

Phillips, D., D. Jordan, S. Morris, I. Jenson, and J. Sumner. 2008. A national survey of the microbiological quality of retail raw meats in Australia. *J. Food Prot*. 71:1232–1236.

Pothuri, P., D. L. Marshall, and K. W. McMillin. 1995. Combined effects of packaging atmosphere and lactic acid on growth and survival of *Listeria monocytogenes* in crayfish tail meat at 4°C. *J. Food Prot.* 59:253–256.

Rahmati, T., and R. Labbe. 2008. Levels and toxigenicity of *Bacillus cereus* and *Clostridium perfringens* from retail seafood. *J. Food Prot*. 71:1178–1185.

Rangel, J. M., P. H. Sparling, C. Crowe, P. M. Griffin, and D. L. Swerdlow. 2005. Epidemiology of *Escherichia coli* O157:H7 outbreaks, United States, 1982–2002. *Emerg. Infect. Dis.* 11:603–609.

Riedo, F. X., R. W. Pinner, M. L. Tosca, M. L. Cartter, L. M. Graves, M. W. Reeves, R.E. Weaver, B. D. Plikaytis, and C. V. Broome. 1994. A point-source foodborne listeriosis outbreak: documented incubation period and possible mild illness. *J. Infect. Dis.* 170:693–696.

Rijpens, N. P., G. Jannes, and L. M. F. Herman. 1996. Incidence of *Listeria* spp. and *Listeria monocytogenes* in ready-to-eat chicken and turkey products determined by polymerase chain reaction and line probe assay hybridization. *J. Food Prot.* 60:548–550.

Riley, L.W., R. S. Remis, S. D. Helgerson, H. B. McGee, J. G. Wells, B. R. Davis, R. J. Hebert, E. S. Olcott, L. N. Johnson, N. T. Hargrett, P. A. Blake, and M. L. Cohen. 1983. Hemorrhagic colitis associated with a rare *Escherichia coli* serotype. *N. Engl. J. Med.* 308:681–685.

Roberts, D. 1994. *Listeria monocytogenes* and food: the U.K. approach. *Dairy Food Environ. Sanit.* 14:198, 200, 202–204.

Rocourt, J., P. BenEmbarek, H. Toyofuku, and J. Schlundt. 2003. Quantitative risk assessment of *Listeria monocytogenes* in ready-to-eat foods: the FAO/WHO approach. *FEMS Immunol. Med. Microbiol.* 35:263–267.

Roy, R.J., F.F. Busta, and D.R. Thompson. 1981. Thermal inactivation of *Clostridium perfringens* after growth at several constant and linearly rising temperatures. *J. Food Sci.* 46:1586–1591.

Rushdy, A. A., J. M. Stuart, L. R. Ward, J. Bruce, E. J. Threlfall, P. Punia, and J. R. Bailey. 1988. National outbreak of *Salmonella senftenberg* associated with infant food. *Epidemiol. Infect.* 120:125–128.

Ryan, K. J., and C. G. Ray. 2004. *Sherris medical microbiology* (4th ed.). New York, McGraw-Hill. 362–368.

Sabah, J. R., V. K. Juneja, and D. Y. C. Fung. 2003. Effect of spices and organic acids on the growth of *Clostridium perfringens* from spore inocula during cooling of *sous-vide* cooked ground beef products. Presented at IFT Annual Meeting, Chicago, IL.

Sabah, J. R., H. Thippareddi, J. L. Marsden, and D. Y. C. Fung. 2003. Use of organic acids for the control of *Clostridium* perfringens in cooked vacuum-packaged restructured roast beef during an alternative cooling procedure. *J. Food Prot.* 66:1408–1412.

Sagoo, S. K., C. L. Little, and R. T. Mitchell. 2001. The microbiological examination of ready-to-eat organic vegetables from retail establishments in the United Kingdom. *Lett. Appl. Microbiol.* 33:434–439.

Sagoo, S. K., C. L. Little, L. Ward, I. A. Gillespie, and R.T. Mitchell. 2003. Microbiological study of ready-to-eat salad vegetables from retail establishments uncovers a national outbreak of salmonellosis. *J. Food Prot.* 66:403–409.

Saito, M. 1990. Production of enterotoxin by *Clostridium perfringens* derived from humans, animals, foods, and the natural environment in Japan. *J. Food Prot.* 53:115–118.

Salamina, G., E. D. Donne, A. Niccolini, G. Poda, D. Cesaroni, M. Bucci, R. Fini, M. Maldini, A. Schuchat, B. Swaminathan, W. Bibb, J. Rocourt, N. Binkin, and S. Salmaso. 1996. A foodborne outbreak of gastroenteritis involving *Listeria monocytogenes. Epidemiol. Infect.* 117429–117436.

Sallami, L., M. Marcotte, F. Naim, B. Ouattara, C. Leblanc, and L. Saucier. 2006. Heat inactivation of *Listeria monocytogenes* and *Salmonella enterica* serovar Typhi in a typical bologna matrix during an industrial cooking-cooling cycle. *J. Food Prot.* 69:3025–3030.

Samelis, J., G. K. Bedie, J. N. Sofos, K. E. Belk, J. A. Scanga, and G. C. Smith. 2002. Control of *Listeria monocytogenes* with combined antimicrobials after post-process contamination and extended storage of frankfurters at 4°C in vacuum packages. *J. Food Prot.* 65:299–307.

Samelis, J., J. N. Sofos, M. L. Kain, J. A. Scanga, K. E. Belk, and G. C. Smith. 2001. Organic acids and their salts as dipping solutions to control *Listeria monocytogenes* inoculated following processing of sliced pork bologna stored at 4°C in vacuum packages. *J. Food Prot.* 64:1722–1729.

Sarker, M. R., R. P. Shivers, S. G. Sparks, V. K. Juneja, and B. A. McClane. 2000. Comparative experiments to examine the effects of heating on vegetative cells and spores of *Clostridium perfringens* isolates carrying plasmid genes versus chromosomal enterotoxin genes. *Appl. Environ. Microbiol*. 66:3234–3240.

Schalch, B., H. Eisgruber, and A. Stolle. 1995. Microwave reheating of vacuum-packaged cooked sausage cold cuts for shelf life improvement. *Fleischerei.* 45:3–4.

Schlech, W. F., P. M. Lavigne, R. A. Bortolussi, A. C. Allen, E. V. Haldane, A. W. Hightower, S. E. Johnson, S. H. King, E. S. Nicholls, and C. V. Broome. 1983. Epidemic listeriosis—evidence for transmission by food. *N. Eng. J. Med.* 308:203–206.

Scotland, S. M., H. R. Smith, and B. Rowe. 1985. Two distinct toxins active on Vero cells from *Escherichia coli* O157:H7. *Lancet* 2:885–886.

Scotland, S. M., H. R. Smith, G. A. Willshaw, and B. Rowe. 1983. Vero-cytotoxin production in strain of *Escherichia coli* is determined by genes carried on bacteriophage. *Lancet* 2:216.

Seman, D. L., S. C. Quickert, A. C. Borger, and J. D. Meyer. 2008. Inhibition of *Listeria monocytogenes* growth in cured ready-to-eat meat products by use of sodium benzoate and sodium diacetate. *J. Food Prot.* 71:1386–1392.

Shen, Y., Y. Liu, Y. Zhang, J. Cripe, W. Conway, J. Meng, G. Hall, and A. A. Bhagwat. 2006. Isolation and characterization of *Listeria monocytogenes* isolates from ready-to-eat foods in Florida. *Appl. Envrion. Microbiol.* 72:5073–5076.

Sheridan, J. J., R. L. Buchanan, and T. J. Montville (eds.). 1996. *HACCP: An integrated approach to assuring the microbiological safety of meat and poultry.* Food & Nutrition Press, Trumbull, CT.

Sheridan, J. J. 2007. Sources of contamination during slaughter and measures for control. *J. Food Safety*. 18: 321–339.

Shigehisa, T., T. Nakagami, and S. Taji. 1985. Influence of heating and cooling rates on spore germination and growth of *Clostridium perfringens* in media and in roast beef. *Jpn. J. Vet. Sci*. 47:259–267.

Sim, J., D. Hood, L. Finnie, M. Wilson, C. Graham, M. Brett, and J. A. Hudson. 2002. Series of incidents of *Listeria monocytogenes* non-invasive febrile gastroenteritis involving ready-to-eat meats. *Lett. Appl. Microbiol.* 35:409–413.

Sivarooban, T., N. S. Hettiarachchy, and M. G. Johnson. 2007. Inhibition of *Listeria monocytogenes* using nisin with grape seed extract on turkey frankfurters stored at 4 and 10°C. *J. Food Prot.* 70:1017–1020.

Smart, J. L., T. A. Roberts, M. F. Stringer, and N. Shah. 1979. The incidence and serotypes of *Clostridium perfringens* on beef, pork and lamb carcasses. *J. Appl. Bacteriol.* 46:377–383.

Smith, H. W., P. Green, and Z. Parsell. 1983. Vero cell toxins in *Escherichia coli* and related bacteria: transfer by phage and conjugation and toxic action in laboratory animals, chickens and pigs. *J. Gen. Microbiol.* 129:3121–3137.

Smith, L., and B. L. Williams. 1984. Clostridia, pp. 101–136. In *The pathogenic anaerobic bacteria*. Charles C Thomas, Springfield, IL.

Smith-Simpson, S., and D. W. Schaffner. 2005. Development of a model to predict growth of *Clostridium perfringens* in cooked beef during cooling. *J. Food Prot.* 68:336–341.

Smittle, R. B. 2000. Microbiological safety of mayonnaise, salad dressings and sauces reduced in the United States: a review. *J. Food Protect.* 63:1144–1153.

Sommers, C. H., and G. Boyd. 2006. Radiation sensitivity and postirradiation growth of foodborne pathogens on a ready-to-eat frankfurter on a roll product in the presence of modified atmosphere and antimicrobials. *J. Food Prot.* 69:2436–2440.

Smoot, L. M., and M. D. Pierson. 1997. Indicator microorganisms and microbiological criteria, pp. 66–80. In M. P. Doyle, L. R. Beuchat, and T. J. Montville (eds.), *Food microbiology: fundamentals and frontiers*. ASM Press, Washington, D.C.

Solomon, E. B., H. J. Pang, and K. R. Matthews. 2003. Persistence of *Escherichia coli* O157:H7 on lettuce plants following spray irrigation with contaminated water. *J. Food Prot.* 66:2198–2202.

Sramova, H., D. Dedicova, P. Petras, and C. Benes. 1991. Epidemic occurrence of alimentary bacterial infections in the Czech Republic, 1979-1989. *Ceskosl. Epidemiol. Mikrobiol. Imunol.* 40:74–84.

Steele, F.M., and K.H. Wright. 2001. Cooling rate effect of outgrowth of *Clostridium perfringens* in cooked, ready-to-eat turkey breast roasts. *Poultry Sci.* 80:813–816.

Stopforth, J. D., T. Mai, B. Kottapalli, and M. Samadpour. 2008. Effect of acidified sodium chlorite, chlorine, and acidic electrolyzed water on *Escherichia coli* O157:H7, *Salmonella*, and *Listeria monocytogenes* inoculated onto leafy greens. *J. Food Prot.* 71:625–628.

Szabo, E. A., and M. E. Cahill. 1999. Nisin and ALTA™ 2341 inhibit the growth of *Listeria monocytogenes* on smoked salmon packaged under vacuum or 100% $CO_2$. *Lett. Appl. Microbiol.* 28:373–377.

Taormina, P. J., G. W. Bartholomew, and W. J. Dorsa. 2003. Incidence of *Clostridium perfringens* in commercially produced cured raw meat product mixtures and behavior in cooked products during chilling and refrigerated storage. *J. Food Prot.* 66:72–81.

Taormina, P. J., L. R. Beuchat, and L. Slutsker. 1999. Infections associated with eating seed sprouts: an international concern. *Emerg Infect Dis.* 5:626–634.

Tassou, C. C., F. J. Samaras, J. S. Arkoudelos, and C. G. Mallidis. 2009. Survival of acid-adapted or non-adapted *Salmonella Enteritidis, Listeria monocytogenes* and *Escherichia coli* O157:H7, in traditional Greek salads. *Int. J. Food Sci. Technol.* 44:279–287.

Teufel, P. 1994. European perspectives on *Listeria monocytogenes*. *Dairy Food Environ. Sanit.* 14:212–214.

Thippareddi, H., V. K. Juneja, R. K. Phebus, J. L. Marsden, and C. L. Kastner. 2003. Control of *Clostridium perfringens* germination and outgrowth by buffered sodium citrate during chilling of ground roast beef and injected pork. *J. Food Prot.* 66:376–381.

Tilden, J., W. Young, C. Custer, A. McNamara, B. Boesl, M. Lambert-Fair, J. Majkowski, D. Vugia, S. B. Werner, J. Hollingsworth, and J. G. Morris, Jr. 1996. A new route of transmission for *Escherichia coli*: infection from dry fermented salami. *Am. J. Public Health* 86:1142–1145.

Todar, K. 2008. *Salmonella* and salmonellosis, pp. 1–5. In *Todar's on-line textbook of bacteriology*. http://www.cgmh.org.tw/chldhos/intr/c4a00/academy/bugs/salchole.html. Accessed May 2009.

Todd, E.C.D. 1989. Costs of acute bacterial foodborne disease in Canada and the United States. *Int. J. Food Microbiol.* 9:313–326.

Toth, I., T. J. Barrrett, M. L. Cohen, H. S. Rumschlag, J. H. Green, and I. K. Wachsmuth. 1991. Enzyme-linked immunosorbent assay for products of the 60 megadalton plasmid of *Escherichia coli* serotype O157:H7. *J. Clin. Micobiol.* 29:1016–1019.

Tschirdewahn, B., S. Notermans, K. Wernars, and F. Untermann. 1991. The presence of enterotoxigenic *Clostridium perfringens* strains in feces of various animals. *Int. J. Food Microbiol.* 14:175–178.

Tuomi, S., M. E. Matthews, and E. H. Marth. 1974. Behavior of *Clostridium perfringens* in precooked chilled ground beef gravy during cooling, holding, and reheating. *J. Milk Food Technol.* 37:494–498.

Tzipori, S., H. Karch, I. K. Wachsmuth, R. M. Robins-Browne, A. D. O'Brien, H. Lior, M. L. Cohen, J. S. Smithers, and M. M. Levine. 1987. Role of a 60-megadalton plasmid and Shiga-like toxins in the pathogenesis of infections caused by enterohemorrhagic *Escherichia coli* O157:H7 in gnotobiotic piglets. *Infect. Immun.* 55:3117–3125.

Uhart, M., S. Ravishankar, and N. D. Maks. 2004. Control of *Listeria monocytogenes* with combined antimicrobials on beef franks stored at 4°C. *J. Food Prot.* 67:2296–2301.

Ukuku, D. O., and W. Fett. 2002. Behavior of *Listeria monocytogenes* inoculated on cantaloupe surfaces and efficacy of washing treatments to reduce transfer from rind to fresh-cut pieces. *J. Food Prot.* 65:924–930.

U.S. Food and Drug Administration. 2004. Press release: Paramount farms expands recall of raw almonds. http://www.fda.gov/oc/po/firmrecalls/paramountexp05_04.html. Accessed May 2009.

U.S. Food and Drug Administration. 2007. FDA finalizes report on 2006 spinach outbreak. http://www.fda.gov/bbs/topics/NEWS/2007/NEW01593.html. Accessed April 2009.

U.S. Food and Drug Administration, Center for Food Safety and Applied Nutrition. 2007. Food Safety: A to Z Reference Guide—*Salmonella*. http://www.cfsan.fda.gov/~dms/a2z-f.html Accessed May 2009.

U.S. Food and Drug Administration, Center for Food Safety and Applied Nutrition. 2009. Foodborne pathogenic microorganisms and natural toxins handbook. *Salmonella* spp. http://www.foodsafety.gov/~mow/chap1.html. Accessed May 2009.

U.S. Food and Drug Administration, Economic Research Service. 2001. Lettuce: in & out of the bag. http://www.ers.usda.gov/publications/AgOutlook/April2001/AO280d.pdf.10. Accessed May 2009.

U.S. Food and Drug Administration, Food Safety and Inspection Service (USDA-FSIS). 2001. Performance standards for the production of certain meat and poultry products; final rule. *Fed. Regist*. 64:732–749.

Uyttendaele, M., P. De Troyb, and J. Debevere. 1999. Incidence of *Listeria monocytogenes* in different types of meat products on the Belgian retail market. *Int. J. Food Microbiol.* 53:75–80.

Viji, V., E. Ailes, C. Wolyniak, F. J. Angulo, and K. C. Klontz. 2006. Recalls of spices due to bacterial contamination monitored by the U.S. Food and Drug Administration: the predominance of Salmonellae. *J. Food Prot.* 69:233–237.

Vugia, D. J., M. Samuel, M. M. Farley, R. Marcus, B. Shiferaw, S. Shallow, K. Smith, F. J. Angulo. 2004. Invasive *Salmonella* infections in the United States, FoodNet, 1996–1999: incidence, serotype distribution, and outcome. *Clin. Infect. Dis.* 38:S149–S156.

Waldroup, A. L. 1996. Contamination of raw poultry with pathogens. *World Poultry Sci. J.* 52:7–25.

Walker, S. J., P. Archer, and J. G. Banks. 1990. Growth of *L. monocytogenes* at refrigeration temperatures. *J. Appl. Bacteriol.* 68:157–162

Wells, J. G., B. R. Davis, I. K. Wachsmuth, L. W. Riley, R. S. Remis, R. Sokow, and G. K. Morris. 1983. Laboratory investigation of hemorrhagic colitis outbreaks associated with a rare *Escherichia coli* serotype. *J. Clin. Microbiol.* 18:512–520.

Wen, Q., and B. McClane. 2004. Detection of enterotoxigenic *Clostridium perfringens* type A isolates in American retail foods. *Appl. Environ. Microbiol*. 70:2685–2691.

Whittman, T. S., M. L. Wolfe, I. K. Wachsmuth, F. Orskov, I. Orskov, and R. A. Wilson. 1993. Clonal relationship among *Escherichia coli* strains that cause hemorrhagic colitis and infantile diarrhea. *Infect. Immun.* 61:1619–1629.

Willardsen, R. R., F. F. Busta, C. E. Allen, and L. B. Smith. 1978. Growth and survival of *Clostridium perfringens* during constantly rising temperatures. *J. Food Sci.* 43:470–475.

Williams, R. C., S. Isaacs, M. L. Decou, E. A. Richardson, M. C. Buffett, R. W. Slinger, M. H. Brodsky, B. W. Ciebin, A. Ellis, J. Hockin, and the *E. coli* O157:H7 Working Group. 2000. Illness outbreak associated with *Escherichia coli* O157:H7 in Genoa salami. *Can. Med. Assoc. J.* 162:1409–1413.

Winthrop, K. L., M. S. Palumbo, J. A. Farrar, J. C. Mohle-Boetani, S. Abbott, M. E. Beatty, G. Inami, and S. B. Werner. 2003. Alfalfa sprouts and *Salmonella* Kottbus infection: a multistate outbreak following inadequate seed disinfection with heat and chlorine. *J. Food Prot*. 66:13–17.

Zaika, L. L. 2003. Influence of NaCl content and cooling rate on outgrowth of *Clostridium perfringens* spores in cooked ham and beef. *J. Food Prot*. 66:1599–1603.

Zeitoun, A. A., and J. M. Debevere. 1991. Inhibition, survival, and growth of *Listeria monocytogenes* on poultry as influenced by buffered lactic acid treatment and modified atmosphere packaging. *Int. J. Food Microbiol.* 14:161–169.

Zhao, T., and M. P. Doyle. 1994. Fate of enterohemorrhagic *Escherichia coli* O157:H7 in commercial mayonnaise. *J. Food Prot.* 57:780–783.

Zhu, M. J., A. Mendonca, H. A. Ismail, and D. U. Ahn. 2009. Fate of *Listeria monocytogenes* in ready-to-eat turkey breast rolls formulated with antimicrobials following electron-beam irradiation. *Poult. Sci.* 88:205–213.

# chapter 2

# Delicatessen salads

*Cheng-An Hwang*

*Contents*



## 2.1 Introduction

Delicatessen or deli-type salads are a category of refrigerated ready-to-eat food product. They are increasingly popular food items for many meal occasions. With various flavors and selections, deli salads are a favorable food choice for every season, particularly in warm weather. Deli salads are available from many places where foods are sold or served, such as supermarkets, deli shops, fast-food and full-service restaurants, and institutional foodservice establishments. Deli salads are also food dishes that can be easily prepared at home. Their variety, availability, and convenience have increased the consumption of deli salads. The estimated annual consumption of deli salads is 5.63 billion servings in the United States alone. Deli salads commonly contain at least two major ingredients: a food

component and mayonnaise or salad dressings. The variety of deli salads are as many as the food ingredients that can be used in salads, which includes almost anything that is edible. There are the well-known and popular salads such as potato salad, macaroni salad, ham salad, seafood salad, and coleslaw, and there are regional, ethnic, or newly created salads such as Cajun-style seafood salad, deviled egg salad, Mediterranean seafood, spicy Mexican bean, Japanese rice wine vinaigrette, and Greek pasta salad (Decker, 2001). In addition to the variety of differences in terms of the main food components, deli salads in a variety of low-fat, low-salt, and low-calorie choices for health-conscious consumers are also increasingly available in the marketplaces. Deli salads provide some of the greatest variety and flavor of any food category. Deli salads in marketplaces come from off-site locations or are prepared on-site. The salads may come from a manufacturer, which prepares, packs, and ships the products to the marketplaces. The marketplace may produce salads on-site from individual or pre-packed ingredients. Salads prepared off-site are generally produced in larger quantity, cost less, and have longer shelf lives than those prepared on-site. In general, deli salads have a relatively shorter shelf life than other types of ready-to-eat food products. Although deli salads may be formulated with preservatives to have longer shelf lives, it is generally recommended that deli salads like potato salad, macaroni salad, and coleslaw be consumed within 3 to 5 days after preparation. Deli salads should be kept at refrigerated temperature, and they should be consumed within 2 hours of removal from refrigeration temperature. The consumption of deli salads has increased markedly in recent years. These products are potential sources of pathogenic microorganisms, and their microbial safety is a health concern.

The potential for mayonnaise-based salads to become contaminated with pathogenic microorganisms can be high during preparation and service of the products. Salad ingredients may also be held for long periods of time under improper storage temperatures, which gives microorganisms appropriate conditions to grow (Albrecht et al., 1995). Handling of products by customers after purchase also increases the potential of further contamination of the product or allowing contaminated microorganisms to grow. Since salads are consumed without prior heating or cooking, the contamination of salads with pathogenic microorganisms is a health hazard to consumers. Outbreaks of foodborne illness have been linked to the consumption of coleslaw contaminated with *Listeria monocytogenes* (Schlech et al., 1983), potato salad contaminated with *Shigella flexneri* (Lew et al., 1991), tuna salad contaminated with *Clostridium perfringens* (Khatib et al., 1994), and salads cross-contaminated with undercooked roast beef (Rodrique et al., 1995). Outbreaks have also been attributed to

contaminated tossed salads (Dunn et al., 1985) and starch-based salads (Bean and Griffin, 1990).

## 2.2 *Pathogens commonly associated with salad ingredients and deli salads*

A wide range of pathogenic microorganisms may be present in salad ingredients or deli salads. Pathogens that are commonly associated with deli salads or salad ingredients or have been implicated in foodborne illnesses associated with the consumption of deli salads or salad ingredients are *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella* spp., *Staphylococcus aureus, Campylobacter jejuni,* and *Aeromonas* spp.

### *2.2.1* Escherichia coli *O157:H7*

*E. coli* is a normal inhabitant of the intestines of all animals, including humans. One of the pathogenic strains, enterohemorrhagic *E. coli* (EHEC) is recognized as a dangerous human pathogen causing bloody diarrhea and producing life-threatening complications, such as hemolytic uremic syndrome (HUS) and thrombotic thrombocytopenic purpura in some individuals. HUS is the leading cause of renal failure and death in young children. *E. coli* serotype O157:H7 is a strain of EHEC that produces large quantities of one or more shiga-like toxins (verotoxin) that cause gastroenteritis in humans. Ground beef or undercooked meat products have been implicated in several foodborne outbreaks caused by *E. coli* O157:H7. This pathogen has also been implicated in outbreaks of foodborne illness associated with the consumption of alfalfa sprouts, unpasteurized fruit juices, dry-cured salami, lettuce, game meat, and cheese curds. As few as 10 viable cells of *E. coli* O157:H7 may cause illness. *E. coli* O157:H7 has been isolated from the feces of livestock, wild birds, and fruit flies. Meat, fruits, and vegetables may be contaminated with *E. coli* O157:H7 by these carriers (ICMSF, 1996a). Once *E. coli* O157:H7 contaminates the produce, it may proliferate despite the acidity of the product (Stopforth et al., 2004).

The survival of *E. coli* O157:H7 in food products with high acidity (low pH) such as apple cider, commercial mayonnaise, and related acidified foods has been examined (Abdul-Raouf et al., 1993; Miller and Kaspar, 1994; Weagant et al., 1994; Zhao et al., 1993; Zhao and Doyle, 1994). Some of the results showed that *E. coli* O157:H7 was able to survive in acidic environments. *E. coli* O157:H7 demonstrated a remarkable tolerance to the acidic conditions of mayonnaise and salad dressings, acidified laboratory media (Buchanan and Edelson, 1996; Erickson and Jenkins, 1991), dry salami (Cheville et al., 1996), apple cider (Miller and Martin, 1990),

and artificial gastric juice (Arnold and Kaspar, 1995). Hathcox et al. (1995) reported that *E. coli* O157:H7 could survive for 93 days in mayonnaise stored at 5°C. One enterohemorrhagic *E. coli* O157:H7 outbreak was linked to commercial real mayonnaise or salad bar mayonnaise-based dressings that were cross-contaminated with EHEC-contaminated beef juice (Anonymous, 1993a,b).

### 2.2.2 Listeria monocytogenes

Studies have suggested that 1% to 10% of humans may be intestinal carriers of *L. monocytogenes*, and that at least 37 mammalian species as well as at least 17 species of birds and some species of fish and shellfish are also carriers of this pathogen. In addition, *L. monocytogenes* has been isolated from soil, silage, and other environmental sources. *L. monocytogenes* is quite hardy and resistant to adverse effects of freezing, drying, and heat. Listeriosis is caused by *L. monocytogenes*. Symptoms of listeriosis include fever, nausea, vomiting, diarrhea, septicemia, meningitis, and it may cause spontaneous abortion or stillbirth. The infective dose of *L. monocytogenes* is unknown. *L. monocytogenes* has been implicated in illnesses associated with the consumption of raw milk, under-pasteurized fluid milk, cheeses (particularly soft-ripened varieties), ice cream, raw vegetables, fermented raw-meat sausages, raw and cooked poultry and meat, and raw and smoked fish. Due to its ability to grow at temperatures as low as 3°C, *L. monocytogenes* is a pathogen of concern for refrigerated food products (ICMSF, 1996a). *L. monocytogenes* is ubiquitous and can be found in soil, dust, sewage, and water, which may contaminate food sources of meat, poultry, dairy products, fruits, and vegetables commonly used as ingredients in deli salads. An outbreak of listeriosis has been linked to the consumption of coleslaw contaminated with *L. monocytogenes* (Schlech et al., 1983). The U.S. Food and Drug Administration has completed several Class I recalls of various mayonnaise-based salads because of contamination with *L. monocytogenes*. These salads include potato salad, ham salad, chicken salad, vegetable salad mix, pasta salad, coleslaw, and crab salad.

### 2.2.3 Salmonella *spp.*

*Salmonella* spp. are widespread in nature, and environmental sources of this organism include water, soil, insects, animal feces, raw meats, raw poultry, and raw seafood. As few as 15 to 20 cells of *Salmonella* spp. may cause illness (salmonellosis) with symptoms such as nausea, vomiting, abdominal cramps, diarrhea, fever, and headache. The microorganisms have been isolated from foods such as raw meats, poultry, eggs, milk and dairy products, fish, shrimp, sauces and salad dressings, cream-filled

desserts and toppings, peanut butter, cocoa, and chocolate. *Salmonella* species are often found in eggs, a main ingredient in mayonnaise-based salad dressings. One serotype, S. Typhimurium, was associated with an outbreak caused by commercially processed egg salad in the United States. A total of 18 cases of infection with S. Typhimurium were linked to kits for making egg salad that were distributed by a vendor to a supermarket chain. This was the first reported S. Typhimurium outbreak associated with a commercially processed hardboiled egg product (CDC, 2004). *Salmonella* spp. are the most common pathogen implicated in foodborne illness associated with the consumption of fresh produce such as lettuce, sprouts, cauliflower, spinach, melon, and tomatoes (Thunberg et al., 2002; Sivapalasingam et al., 2004). Many of these produce items are used as ingredients in deli salads.

### *2.2.4* Staphylococcus aureus

*S. aureus* is widely distributed in the environment. Staphylococcal bacteria can be found on the mucous membranes and skin of humans. Therefore, these organisms may be transferred from humans to food. Some strains are capable of producing a heat-stable toxin that causes illness with symptoms of headache, vomiting, and abdominal cramping. A toxin dose of less than 1.0 µg, approximately *S. aureus* $>10^5$ cfu/g, in contaminated food may cause illness (ICMSF, 1996a). Foods that are frequently incriminated in *S. aureus* foodborne illness include meat and meat products, poultry and egg products, bakery products, sandwich fillings, and milk and dairy products. Salads that have been implicated in *S. aureus* foodborne illness include egg, tuna, chicken, potato, and macaroni salads (Bergdoll, 1990).

### *2.2.5* Campylobacter jejuni

*C. jejuni* is a microaerophilic organism capable of growth in environments with reduced levels of oxygen. It is the most common cause of gastrointestinal illness worldwide and is the leading cause of bacterial diarrheal illness in the United States (Evans et al., 2003). A dose of *C. jejuni* at 400 to 500 cells may cause illness in some individuals. *C. jejuni* is frequently isolated from raw chicken. Surveys show that 20% to 100% of retail chickens are contaminated. Outbreaks are often linked to poultry or cross-contamination from poultry products (Gillespie et al., 2000). *C. jejuni* has been isolated from spinach, lettuce, radish, parsley, green onion, potato, and mushroom (Kumar et al., 2001; Park and Sanders, 1992). Epidemiological evidence suggests that salad vegetables are the second-highest risk factor for *Campylobacter* infection after poultry (Evans et al., 2003). Illness caused by *C. jejuni* has been linked to the consumption of

lettuce, sweet potatoes, cucumber, melon, and strawberries (Brandl et al., 2004), which are ingredients used in many varieties of deli salads.

### 2.2.6 Aeromonas *spp.*

*Aeromonas* spp. are ubiquitous in water, soil, feces, and on vegetation (McMahon and Wilson, 2001). *Aeromonas* has been isolated from a wide range of fresh produce including sprouted seeds, asparagus, broccoli, cauliflower, carrot, celery, cherry tomatoes, cucumber, lettuce, mushroom, pepper, turnip, and watercress (Merino et al., 1995, McMahon and Wilson 2001; Neyts et al., 2001; Isonhood and Drake, 2002; Heaton and Jones, 2008). Growth of *Aeromonas* can occur on shredded lettuce, chicory (Jacxens et al., 1999), and tomatoes (Velazquez et al., 1998*). A. hydrophila* is the species most isolated from fresh vegetables often linked to disease in humans (Callister and Aggar, 1987). Food isolates of *Aeromonas* have been shown to tolerate low pH and to grow at refrigeration temperatures. There are no documented cases of foodborne illness caused by *A. hydrophila* associated with consumption of deli salads. The high prevalence of *A. hydrophila* in vegetables and its ability to survive low pH and grow rapidly at the refrigerated temperature make the microorganism a concern for consumers of deli salads.

## 2.3 *Microbiological quality of deli salads*

The microbiological quality of deli salads is influenced by the microbiological quality of ingredients used for salads and the practice of processing, storage, and handling of the product during retailing or serving. Food components used in salads include meat, seafood, poultry, fruits, and vegetables. A wide range of microorganisms are commonly associated with these food ingredients and have been reported extensively. The potential for mayonnaise-based salads to become contaminated with spoilage, pathogenic microorganisms, or both, can be high because of the extensive handling of the food ingredients in salad preparation by workers and the handling of the salads during service by foodservice personnel. Also, salad ingredients and the finished products may be held for long periods of time under improper temperatures, giving microorganisms appropriate conditions for growth (Albrecht et al., 1995).

This section presents the microbiological quality of the ingredients commonly used in salad preparation. The microbiological quality of the ingredients directly affects the quality of the prepared salads, since there is no terminal processing step in salad manufacturing processes that is capable of eliminating microorganisms present in the ingredients.

### *2.3.1 Mayonnaise and salad dressings*

Mayonnaise or salad dressing is one of the main ingredients used in the preparation of deli salads. According to the "standard of identity" classification of the U.S. Food and Drug Administration, mayonnaise must contain ≥65% oil and ≥0.25% acetic acid and be produced by using egg as an emulsifier, and with a final pH ≤4.1. Mayonnaise made with unpasteurized eggs must have a final pH ≤4.1 and contain ≥1.4% acetic acid in the aqueous phase, and the finished product must be held for 72 h before shipping. This regulation is to ensure that the product contains no viable *Salmonella* spp. Commercial mayonnaise and mayonnaise-dressing manufacturers use pasteurized liquid or frozen eggs exclusively. The liquid eggs are required to be pasteurized between 55.6°C and 63.3°C for 3.5 to 6.2 min depending on blend composition and addition of ingredients such as salt or sugar (CFR 7:59.500–59.580). Mayonnaise made by major producers in the United States contains approximately 0.31% acetic acid. Unlike mayonnaise, salad dressing is not a product with "standard of identity" classification, so it is not legally defined. Generally, salad dressing contains ≥30% oil and with a final pH that is higher than that of mayonnaise. Due to their low pH (<4.6), both mayonnaise and salad dressing are classified as acid foods (CFR 21:114).

Although pasteurized eggs are commonly used for manufacturing commercial mayonnaise, pathogens such as *Salmonella*, *L. monocytogenes*, and *S. aureus* may be present in pasteurized eggs and in the final product through contamination during processing. The high acidity (low pH) derived from ingredients in mayonnaise such as vinegar and other types of acidulants is the most important and significant factor that affects the survival of pathogenic microorganisms in mayonnaise and salad dressings. The low pH is derived primarily from acetic acid and, to a lesser extent, lactic acid and citric acid (Smittle, 2000). Erickson et al. (1995) examined the survival of *E. coli* O157:H7 in commercial real mayonnaise and reduced-calorie and reduced-fat mayonnaise dressings (pH 3.1–3.9) at 25°C. They reported that *E. coli* O157:H7 was inactivated in mayonnaise with pH ≤3.9, indicating that the acidity in mayonnaise quickly inactivated *E. coli* O157:H7; therefore, commercial real mayonnaise, mayonnaise-based products, and reduced-calorie and reduced-fat mayonnaise dressings had a low possibility of being sources of *E. coli* O157:H7. Similar studies have also been conducted to examine the survival of *L. monocytogenes*, *Salmonella* spp., *S. Enteritidis*, *S. aureus*, and *Y. enterocolitica* in mayonnaise, salad dressing, and sauces. These pathogens were unable to survive in a variety of mayonnaises, dressings, and sauces (Brackett, 1986; Gomez-Lucia et al., 1987; Perales and Garcia, 1990; Erickson and Jenkins, 1991; Glass and Doyle, 1991; Raghubeer et al., 1994; Hathcox et al., 1995; Cheville

et al., 1996, Gahan et al., 1996; Zhao and Doyle, 1994, Glass et al., 1992; Beuchat et al., 2004). Results from studies on the survival of pathogens in mayonnaise and salad dressing indicated that foodborne pathogens die off at various rates, depending on the organism (e.g., *E. coli* O157:H7 died off slower than other pathogens), acid type and concentration, storage temperature, organism adaptability, and pH (Smittle, 2000). In addition to the acidity, the presence of lysozyme in mayonnaise made with whole eggs may have an antimicrobial effect on *L. monocytogenes*, *S. aureus*, *Clostridium perfringens* (Paul and Potter, 1978; Erickson et al., 1991), and Gram-negative bacteria (Peterson and Hartsell, 1955). Glass and Doyle (1991) also speculated on a synergistic effect of acetic acid and lysozyme or other antimicrobial substances in egg whites on inactivating *Salmonella* in mayonnaise.

To examine the potential sources of contamination of mayonnaise, Erickson et al. (1995) evaluated the risk of EHEC contamination during commercial mayonnaise production by pasteurized liquid eggs and wet processing areas. Egg samples and environmental swab samples (floor drains, water and/or lubricant drip pans, and conveyor belts) were collected from three mayonnaise and/or mayonnaise dressing processing plants and tested for the presence of *E. coli* O157:H7. There was no EHEC recovered from the egg samples (253 samples from 188 egg lots), and no *E. coli* or EHEC was recovered from 114 composite environmental swab samples. The study concluded that EHEC was not present in properly pasteurized liquid eggs and in processing areas that were properly cleaned and sanitized. The conclusions from this study and those of Weagant et al. (1994) and Zhao and Doyle (1994) indicated that commercial mayonnaise plants which use pasteurized eggs and follow good manufacturing practices and sanitation programs have a low risk for EHEC contamination.

There were few documented foodborne illnesses associated with the consumption of commercially prepared mayonnaise, salad dressings, or sauces. This indicates that these products generally would not contain infectious doses of pathogenic microorganisms during their shelf life. While commercial mayonnaise and salad dressings are microbiologically safe, cautions should be taken when these types of products are produced in house or are homemade. Mayonnaise or salad dressing produced from scratch in stores may deviate from the guidelines for products produced commercially. Microbial contamination of the products could occur if unpasteurized eggs are used and good manufacturing practice is not properly followed. The contaminated microorganisms could survive or even multiply in the products if there is an insufficient quantity of acids in the products to inhibit or inactivate the microorganisms. The presence of pathogenic microorganisms in these products could cause food

safety concerns. Therefore, non-commercial mayonnaise or salad dressing should be prepared and processed according to the guidelines and good manufacturing practice (Anonymous, 2008).

### *2.3.2 Meat, poultry, and seafood*

Meat, poultry, and seafood are common food components added to salads. The microbiological quality of these food products has been extensively studied and reported (ICMSF, 1996b). The pathogens that are likely to be found on livestock (cattle, sheep, and swine) and poultry (chicken and turkey) are *E. coli* O157:H7, *Salmonella*, *Campylobacter*, *L. monocytogenes*, *Yersinia enterocolitica,* and *Clostridium perfringens*. These microorganisms are often carried over to the processed products due to inadequate processes, for example, heating, to eliminate these pathogens or through cross-contamination via processing equipment or from the environment. These pathogens have been implicated in outbreaks of foodborne illness associated with the consumption of meat and poultry products. Consumption of meat and poultry products contaminated with pathogenic microorganisms contributes to the majority of foodborne illness. In the United States, poultry products were implicated in 20% of the 1,426 foodborne illness outbreaks that occurred between 1992 and 1999. Chicken was implicated in almost 75% of the outbreaks and turkey in over 20% of the outbreaks. *Salmonella* spp. were implicated in 30% of the outbreaks, and *C. perfringens* and *Campylobacter* were implicated in 21% and 6% of the outbreaks, respectively (Kessel et al., 2001). In a survey conducted in Canada, a total of 800 meat and poultry products, including raw ground beef, chicken legs, pork chops, and ready-to-eat fermented sausage, roast beef, processed turkey breast, chicken wieners, and beef wieners, from the retail marketplace in Edmonton, Alberta, were analyzed for *Salmonella*, *Campylobacter* spp., *L. monocytogenes,* and shiga toxin-producing *E. coli*. *Salmonella* and *Campylobacter* were found in 30% and 62% of raw chicken legs, respectively. *L. monocytogenes* was found in 52% of raw ground beef, 34% of raw chicken legs, 24% of raw pork chops, 4% of fermented sausages, 3% of processed turkey breast, 5% of beef wieners, and 3% of chicken wieners. One ground beef sample was positive for shiga toxin-producing *E. coli* O22:H8 (Bohaychuk et al., 2006). In New Zealand, 1,011 samples of beef, unweaned veal, chicken, lamb and mutton, and pork were collected from retail between August 2003 and June 2004, and tested for the presence of *C. jejuni* and *C. coli*. *C. jejuni* and *C. coli* were found in 89.1% of chicken, 9.1% of pork, 3.5% of beef, and 6.9% of lamb and mutton samples. *C. jejuni* was in 95% (246/259) of the positive samples (Wong et al., 2007). Seafood such as shrimp, crab meat, and surimi are food components in

many varieties of seafood salads. *Vibrio parahaemolyticus* is the pathogenic microorganism most commonly associated with seafood and has been linked to outbreaks of foodborne illness associated with the consumption of cooked shrimp, crab, and lobster (ICMSF, 1996b).

### 2.3.3 Non-meat ingredients

Non-meat food ingredients used in salad preparation are mostly fruits and vegetables. Consumption of fruit and vegetable products is considered as a potential risk to be infected with pathogens such as *L. monocytogenes*, *Salmonella,* and *E. coli* O157:H7. Outbreaks of foodborne illness have been linked to the consumption of lettuce, spinach, sprouts, green onions, and tomatoes, to name a few. Microbial contamination can be introduced to fruits and vegetables by irrigation water, insects, animals, birds, farm workers, processing equipment, and environmental cross-contamination (Heaton and Jones, 2008). Fruits and vegetables (produce) are ingredients frequently used in preparation of deli salads. The microbiological quality of the produce hence contributes to the microbiological quality of the finished salads. In the United States, it was estimated that 3% to 8% of the outbreaks of gastrointestinal illness were linked to produce (Madden, 1992). Fresh produce has been known to be contaminated with various pathogenic microorganisms and cause foodborne illnesses (Beuchat, 1996; Tauxe et al., 1997). Albrecht et al. (1995) examined the vegetable salad ingredients (lettuce, tomatoes, broccoli, and cauliflower) from three deli shops in grocery stores, and found the total aerobic counts ranged from 5.5 to 6.6 $\log_{10}$ cfu/g and coliform counts ranged from 4.9 to 6.3 $\log_{10}$ cfu/g. In an inoculation study, fresh broccoli was processed into florets and inoculated with *E. coli* ATCC 23742, and then the floret was subjected to three washing treatments. When a chlorine wash solution was used, it only slightly reduced the aerobic microbial load and the inoculated coliform population on the broccoli. The results indicated that vegetables commonly used for salad dishes are likely to contain high levels of microorganisms and washing the vegetables may not significantly reduce the microbial load on the vegetable. The high microbial loads in vegetables have also been reported by Albrecht et al. (1995), Hagenmaier and Baker (1998), Lin et al. (1996), and Johnston et al. (2005). Fruits and vegetables have also been known to be contaminated with various pathogenic microorganisms and cause foodborne illness (Beuchat, 1996; Tauxe et al., 1997; Johnston et al., 2005). In Spain, 300 samples of fresh and minimally processed fruit and vegetables and sprouts from several retail establishments were tested for microbiological quality in 2005 to 2006. The samples included ready-to-eat fruits, whole fresh vegetables, sprouts, and ready-to-eat salads containing one to six types of vegetables. Results showed that fresh-cut grated carrot,

arugula, and spinach contained >7.4 $\log_{10}$ cfu/g, fresh-cut endive and lettuce contained >6.2 log cfu/g, and sprouts contained >7.9 $\log_{10}$ cfu/g aerobic mesophilic bacteria. Of the samples analyzed, 40% of the samples were positive for *E. coli*, 1.3% were positive for *Salmonella,* and 0.7% were positive for *L. monocytogenes* (Abadias et al., 2008). These reports indicate that fruits and vegetables are significant sources of microbial contamination for salad when used as ingredients.

In England and Wales from 1992 to 2000, 83 outbreaks of foodborne illness were associated with the consumption of salad vegetables or fruits. A total of 3,438 people were infected, 69 were hospitalized, and one person died. Most outbreaks were linked to commercial catering (67.5%). The pathogen most implicated in the outbreaks was *Salmonella* (41.0%), such as in lettuce contaminated with *S. enterica* serovar Typhimurium DT 104, and S. Typhimurium DT 204b (Long et al., 2002). Fresh produce contaminated with *L. monocytogenes* causing enteric infection has long been recognized (Blakeman, 1985). Harvey and Gilmour (1993) suggested the contamination probably came from the environment and occurred during processing. The prevalence rates of *L. monocytogenes* in fresh produce in many countries have been reported. The prevalence data were varied among the locations and countries where the studies were conducted. Arumugaswamy et al. (1994) reported that *L. monocytogenes* was isolated from bean sprouts (85%), leafy vegetables (22%), and cucumbers (80%) in Malaysia. In Pakistan, 6.7% of cucumbers tested positive for *L. monocytogenes* (Beuchat, 1996). In two studies in Canada, *L. monocytogenes* were found in 2.2% (Schlech et al., 1983) and 6.7% (Odumeru et al., 1997) of the cabbage samples. In the United States, the prevalence rate was 1.1% in cabbage samples (Heisick et al., 1989). *L. monocytogenes* was also found in potatoes (27.1%) and radishes (14.4% and 36.8%) in studies conducted in the United States (Beuchat, 1996). Szabo et al. (2000) surveyed 120 bagged lettuce samples in Australia and reported that *L. monocytogenes* was positive in 2.5% samples. Thunberg et al. (2002) isolated *L. monocytogenes* from potatoes (50%) and field cress (18%) purchased at farmers' markets. Crepet et al. (2007) reported that prevalence of *L. monocytogenes* on salad vegetables was usually <5%, with lower prevalence on leafy vegetables than on sprouted seeds, carrots, cabbage, celery, and spinach. Root vegetables seem to have a higher prevalence rate of *L. monocytogenes* than leafy vegetables do. Heisick et al. (1989) suggested that this might be due to the increased contact of the vegetables with soil. *L. monocytogenes* is a psychrotrophic microorganism and is capable of growing during storage if present on fresh produce (Wonderling et al., 2004). Beuchat and Brackett (1990) showed that *L. monocytogenes* was capable of growth on lettuce when exposed to processing conditions. Although *Listeria* contamination of fresh produce and survival up to the points of service seems likely,

outbreaks linked to fresh produce are infrequent and tend to be limited to vulnerable groups. The two documented outbreaks which occurred in 1979 and 1981, respectively, were attributed to cabbage (in coleslaw) and salad items (celery, lettuce, and tomatoes) served as part of hospital meals (Anonymous, 2001).

## 2.4 Deli salads

Results from surveys of microbiological quality of deli salads generally indicate that high levels of microorganisms are present in salad products. Christensen and King (1971) found bacterial counts in chicken salad ranging from 4.0 to 7.0 $\log_{10}$ cfu/g and in coleslaw ranging from 2.7 to 4.5 $\log_{10}$ cfu/g. In a study with deli salads obtained from various manufacturers, shrimp and egg salads were found to have bacterial levels of 6.1 $\log_{10}$ cfu/g and 4.1 to 6.8 $\log_{10}$ cfu/g, respectively (Fowler and Clark, 1975). A study of ready-to-eat foods at retail stores in Japan found bacterial counts of 3.5, 3.4, and 3.7 log cfu/g in potato salad, macaroni salad, and coleslaw, respectively (Kaneko et al., 1999). Deli salads obtained from butcher shops in Germany were found to have bacterial counts ranging from 5.0 to 8.0 $\log_{10}$ cfu/g (Becker et al., 2002).

Bornemeier et al. (2003) examined mayonnaise-based salads in grocery store delis for potential contamination with *S. aureus* and *L. monocytogenes.* They examined potato (pH 4.1–4.6), macaroni (pH 4.0–4.5), and krab (surimi, pH 4.5–5.8) salads for total aerobic plate counts, *L. monocytogenes,* and *S. aureus.* The salads had total aerobic plate counts of 3.0 to 3.8 $\log_{10}$ cfu/g and *S. aureus* counts of 1.4 to 2.5 $\log_{10}$ cfu/g. Coagulase-positive *S. aureus* was found in krab salad and macaroni salad, while *L. monocytogenes* was not found in any sample. Results of this study also indicate that temperature conditions for all three salads and the pH range for krab salad could support growth of pathogenic microorganisms. In a study examining microbiological quality of commercially processed deli salads, Montville and Schaffner (2004) reported that deli salads containing poultry or eggs (chicken salad, egg salad, turkey salad) had bacterial counts of 3.3 $\log_{10}$ cfu/g, and shrimp salad and tuna salad had counts of 3.7 and 3.0 $\log_{10}$ cfu/g, respectively. In Spain, American salad (40% white cabbage, 15% carrot, 12% onion; mayonnaise, mustard guar, xanthan gums, potassium sorbate, and sodium benzoate, pH 4.4) produced by a processing factory located in Navarra, Spain, was tested for the presence of *Bacillus cereus*. The results showed that *B. cereus* was positive in 8.3% of the samples (Valero et al., 2007).

## *2.5 Survival of foodborne pathogens in salads*

The behaviors of common pathogens in deli salads have been examined. Guentert et al. (2005) inoculated commercially produced pasteurized chicken salad (pH 4.0, 4.6, and 5.2) with *L. monocytogenes* and stored the samples at 5.0°C, 7.2°C, and 21.1°C for up to 119 days. At 21.1°C, a 6-$\log_{10}$ reduction was seen after 14 days at pH 4.0, after 52 days at pH 4.6, and after 38 days at pH 5.2. Inactivation occurred in all pH levels and at all temperatures, particularly at lower storage temperatures. The researchers concluded that the *L. monocytogenes* was unable to survive in the salad due to the low pH. Arias et al. (2001) examined the survival of *E. coli* O157:H7 in salads made of meat, chopped cabbage, or poultry mixed with various quantities of commercial mayonnaise (0%, 18%, 37%, and 56% w/w) and stored at 12°C for 24, 48, and 72 h. They reported that *E. coli* O157:H7 counts increased in all samples during the storage and concluded that *E. coli* O157:H7 was capable of surviving and growing in meat, cabbage, and poultry mixed with mayonnaise. Several studies have also shown that salads and sandwiches prepared with various concentrations of mayonnaise would support the growth of pathogens. Doyle et al. (1982) reported growth of *Salmonella* and *S. aureus* in chicken and ham salads made with mayonnaise at pH 5.6 and 5.2, respectively. Abdul-Raouf et al. (1993) reported that *E. coli* O157:H7 was not inactivated in beef salads (pH 5.6–6.1) stored at 5°C, and growth of *E. coli* O157:H7 occurred in the salad stored at 21°C and 30°C. Growth of *L. monocytogenes* was observed in a chicken salad (pH 5.7) prepared with commercial mayonnaise after 7 days of storage at 4°C (Erickson et al., 1993). While mayonnaise and some food ingredients in salads, for example, fruits and vegetables, added to salads are acidic, some ingredients such as egg and egg yolks (pH 6.0–7.0), egg whites (pH 9.07), garlic (pH 5.3–5.8), onions (pH 5.3–5.8), and meat, poultry, and seafood (pH 6.0–7.5) are considered low-acid foods (ICMSF, 1996b). Studies have been conducted to examine the behaviors of *L. monocytogenes* in salads with food components that were low acid and contaminated with the pathogen (Hwang, 2005; Hwang and Tamplin, 2005; Hwang and Marmer, 2007). Seafood (shrimp and imitation crab meat), ham, potato, egg, and pasta were inoculated with *L. monocytogenes* before mixing with pH-adjusted mayonnaise (pH 3.6–5.2), and the salads were stored at 4°C to 16°C. The results showed that *L. monocytogenes* was able to grow in all salad samples except in some samples of potato salad. It is speculated that the food components (higher pH, $a_w$, and nutrient contents) buffered the acidity of mayonnaise and provided a favorable growth condition for *L. monocytogenes*.

## 2.6 Controls

Food preservatives have been used in deli salads to enhance microbiological quality. The preservatives are mainly used to eliminate or inhibit the growth of *L. monocytogenes*. NovaGARD CB1, manufactured by Rhodia Co., is an antimicrobial ingredients system used to reduce or eliminate *L. monocytogenes* in meat-containing deli salads and sauces. It is a blend of cultured dextrose, sodium diacetate, egg white lysozyme, and nisin preparation that is recommended for use in coleslaw, chicken, tuna, and seafood salads to inhibit the growth of *L. monocytogenes* (Anonymous, 2004). PURAC Fresh, manufactured by PURAC Co., is a product for deli salads for *Listeria* inhibition, shelf life extension, pH regulation, and flavor improvement. PURAC Fresh is a liquid lactic acid–based product and is used in deli salads, such as potato, chicken, tuna, and pasta salads, to control the growth of *Listeria monocytogenes* (Anonymous, 2009).

Following good manufacturing practice, deli salads manufactured or prepared with mayonnaise or salad dressing and ingredients of reasonably good microbiological quality are pathogen-free without any food safety concerns. The safety of the salads is mainly contributed by the high acidity of mayonnaise that inhibits the growth of the few viable microorganisms that may be introduced by the food ingredients. Since the high acidity of mayonnaise may be diluted by low-acid foods, salad producers should recognize that mayonnaise or salad dressing cannot be solely relied upon to ensure the microbiological quality and safety of salad products. Good manufacturing, sanitation, and product handling practices must be followed to minimize cross-contamination, in addition to proper handling of the products during distribution and storage. In the marketplace and consumers' homes, deli salads must be kept at refrigerated temperature and handled with stringent hygienic practices to prevent cross-contamination by other food products and the environment.

## References

Abadias, M., J. Usall, M. Anguera, C. Solsona, and I. Vinas. 2008. Microbiological quality of fresh, minimally-processed fruit and vegetables, and sprouts from retail establishments. *Int. J. Food Microbiol.* 31(123):121–129.

Abdul-Raouf, U.M., L.R. Beuchat, and M.S. Ammar. 1993. Survival and growth of *Escherichia coli* O157:H7 in ground, roasted beef as affected by pH, acidulants, and temperature. *Appl. Environ. Microbiol.* 59:2364–2368.

Albrecht, J.A., F.L. Hamouz, S.S. Sumner, and V. Melch. 1995. Microbial evaluation of vegetable ingredients in salad bars. *J. Food Prot.* 58:683–685.

Anonymous. 1993a. Food vehicle in Oregon 0157 outbreak is a stunner. *Food Prot. Rep*. May, p. 2–3.

Anonymous. 1993b. Cross-contamination/different strain in Oregon *E. coli* cases. *Food Chem. News*. April 19, p. 32.

Anonymous. 2001. Analysis and evaluation of preventive control measures for the control and reduction/elimination of microbial hazards on fresh and fresh-cut produce. Chapter IV. Outbreaks associated with fresh produce: incidence, growth, and survival of pathogens in fresh and fresh-cut produce. US Food and Drug Administration. Rockville, MD. http://www.cfsan.fda.gov/~comm/ift3-4o.html.

Anonymous. 2004. Safe salads. http://findarticles.com/p/articles/mi_m3289/is_1_173/ai_112593911.

Anonymous. 2009. Deli salads. http://www.purac.com/purac_com/c236e6dab85f23813e2543c4a8a32299.php.

Arias, M.L., R. Monge-Rojas, F. Antillón, and C. Chaves. 2001. Growth and survival of *Escherichia coli* O157:H7 in meat, poultry and vegetables mixed with different concentrations of mayonnaise. *Rev. Biol. Trop.* 49:1207–1212.

Arnold, K.W., and C.W. Kaspar. 1995. Starvation- and stationary-phase-induced acid tolerance in *Escherichia coli* O157:H7. *Appl. Environ. Microbiol*. 61:2037–2039.

Arumugaswamy, R.K., G.R. Rahamat Ali, and N.B. Abd Hamid. 1994. Prevalence of *Listeria monocytogenes* in foods in Malaysia. *Int. J. Food Microbiol*. 23:117–121.

Bean, N.H., and P.M. Griffin. 1990. Foodborne disease outbreaks in the United States, 1973–1987: pathogens, vehicles and trends. *J. Food Prot*. 53:804–817.

Becker, B., B. Trierweiler, J. Fechler, T. Bohme, W.E.L. Spiess, and W.H. Holzapfel. 2002. Attended chilling cabinets in the food retail sale–3. Hygienic quality of delicatessen salads. *Fleischwirtschaft* 82:104–107.

Bergdoll, M.S. 1990. Staphylococcal food poisoning, p. 96. In D.O. Cliver (ed.), *Foodborne Disease*. Academic Press, New York.

Beuchat, L.R. 1996. Pathogenic microorganisms associated with fresh produce. *J. Food Prot*. 59:204–216.

Beuchat, L.R., and R.E. Brackett. 1990. Survival and growth of *Listeria monocytogenes* on lettuce as influenced by shredding, chlorine treatment, modified atmosphere packaging and temperature. *J. Food Sci.* 55:755–758.

Beuchat, L.R., J.-H. Rye, B.B. Adler, and M.D. Harrison. 2004. Death of *Salmonella*, *Escherichia coli* O157:H7, and *Listeria monocytogenes* in shelf-stable, dairy-based, pourable salad dressings. *J. Food Prot.* 69:801–814.

Blakeman, J.P. 1985. Ecological succession of leaf surface microorganisms in relation to biological control, pp. 6–30. In C.E. Windels and S.E. Lindow (eds.), *Biological Control on the Phylloplane.* American Phytopathological Society, St. Paul, MN.

Bohaychuk V.M., G.E. Gensler, R.K. King, K.I. Manninen, O. Sorensen, J.T. Wu, M.E. Stiles, and L.M. McMullen. 2006. Occurrence of pathogens in raw and ready-to-eat meat and poultry products collected from the retail marketplace in Edmonton, Alberta, Canada. *J. Food Prot*. 69:2176–2182.

Bornemeier, V.L., J.A. Albrecht, and S.S. Sumner. 2003. Survey of mayonnaise-based salads for microbial safety and quality. *Food Prot. Trends* 23:387–392.

Brackett, R.E. 1986. Growth and survival of *Yersinia enterocolitica* at acidic pH. *Int. J. Food Microbiol*. 3:243–251.

Brandl, M.T., A.F. Haxo, A.H. Bates, and R.E. Mandrell. 2004. Comparison of survival of *Campylobacter jejuni* in the phyllosphere with that in the rhizosphere of spinach and radish plants. *Appl. Environ. Microbiol.* 70:1182–1189.

Buchanan, R.L., and S.G. Edelson. 1996. Culturing enterohemorrhagic *Escherichia coli* in the presence and absence of glucose as a sample means of evaluating the acid tolerance of stationary-phase cells. *Appl. Environ. Microbiol*. 62:4009–4013.

Buckalew, J.J., D.W. Schaffner, and M. Solberg. 1996. Surface sanitation and microbiological food quality of a university foodservice operation. *J. Food Serv. Sys*t. 9:25–39.

Callister, S.M., and W.A. Aggar. 1987. Enumeration and chracterisation of *Aeromonas hydrophila* and *Aeromonas caviae* isolated from grocery store produce. *Appl. Environ. Microbiol*. 53:249–253.

CDC (Centers for Disease Control and Prevention). 2004. *Salmonella* serotype Timphimurium outbreak associated with commercially processed egg salad—Oregon, 2003. MMWR *Morb. Mortal. Wkly. Rep*. 53:1132–1134.

CFR (Code of Federal Regulations). Title 7, Parts 59.500 to 59.580. Sanitary egg processing and facility requirements. U.S. Government Printing Office, Washington, D.C.

CFR. Title 21, Part 114. Acidified foods. U.S. Printing Office, Washington, D.C.

Cheville, A.M., K.W. Arnold, C. Buchrieser, C.M. Cheng, and C.W. Kaspar. 1996. rpoS regulation of acid, heat, and salt tolerance in *Escherichia coli* O157:H7. *Appl. Environ. Microbiol*. 62:1822–1824.

Christiansen, L.N., and N.S. King. 1971. The microbial content of some salads and sandwiches at retail outlets. *J. Milk Food Technol*. 34:289–293.

Crepet, A., I. Albert, C. Dervin, and F. Carlin. 2007. Estimation of microbial contamination of food from prevalence and concentration data: application to *Listeria monocytogenes* in fresh vegetables. *Appl. Environ. Microbiol.* 73:250–258.

Decker, K.J. 2001. Deli salads grow up. http://www.foodproductdesign.com/articles/464/464_0501_2.html.

Doyle, M.P., N.L. Bains, J.L. Schoeni, and E.M. Foster. 1982. Fate of *Salmonella typhimurium* and *Staphylococcus aureus* in meat salads prepared with mayonnaise. *J. Food Prot*. 45:152–156.

Dunn, R.A., W.N. Hall, J.V. Altamirano, S.E. Dietrich, B. Robinson-Dunn, and D.R. Johnson. 1985. Outbreak of *Shigella flexneri* linked to salad prepared at a central commissionary in Michigan. *Public Health Rep*. 110:580–586.

Erickson, J.P., and P. Jenkins. 1991. Comparative *Salmonella* spp. and *Listeria monocytogenes* inactivation rates in four commercial mayonnaise products. *J. Food Prot*. 54:913–916.

Erickson, J.P., D.N. McKenna, M.A. Woodruff, and J.S. Bloom. 1993. Fate of *Salmonella* spp., *Listeria monocytogenes*, and spoilage microorganisms in home-style salads prepared with commercial real mayonnaise or reduced calorie mayonnaise dressings. *J. Food Prot*. 56:1015–1021.

Erickson, J.P., J.W. Stamer, M. Hayes, D.N. McKenna, and L.A. Van Alstine. 1995. An assessment of *Escherichia coli* O157:H7 contamination risks in commercial mayonnaise from pasteurized eggs and environmental sources, and behavior in low-pH dressings. *J. Food Prot*. 58:1059–1064.

Evans, M.R., C.D. Ribeiro, and R.L. Salmon. 2003. Hazards of healthy living: bottled water and salad vegetables as risk factors for *Campylobacter* infection. *Emerg. Infect. Dis*. 9:1219–1225.

Fowler, J.L., and W.S. Clark. 1975. Microbiology of delicatessen salads. *J. Milk Food Technol*. 38:111–113.

Gahan, C.G.M., B. O'Driscoll, and C. Hill. 1996. Acid adaptation of *Listeria monocytogenes* can enhance survival in acidic foods and during milk fermentation. *Appl. Environ. Microbiol*. 62:3128–3132.

Gillespie, I., C. Little, and R. Mitchell. 2000. Microbiological examination of cold ready-to-eat sliced meats from catering establishments in the United Kingdom. *J. Appl. Microbiol*. 88:467–474.

Glass, K.A., and M.P. Doyle. 1991. Fate of *Salmonella* and *Listeria monocytogenes* in commercial, reduced-calorie mayonnaise. *J. Food Prot*. 54:691–695.

Glass, K.A., J.M. Loeffelholz, J.P. Ford, and M.P. Doyle. 1992. Fate of *Escherichia coli* O157:H7 as affected by pH or sodium chloride in fermented, dry sausage. *Appl. Environ. Microbiol*. 58:2513–2516.

Gomez-Lucia, E., L. Goyache, J.L. Blanco, J.F.F. Garayzabal, J.A. Orden, and G. Suarez. 1987. Growth of *Staphylococcus aureus* and enterotoxin production in homemade mayonnaise prepared with different pH values. *J. Food Prot*. 50:872–875.

Guentert A.M., R.H. Linton, J.B. Luchansky, and M.A. Cousin. 2005. Behavior of *Listeria monocytogenes* in pH-modified chicken salad during refrigerated storage. *J. Environ. Health* 68:31–38, 43.

Hagenmaier, R.D., and R.A. Baker. 1998. A survey of the microbial population and ethanol content of bagged salad. *J. Food Prot*. 61:357–359.

Harvey, J., and A. Gilmour. 1993. Occurrence and characteristics of *Listeria* in food produced in Northern Ireland. *Int. J. Food Microbiol*. 19:193–205.

Hathcox, A.K., L.R. Beuchat, and M.P. Doyle. 1995. Death of enterohemorrhagic *Escherichia coli* O157:H7 in real mayonnaise and reduced-calorie mayonnaise dressing as influenced by initial population and storage temperature. *Appl. Environ. Microbiol*. 61:4172–4177.

Heaton J.C., and K.K. Jones. 2008. Microbial contamination of fruit and vegetables and the behaviour of enteropathogens in the phyllosphere: a review. *Appl. Microbiol*. 104:613–626.

Heisick, J.E., D.E. Wagner, M.L. Nierman, and J.T. Peeler. 1989. *Listeria* spp. found on fresh market produce. *Appl. Environ. Microbiol*. 55:1925–1927.

Hwang, C.-A. 2005. The effect of mayonnaise pH and storage temperature on the behavior of *Listeria monocytogenes* in ham salad and potato salad. *J. Food Prot*. 68:1628–1634.

Hwang, C.-A., and B.S. Marmer. 2007. Growth of *Listeria monocytogenes* in egg salad and pasta salad formulated with mayonnaise of various pH and stored at refrigerated and abuse temperatures. *Food Microbiol*. 24:211–218.

Hwang, C.-A., and M.L. Tamplin. 2005. The influence of mayonnaise pH and storage temperature on the growth of *Listeria monocytogenes* in seafood salad. *Int. J. Food Microbiol*. 102:277–285.

ICMSF (The International Commission on Microbiological Specifications for Foods). 1996a. *Microorganisms in Foods 5: Characteristics of Microbial Pathogens*. Blackie Academic & Professional. London, United Kingdom.

ICMSF. 1996b. *Microorganisms in Foods 6: Microbial Ecology of Food Commodities*. Blackie Academic & Professional. London, United Kingdom.

Isonhood, J.H., and M. Drake. 2002. *Aeromonas* species in food. *J. Food Prot*. 65:575–582.

Jacxens, L., F. Devlieghere, P. Falcato, and J. Debevere. 1999. Behaviour of *Listeria monocytogenes* and *Aeromonas* spp. on fresh cut produce packaged under equilibrium modified atmosphere. *J. Food Prot*. 62:1128–1135.

Johnston L.M., L.A. Jaykus, D. Moll, M.C. Martinez, J. Anciso, B. Mora, and C.L. Moe. 2005. A field study of the microbiological quality of fresh produce. *J. Food Prot*. 68:1840–1847.

Kaneko, K., H. Hayashidani, Y. Ohtomo, J. Kosuge, M. Kato, K. Takahashi, Y. Shiraki, and M. Ogawa. 1999. Bacterial contamination of ready-to-eat foods and fresh products in retail shops and food factories. *J. Food Prot*. 62:644–649.

Kessel A.S., I.A. Gillespie, S.J. O'Brien, G.K. Adak, T.J. Humphrey, and L.R. Ward. 2001. General outbreaks of infectious intestinal disease linked with poultry, England and Wales, 1992–1999. *Commun. Dis. Public Health* 4:171–177.

Khatib, R., M. Naber, N. Shellum, L. Ashton, K. Knowles, V. Giardina, and F.M. Wilson. 1994. A common source outbreak of gastroenteritis in a teaching hospital. *Infect. Control Hosp. Epidemiol*. 15:534–535.

Kumar, A., R.K. Agarwal, K.N. Bhilegaonkar, B.R. Shome, and V.N. Bachhil. 2001. Occurrence of *Campylobacter jejuni* in vegetables. *Int. J. Food Microbiol*. 67:153–155.

Lew, J.F., D.L. Swerdlow, M.E. Dance, P.M. Griffin, C.A. Bopp, M.J. Gillenwater, T. Mercatante, and R.I. Glass. 1991. An outbreak of shigellosis aboard a cruise ship caused by a multiple-antibiotic-resistant strain of *Shigella flexneri*. *Am. J. Epidemiol*. 134:413–420.

Lin, C., S.Y. Fernandao, and C. Wei. 1996. Occurrence of *Listeria monocytogenes*, *Salmonella* spp., *Escherichia coli* and *E. coli* 0157:H7 in vegetable salads. *Food Control* 7:135–140.

Long S.M., G.K. Adak, S.J. O'Brien, and I.A. Gillespie. 2002. General outbreaks of infectious intestinal disease linked with salad vegetables and fruit, England and Wales, 1992–2000. *Commun. Dis. Public. Health* 5:101–105.

Madden, J.H. 1992. Microbial pathogens in fresh produce—the regulatory perspective. *J. Food Prot*. 55:821–823.

McMahon, M.A.S., and I.G. Wilson. 2001. The occurrence of enteric pathogens and *Aeromonas* species in organic vegetables. *Int. J. Food Microbiol*. 70:155–162.

Merino, S., X. Rubires, S. Knochel, S., and J.M. Tomas. 1995. Emerging pathogens: *Aeromonas* spp. *Int. J. Food Microbiol*. 28:157–168.

Miller, L.G., and C.W. Kaspar. 1994. *Escherichia coli* O157:H7 acid tolerance and survival in apple cider. *J. Food Prot*. 57:460–464.

Miller, M.L., and E.D. Martin. 1990. Fate of *Salmonella enteritidis* and *Salmonella typhimurium* inoculated into an Italian salad dressing with added eggs. *Dairy Food Environ. Sanit*. 10:12–14.

Montville, R., and D.W. Schaffner. 2004. Statistical distributions describing microbial quality of surfaces and foods in food service operations. *J. Food Prot*. 67:162–167.

Neyts, K., G. Huys, M. Uyttendaele, J. Swings, and J. Debevere. 2001. Incidence and identification of mesophilic *Aeromonas* spp. from retail foods. *Lett. Appl. Microbiol*. 31:359–363.

Odumeru, J.A., S.J. Mitchell, and M.D. Alves. 1997. Assessment of the microbiological quality of ready-to-use vegetables for healthcare food services. *J. Food Prot*. 60:954–960.

Park, C.E., and G.W. Sanders. 1992. Occurrence of thermotolerant *Campylobacters* in fresh vegetables sold at farmers' outdoor markets and supermarkets. *Can. J. Microbiol*. 38:313–316.

Paul, M.E., and N.N. Potter. 1978. Bacterial growth in whole egg and egg substitutes including inoculation with *Staphylococcus aureus* and *Clostridium perfringens*. *J. Food Sci*. 43:803–806.

Perales, I., and M.I. Garcia. 1990. The influence of pH and temperature on the behavior of *Salmonella enteritidis* phage type 4 in homemade mayonnaise. *Lett. Appl. Microbiol*. 10:19–22.

Peterson, R.G., and S.E. Hartsell. 1955. The lysozyme spectrum of the Gram-negative bacteria. *J. Infect. Dis.* 96:75–81.

Raghubeer, E.V., J.S. Ke, M.L. Campbell, and R.S. Meyer. 1994. Fate of *Escherichia coli* O157:H7 and other coliforms in commercial mayonnaise and refrigerated salad dressing. *J. Food Prot*. 58:13–18.

Rodrique, D.C., E.E. Mast, K.D. Greene, J.P. Davis, M.A. Hutchinson, J. G. Wells, T.J. Barret, and P.M. Griffin. 1995. A university outbreak of *Escherichia coli* O157:H7 infections associated with roast beef and an unusually benign clinical course. *J. Infect. Dis*. 172:1122–1125.

Schlech, W.K., P.M. Lavigne, R.A. Bortolussi, A.C. Allen, E.V. Haldane, A.J. Wort, A.W. Hightower, S.E. Johnson, S.H. King, E.S. Nicholls, and C.V. Broome. 1983. Epidemic listeriosis—evidence for transmission by food. *N. Engl. J. Med*. 308:203–206.

Sivapalasingam, S., C.R. Friedman, L. Cohen, and R.V. Tauxe. 2004. Fresh produce: a growing cause of outbreaks of foodborne illness in the United States, 1973 through 1997. *J. Food Prot*. 67:2342–2353.

Smittle, R.B. 2000. Microbiological safety of mayonnaise, salad dressings, and sauces produced in the United States: a review. *J. Food Prot*. 63:1144–1153.

Stopforth, J.D, J.S. Ikeda, P.A. Kendall, and J.N. Sofos. 2004. Survival of acid-adapted or nonadapted *Escherichia coli* O157:H7 in apple wounds and surrounding tissue following chemical treatments and storage. *Int. J. Food Microbiol*. 90:51–61.

Szabo, E.A., K.J. Scurrah, and J.M. Burrows. 2000. Survey for psychrotrophic bacterial pathogens in minimally processed lettuce. *Lett. Appl. Microbiol*. 30:456–460.

Tauxe, R., H. Kruse, C. Hedberg, M. Potter, J. Madden, and K. Wachsmuth. 1997. Microbial hazards and emerging issues associated with produce; a preliminary report to the National Advisory Committee on microbiologic criteria for foods. *J. Food Prot*. 60:1400–1408.

Thunberg, R.L., T.T. Tran, R.W. Bennett, and R.N. Matthews. 2002. Microbial evaluation of selected fresh produce obtained at retail markets. *J. Food Prot*. 65:677–682.

Valero, M., L.A. Hernandez-Herrero, and M.J. Giner. 2007. Survival, isolation and characterization of a psychrotrophic *Bacillus cereus* strain from a mayonnaise-based ready-to-eat vegetable salad. *Food Microbiol*. 24:671–677.

Velazquez, L.C., M.E. Scudero, M.S. Digenaro, Y.M. Decortinez, and M.S. Guzman. 1998. Survival of *Aeromonas hydrophila* in fresh tomatoes stored at different temperatures and treated with chlorine. *J. Food Prot*. 61:414–418.

Weagant, S.D., J.L. Bryant, and D.H. Bark. 1994. Survival of *Escherichia coli* O157:H7 in mayonnaise and mayonnaise-based sauces at room and refrigerated temperatures. *J. Food Prot*. 57:629–631.

Wonderling, L.D., B.J. Wilkinson, and D.O. Bayles. 2004. The htrA (degP) gene of *Listeria monocytogenes* 10403S is essential for optimal growth under stress conditions. *Appl. Environ. Microbiol*. 70:1935–1943.

Wong, T.L., L. Hollis, A. Cornelius, C. Nicol, R. Cook, and J.A. Hudson. 2007. Prevalence, numbers, and subtypes of *Campylobacter jejuni* and *Campylobacter coli* in uncooked retail meat samples. *J. Food Prot*. 70:566–573.

Zhao, T., and M.P. Doyle. 1994. Fate of enterohemorrhagic *Escherichia coli* O157:H7 in commercial mayonnaise. *J. Food Prot*. 57:780–783.

Zhao, T., M.P. Doyle, and R.E. Besser. 1993. Fate of enterohemorraghic *Escherichia coli* O157:H7 in apple cider with and without preservatives. *Appl. Environ. Microbiol*. 59:2526–2530.

# chapter 3

# The safety of ready-to-eat dairy products

*Annel K. Greene, Zeynep B. Güzel-Seydim, and Atif Can Seydim*

*Contents*



> Those who cannot remember the past are condemned to repeat it.
>
> **George Santayana (1905)**

## 3.1 Milk as a food

Cattle, sheep, and goats were domesticated in the Near East at least 10,000 years ago. Up until recently, the earliest known uses of milk as a food were believed to have been in the 4th millennium in Britain and the 6th millennium in eastern Europe. However, by analyzing pottery fragments for milk residues, it recently was determined that milk was also in use in

southeastern Europe and northwestern Anatolia as early as the fifth to the seventh millennium. Northwestern Anatolia, in what is now modern-day Turkey, favored bovine milk probably because cattle growth conditions were more favorable in that region. Sheep and goats were common in Anatolia, but their use as dairy animals was less frequent (Evershed et al., 2008).

Milk contains a unique balance of proteins, fats, carbohydrates, and water. It contains nine essential nutrients. In an 8-ounce glass, milk provides 30% of the daily value of calcium, 25% of vitamin D, 16% of protein, 11% of potassium, 10% of vitamin A, 13% of vitamin $B_{12}$, 24% of riboflavin, 10% of niacin, and 20% of phosphorus (National Dairy Council, 2004). Because of the rich balance of readily available carbohydrates and proteins, milk is a highly perishable product susceptible to bacterial growth. Not only is spoilage a concern for palatability and loss of nutrients, but milk also can support the growth and transport of pathogenic microorganisms (Goff, 1995).

In order to prevent passage of foodborne disease entities through milk, ready-to-eat (RTE) dairy products are produced following strict modern sanitary guidelines to keep them microbiologically safe for consumers. Through the past several decades, the dairy industry and public health officials have developed a rigid series of processing and packaging guidelines for dairy processing. These guidelines take into consideration each potential hazard and provide a means of ensuring that no microbial hazard occurs within the RTE product. However, failure to properly follow the sanitary guidelines for processing dairy products or consumption of raw milk can lead to disease transmission.

Raw milk is milk as it comes from the dairy animal—unprocessed. If consumed as raw milk, the product does not follow the complete sanitary safety guidelines recommended by public health agencies for protecting against potential disease transmission via milk products and may pose a risk to consumers. There are groups of raw milk proponents who insist that raw milk and raw milk products possess extraordinary health benefits which hypothetically can cure diseases such as cancer, allergies, digestive disorders, and numerous other conditions. These raw milk proponents often assert that public health agencies and the dairy industry are involved in a conspiracy to rob consumers of these supposed health cures of raw milk (Sheehan, 2005). However, a review of scientific literature reveals that consumption of raw milk circumvents the public health protections afforded by decades of research and development into safe dairy food handling and processing procedures. The purpose of public health is to protect the population from harm. Public health agencies were formed as a result of public outcry over devastating and often fatal disease transmission through foods and water. These

agencies are charged with protecting the masses from harm by using the most modern scientific knowledge available. However, the proponents of raw milk demand that public health agencies ignore the safety of the public and ignore knowledge learned from the past about potential disease transmission via milk—all because of unsubstantiated benefits of raw milk. Modern scientific knowledge is acquired through controlled experimentation and observation of natural phenomenon. Prior to publication in peer-reviewed journals, scientists must submit their research findings for stringent scientific review to ensure scientific accuracy and reproducibility. The purported health benefits of raw milk have not been documented in such "peer-reviewed" scientific literature, whereas the safety of correctly pasteurized dairy products has been proven repeatedly over the past century. Therefore, a comparison of risks to benefits reveals that the safety of properly pasteurizing dairy products to protect the populace far outweighs unproven health benefits claimed due to consumption of raw milk.

A review of history provides lessons on dairy product safety and reasons for not repeating the mistakes of the past.

## *3.2 History*

Viral and bacterial disease in the dairy animal can be transmitted to humans through a number of means, including excretion of milk. Records indicate that as far back as the late 1500s, milk was identified as a potential source of disease in Europe. In Venice, in 1599, the senate banned selling or using milk, butter, or cheese during any epidemic. Public health safety was of such importance that the penalty for violating this law was death! Nearly 100 years later, in 1682, another law required burial of milk because of anthrax (Roadhouse and Henderson, 1941).

As early as the 1830s, the American public became concerned over the safety of milk sold in the larger cities. The practice of producing milk from animals confined within urban environments and fed distillery waste ("swill") was of concern to many consumers. "Swill milk" eventually was outlawed in the United States. As a result, commercial milk production moved to rural areas, and milk was transported into the cities. However, rural milk production was unsanitary, and unrefrigerated transportation to the city compounded the problems. Adulterants such as bicarbonate of soda, sugar, molasses, and chalk were frequently used to mask spoilage. Oftentimes, milk was not even milk but instead a curious blend of ingredients made to appear to be milk. In real milk and adulterated milk though, disease outbreaks continued in the United States and abroad. As each outbreak occurred, public health officials learned more about the nature of the disease transmission (Meckel, 1998).

In 1857, an outbreak of typhoid fever in Penrith, England, was reported and attributed to milk by local physician Dr. Michael Taylor. Ten years later, in 1867, this same Dr. Taylor also observed an outbreak of scarlet fever in the same locale and again concluded that the disease was spread via milk (Hammer, 1948).

In France, by 1864, Louis Pasteur had conducted his famous swan neck flask experiments and proven that microorganisms could be spread through contamination (Anonymous, 1903). A new generation of scientific discovery into the nature of diseases and epidemics emerged thereafter. Unfortunately, after the U.S. Civil War, American government reforms and public health agencies were limited by severe budget cuts brought on by the start of the economic depression in 1873. But by the mid-1870s, American public health officials had better scientific knowledge and were able to begin applying these principles to preventing disease spread. However, many so-called "food reformers" touted wild claims related to food safety based on wives' tales, misinformation, speculation, and unproven theories. Public health officials struggled to educate the public on the scientific principles for food sanitation against this passionate group. Eventually though, in the area of milk sanitation, the body of scientific data was so immense to prove the connection between adulterated milk and infant mortality that even the "food reformers" had to agree with public health officials. Based on this data, a number of laws concerning milk adulteration and designed especially to curb the high infant mortality rate were passed in the 1880s. Although the laws to improve the quality of milk supplies within cities eventually led to milk that was indeed milk and not adulterants designed to mask spoilage, there still was the challenge of bacterial contamination of the milk (Meckel, 1998).

The U.S. Public Health Service reported 238 human disease epidemics caused by milk during the period from 1911 to 1915 and 663 milk-borne disease epidemics in the period from 1918 to 1936 (Roadhouse and Henderson, 1941; Hammer, 1948). These disease epidemics included typhoid fever, paratyphoid fever, strep throat, scarlet fever, diphtheria, dysentery, and gastroenteritis. In 1922, an outbreak of streptococcal sore throat was reported in Portland, Oregon. Raw milk was determined to be the cause of the 487 cases and 22 deaths. Further investigation revealed that the source was a single cow with a single infected quarter of her udder. In 1927, 4,755 people became ill with typhoid fever in Montreal, Canada. Within 4 months, 453 of the patients had died. Improperly pasteurized milk was implicated as the causative agent. An outbreak of diphtheria in Austin, Texas, in the 1930s caused illness in 52 people. The cause of the outbreak was raw milk provided by a single cow that had been infected by a milker with a chronic nasal ulcer caused by *Corynebacterium diphtheriae.* In 1932, 211 out of 626 cases of human brucellosis reported in Iowa

were directly attributable to raw milk consumption. In 1936, an outbreak of scarlet fever in Wellsville, New York, caused 200 illnesses. Again during the investigation, the source was traced to raw milk consumed from one dairy. On this farm, one cow was found to have an infected udder (Roadhouse and Henderson, 1941).

As of 1938, raw milk caused 25% of all food and waterborne disease outbreaks in the United States. During the height of this period of milkborne outbreaks, in 1924, the first edition of the Pasteurized Milk Ordinance (PMO) had been released by the U.S. Public Health Service. State and local government agencies could voluntarily adopt the PMO as a model regulation providing the most up-to-date scientific guidelines for safe processing, packaging, and distribution of dairy products. Pasteurization, a thermal process, was used to decontaminate raw milk and strict procedures for preventing re-contamination were included in the recommended guidelines. Since its inception, there have been 25 revisions/updates to the PMO to incorporate new knowledge. After widespread adoption of the sanitary guidelines set forth in the PMO, now less than 1% of food and waterborne disease outbreaks in the United States are due to dairy foods (U.S. Public Health Service/Food and Drug Administration, 2003). Worldwide, public health agencies have adopted similar guidelines for processing raw milk into a microbiologically safe food (Turkish Ministry of Agriculture and Rural Affairs General Directorate of Protection and Control Vision, 2000; FAO/WHO, 2004; Food Standards Australia New Zealand, 2006; Canadian Food Inspection Service, 2007). If properly followed, the guidelines set forth in the PMO can prevent milkborne disease transmission.

Because of the hazards associated with raw milk consumption, the U.S. Food and Drug Administration banned the sale of raw milk to consumers in 1987 (Bren, 2004; Jayarao et al., 2006). However, intrastate raw milk sales are governed by state regulation, and as of 2004, 27 states allowed sale of raw milk directly to consumers (Headrick et al., 1997; Centers for Disease Control, 2007b). Between 1973 and 1992, 46 disease outbreaks were reported in 21 states due to raw milk consumption. A review of the outbreak trends indicated that states which had banned raw milk sale to consumers had drastically lower rates of milkborne disease (Headrick et al., 1998).

## *3.3 Sources of milk contamination*

The U.S. Centers for Disease Control and Prevention reported that between 1988 and 1992, bacterial pathogens were the cause of 79% of all foodborne outbreaks for which etiology was determined (Centers for Disease Control, 1996). Milk may be contaminated via a number of means. Diseased dairy animals may harbor such organisms as *Brucella abortis*, *Mycobacterium*

*tuberculi, Coxiella burnetti, Salmonella typhi, Corynebacterium diphtheriae,* and other potential pathogens. Mastitis from the Greek "mastos" for breast and "-itis" for inflammation is an infection of the mammary gland (Nieminen et al., 2007). In dairy animals, mastitis may be due to a number of Gram-positive and Gram-negative bacteria including *Staphylococci*, *Micrococci*, *Clostridium*, *Corynebacterium*, *Brucella, Listeria monocytogenes, Pseudomonas aeruginosa, Actinomyces farcinus,* and *Cryptococcus neoformans.*

Microorganisms may be transmitted to the dairy animal from other animals, humans, air, water, soil, and the equipment used for milking. Udders may be contaminated with pathogens from grass, feces, soil, milking equipment, and hands. The animal herself may shed viruses or bacteria. A diseased milker or an unsanitary milking machine may contaminate the milk as it is drawn from the udder. In smaller dairy operations, after collection, the milk is transported via buckets or portable vessels. In larger dairy operations, the milk is pumped via pipes to a holding vessel such as a bulk milk tank. At each transfer, milk may become contaminated by unsanitary equipment. Milk is then transferred from the farm to a milk processing/bottling operation. Any failure to keep the milk tank truck sanitary and to protect the milk against contamination during transport can lead to increased risk of spoilage and/or disease transmission via milk. Upon off-loading at the processing plant, pipes, pumps, and storage tanks must be free of disease-causing contaminants. In the case of raw milk, the product is then bottled without any protective step to destroy potential contaminants which may have been derived during all previous product-handling steps. Any pathogenic bacterial or viral contaminants inherent in the raw milk thus will be transferred in the product to the consumer. However, in the case of pasteurized milk, a thermal process is used to destroy any pathogenic microorganisms that were acquired from the animal udder through to the processing plant. This thermal process is designed to destroy pathogenic contaminants. Continual reevaluation and study of thermal processing conditions ensure that the heat process is sufficient. After the milk is pasteurized, it is critical that each step thereafter is sanitary to prevent post-process contamination. Particular areas of concern for post-process contamination are dirty equipment and utensils, packaging materials, the processing plant environment, personnel with inadequate hygiene/illness, and improper storage. The flow of potential contaminants to the consumer via raw versus pasteurized milk is illustrated in Figure 3.1. Ready-to-eat dairy foods can potentially contain pathogens due to use of raw milk, inefficient heat treatment, post-process contamination, or a combination of these factors. Approximately 45 outbreaks associated with unpasteurized milk and cheese made with raw milk occurred from 1998 to 2005, affecting a total of 1,000 individuals. However, improperly pasteurized milk is equally as dangerous as raw

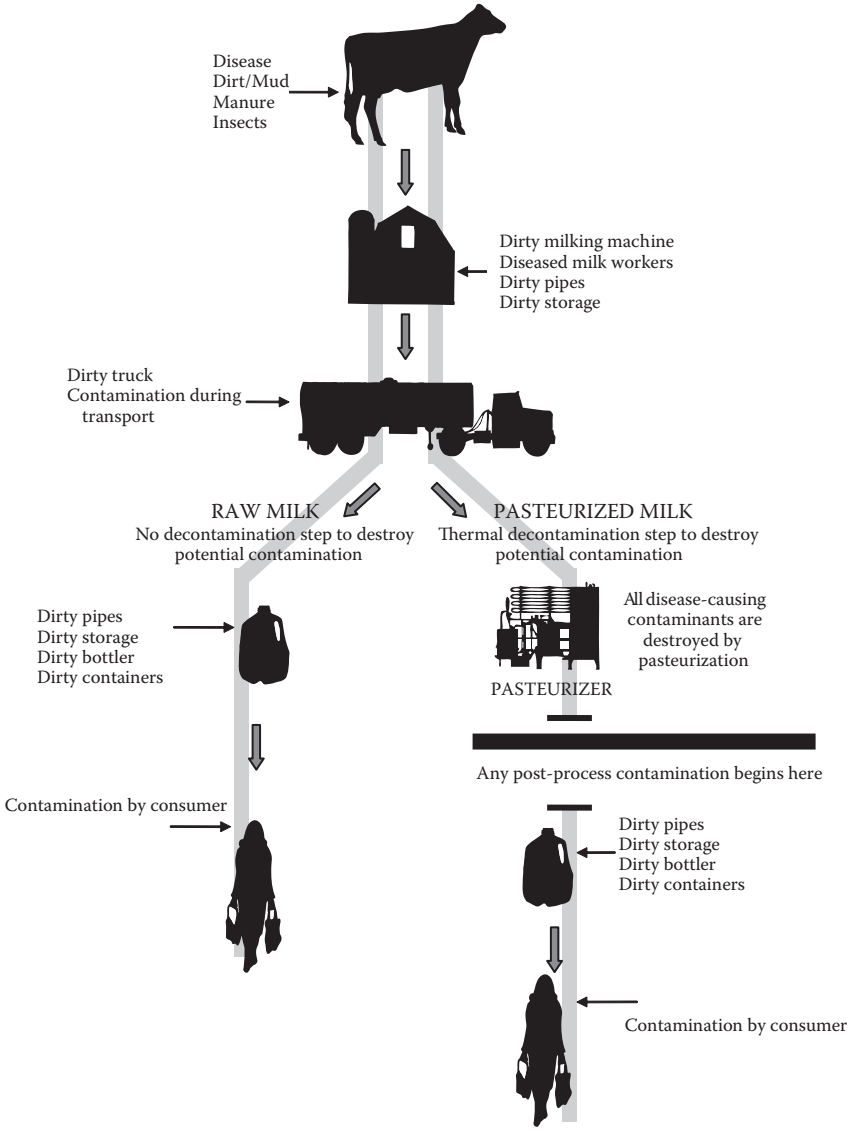


***Figure 3.1*** Contamination risks and potential flow of milkborne disease-causing microorganisms to the consumer in raw (left) versus pasteurized (right) milk. (Illustration by S. D. Chambers and A.K. Greene.)

milk in that the bacteria which were in the raw milk could potentially survive. Similarly, post-process contaminated milk or pasteurized milk that is mixed with raw milk poses potential hazards to consumers in that all of the risks of raw milk have been reintroduced into the product. The protections afforded by pasteurization are lost when raw milk is reintroduced and inoculates the milk with pathogenic bacteria. Most of the dairy-originated outbreaks recorded worldwide have occurred due to either consumption of raw milk or cheeses made using raw milk. However, due to the large scale of modern milk processing, post-process contamination of pasteurized milk has led to the greatest number of illnesses in recent years. Proponents of raw milk claim that the blame for these large-scale outbreaks is due to pasteurization. However, the blame is due to improper pasteurization resulting in the product being contaminated with disease-causing organisms from raw milk or the environment.

## *3.4 Pasteurization*

In an experiment conducted in March through April, 1862, Louis Pasteur and colleague Claude Bernard tested a process of thermal treatment to prevent decomposition of urine and blood (Royal Society of Chemistry, 2008). This process, which is now known as pasteurization, proved useful in the dairy industry for decontaminating products from bacterial and viral adulteration. Pasteurization of milk proved to be the ideal food safety measure. The process effectively destroys pathogenic bacteria and viruses and, if used in conjunction with practices that prevent post-process contamination, it prevents milkborne disease transmission. Pasteurization does not significantly change the flavor or color of the product, and because of the fluid nature of milk, it is easy to apply. Pasteurization is not sterilization; instead, pasteurization is designed to destroy all pathogenic contaminants, and during this process a percentage of spoilage microorganisms are also destroyed.

The time and temperature relationships used in the process were determined by lengthy thermal death time studies. Therefore, commercial pasteurization techniques were established to provide the minimum temperature and associated processing time required to destroy known pathogens which may be in raw milk (Table 3.1). Every particle of milk must be heated to these time temperature requirements in a processing device that meets standard sanitary requirements as set forth in the regulations. Dairy products containing higher fats and solids require additional processing rigor to assure sufficient lethality to inherent microorganisms. Pasteurization is but one tool used in the complete process of sanitary production of safe milk. Sanitary guidelines set forth in the PMO

***Table 3.1*** Minimum Pasteurization Temperature and Time Requirements

| Temperature | Time |
|---|---|
| 63°C (145°F)* | 30 minutes |
| 72°C (161°F)* | 15 seconds |
| 89°C (191°F) | 1.0 second |
| 90°C (194°F) | 0.5 seconds |
| 94°C (201°F) | 0.1 seconds |
| 96°C (204°F) | 0.05 seconds |
| 100°C (212°F) | 0.01 seconds |

* Processing parameters most commonly used by commercial dairy plants in the United States.

*Source:* From U.S. Public Health Service/Food and Drug Administration, 2003.

***Table 3.2*** Grade "A" Raw Milk and Milk Products for Pasteurization, Ultra-Pasteurization, or Aseptic Processing for Cow's Milk

| | |
|---|---|
| Temperature | Cooled to 10°C (50°F) or less within 4 hours or less of the commencement of the first milking, and to 7°C (45°F) or less within 2 hours after the completion of milking, provided that the blend temperature after the first milking and subsequent milkings does not exceed 10°C (50°F). |
| Bacterial limits | Individual producer milk not to exceed 100,000 per mL prior to commingling with other producer milk. Not to exceed 300,000 per mL as commingled milk prior to pasteurization. |
| Drugs | No positive results on drug residue detection methods as referenced in Section 6. |
| Laboratory somatic cell count | Individual producer milk not to exceed 750,000 per mL. |

*Source:* From U.S. Public Health Service/Food and Drug Administration, 2003.

require cleanliness, refrigeration, and hygienic handling of milk prior to pasteurization (Table 3.2).

Anecdotal evidence indicates that the established time-temperature relationships are sufficient to destroy all pathogenic microorganisms which may be contained within raw milk. Through the years, there have been outbreaks associated with pasteurized products, but in every case, the cause was traced to improper pasteurization or post-process re-contamination of the product. Decades of data have proven that pasteurization destroys and decontaminates raw milk, and if sanitary guidelines

are followed to protect the product from post-process contamination, then foodborne illness from milk should be prevented. However, there is considerable evidence of foodborne illness from raw milk, from improperly pasteurized milk, and from post-process contaminated milk. The same is true of other dairy products such as flavored milk, cheese, yogurt, and ice cream. Butter has been implicated in only a very few foodborne disease outbreaks (Hammer, 1948).

## 3.5 *Disease outbreaks associated with fluid milk products*

Several pathogenic microorganisms have potential to be present in raw and improperly processed dairy foods. These organisms can cause a variety of moderate to severe diseases ranging from gastroenteritis to chronic diarrhea to death (Centers for Disease Control, 1984d, 2001b). A number of disease outbreaks have been reported in the United States and Canada attributable to fluid milk products (Table 3.3).

Enterohemorrhagic *Escherichia coli* are Gram-negative short rods that are capable of producing one or more potent toxins that can cause severe disease in humans. The most notable of these strains is *E. coli* O157:H7. The illness manifests as a severe abdominal cramping and watery diarrhea followed by severely bloody diarrhea. Vomiting may occur, and fever is usually absent or minor. The disease can become severe and involve organs such as the kidney, but normally it is self-limiting and resolves on average by 8 days. Research data is incomplete, but it is believed that the infective dose for enterohemorrhagic *E. coli* may be as low as 10 cells (U.S. Food and Drug Administration Center for Food Safety and Applied Nutrition, 2009). Cattle have been reported as a source of *E. coli* O157:H7 (Lahti et al., 2002).

Allerberger et al. (2001) reported *Escherichia coli* O157 infection in a 9-year-old boy who had been fed one sample of raw goat milk by a farmer during a school visit. He became ill with bloody diarrhea and was hospitalized. After discharge, he remained an asymptomatic carrier of *Escherichia coli* O157:H-. Unfortunately, his younger sister (27 months) who had not consumed the raw milk, later became ill with the same sorbitol nonfermenting, Shiga toxin 2c-producing, *E. coli* O157:H- strain. Medical and public health personnel concluded that the younger sister became ill due to person-to-person transfer of the disease which was of raw milk origin.

In 2005, an outbreak of *E. coli* O157:H7 was reported in Washington due to raw milk obtained through a "cow share" program with five dairy cows. Sale of raw milk was legal in the state of Washington via licensed, inspected farms meeting state sanitary standards. Milk provided via this

*Table 3.3* Milkborne Illness Caused by Fluid Milk Products

| Year | Location | Causative Organism | Number of People Ill | Age Range of Ill | Number of Fatalities | Reason | Reference |
|---|---|---|---|---|---|---|---|
| | | | **Illness Due to Raw Milk** | | | | |
| 1980 | Montana | *Salmonella* Typhimurium | 105 | 3 weeks to 71 years old | 0 | Raw milk | Centers for Disease Control, 1981b |
| 1981 | California | *Salmonella* Saint-Paul | 1 | Infant | 0 | Certified raw milk | Centers for Disease Control, 1981a |
| 1981 | Oregon | *Campylobacter jejuni* | 91 | Unknown | 0 | Raw milk | Centers for Disease Control, 1981a |
| 1981 | Kansas | *Campylobacter jejuni* | 43 | Unknown | 0 | Raw milk from commercial dairy | Centers for Disease Control, 1981c |
| 1981 | Washington | *Salmonella* Dublin | 18 | 8 months to 71 years old | 0 | Raw milk from commercial dairy | Centers for Disease Control, 1981d |
| 1983 | Pennsylvania | *Campylobacter jejuni* | 57 | School children and adults in two outbreaks | 0 | Raw milk from farm tour | Centers for Disease Control, 1983a |
| 1984 | Minnesota | Unknown | 122 | Unknown | 0 | Raw milk | Osterholm et al. 1986 |

*(continued on next page)*

***Table 3.3 (continued)*** Milkborne Illness Caused by Fluid Milk Products

| Year | Location | Causative Organism | Number of People Ill | Age Range of Ill | Number of Fatalities | Reason | Reference |
|---|---|---|---|---|---|---|---|
| 1984 | California | *Campylobacter jejuni* | 12 | 9 children and 3 adults | 0 | Certified raw milk, ice cream, and kefir | Centers for Disease Control, 1984e |
| 1985 | California | *Campylobacter jejuni* | 23 | Unknown | 0 | Raw milk | Centers for Disease Control, 1986 |
| 1986 | U.S. | *Escherichia coli* O157:H7 | 2 | 5 and 13 months | 0 | Raw milk | Martin et al., 1986 |
| 2001 | Canada | *Escherichia coli* O157:H7 | 5 | Unknown | 0 | Raw goat's milk | Public Health Agency of Canada, 2002 |
| 2001 | Wisconsin | *Campylobacter jejuni* | 75 | 2 to 63 years old | 0 | Raw milk from cow leasing program | Centers for Disease Control, 2002 |
| 2002 | Indiana, Illinois, Ohio | *Salmonella* Typhimurium | 62 | 1 to 70 years old | 0 | Raw milk | Centers for Disease Control, 2003; Mazurek et al., 2004 |
| 2003 | Utah | *Campylobacter jejuni* | 13 | 11 to 50 years old | 0 | Raw milk served at high school dinner | Peterson, 2003 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 2005 | Washington, Oregon | *Escherichia coli* O157:H7 | 4 | Unknown | 0 | Raw milk from cow share program | Centers for Disease Control, 2007a |
| 2007 | Pennsylvania | *Salmonella* Typhimurium | 8 | Unknown | 0 | Raw milk | Centers for Disease Control, 2007b |
| | | **Illness Due to Improperly Pasteurized or Post-Process Contaminated Milk** | | | | | |
| 1978 | New York | *Yersinia enterocolitica* | 38 | School children | 0 | Improper procedures: contaminated chocolate mixed into milk post-pasteurization | Black et al., 1978 |
| 1983 | Massachusetts | *Listeria monocytogenes* | 49 | 7 fetuses, 42 immuno-compromised adults | 14 | Improper procedures: pasteurized milk contaminated with raw milk | Fleming et al., 1985 |
| 1984 | Kentucky | *Salmonella* Typhimurium | 16 | Unknown | 0 | Improper pasteurization procedures | Centers for Disease Control, 1984c |

*(continued on next page)*

***Table 3.3 (continued)*** Milkborne Illness Caused by Fluid Milk Products

| Year | Location | Causative Organism | Number of People Ill | Age Range of Ill | Number of Fatalities | Reason | Reference |
|---|---|---|---|---|---|---|---|
| 1985 | Illinois | *Salmonella* Typhimurium | 168,000 to 198,000 estimated; 16,000 cases confirmed by culture | | | Improper procedures: pasteurized milk contaminated with raw milk | Centers for Disease Control, 1985; Ryan et al., 1987 |
| 1994 | Illinois | *Listeria monocytogenes* | 45 | 3 to 79 years old | 0 | Improper procedures: post-process contamination of chocolate milk | Dalton et al., 1997 |
| | | | **Illness Due to Pasteurized Milk** | | | | |
| 1982 | Tennessee, Arkansas, Mississippi | *Yersinia enterocolitica* | 172 | 6 weeks to 73 years old | 0 | Pasteurized milk; no identifiable cause of contamination determined | Tacket et al., 1984 |

*Source:* From the Milk Quality Improvement Program, Department of Food Science, Cornell University, 2007.

cow share program was consumed by 140 people, and 13% of those became ill. Of these 18 patients, 94% of them had diarrhea, 72% had bloody diarrhea, and 72% experienced abdominal cramps. *E. coli* O157:H7 was isolated from raw milk samples and from one patient (Centers for Disease Control, 2007a).

*Campylobacter jejuni* is a microaerophilic, Gram-negative motile rod-shaped bacteria which is now identified as the foremost cause of foodborne bacterial diarrheal disease in the United States. The disease manifests 2 to 5 days post-ingestion as diarrhea which may be bloody, along with nausea, abdominal pain, fever, headache, and muscle aches. The symptoms normally occur for 7 to 10 days duration. The illness is caused by an infection due to a low infective dose as low as 400 to 500 bacteria. Although rare, infections can lead to further complications including arthritis, severe kidney damage via hemolytic uremic syndrome, or, if septicemia proceeds, any organ may be involved. Infections caused by *C. jejuni* are fatal in approximately 1 out of every 1000 cases (U.S. Food and Drug Administration Center for Food Safety and Applied Nutrition, 2009). Doyle and Roman (1982) reported that *C. jejuni* was present in 0.9% of raw milk samples obtained from nine farms and from 64% of dairy cows. With this data, Doyle and Roman reiterated the need for proper pasteurization of milk and avoidance of raw milk consumption.

In 1981, an outbreak of gastrointestinal illness was caused by *C. jejuni* in the Atlanta, Georgia, suburbs. Correlation data indicated the source was raw milk, and although *C. jejuni* was isolated from cows at the raw milk source, the organism was never isolated in the milk (Potter et al., 1983).

In 1984, an outbreak of *C. jejuni* illness involving kindergarten children and adults occurred in California after a group field trip to a local certified raw milk bottling plant. During the tour, the group was served certified raw milk, ice cream, and kefir. Between 3 to 6 days later, 9 children and 3 adults had become ill with fever and gastroenteritis. Stool samples indicated *C. jejuni.* Since only those who visited the raw milk facility became ill, it is believed that raw milk was the mode of disease transmission (Centers for Disease Control, 1984e).

In April, 1986, an outbreak of *C. jejuni* illness occurred in elementary school children. Milk was implicated as the source, and further investigation revealed that milk had been improperly vat pasteurized at 135ºF (57.2ºC) for 25 minutes rather than at the required 145ºF (62.8ºC) for 30 minutes. As a result of this violation of pasteurization regulations, the children suffered diarrhea, cramps, nausea, fever, vomiting, and bloody stools (U.S. Food and Drug Administration Center for Food Safety and Applied Nutrition, 2009). In another outbreak, 120 cases of *C. jejuni* infections were caused by drinking raw milk in Kansas (Centers for Disease Control, 1996).

In late 2001, an outbreak of *C. jejuni* occurred in northwestern Wisconsin due to consumption of raw milk. The 75 reported cases involved patients ranging from 2 to 63 years old with a median age of 30 years. The patients suffered diarrhea, abdominal cramps, fever, and nausea. Approximately one-fourth of the patients had grossly bloody diarrhea. Raw milk sale is illegal in Wisconsin. However, the owners of the dairy had circumvented the law by devising a "cow leasing" program in which consumers leased all or part of a cow. In return, the dairy farm operators milked the cows and stored the milk in a commingled bulk tank. "Owners" received their milk via home delivery or by picking it up at the farm (Centers for Disease Control, 2002).

*Salmonella* is another rod-shaped, motile, Gram-negative organism which causes foodborne infection. Symptoms include nausea, vomiting, abdominal cramps, diarrhea, fever, and headache and last for 1 to 2 days or longer. The infective dose may be as low as 15 to 20 cells (U.S. Food and Drug Administration Center for Food Safety and Applied Nutrition, 2009).

In 1981 and 1982, 46 and 70 cases of *Salmonella* Dublin illness, respectively, were reported to health agencies in California and in each year 24% of the patients had consumed certified raw milk. In 1983, there were 123 cases of *S.* Dublin illness, and 44% reported consuming certified raw milk. A risk assessment performed at this time revealed that the risk of *S.* Dublin illness was 458.3 per million population for people drinking 1 pint daily of raw milk versus 2.9 per million population for people who did not consume any raw milk. Therefore, consuming raw milk increased risk of *S.* Dublin illness by a factor of 158. This was equivalent to a 15-fold higher correlation between raw milk consumption and *S.* Dublin illness than the correlation between cigarette smoking and lung cancer (Centers for Disease Control, 1984a).

In April 1984, an outbreak of gastroenteritis was caused by *Salmonella* Typhimurium at a convent in western Kentucky. Milk was implicated as the cause, and further investigation revealed that improper pasteurization at 130°F (54.5°C) for 30 minutes rather than the required 145°F (62.8°C) for 30 minutes had allowed the pathogens to survive (Centers for Disease Control, 1984c).

In March and April 1985, the largest known outbreak of Salmonella illness in the United States was caused by contaminated pasteurized 2% fat milk from a processing plant in Illinois. More than 16,000 confirmed cases of salmonellosis occurred in six states. In total, between 168,000 and 198,000 cases of salmonellosis were estimated to have been caused by this milkborne outbreak. Investigators determined that the pasteurization equipment had been modified, resulting in the pasteurized milk being contaminated with raw milk (Centers for Disease Control, 1985a).

In 2007, the Pennsylvania Department of Health and the Pennsylvania Department of Agriculture received reports of illness from raw milk. Investigation revealed two separate incidents of diarrheal illness caused by *S.* Typhimurium due to consumption of raw milk from the same dairy. A total of 275 customers purchased raw milk from this dairy, and of the cases reported to authorities, 29 people were confirmed to have been made ill by the raw milk. Raw milk sales are allowed in Pennsylvania from permitted raw milk farms provided warning notices describing the potential hazards of raw milk are posted. In addition to fluid milkborne illness, it was found that milk from this same dairy had been used to make queso fresco cheese which was illegally sold unlabeled in a grocery store. Fourteen percent of the victims became ill from the raw milk cheese. After the raw milk dairy's permit was suspended, another outbreak of *S.* Typhimurium occurred. Investigation revealed that the dairy had continued to distribute raw milk to the public even though its raw milk license was suspended. The raw milk license for this dairy was then revoked. The victims ranged in age from 5 months to 76 years with a median age of 6 years old (Centers for Disease Control, 2007b).

In a survey of pathogenic bacteria in raw milk in Pennsylvania, 13% of all raw milk samples contained one or more disease-causing bacteria. *C. jejuni* was present in 2% of all raw milk samples, Shiga toxin–producing *E. coli* was found in 2.4% of the samples, and *Listeria monocytogenes* was found in 28% of the samples. *Salmonella* was found in 6% of the raw milk samples (Jayarao et al., 2006).

*Yersinia enterocolitica* is a small, Gram-negative rod bacterium which causes diarrhea and/or vomiting, fever, and severe abdominal pain. The abdominal pain can mimic appendicitis and, thus, is caused "pseudoappendicitis." In a survey of pathogenic bacteria in raw milk in Pennsylvania, *Y. enterocolitica* was isolated from 1.2% of the samples (Jayarao et al., 2006).

In 1976, an outbreak of yersiniosis occurred in Oneida County, New York, involving school children. This was the first reported yersiniosis incident in the United States in which a food vehicle was identified. It was later determined that chocolate mix was added to previously pasteurized milk and not re-pasteurized (Black et al., 1978).

An outbreak of *Y. enterocolitica* O:8 occurred in October 1995 in Vermont and New Hampshire. Bottled pasteurized milk was identified as the source of the infection. No deficiencies were identified in the equipment or procedures, but post-process contamination was suspected as a pig on the farm tested positive for the same strain of *Y. entercolitica*. Ten people, ranging in age from 6 months to 44 years old, became ill. Three people were hospitalized, and one underwent an appendectomy (Ackers et al., 2000).

## 3.6 *Disease outbreaks associated with cheese products*

Cheeses made from raw milk pose risks to consumers. In recent years, a higher incidence of foodborne outbreaks in the United States has been associated with the Mexican-type cheeses that were homemade, produced, and sold without regulatory permits, or purchased during international travels. It was reported that the production of Latin-style cheeses increased from 68.8 million lbs. in 1997 to 190.5 million lbs. in 2007 in the United States (National Agricultural Statistical Services, 2008).

West (2008) delineated the differences between raw milk cheese advocates and pasteurized milk cheese advocates. He described raw milk advocates as "value based" and pasteurization advocates as "scientific based" to indicate the difference in judgment via desire versus reproducible fact. Evaluation of the cheeses associated with illness indicated that the most common product was fresh cheese made with raw milk and possessing a relatively high pH, high water activity, and low salt content. These conditions are favorable for growth of pathogenic microorganisms. Proponents of raw milk cheese claim that the final product is tastier and healthier than pasteurized milk cheese. However, cheeses made from raw milk have been the source of several foodborne outbreaks in the United States (Table 3.4). MacDonald et al. (2005) reported 5 stillbirths, 3 premature births, and 3 infected newborns due to listeriosis in Winston-Salem, North Carolina, after the mothers consumed Mexican-style soft cheese made from raw milk (Centers for Disease Control, 2001a). Makino et al. (2005) reported an outbreak of foodborne listeriosis due to cheese in Japan, during 2001. Results of an investigation revealed extensive environmental contamination of the cheese processing plant with *L. monocytogenes*. Eighty-six people became ill during the outbreak.

The U.S. Centers for Disease Control reported that Group C streptococcal infections were identified in northern New Mexico due to consumption of queso blanco, which was produced in a small farm from raw cow milk (Centers for Disease Control, 1983b).

Multidrug-resistant *Salmonella* Typhimurium DT104 was linked to foodborne disease outbreaks after the consumption of unpasteurized, homemade Mexican-style queso fresco in Yakima County, Washington, and in northern California (Cody et al., 1999; Villar et al., 1999). An outbreak of multidrug-resistant *Salmonella enteric* serotype Newport infections was associated with consumption of unpasteurized Mexican-style aged cheese in Illinois from March 2006 to April 2007 (Centers for Disease Control, 2008).

Shiga toxin–producing *E. coli* is prevalent in dairy cattle and their products (Hussein and Sakuma, 2005). Many countries allow production

***Table 3.4*** Milkborne Illness Caused by Milk Products: Cheese and Ice Cream

| Year | Location | Causative Organism | Number of People Ill | Age Range of Ill | Number of Fatalities | Reason | Reference |
|---|---|---|---|---|---|---|---|
| | | | **Illness Due to Cheese and Ice Cream** | | | | |
| 1973 | Unreported | *Brucella melitensis* | 3 | Unknown | 0 | Fresh Mexican cheese made with raw milk | Eckman, 1975 |
| 1983 | New Mexico | Group C *Streptococcus* | 16 | Unknown | 0 | Homemade fresh Mexican-style cheese from contaminated raw milk | Centers for Disease Control, 1983b |
| 1984 | Canada | *Salmonella Typhimurium* | | | | Cheese | Centers for Disease Control, 1984b |
| 1985 | California | *Listeria monocytogenes* | 141 | 93 pregnant/ neonatal, 38 immuno-compromised adults, 5 elderly | 10 neonatal; 20 fetal | Mexican-style soft cheese contaminated with raw milk | Centers for Disease Control, 1985b; Linnan et al., 1988 |

*(continued on next page)*

***Table 3.4 (continued)*** Milkborne Illness Caused by Milk Products: Cheese and Ice Cream

| Year | Location | Causative Organism | Number of People Ill | Age Range of Ill | Number of Fatalities | Reason | Reference |
|---|---|---|---|---|---|---|---|
| 1994 | Minnesota source, nationwide distribution and outbreak | *Salmonella enteritidis* | 224,000 estimated ill; 593 confirmed by culture | | | Ice cream products: pasteurized ice cream premix contaminated with raw eggs during shipping | Centers for Disease Control, 1994; Hennessey et al., 1996 |
| 2001 | North Carolina | *Listeria monocytogenes* | 12 | 10 pregnant women | | Homemade fresh Mexican-style cheese from contaminated raw milk | Centers for Disease Control, 2001a |
| 2004 | New York | *Mycobacterium bovis* | 35 cases of human tuberculosis | | | Fresh cheese from Mexico (pasteurization status unknown) | Centers for Disease Control, 2005 |
| 2007 | Kansas | *Campylobacter jejuni* | 68 | Unknown | Unknown | Raw milk cheese | Kansas Department of Health and Environment, 2007 |

*Source:* From the Milk Quality Improvement Program, Department of Food Science, Cornell University, 2007.

of raw milk cheese provided that it is aged for a certain length of time. It has always been believed that the process of aging eliminates pathogenic bacteria. Castellano cheese is an uncooked and hard/semi-hard Spanish-type cheese made from raw ewe's milk. Caro and Garcia-Armesto (2007) analyzed 83 Castellano cheese samples with different aging times (2.5, 6, and 12 months) and were able to detect *E. coli* O157:H7 and other Shiga toxin–producing *E. coli* in 2.4% of the samples, including one of the 12-month aged cheese samples.

In June 1998, eight laboratory-confirmed and four suspected *E. coli* O157:H7 cases were reported in west-central Wisconsin. The causative agent was determined to be fresh cheese curds (less than 60 days old) made from raw milk at a dairy processing plant (Centers for Disease Control, 2000).

Vernozy-Rozand et al. (2005) stated that in France, goat milk cheeses are usually produced from raw milk. They conducted a study on the growth and survival of *E. coli* O157:H7 in raw goat milk cheeses. Raw goat milk inoculated with *E. coli* O157:H7 was used to make experimental cheeses. It was determined that while the number of the *E. coli* O157:H7 population decreased, viable cells were still present after 42 days of ripening.

A large regional outbreak of gastroenteritis occurred in Spain in 1995–1996 due to consumption of pasteurized fresh cheese. The source of this outbreak was traced to post-pasteurization contamination with *Shigella sonnei* (Garcia-Fulgueiras et al., 2001).

Botulism outbreaks due to the consumption of dairy products are rare. An outbreak of botulism occurred after consumption of tiramisu, a traditional uncooked Italian dessert made with mascarpone cheese (Aureli et al., 2000). In this outbreak, eight cases occurred in three different cities in southern Italy. Symptoms included abdominal pain, vomiting, nausea, diplopia, and difficulty in speaking after consumption of the tiramisu. Mascarpone cheese is a soft, high pH, high moisture, low salt cheese. In this outbreak, the time between the consumption of dessert and the start of the gastrointestinal symptoms was 8 to 72 h; neurological symptoms occurred thereafter. One victim died after 37 days of hospitalization. The remaining patients recovered after 37 to 78 days hospitalization. *C. botulinum* Type A spores were found in 5 out of 34 mascarpone cheese samples collected from the stores. However, samples of the cheese obtained from the processing plant did not contain spores. Cheese was made from pasteurized milk. However, investigators concluded that with a pH of 6.1 to 6.2 and water activity of 0.945 to 0.988, post-process contamination and possible temperature abuse were involved (Aureli et al., 2000).

In yet another case, cheese sauce caused illness in 5 people and the death of another in Georgia. The causative agent was identified as *C. botu-*

*linum* contamination of the cheese sauce which had been opened and left unrefrigerated at 22°C for a length of time (Townes et al., 1996).

After a nationwide outbreak of *Salmonella paratyphi* in France was traced to unpasteurized goat's milk cheese, 30 tons of cheese was destroyed. The pathogen was detected in cheese samples from the cheese manufacturing plant (Desenclos et al., 1996).

Unpasteurized goat cheese produced in Mexico and sold by unlicensed vendors from their cars caused an outbreak of *Brucella melitensis* infections in Texas. The 29 victims were Mexican immigrants. Fourteen people were hospitalized, and one person died (Centers for Disease Control, 1983c, 1990).

In a report by do Carmo et al. (2002), a foodborne illness outbreak was described as due to enterotoxigenic strains of *Staphylococcus* present in Minas cheese and raw milk in Brazil.

## 3.7 *Disease outbreaks associated with other dairy products*

Usually low pH fermented dairy products like yogurt are not conducive for growth of foodborne pathogens such as *C. botulinum* and *E. coli* O157. However, an outbreak of botulism occurred in Bitlis, in the eastern part of Turkey, due to the consumption of a traditional type of concentrated yogurt. Ten members of one family suffered symptoms similar to those of botulism: dry mouth, difficulty in talking and swallowing, weakness, headache, and blurred vision. All of the family members were administered botulinum antitoxin. All ten were hospitalized, yet two of the family members died. Type A botulinal toxin was found in the patients' blood serum and attributed to consumption of yogurt. "Suzme yogurt" is a condensed yogurt which is traditionally produced in small villages. After yogurt production, excess free water is drained from the yogurt by hanging it in cloth bags. The concentrated yogurt is then placed into a plastic container. In this case, the container was buried in the ground for 2 months before consumption. The container was damaged and the yogurt contaminated with soil (Akdeniz et al., 2007).

An outbreak of verocytotoxin-producing *E. coli* O157 infection was associated with yogurt consumption in England. During this outbreak 11 people became ill due to post-pasteurization contamination (Morgan et al., 1993).

*Mycobacterium avium* subspecies *paratuberculosis* is the etiologic agent of Johne's disease, which causes chronic inflammatory bowel disease in ruminants. This same microorganism has been associated with the chronic gastrointestinal Crohn's disease in humans (Beier and Pillai,

2007). From 2001 to 2004, 35 cases of *M. bovis* infection were reported in New York City. A 15-year-old boy died due to peritoneal tuberculosis caused by *Mycobacterium bovis*. *M. bovis* is a pathogen that mainly affects cattle. However, humans may become infected by consumption of unpasteurized dairy products (Centers for Disease Control, 2005).

The high fat, protein, sugar, and water content and neutral pH of ice cream creates a desirable environment for bacterial growth. Foodborne disease outbreaks from ice cream have been usually associated with use of raw milk, unpasteurized eggs, or environmental post-process contamination. In July 1986, Kraft, Inc. voluntarily recalled 25,000 cases (approximately 1.8 million squares) of its Polar B'ar ice cream bars made at its Richmond, Virginia, processing plant. The concern prompting the recall was for *Listeria monocytogenes* contamination (Farber and Peterkin, 1991). According to Ryser and Marth (1999), more than 50 recalls of dairy products have been conducted since 1985 for *Listeria*. However, they point out that no cases of listeriosis have been attributed to dairy foods in the United States. In late 1994, an outbreak of *Salmonella enteritidis* foodborne illness was reported to the Minnesota Department of Health. An investigation revealed that victims had consumed Schwann's brand of ice cream. The source of the infection was traced to a tank truck that had been used to haul unpasteurized eggs immediately prior to carrying pasteurized ice cream mix from one processing plant to another. In violation of regulations, the ice cream mix was not re-pasteurized after transport. The mistake resulted in more than 24,000 people infected with salmonellosis (Hennessy et al., 1996).

## *3.8 Market and processing environment studies*

A number of market studies have been conducted to determine potential pathogenic bacterial content of RTE dairy foods and raw ingredients. Surveys, reviews, modeling, and genotyping all improve the base of knowledge about how a pathogen is transmitted via dairy foods to consumers (Farber and Losos, 1988; Donnelly, 1990; El-Gazzar and Marth, 1992; Holm et al., 2002; Hussein and Sakuma, 2005; Hamdi et al., 2007; Ho et al., 2007; Hong et al., 2007; El-Sharoud et al., 2008).

Clark et al. (2006) conducted a study on the prevalence of viable *M. avium* subspecies *paratuberculosis* and its genetic components, *IS*200 and *hsp*X, in 98 cheese curd samples manufactured from pasteurized milk. Prescreen results indicated that samples did not have the viable cells; however, 5% of the curd samples contained both of the genetic components. Further analysis by polymerase chain reaction (PCR) indicated that 1% of the samples tested positive with both primers after the PCR confirmatory slant rinse. In Switzerland, 143 cheese samples made from raw

milk were analyzed for *Mycobacterium avium* subsp. *paratuberculosis* using cultivation and real-time PCR. No viable cells were found, but F-57 based real-time PCR identified 4.2% of the samples as positive for the presence of the organism's genetic material (Stephan et al., 2007).

Ayele et al. (2005) studied *M. avium* subsp. *paratuberculosis* cultured from raw and commercially pasteurized cow milk in the Czech Republic. In this study, 19.7% of the raw milk from herds infected with *M. avium* subsp. *paratuberculosis* tested positive for the organism. Disturbingly, upon pasteurizing the milk from the contaminated herds, 1.6% to 2% of cartons of commercially and locally pasteurized milk, respectively, tested positive for the organism. These findings indicate that a review of pasteurization standards may be required in areas where dairy animals are infected with *M. avium* subsp. *paratuberculosis.*

Callaway et al. (2005) measured the prevalence and variety of *Salmonella* species in lactating dairy cattle in four U.S. states. The researchers concluded that 27% to 31% of U.S. dairy herds are infected with *Salmonella*.

In a total of 2,948 dairy products collected from different regions of Italy, none tested positive for the presence of verocytotoxin (VTEC) *E. coli* O157, even though 1,814 samples were produced from unpasteurized milk (Conedera et al., 2004). In Lima, Peru, approximately 8% of soft cheese samples tested contained Shiga toxin–producing *E. coli* O157:H7 (Mora et al., 2007). In a study in Ireland, *E. coli* O157:H7 and other VTEC *E. coli* strains were detected in raw milk filters from 74 milk production facilities. Five *E. coli* VTEC O157:H7, 17 *E. coli* O26 (four of which were VTEC), and 22 non-virulent *E. coli* O157 strains were determined using PCR (Murphy et al., 2007).

Muehlherr et al. (2003) tested 407 samples of bulk tank milk from farms in Switzerland. Of these samples, 344 were of goat milk and 63 were of ewe milk. No *Campylobacter* spp. and *Salmonella* spp. were detected in any of the samples, but *Enterobacteriaceae* were detected in 61.6% of the goat's milk samples and 71.4% of the ewe's milk samples. *S. aureus* was present in 31.7% samples of goat's milk and 33.3% samples of ewe's milk. Using PCR testing, the researchers determined that 16.3% of the goat's milk and 12.7% of the ewe's milk samples were positive for Shiga toxin–producing *E. coli,* and 23.0% of the goat's milk and 23.8% of the ewe's milk were presumptively positive for *M. avium* subsp. *paratuberculosis*. These results also indicate the hazard of consuming raw goat's or raw ewe's milk products.

*Y. enterocolitica* has the ability to grow in milk at refrigeration temperatures. Lovett et al. (1982) reported that there are heat-resistant strains. Fortunately, the pasteurization process recommended by U.S. Public Health Service exceeds the temperature required for destruction of these heat-stable strains. In another study, the prevalence of *Y. enterocolitica* in

cheese samples produced from raw milk in Ontario, Canada, was reported. The researchers found that 18.2% of the raw milk and 9.2% of the raw milk cheese curd samples contained *Y. enterocolitica* (Schiemann, 1978, 1987).

Jayarao and Henning (2001) studied the prevalence of foodborne pathogens in bulk tank milk and determined that 26.7% of all bulk milk tank samples contained one or more pathogenic bacterial species. They determined that 9.2% of the samples contained *C. jejuni*, 3.8% contained Shiga toxin–producing *E. coli*, 4.6% contained *L. monocytogenes*, 6.1% contained *Salmonella* spp., and 6.1% contained *Y. enterocolitica.* In 2004, Van Kessel et al. examined the prevalence of Salmonellae, *L. monocytogenes*, and fecal coliforms in U.S. dairy farm bulk tank milk. In the study, samples were collected from 861 bulk tank milk samples in 21 states. Results indicated 95% of the samples contained coliform bacteria, 2.6% contained *Salmonella*, and 6.5% contained *L. monocytogenes.* Karns et al. (2005) used real-time PCR as an improved method for determining *Salmonella enterica* in raw milk samples from bulk milk tanks from 854 farms in 21 states. Using this improved sensitivity technique, they determined that 11.8% of the raw milk samples were contaminated with *Salmonella enterica,* opposed to only 2.6% detected by using conventional culture techniques. Kagkli et al. (2007) reported contamination of milk with enterococci and coliforms from bovine feces. Konuk et al. (2007) isolated Mycobacteria from raw milk samples in the Afyonkarahisar district of Turkey. Clearly all of these studies indicate the extreme risk posed by consumption of raw milk. The U.S. Centers for Disease Control indicate that people who drink raw milk have a 158 time greater likelihood of being stricken by illness from *Salmonella* Dublin than people who drink properly pasteurized milk (Vasavada, 1988).

Growth of *Staphylococcus aureus* and resultant enterotoxin production was investigated during processing of uncooked, semi-hard, raw milk cheese (Delbes et al., 2006). In the samples, coagulase positive staphylococci were detected at 2.82 to 6.84 log cfu/g after 24 hours. The researchers reported that the higher the initial coagulase levels in the cheese, the higher the chance of enterotoxin being present in the cheese (Delbes et al., 2006). In another study, it was reported that 33 fresh, soft, semi-hard, and hard raw milk cheese samples made with bovine and caprine raw milk by small- and large-scale Italian producers contained S. *aureus* and staphylococcal enterotoxins (SEs) (Cremonisi et al., 2007). Angelidis et al. (2005) analyzed 42 RTE Greek cheese samples and while neither *Salmonella* spp. nor *L. monocytogenes* were detected, three samples tested positive for *E. coli* and/or *S. aureus*. Fremaux et al. (2006) studied the dissemination and persistence of Shiga toxin–producing *Escherichia coli* strains on French dairy farms. Shiga toxin (Stx) genes were present in 25% of fecal samples

and 20% of environmental samples from the dairy farms. Some of the toxigenic material persisted for up to 12 months in the farm environment. This study indicates the risk of contamination of raw milk from the dairy farm environment.

Kongo et al. (2006) investigated the presence of *L. monocytogenes* in a raw milk cheese known as Sao Jorge in the Azores Islands, Portugal. A total of 357 raw milk, curd, and cheese samples were collected over the course of 1 year. Among the samples, *L. monocytogenes* was identified by using PCR analysis and ribotyping in 2 of the raw milk samples. Boerlin et al. (2003) reported that 11% of healthy dairy cows in Switzerland had antibodies to listeriolysin O and internalin A antigens due to listeriosis in the cows. It was also reported that listeriosis in cows was associated with consumption of corn silage as well as related to the level of dairy farm sanitation. In Portugal, 213 *Listeria* spp. isolates were obtained from eight dairies; *L. monocytogenes* serotype 4b was the most prevalent as determined by PCR serotyping (Chambel et al., 2007). A study was conducted in Brazil on the presence of *L. monocytogenes* in 50 pasteurized milk samples (nine commercial brands) and 55 Minas Frescal cheese samples (10 commercial brands) (Brito et al., 2008). According to the results, all pasteurized milk samples tested negative while 60% of the cheese samples from one brand tested positive for *L. monocytogenes*. Due to these results, samples were collected directly from the specific processing plant which had produced the contaminated cheeses. The processing plant was later renovated to prevent contamination problems recurring (Brito et al., 2008).

Even though cheeses produced from raw milk have been reported to be an important source of *L. monocytogenes,* post-pasteurization contamination can also pose hazards. A total of 357 fresh cheese and processing plant environmental (food contact surfaces, drains, floors, walls, etc.) samples were collected from three Latin-style cheese processing plants in New York. This plant used pasteurized milk for production of the cheese. *L. monocytogenes* was isolated from 6.3% of the cheese samples. However, 11% of environmental samples were positive for *L. monocytogenes*, which indicated that post-process contamination occurred. The authors also revealed that ribotype DUP-1044A subtype of *L. monocytogenes,* which has been previously linked in listeriosis outbreaks, was isolated from 20 of 36 isolates (Kabuki et al., 2004). Carvallo et al. (2007) reported low prevalence (3%) of *L. monocytogenes* in Minas Frescal cheese made from pasteurized milk.

In 1988, D'Aoust et al. studied the thermal inactivation of *Campylobacter* species, *Y. enterocolitica*, and hemorrhagic *E. coli* O157:H7 in fluid milk. This study confirmed that high temperature short time (HTST) pasteurization temperatures and minimum holding times were sufficient to destroy the then emerging pathogens hemorrhagic *E. coli* O157:H7, *Y. enterocolitica*, and

*Campylobacter* spp. *(C. fetus, C. coli,* and *C. jejuni).* However, the researchers reiterated that stringent thermal process control is critical to ensuring microbiological safety of RTE dairy products (D'Aoust et al., 1988).

In 1992, Cotton and White conducted a survey of *L. monocytogenes, Y. enterocolitica,* and *Salmonella* in milk and ice cream processing dairy plant environments. No *Salmonella* spp. were isolated from any of the environmental samples, but 6.8% of the samples were positive for *Y. enterocolitica*, 6.5% were positive for *L. monocytogenes,* and 9.3% were positive for *Listeria* spp. other than *L. monocytogenes*. This indicates the critical need for tight environmental control within the processing plant environment to prevent post-process contamination (Cotton and White, 1992). In a more recent survey, Frye and Donnelly (2005) reported a low incidence of *L. monocytogenes* in pasteurized fluid milk.

During a 1-year-long study in France, the prevalence and characterization of Shiga toxin–producing *E. coli* isolated from cattle, food, and children was investigated (Pradel et al., 2000). The study results indicated that 101 of 603 cheese samples were positive for the Stx gene which was likely to be pathogenic for humans. Hemolytic uremic syndrome, hemorrhagic colitis, and uncomplicated diarrhea have been reported associated with the Stx gene. The dominant serotype isolated in the study was O157:H7. Stx positive samples showed a seasonal pattern with the highest number recorded during April to September. The researchers determined that many of the isolates belonged to serogroups that have specific properties which can allow them to survive in meat and cheese (Pradel et al., 2000). Lekkas et al. (2006) reported the survival of *Escherichia coli* O157:H7 in Galotyri cheese stored at 4°C and 12°C.

*Streptococcus equi* subsp. *zooepidemicus* belongs to Group C streptococci and rarely causes infections in humans. However, it was isolated from fresh goat cheese made from unpasteurized milk and throat swabs of three people who had consumed the cheese (Kuusi et al., 2006).

A study was conducted on the microbial quality of retail cheeses made from raw, thermized, or pasteurized milk in the United Kingdom by Little et al. (2008). Their results indicated that cheese samples made from raw or thermized milk had at least 4 log of *S. aureus* and *E. coli.* Cheese samples made from pasteurized milk also were contaminated with *S. aureus* and *E. coli* at 3 log. The researchers emphasized the importance of good manufacturing techniques. Mariani et al. (2006) demonstrated that the wooden shelves used in cheese aging rooms were one source of contamination with microorganisms such as micrococci-corynebacteria, yeasts, and molds and at lower rates with enterococci, staphylococci, and pseudomonads.

Zhou et al. (2007) evaluated Chinese commercial pasteurized milk obtained from the chain supermarkets in Wuhan, China, in spring and in fall of 2006. Of the spring samples, 71.4% contained *Bacillus cereus,*

and 33.3% of the fall samples also contained the bacterium. PCR analyses indicated enterotoxin genes present in the bacteria.

## 3.9 Emerging techniques to prevent post-pasteurization contamination

Technological developments have resulted in significant alternatives to conventional methods of thermal processing. Technologies such as high pressure processing, pulsed electric field, irradiation, ultrasound, and bacteriocin applications are of specific interest for the food industry as a means to provide food safety while preventing undesirable sensory changes in food texture, color, and flavor. Improved pathogen testing methods allow more sensitive detection in shorter time.

López-Pedemonte et al. (2007) determined the effects of high hydrostatic pressure (HHP) application in cheese samples experimentally contaminated with two strains of *L. monocytogenes* (NCTC 11994 and Scott A). Pressures of 300, 400, and 500 MPa were applied at 5°C and 20°C. During the subsequent 30-day storage of the cheese samples, HHP treated cheese had significantly reduced levels of *L. monocytogenes,* especially the samples treated with the higher pressure applications (400–500 MPa). It was also suggested that a combination of high pressure treatments with bacteriocins would help to produce safe cheese from raw milk (López-Pedemonte et al., 2007; Rodríguez et al., 2005). Arqués et al. (2006) made Spanish semi-soft La Serena cheese using high pressure treatment. Their results indicated that 300 to 400 MPa applications were effective in decreasing coliforms, *S. aureus,* and enterococci.

López-Pedemonte et al. (2007) studied HHP at mild temperatures with addition of nisin on improving safety of soft-type cheeses. They determined that 400 MPa HHP with 1.56 mg/L nisin application caused a 2.4 $\log_{10}$ cfu/g inhibition of *B. cereus* ATCC 9139 in cheese samples. In another study, high pressure treatment (300–500 MPa) was combined with different bacteriocin-producing lactic acid bacteria as an adjunct culture in cheese production (Rodríguez et al., 2005). Raw milk was inoculated with *E. coli* O157:H7 and one of several different bacteriocin-producing cultures. High pressure treatments were applied at Day 2 after cheese production at 300 MPa for 10 min and 500 MPa for 5 min, and at Day 50 at 300 MPa for 10 min and 500 MPa for 5 min. The combined applications, and especially HP at 300 MPa on Day 50 with addition of nisin-A, bacteriocin TAB 57, enterocin I, or enterocin AS-48–producing LAB, inhibited *E. coli* O157:H7 in 2-month aged cheese (Rodríguez et al., 2005). Arqués et al.

(2005) inactivated *S. aureus* in raw milk cheese using a combination of high pressure treatment and bacteriocin-producing lactic acid bacteria.

Bacteriocins from food grade cultures are effective in inhibiting foodborne pathogens, and preventing or reducing food spoilage (Nissen et al., 2001). Bacteriocin applications have been well studied in dairy foods. Broad spectrum bacteriocin lacticin 3147, which is produced by *Lactococcus lactis,* was used against *L. monocytogenes* on the surface of smear ripened cheese (O'Sullivan et al., 2006). Cheese samples were experimentally contaminated with *L. monocytogenes* and inoculated with lacticin 3147-producing and non-producing lactic cultures. Although the application of lacticin 3147–producing culture did not provide total elimination of the *L. monocytogenes,* it did reduce the population. It was proposed that this technique might improve the safety of smear-ripened cheeses, which usually have only low levels of post-pasteurization contamination. Bizani et al. (2008) used the bacteriocin-like peptide cerein to inhibit *L. monocytogenes* in dairy products. McAuliffe et al. (1999) had similar results in cottage cheese using lacticin 3147.

The effect of irradiation on the microbial and sensory properties of ice cream samples was studied by Kamat et al. (2000). Ice cream samples were irradiated at 1, 2, 3, 4, and 5 kGy. High irradiation levels adversely affected the sensory properties of the ice cream and caused off-flavors. However, the 1-kGy irradiation treatment had acceptable sensory properties, and researchers indicated this was a viable method for producing microbiologically safe ice cream.

Rajagopal et al. (2005) determined that the combination of low pressure carbon dioxide and refrigeration improved the microbial quality of raw milk and reduced pathogen populations.

In the past few decades, advances in packaging science have helped in the challenge of assuring food safety. New biodegradable and antimicrobial food packaging materials containing spice extracts and bacteriocins (Seydim and Sarikus, 2006) and modified atmosphere packaging are important developments for dairy product safety (Robertson, 2005). Duan et al. (2007) reported that chitosan lysozyme coatings were effective against *L. monocytogenes*, *Pseudomonas fluorescens,* and *E. coli* on mozzarella cheese surfaces.

New methodologies for detecting pathogens are being rapidly developed (Bhaduri and B. Cottrell, 1998; Abubakar et al., 2007; Beutin et al., 2007). Karns et al. (2007) developed PCR methods for analyzing presence of *E. coli* O157:H7 and *E. coli* virulence factors in U.S. bulk tank milk. Test methods such as these allow quick monitoring of product to ensure pathogen control and elimination. These tools provide more rapid identification of causative

agents and will allow more effective testing of the finished product before it leaves the processing facility. Risk assessment studies improve knowledge for preventing exposure of consumers to pathogenic organisms (Van Gerwen et al., 2000; McLauchlin et al., 2004; Clough et al., 2006).

The most effective tool in the prevention of foodborne disease outbreaks is education. To help prevent outbreaks, Washington State University conducted a project to develop queso fresco using pasteurized milk. They then educated people on proper safe cheese production via a series of workshops directed toward older Hispanic women. These women were selected so they could then later train others in their communities. More than 250 individuals attended the workshops between 1997 and 1998. Follow-up surveys indicated that the individuals continued to use pasteurized milk for the production of queso fresco (Bell et al., 1999).

## 3.10 *Ensuring the safety of ready-to-eat dairy products*

A review of literature reveals that dairy products made from raw milk pose a significant risk of causing foodborne illness. Because raw milk products now represent a minute portion of total commercial dairy production, outbreaks are usually confined regionally near the source. However, in the larger commercial dairy processing industry, which may have sales in multiple states or regions, failure to properly pasteurize products and to subsequently protect those products from post-process contamination can lead to widespread foodborne illness outbreaks. The tragic outbreaks that have resulted from failure to properly pasteurize the products indicate the importance and significance of the pasteurization process as a protective measure. The failures can be equated to what would have happened if the milk was sold as raw milk or raw milk products.

The good manufacturing practices set forth in the Grade A Pasteurized Milk Ordinance and similar documents have been proven repeatedly to be the proper sanitation techniques and controls to ensure safe dairy product production. Proper adherence to these guidelines is essential to prevent contamination. In addition to processing the foods in accordance with the guidelines, manufacturers test finished dairy products for contamination. Total quality control assurance programs are significant in ensuring safety. The Hazard Analysis Critical Control Point (HACCP) system was developed in the 1960s by the Pillsbury Corporation for the U.S. National Aeronautics and Space Administration (NASA) and the U.S. military. It was first developed for ensuring absolute food safety for astronauts in space but has since been applied to food plants to effectively detect hazards/errors in processing environments and throughout the entire route to customers.

HACCP has had a significant effect on improving food safety. Many other countries have followed the U.S. approach and in the European Union, HACCP application is now mandatory for food plants. Determination of critical control points is crucial in dairy plants and for dairy product safety. Zero tolerance regulations for pathogens such as *L. monocytogenes* and *E. coli* O157:H7 have created pressure on the food processors to continue to improve dairy safety. Farm to plate quality programs are effective in preventing foodborne illness in RTE dairy products (Reed and Grivetti, 2000; Allard, 2002; Valeeva et al., 2005; Beier and Pillai, 2007).

Furthermore, in Europe, International Organization for Standardization (ISO) representatives have established quality assurance standards for food processing plants. These standards include: ISO 9000–2000 Fundamental Quality Management System and Vocabulary, ISO 9001:2000 Requirements for a Quality Management System, and ISO 19011:2002 Principles for Auditing Quality and Environmental Management Systems. The generalized control mechanisms critical to total quality assurance in the safety of RTE dairy products is illustrated in Figure 3.2.

## *3.11 Raw milk versus pasteurized milk products and the safety of ready-to-eat dairy foods*

Numerous individuals and groups actively campaign for the legalization of raw milk sales. These groups claim that peer-reviewed scientific literature supports their arguments that raw milk is safe and contains profound nutritional and/or medicinal values not found in pasteurized milk. However, a review of the references provided on a raw milk website reveals that many of the journal articles do not exist, are misquoted, or are misinterpreted. A review of their arguments indicates that the authors often do not take into account all aspects of raw milk as a vehicle for disease transmission (Bren, 2004; Weston A. Price Foundation, 2007, 2008). In the Weston A. Price Foundation's own documents, it is stated "That the consumption of raw milk carries some risk is undeniable" (Weston A. Price Foundation, 2007). On this point, public health officials agree. And it is based on this knowledge that public health officials attempt to limit and/or prevent disease transmission via milk. According to anecdotal data available through scientifically researched and observed disease outbreaks, public health officials and food microbiologists agree that the best known scientific method of preventing milkborne disease outbreaks is to properly pasteurize milk and to properly follow sanitary guidelines for bottling and distributing the pasteurized product. The key to success in this sanitary process is to *properly* follow the guidelines and to scientifically review any outbreak to ensure that the guidelines were adequate. If guidelines are found inadequate, modifications must be made.

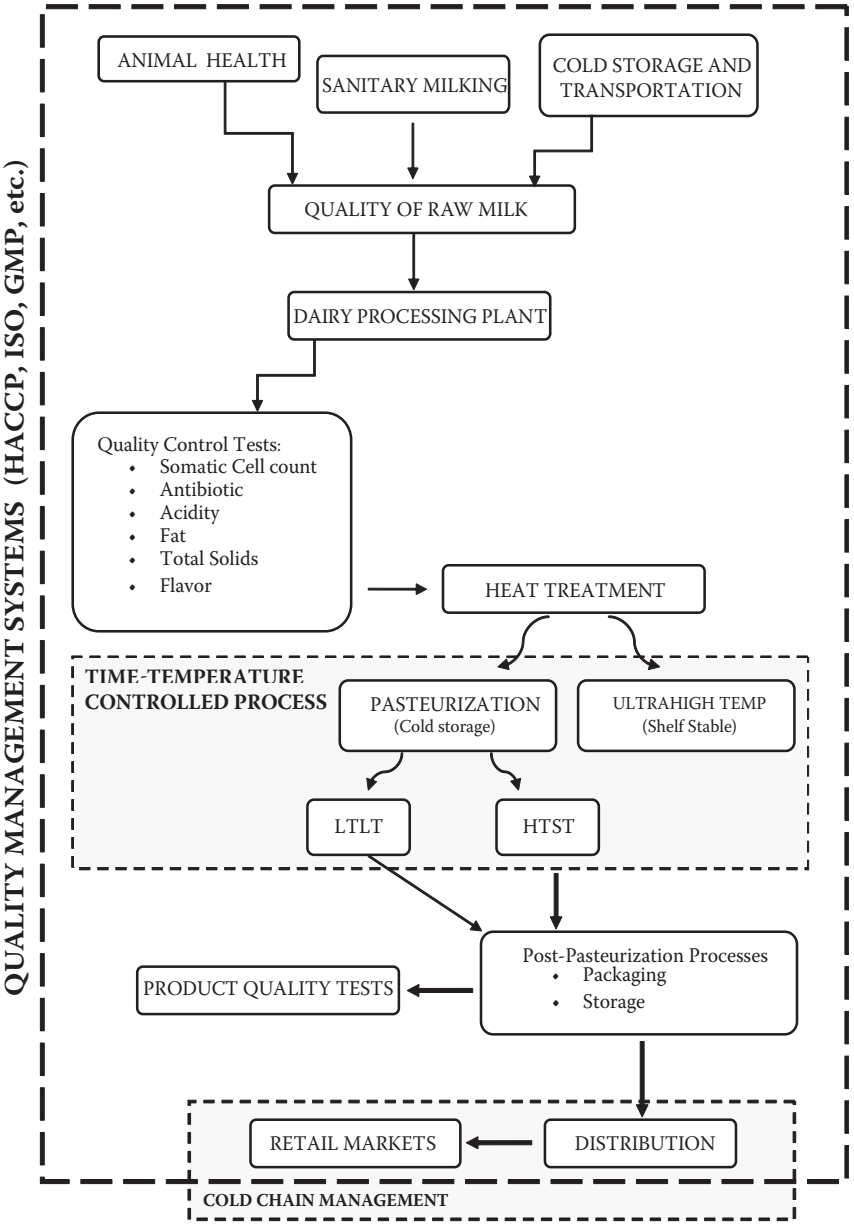


***Figure 3.2*** Control mechanisms for assurance of dairy safety. (Illustration by A.C. Seydim and Z.B. Güzel-Seydim.)

The lessons of the past recorded in public health history clearly indicate that consumption of raw milk and raw milk products increases the chance of contracting disease. Analyses of raw milk repeatedly have indicated fecal and environmental contaminants can and do occur. Proper pasteurization of raw milk has been proven to destroy these pathogens. Using care to ensure that no post-pasteurization contamination occurs, results in microbiologically safe RTE dairy foods. However, failure to follow sanitary processing guidelines and good manufacturing practices can result in hazardous dairy products. Strict vigilance to total quality control is essential for providing consumers with safe RTE dairy products.

Proponents of raw milk have pushed forward such bills as the California Senate Bill 201, dubbed "The Fresh Raw Milk Act of 2008," which support and promote raw milk (Weston A. Price Foundation, 2008). Although the bill sets forth regulations on use of HACCP and other strict quality control measures, the risk from consuming raw milk remains. Unfortunately, "those who cannot remember the past are condemned to repeat it" (Santayana, 1905).

## *Acknowledgments*

The authors wish to thank Ms. Rayleen A. Hendrix, Mr. Steven D. Chambers, and Ms. Andrea Kesler for their kind assistance.

## *References*

Abubakar, I., L. Irvine, C.F. Aldus, G.M. Wyatt, R. Fordham, S. Schelenz, L. Shepstone, A. Howe, M. Peck, and P.R. Hunter. 2007. A systematic review of the clinical, public health and cost-effectiveness of rapid diagnostic tests for the detection and identification of bacterial intestinal pathogens in faeces and food. *Health Technol. Assess.* 11(36): Executive Summary. http://www.hta.ac.uk/execsumm/summ1136.shtml

Ackers, M.-L., S. Schoenfeld, J. Markman, M.G. Smith, M.A. Nicholson, W. DeWitt, D.N. Cameron, P.M. Griffin, and L. Slutsker. 2000. An outbreak of *Yersinia enterocolitica* O:8 infections associated with pasteurized milk. *J. Infect. Dis.* 181:1834–1837.

Akdeniz, H., T. Buzgan, M. Tekin, H. Karsen, and M.K. Karahocagil. 2007. An outbreak of botulism in a family in Eastern Anatolia associated with eating suzme yoghurt buried under soil. *Scand. J. Infect. Dis.* 39(2):108–114.

Allard, D.G. 2002. The 'farm to plate' approach to food safety—everyone's business. *Can. J. Infect. Dis.* 13(3):185–190.

Allerberger, F., M. Wagner, P. Schweiger, H-P. Rammer, A. Resch, M.P. Dierich, A.W. Friedrich, and H. Karch. 2001. *Escherichia coli* O157 infections and unpasteurised milk. *Eurosurveillance* 6(10):379. http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=379

Angelidis, A.S., E.N. Chronis, D.K. Papageorgiou, I.I. Kazakis, K.C. Arsenoglou, and G.A. Stathopoulos. 2005. Non-lactic acid, contaminating microbial flora in ready-to-eat foods: a potential food-quality index. *Food Micro.* 23(1):95–100.

Anonymous. 1903. *Merck's Archives: A Journal of Materia Medica and Therapeutics for the General Practitioner—Volume 5*. Merck & Company: New York.

Arqués, J.L., S. Garde, P. Gaya, M. Medina, and M. Nuñez. 2006. Short communication: inactivation of microbial contaminants in raw milk La Serena cheese by high-pressure treatments. *J. Dairy Sci.* 89:888–891.

Arqués, J.L., E. Rodríguez, P. Gaya, M. Medina, B. Guamis, and M. Nuñez. 2005. Inactivation of *Staphylococcus aureus* in raw milk cheese by combinations of high-pressure treatments and bacteriocin–producing lactic acid bacteria. *J. Appl. Micro.* 98(2):254–260.

Aureli, P., M. Di Cunto, A. Maffei, G. De Chiara, G. Franciosa, L. Accorinti, A.M. Gambardella, and D. Greco. 2000. An outbreak in Italy of botulism associated with a dessert made with mascarpone cream cheese. *Eur. J. Epidemiol.* 16:913–918.

Ayele, W.Y., P. Svastova, P. Roubal, M. Bartos, and I. Pavlik. 2005. *Mycobacterium avium* subspecies *paratuberculosis* cultured from locally and commercially pasteurized cow's milk in the Czech Republic. *Appl. Environ. Microbiol.* 71(3):1210–1214.

Beier, R.C. and S.D. Pillai. 2007. *Future Directions in Food Safety. Infectious Diseases: Foodborne Diseases*, Ed. S. Simjee. Humana Press Inc.: Totowa, NJ. 511–530.

Bell, R.A., V.N. Hillers, and T.A. Thomas. 1999. Hispanic grandmothers preserve cultural traditions and reduce foodborne illness by conducting safe cheese workshops. *J. Amer. Diet. Assoc*. 99(9):1114–1116.

Beutin, L., A. Miko, G. Krause, K. Pries, S. Haby, K. Steege, and N. Albrecht. 2007. Identification of human-pathogenic strains of Shiga toxin–producing *Escherichia coli* from food by a combination of serotyping and molecular typing of Shiga toxin genes. *Appl. Environ. Microbiol.* 73(15):4769–4775.

Bhaduri, S. and B. Cottrell. 1998. A simplified sample preparation method from various foods for PCR detection of pathogenic *Yersinia enterocolitica*: a possible model for other food pathogens. *Mol. Cell. Probes.* 12(2):79–83.

Bizani, D., J.A.C. Morrissy, A.P.M. Dominguez, and A. Brandelli. 2008. Inhibition of *Listeria monocytogenes* in dairy products using the bacteriocin-like peptide cerein 8A. *Int. J. Food Micro.* 121(2):229–233.

Black, R.E., R.J. Jackson, T. Tsai, M. Medvesky, M. Shayegani, J.C. Feeley, K.I. MacLeod, and A.H. Wakelee. 1978. Epidemic *Yersinia enterocolitica* infection due to contaminated chocolate milk. *N. Engl. J. Med.* 298:76–79.

Boerlin, P., F. Boerlin-Petzold, and T. Jemmi. 2003. Use of listeriolysin O and internalin A in a seroepidemiological study of Listeriosis in Swiss dairy cows. *J. Clin. Micro.* 41(3):1055–1062.

Bren, L. 2004. Got Milk? Make Sure It's Pasteurized. *FDA Consumer Magazine.* September–October 2004. http://www.fda.gov/fdac/features/2004/504_milk.html

Brito, J.R.F., E.M.P. Santos, E.F. Arcuri, C.C. Lange, M.A.V.P. Brito, G.N. Souza, M.M.P.O. Cerqueira, J.M.S. Beltran, J.E. Call, Y. Liu, A.C.S. Porto-Fett, and J.B. Luchansky. 2008. A retail survey of Brazilian milk and Minas Frescal cheese and a contaminated dairy plant to establish the prevalence, relatedness, and sources of *Listeria monocytogenes*. *Appl. Environ. Microbiol.* 74(15):4954–4961.

Callaway, T.R., J.E. Keen, T.S. Edrington, L.H. Baumgard, L. Spicer, E.S. Fonda, K.E. Griswold, T.R. Overton, M.E. VanAmburgh, R.C. Anderson, K.J. Genovese, T.L. Poole, R.B. Harvey, and D.J. Nisbet. 2005. Fecal prevalence and diversity of *Salmonella* species in lactating dairy cattle in four states. *J. Dairy Sci.* 88:3603–3608.

Canadian Food Inspection Service. 2007. *Dairy Establishment Inspection Manual.* http://www.inspection.gc.ca/english/fssa/dailai/man/contentse.shtml

do Carmo, L.S., R.S. Dias, V.R. Linardi, M.J. de Sena, D.A. dos Santos, M.E. de Faria, E.C. Pena, M. Jett, and L.G. Heneine. 2002. Food poisoning due to enterotoxigenic strains of *Staphylococcus* present in Minas cheese and raw milk in Brazil. *Food Micro.* 19:9–14.

Caro, I. and M.R. Garcia-Armesto. 2007. Occurrence of Shiga toxin–producing *Escherichia coli* in a Spanish raw ewe's milk cheese. *Int. J. Food Micro.* 116(3):410–413.

Carvallo, J.D.G., W.H. Viotto, and A.Y. Kuaye. 2007. The quality of Minas Frescal cheese produced by different technological processes. *Food Control.* 18:262–267.

Centers for Disease Control and Prevention. 1981a. Raw-milk associated illness—Oregon, California. *MMWR.* 30:90–97. U.S. Dept. of Health and Human Services, Centers for Disease Control and Prevention, Atlanta, GA.

Centers for Disease Control and Prevention. 1981b. Salmonellosis associated with raw milk—Montana. *MMWR*. 30:211–212. U.S. Dept. of Health and Human Services, Centers for Disease Control and Prevention, Atlanta, GA.

Centers for Disease Control and Prevention (CDC). 1981c. Outbreak of *Campylobacter enteritis* associated with raw milk—Kansas. *MMWR*. 30:218–220. U.S. Dept. of Health and Human Services, Centers for Disease Control and Prevention, Atlanta, GA.

Centers for Disease Control and Prevention (CDC). 1981d. *Salmonella* Dublin associated with raw milk—Washington State. *MMWR.* 30:373–374. U.S. Dept. of Health and Human Services, Centers for Disease Control and Prevention, Atlanta, GA.

Centers for Disease Control and Prevention. 1983a. Campylobacteriosis associated with raw milk consumption—Pennsylvania. *MMWR* 32(26):337–338, 344. U.S. Dept. of Health and Human Services, Centers for Disease Control and Prevention, Atlanta, GA.

Centers for Disease Control and Prevention. 1983b. Group C Streptococcal infections associated with eating homemade cheese—New Mexico. *MMWR* 32(39):510, 515–516. U.S. Dept. of Health and Human Services, Centers for Disease Control and Prevention, Atlanta, GA.

Centers for Disease Control and Prevention. 1983c. Epidemiologic notes and reports Brucellosis—Texas. *MMWR* 32(42):548–553. U.S. Dept. of Health and Human Services, Centers for Disease Control and Prevention, Atlanta, GA.

Centers for Disease Control and Prevention. 1984a. *Salmonella* Dublin and raw milk consumption—California. *MMWR* 33(14):196–198. U.S. Dept. of Health and Human Services, Centers for Disease Control and Prevention, Atlanta, GA.

Centers for Disease Control and Prevention. 1984b. Salmonellosis associated with cheese consumption—Canada. *MMWR* 33(27):387. U.S. Dept. of Health and Human Services, Centers for Disease Control and Prevention, Atlanta, GA.

Centers for Disease Control and Prevention. 1984c. Salmonellosis from inadequately pasteurized milk—Kentucky. *MMWR* 33(36):505–506. U.S. Dept. of Health and Human Services, Centers for Disease Control and Prevention, Atlanta, GA.

Centers for Disease Control and Prevention. 1984d. Epidemiologic notes and reports chronic diarrhea associated with raw milk consumption—Minnesota. *MMWR* 33(37):521–522, 527–528. U.S. Dept. of Health and Human Services, Centers for Disease Control and Prevention, Atlanta, GA.

Centers for Disease Control and Prevention. 1984e. Epidemiologic notes and reports *Campylobacter* outbreak associated with certified raw milk products—California. *MMWR* 33(39):562. U.S. Dept. of Health and Human Services, Centers for Disease Control and Prevention, Atlanta, GA.

Centers for Disease Control and Prevention. 1985a. Milk-borne Salmonellosis—Illinois. *MMWR* 34(14):200. U.S. Dept. of Health and Human Services, Centers for Disease Control and Prevention, Atlanta, GA.

Centers for Disease Control and Prevention. 1985b. Epidemiologic notes and reports Listeriosis outbreak associated with Mexican-style cheese—California. *MMWR*. 34:357–359. U.S. Dept. of Health and Human Services, Centers for Disease Control and Prevention, Atlanta, GA.

Centers for Disease Control and Prevention. 1986. Epidemiologic notes and reports *Campylobacter* outbreak associated with raw milk provided on a dairy tour—California. *MMWR*. 35:311–312. U.S. Dept. of Health and Human Services, Centers for Disease Control and Prevention, Atlanta, GA.

Centers for Disease Control and Prevention. 1990. Foodborne disease outbreaks, 5-year summary, 1983–87. *MMWR* 39(SSO1):15–23. U.S. Dept. of Health and Human Services, Centers for Disease Control and Prevention, Atlanta, GA.

Centers for Disease Control and Prevention. 1994. Emerging infectious diseases outbreak of *Salmonella enteritidis* associated with nationally distributed ice cream products—Minnesota, South Dakota, and Wisconsin, 1994. *MMWR* 43(40):740–741. U.S. Dept. of Health and Human Services, Centers for Disease Control and Prevention, Atlanta, GA.

Centers for Disease Control and Prevention. 1996. Surveillance for foodborne-disease outbreaks—United States, 1988–1992. *MMWR* 45:SS–5. U.S. Dept. of Health and Human Services, Centers for Disease Control and Prevention, Atlanta, GA.

Centers for Disease Control and Prevention. 2000. Outbreak of *Escherichia coli* O157:H7 infection associated with eating fresh cheese curds—Wisconsin, June 1998. *MMWR* 49(40):911–913. U.S. Dept. of Health and Human Services, Centers for Disease Control and Prevention, Atlanta, GA.

Centers for Disease Control and Prevention. 2001a. Outbreak of Listeriosis associated with homemade Mexican-style cheese—North Carolina, October 2000–January 2001. *MMWR* 50(26):560–570. U.S. Dept. of Health and Human Services, Centers for Disease Control and Prevention, Atlanta, GA.

Centers for Disease Control and Prevention. 2001b. Outbreaks of *Escherichia coli* O157:H7 infections among children associated with farm visits—Pennsylvania and Washington, 2000. *MMWR* 50(15):293–297. U.S. Dept. of Health and Human Services, Centers for Disease Control and Prevention, Atlanta, GA.

Centers for Disease Control and Prevention. 2002. Outbreak of *Campylobacter jejuni* infections associated with drinking unpasteurized milk procured through a cow-leasing program—Wisconsin, 2001. *MMWR* 51(25):548–549. U.S. Dept. of Health and Human Services, Centers for Disease Control and Prevention, Atlanta, GA.

Centers for Disease Control and Prevention. 2003. Multistate outbreak of *Salmonella* serotype Typhimurium infections associated with drinking unpasteurized milk—Illinois, Indiana, Ohio, and Tennessee, 2002–2003. *MMWR* 52:613–615. U.S. Dept. of Health and Human Services, Centers for Disease Control and Prevention, Atlanta, GA.

Centers for Disease Control and Prevention. 2005. Human tuberculosis caused by *Mycobacterium bovis*—New York City, 2001–2004. *MMWR* 54(24):605–608. U.S. Dept. of Health and Human Services, Centers for Disease Control and Prevention, Atlanta, GA.

Centers for Disease Control and Prevention. 2007a. *Escherichia coli* O157:H7 infection associated with drinking raw milk—Washington and Oregon, November–December 2005. *MMWR* 56(08):165–167. U.S. Dept. of Health and Human Services, Centers for Disease Control and Prevention, Atlanta, GA.

Centers for Disease Control and Prevention. 2007b. Typhimurium infection associated with raw milk and cheese consumption—Pennsylvania, 2007. *MMWR* 56(44):1161–1164. U.S. Dept. of Health and Human Services, Centers for Disease Control and Prevention, Atlanta, GA.

Centers for Disease Control and Prevention. 2008. Outbreak of multidrug-resistant *Salmonella enterica* serotype Newport infections associated with consumption of unpasteurized Mexican-style aged cheese—Illinois, March 2006–April 2007. *MMWR* 57(16):432–435. U.S. Dept. of Health and Human Services, Centers for Disease Control and Prevention, Atlanta, GA.

Chambel, L., M. Sol, I. Fernandes, M. Barbosa, I. Zilhão, B. Barata, S. Jordan, S. Perni, G. Shama, A. Adrião, L. Faleiro, T. Requena, C. Peláez, P.W. Andrew, and R. Tenreiro. 2007. Occurrence and persistence of *Listeria* spp. in the environment of ewe and cow's milk cheese dairies in Portugal unveiled by an integrated analysis of identification, typing and spatial-temporal mapping along production cycle. *Int. J. Food Micro.* 116(1):52–63.

Clark, Jr., D.L., J.L. Anderson, J.J. Koziczkowski, and Jay L.E. Ellingson. 2006. Detection of *Mycobacterium avium* subspecies *paratuberculosis* genetic components in retail cheese curds purchased in Wisconsin and Minnesota by PCR. *Mol. Cell. Probes*. 20(3–4):197–202.

Clough, H.E., D. Clancy, and N.P. French. 2006. Vero-cytotoxigenic *Escherichia coli* O157 in pasteurized milk containers at the point of retail: a qualitative approach to exposure assessment. *Risk Anal.* 26(5):1291–1309.

Cody, S.H., S.L. Abbott, A.A. Marfin, B. Schulz, P. Wagner, K. Robbins, J.C. Mohle-Boetani, and D.J. Vugia. 1999. Two outbreaks of multidrug-resistant *Salmonella* serotype Typhimurium DT104 infections linked to raw-milk cheese in Northern California. *J. Amer. Med. Assoc.* 281(19):1805–1810.

Conedera, G., P. Dalvit, M. Martini, G. Galiero, M. Gramaglia, E. Goffredo, G. Loffredo, S. Morabito, D. Ottaviani, F. Paterlini, G. Pezzotti, M. Pisanu, P. Semprini, and A. Caprioli. 2004. Verocytotoxin–producing *Escherichia coli* O157 in minced beef and dairy products in Italy. *Int. J. Food Micro.* 96(1):67–73.

Cotton, L.N. and C.H. White. 1992. *Listeria monocytogenes, Yersinia enterocolitica,* and *Salmonella* in dairy plant environments. *J. Dairy Sci.* 75:51–57.

Cremonesi, P., G. Perez, G. Pisoni, P. Moroni, S. Morandi, M. Luzzana, M. Brasca, and B. Castiglioni. 2007. Detection of enterotoxigenic *Staphylococcus aureus* isolates in raw milk cheese. *Letters in Appl. Micro.* 45(6):586–591.

Currier, R.W. 1981. Raw milk and human gastrointestinal disease: problems resulting from legalized sale of "certified raw milk." *J. Pub. Health Pol.* 2(3):226–234.

Dalton, C.B., C.C. Austin, J. Sobel, P.S. Hayes, W.F. Bibb, L.M. Graves, B. Swaminathan, M.E. Proctor, and P.M. Griffin. 1997. An outbreak of gastroenteritis and fever due to *Listeria monocytogenes* in milk. *N. Engl. J. Med.* 336(2):100–106.

D'Aoust, J.-Y., C.E. Park, R.A. Szabo, E.C.D. Todd, D.B. Emmons, and R.C. McKellar. 1988. Thermal inactivation of *Campylobacter* species, *Yersinia enterocolitica*, and hemorrhagic *Escherichia coli* O157:H7 in fluid milk. *J. Dairy Sci.* 71:3230–3236.

Delbes, C., J. Alomar, N. Chougui, J.F. Martin, and M.C. Montrel. 2006. *Staphylococcus aureus* growth and enterotoxin production during the manufacture of uncooked, semihard cheese from cows' raw milk. *J. Food Prot.* 69(9):2161–2167.

Desenclos, J.-C., P. Bouvet, E. Benz-Lemoine, F. Grimont, H. Desqueyroux, I. Rebiere, and P.A. Grimont. 1996. Large outbreak of *Salmonella enterica* serotype paratyphi B infection caused by a goats' milk cheese, France, 1993: a case finding and epidemiological study. *BMJ* 312:91–94.

Donnelly, C.W. 1990. Concerns of microbial pathogens in association with dairy foods. *J. Dairy Sci.* 73:1656–1661.

Doyle, M.P. and D.J. Roman. 1982. Prevalence and survival of *Campylobacter jejuni* in unpasteurized milk. *Appl. Environ. Micro.* 44(5):1154–1158.

Duan, J., S.-I. Park, M.A. Daeschel, and Y. Zhao. 2007. Antimicrobial chitosan-lysozyme (CL) films and coatings for enhancing microbial safety of mozzarella cheese. *J. Food Sci.* 72(9):M355–M362.

Eckman, M.R. 1975. Brucellosis linked to Mexican cheese. *JAMA* 232:636–637.

El-Gazzar, F.E. and E.H. Marth. 1992. Salmonellae, salmonellosis, and dairy foods: a review. *J. Dairy Sci.* 75:2327–2343.

El-Sharoud, W.M., M.Z. El-Din, D.M. Ziada, S.F. Ahmed, and J.D. Klena. 2008. Surveillance and genotyping of *Enterobacter sakazakii* suggest its potential transmission from milk powder into imitation recombined soft cheese. *J. Appl. Micro.* Doi:10.1111/j.1365–2672.2008.03777.x.

Evershed, R.P. , S. Payne, A.G. Sherratt, M.S. Copley, J. Coolidge, D. Urem-Kotsu, K. Kotsakis, M. Özdoğan, A.E. Özdoğan, O. Nieuwenhuyse, P.M.M.G. Akkermans, D. Bailey, R.-R. Andeescu, S. Campbell, S. Farid, I. Hodder, N. Yalman, M. Özbaşaran, E. Bıçakçı, Y. Garfinkel, T. Levy, and M.M. Burton. 2008. Earliest date for milk use in the Near East and southeastern Europe linked to cattle herding. *Nature* advance online publication 6 August 2008 doi:10.1038/nature07180.

FAO/WHO. 2004. *Code of Hygienic Practice for Milk and Milk Products* CAC/RCP 57. http://www.codexalimentarius.net/

Farber, J.M. and J.Z. Losos. 1988. *Listeria monocytogenes*: a foodborne pathogen. *CMAJ* 138:413–418.

Farber, J.M. and P.I. Peterkin. 1991. *Listeria monocytogenes*, a food-borne pathogen. *Microbiol. Rev.* 55(3):476–511.

Fleming, D., S.L. Cochi, K.L. MacDonald, J. Brondum, P.S. Hayes, B.D. Plikaytis, M.B. Holmes, A. Audrier, C.V. Broome, and A.L. Reingold. 1985. Pasteurized milk as a vehicle of infection in an outbreak of listeriosis. *New Engl. J. Med.* 312:404–407.

Food Standards Australia New Zealand. 2006. *Proposal P296—Primary Production & Processing Standard for Dairy.* http://www.foodstandards.gov.au/standardsdevelopment/proposals/proposalp296primaryp2806.cfm

Fremaux, B., S. Raynaud, L. Beutin, and C. Vernozy Rozand. 2006. Dissemination and persistence of Shiga toxin–producing *Escherichia coli* (STEC) strains on French dairy farms. *Vet. Micro.* 117(2–4):180–191.

Frye, C. and C.W. Donnelly. 2005. Comprehensive survey of pasteurized fluid milk produced in the United States reveals a low prevalence of *Listeria monocytogenes*. *J. Food Prot.* 68(5):973–979.

Garcia-Fulgueiras, A., S. Sanchez, J.J. Guillen, B. Marsilla, A. Aladuena and C. Navarro. 2001. A large outbreak of *Shigella sonnei* gastroenteritis associated with consumption of fresh pasteurized milk cheese. *Eur. J. Epidem.* 17:533–538.

Goff, D. 1995. *Dairy Science and Technology Education Series.* University of Guelph, Canada, http://www.foodsci.uoguelph.ca/dairyedu/home.html

Hamdi, T.M., M. Naïm, P. Martin, and C. Jacquet. 2007. Identification and molecular characterization of *Listeria monocytogenes* isolated in raw milk in the region of Algiers (Algeria). *Int. J. Food Micro.* 116(1):190–193.

Hammer, B.W. 1948. *Dairy Bacteriology.* John Wiley & Sons, Inc.: New York.

Headrick, M.L., S. Korangy, N.H. Bean, F.J. Angulo, S.F. Altekruse, M.E. Potter, and K.C. Klontz. 1998. The epidemiology of raw milk-associated foodborne disease outbreaks reported in the United States, 1973 through 1992. *Am. J. Public Health* 88:1219–1221.

Headrick, M.L., B. Timbo, K.C. Klontz, and S.B. Werner. 1997. Profile of raw milk consumers in California. *Public Health Rep.* 112(5):418–422.

Hennessey, T., C.W. Hecberg, L. Slutsker, K.E. White, J.M. Besser-Wiek, M.E. Moen, J. Feldman, W.W. Coleman, L.M. Edmonson, K.L. MacDonald, M.T. Osterholm, and The Investigation Team. 1996. A national outbreak of *Salmonella enteritidis* infections from ice cream. *N. Engl. J. Med.* 334:1281–1286.

Ho, A.J., V.R. Lappi, and M. Wiedmann. 2007. Longitudinal monitoring of *Listeria monocytogenes* contamination patterns in a farmstead dairy processing facility. *J. Dairy Sci.* 90:2517–2524.

Holm, S., R.B. Toma, W. Reiboldt, C. Newcomer, and M. Calicchia. 2002. Cleaning frequency and the microbial load in ice-cream. *Int. J. Food Sci. Nutr.* 53(4):337–342.

Hong, E., M. Doumith, S. Duperrier, I. Giovannacci, A. Morvan, P. Glaser, C. Buchrieser, C. Jacquet, and P. Martin. 2007. Genetic diversity of *Listeria monocytogenes* recovered from infected persons and pork, seafood, and dairy products on retail sale in France during 2000 and 2001. *Int. J. Food Micro.* 114(2):187–194.

Hussein, H.S. and T. Sakuma. 2005. Invited review: Prevalence of Shiga toxin–producing *Escherichia coli* in dairy cattle and their products. *J. Dairy Sci.* 88:450–465.

Jayarao, B.M., S.C. Donaldson, B.A. Straley, A.A. Sawant, N.V. Hegde, and J.L. Brown. 2006. A survey of foodborne pathogens in bulk tank milk and raw milk consumption among farm families in Pennsylvania. *J. Dairy Sci.* 89:2451–2458.

Jayarao, B.M. and D.R. Henning. 2001. Prevalence of foodborne pathogens in bulk tank milk. *J. Dairy Sci.* 84:2157–2162.

Kabuki, D.Y., A.Y. Kuaye, M. Wiedmann, and K.J. Boor. 2004. Molecular subtyping and tracking of *Listeria monocytogenes* in Latin-style fresh-cheese processing plants. *J. Dairy Sci.* 87:2803–2812.

Kagkli, D.M., M. Vancanneyt, P. Vandamme, C. Hill, and T.M. Cogan. 2007. Contamination of milk by enterococci and coliforms from bovine faeces. *J. Appl. Micro.* 103(5):1393–1405.

Kamat, A., R. Warke, M. Kamat, and P. Thomas. 2000. Low-dose irradiation as a measure to improve microbial quality of ice cream. *Int. J. Food Micro.* 62(1–2):27–35.

Kansas Department of Health and Environment. 2007. Outbreak of *Campylobacter jejuni* infections associated with consumption of cheese made from raw milk—Western Kansas, 2007. http://www.kdheks.gov/epi/download/Western_KS_OCT07_Campylobacter.pdf

Karns, J.S., J.S. Van Kessel, B.J. McCluskey, and M.L. Perdue. 2005. Prevalence of *Salmonella enterica* in bulk tank milk from US dairies as determined by polymerase chain reaction. *J. Dairy Sci.* 88:3475–3479.

Karns, J.S., J.S. Van Kessel, B.J. McCluskey, and M.L. Perdue. 2007. Incidence of *Escherichia coli* O157:H7 and *E. coli* virulence factors in US bulk tank milk as determined by polymerase chain reaction. *J. Dairy Sci.* 90:3212–3219.

Kongo, J.M., F.X. Malcata, A.J. Ho, and M. Wiedmann. 2006. Detection and characterization of *Listeria monocytogenes* in Sao Jorge (Portugal) cheese production. *J. Dairy Sci.* 89:4456–4461.

Konuk, M., E. Korcan, S. Dülgerbaki, and M. Altındiş. 2007. Isolation and identification of Mycobacteria from raw milk samples in Afyonkarahisar district of Turkey. *Int. J. Food Micro*. 115(3):343–347.

Kuusi, M., E. Lahti, A. Virolainen, M. Hatakka, R. Vuento, L. Rantala, J. Vuopio-Varkila, E. Seuna, M. Karppelin, M. Hakkinen, J. Takkinen, V. Gindonis, K. Siponen, and K. Huotari. 2006. An outbreak of *Streptococcus equi* subspecies *zooepidemicus* associated with consumption of fresh goat cheese. *BMC Infect. Dis.* 6:36.

Lahti, E., M. Eklund, P. Ruutu, A. Siitonen, L. Rantala, P. Nuorti, T. Honkanen-Buzalski. 2002. Use of phenotyping and genotyping to verify transmission of *Escherichia coli* O157:H7 from dairy farms. *Eur. J. Clin. Microbiol. Infect. Dis.* 21:189–195.

Lekkas, C., A. Kakouri, E. Paleologos, L.P. Voutsinas, M.G. Kontominas, and J. Samelis. 2006. *Food Micro.* 23(3):268–276.

Linnan, M.J., L. Mascola, X.D. Lou, V. Goulet, S. May, C. Salminen, D.W. Hird, M.L. Yonekura, P. Hayes, R. Weaver, A. Audurier, B.D. Plikaytis, S.L. Fannin, A. Kleks, and C.V. Broome. 1988. Epidemic Listeriosis associated with Mexican-style cheese. *N. Engl. J. Med*. 319:823–828.

Little, C.L., J.R. Rhoades, S.K. Sagoo, J. Harris, M. Greenwood, V. Mithani, K. Grant, and J. McLauchlin. 2008. Microbiological quality of retail cheeses made from raw, thermized or pasteurized milk in the UK. *Food Micro.* 25(2):304–312.

López-Pedemonte, T., A. Roig-Sagués, S. De Lamo, M. Hernandez-Herrero, and B. Guamis. 2007. Reduction of counts of *Listeria monocytogenes* in cheese by means of hydrostatic pressure. *Food Micro.* 24(1):59–66.

Lovett, J., J.G. Bradshaw, and J.T. Peeler. 1982. Thermal inactivation of *Yersinia enterocolitica* in milk. *Appl. Environ. Microbiol*. 44(2):517–519.

MacDonald, P.D.M., R.E. Whitwam, J.D. Boggs, J.N. MacCormack, K.L. Anderson, J.W. Reardon, J.R. Saah, L.M. Graves, S.B. Hunter and J. Sobel. 2005. Outbreak of listeriosis among Mexican immigrants as a result of consumption of illicitly produced Mexican-style cheese. *Clin. Infect. Dis.* 40:677–682.

Makino, S.-I., K. Kawamoto, K. Takeshi, Y. Okada, M. Yamasaki, S. Yamamoto, and S. Igimi. 2005. An outbreak of food-borne listeriosis due to cheese in Japan, during 2001. *Int. J. Food Micro.* 104(2):189–196.

Mariani, C., R. Briandet, J.-F. Chamba, E. Notz, A. Carnet-Pantiez, R.N. Eyoug, and N. Oulahal. 2006. Biofilm ecology of wooden shelves used in ripening the French raw milk smear cheese Reblochon de Savoie. *J. Dairy Sci.* 90:1653–1661.

Martin, M.L., L.D. Shipman, J.G. Wells, M.E. Potter, K. Hedberg, I.K. Wachsmuth, and R.V. Tauxe. 1986. Isolation of *Escherichia coli* O157:H7 from dairy cattle associated with two cases of haemolytic uraemic syndrome. *Lancet* 2:1043.

Mazurek, J., E. Salehi, D. Propes, J. Holt, T. Bannerman, L.M. Nicholson, M. Bundesen, R. Duffy, and R.L. Moolenaar. 2004. A multistate outbreak of *Salmonella enterica* serotype Typhimurium infection linked to raw milk consumption—Ohio, 2003. *J. Food Prot.* 67:2165–2170.

McAuliffe, O., C. Hill, and R.P. Ross. Inhibition of *Listeria monocytogenes* in cottage cheese manufactured with a lacticin 3147–producing starter culture. *J. Appl. Micro.* 86(2):251–256.

McLauchlin, J., R.T. Mitchell, W.J. Smerdon, and K. Jewel. 2004. *Listeria monocytogenes* and listeriosis: a review of hazard characterisation for use in microbiological risk assessment of foods. *Int. J. Food Micro.* 92(1):15–33.

Meckel, R.A. 1998. *Save the Babies: American Public Health Reform and the Prevention of Infant Mortality 1850–1929.* The University of Michigan Press: Ann Arbor.

Milk Quality Improvement Program, Department of Food Science, Cornell University. 2007. Disease Outbreaks Associated with Milk Products. http://www.milkfacts.info/Milk%20Microbiology/Disease%20Outbreaks.htm

Mora, A., S.L. León, M. Blanco, J.E. Blanco, C. López, G. Dahbi, A. Echeita, E.A. González, and J. Blanco. 2007. Phage types, virulence genes and PFGE profiles of Shiga toxin–producing *Escherichia coli* O157:H7 isolated from raw beef, soft cheese and vegetables in Lima (Peru). *Int. J. Food Micro.* 114(2):204–210.

Morgan, D., C.P. Newman, D.N. Hutchinson, A.M. Walker, B. Rowe, and F. Majid. 1993. Verotoxin producing *Escherichia coli* O157 infections associated with the consumption of yogurt. *Epidemiol. Infect.* 111:181–187.

Muehlherr, J.E., C. Zweifel, S. Corti, J.E. Blanco, and R. Stephan. 2003. Microbiological quality of raw goat's and ewe's bulk-tank milk in Switzerland. *J. Dairy Sci.* 86:3849–3856.

Murphy, M., J.F. Buckley, P. Whyte, M. O'Mahony, W. Anderson, P.G. Wall, and S. Fanning. 2007. Surveillance of dairy production holdings supplying raw milk to the farmhouse cheese sector for *Escherichia coli* O157, O26 and O111. *Zoonoses and Public Health* 54(9–10):358–365.

National Agricultural Statistical Services. 2008. http://www.nass.usda.gov

National Dairy Council. 2004. Milk's Unique Nutrient Package: Benefits for Bones and Beyond. http://www.nationaldairycouncil.org/NationalDairyCouncil/Nutrition/Nutrients/Dairys+Nutrient+Package.htm

Nieminen, T., N. Rintaluoma, M. Andersson, A.-M. Taimisto, T. Ali-Vehmas, A. Seppälä, O. Priha, and M. Salkinoja-Salonen. 2007. Toxinogenic *Bacillus pumilus* and *Bacillus licheniformis* from mastitic milk. *Vet. Micro.* 124(3–4):329–339.

Nissen, H., H. Holo, L. Axelsson, and H. Blom. 2001. Characterization and growth of *Bacillus* spp. in heat-treated cream with and without nisin. *J. Appl. Micro.* 90(4):530–534.

Osterholm, M.T., K.L. Macdonald, K.E. White, J.G. Wells, J.S. Spika, M.E. Potter, J.C. Forfang, R.M. Sorenson, P.T. Milloy, and P.A. Blake. 1986. An outbreak of a newly recognized chronic diarrhea syndrome associated with raw milk consumption. *JAMA* 256:484–490.

O'Sullivan, L., E.B. O'Connor, R.P. Ross, and C. Hill 2006. Evaluation of live-culture–producing lacticin 3147 as a treatment for the control of *Listeria monocytogenes* on the surface of smear-ripened cheese. *J. Appl. Micro.* 100(1):135–143.

Peterson, M.C. 2003. *Campylobacter jejuni enteritis* associated with consumption of raw milk. *J. Environ. Health* 65:20–21.

Potter, M.E., M.J. Blaser, R.K. Sikes, A.F. Kaufmann, and J.G. Wells. 1983. Human *Campylobacter* infection associated with raw milk. *Am. J. Epidem.* 117(4):475–483.

Pradel, N., V. Livrelli, C. De Champs, J.B. Palcaus, A. Reynaud, F. Scheutz, J. Sirot, B. Joly, and C. Forestier. 2000. Prevalence and characterization of Shiga toxin–producing *Escherichia coli* isolated from cattle, food, and children during a one year prospective study in France. *J Clin. Micro.* 38(3):1023–1031.

Public Health Agency of Canada. 2002. *Escherichia coli* O157:H7 outbreak associated with the ingestion of unpasteurized goat's milk in British Columbia, 2001. Canada Communicable Disease Report. http://www.phac-aspc.gc.ca/publicat/ccdr-rmtc/02vol28/dr2801eb.html

Rajagopal, M., B.G. Werner, and J.H. Hotchkiss. 2005. Low pressure $CO_2$ storage of raw milk: microbiological effects. *J. Dairy Sci.* 88:3130–3138.

Reed, B.A. and L.E. Grivetti. 2000. Controlling on-farm inventories of bulk-tank raw milk—an opportunity to protect public health. *J. Dairy Sci.* 83:2988–2991.

Roadhouse, C.L. and J.L Henderson. 1941. *The Market Milk Industry.* McGraw-Hill Book Company, Inc.: New York.

Robertson, G. 2005. *Food Packaging: Principles and Practice*, 2nd ed. Taylor & Francis: Boca Raton, FL.

Rodríguez, E., J.L. Arqués, M. Nunez, P. Gaya, and M. Medina. 2005. Combined effect of high-pressure treatments and bacteriocin–producing lactic acid bacteria on inactivation of *Escherichia coli* O157:H7 in raw-milk cheese. *Appl. Environ. Microbiol.* 71(7):3399–3404.

Royal Society of Chemistry. 2008. March/April: Pasteur & Bernard Test Pasteurisation. http://www.rsc.org/Library/LICHelp/HistoricalChemistry/March.asp Accessed December 2008.

Ryan, C.A., M.K. Nichels, N.T. Hargrett-Bean, M.E. Potter, T. Endo, L. Mayer, C.W. Langkop, C. Gibson, R.C. McDonald, R.T. Kenney, N.D. Puhr, P.J. McDonnell, R.J. Martin, M.L. Cohen, and P.A. Blake. 1987. Massive outbreak

of antimicrobial-resistant Salmonellosis traced to pasteurized milk. *JAMA* 258:3269–3274.

Ryser, E.T. and E.H. Marth. 1999. *Listeria, Listeriosis, and Food Safety: Listeriosis, and Food Safety.* CRC Press: Boca Raton, FL.

Santayana, G. 1905. *The Life of Reason—Part 1. Reason in Common Sense*. Charles Scribner's Sons: New York.

Schiemann, D.A. 1978. Association of *Yersinia enterocolitica* with the manufacture of cheese and occurrence in pasteurized milk. *Appl. Environ. Microbiol*. 36(2):274–277.

Schiemann, D.A. 1987. *Yersinia enterocolitica* in milk and dairy products. *J. Dairy Sci.* 70:383–391.

Seydim, A.C. and G. Sarikus. 2006. Antimicrobial activity of whey protein based edible films incorporated with oregano, rosemary and garlic essential oils. *Food Res. Int.*:40 (7):639–644.

Sheehan, J.F. 2005. On the safety of raw milk (with a word about pasteurization). 2005 National Conference for Interstate Milk Shippers. http://www.cfsan.fda.gov/~ear/milksafe.html

Stephan, R., S. Schumacher, T. Tasara, and I.R. Grant. 2007. Prevalence of *Mycobacterium avium* subspecies *paratuberculosis* in Swiss raw milk cheeses collected at the retail level. *J. Dairy Sci.* 90:3590–3595.

Tacket, C.O., J.P. Narain, R. Sattin, J.P. Lofgren, C. Konigsberg, Jr., R.C. Rendtorff, A. Rausa, B.R. Davis, M.L. Cohen. 1984. A multistate outbreak of infections caused by *Yersinia enterocolitica* transmitted by pasteurized milk. *JAMA* 251:483–486.

Townes, J.M., P.R. Cieslak, C.L. Hatheway, H.M. Solomon, J.T. Holloway, M.P. Baker, C.F. Keller, L.M. McCroskey, and P.M. Griffin. 1996. An outbreak of type A botulism associated with a commercial cheese sauce. *Annals Int. Med.* 125(7):558–563.

Turkish Ministry of Agriculture and Rural Affairs General Directorate of Protection and Control Vision. 2000. Turkish Food Codex (Turk Gida Kodeksi). http://www.kkgm.gov.tr/TGK/Teblig/2000-6.html

U.S. Food and Drug Administration Center for Food Safety and Applied Nutrition. 2009. *Foodborne Pathogenic Microorganisms and Natural Toxins Handbook: The "Bad Bug Book."* http://www.foodsafety.gov/~mow/intro.html or http://vm.cfsan.fda.gov/~MOW/intro.html

U.S. Public Health Service/Food and Drug Administration. 2003. *Grade "A" Pasteurized Milk Ordinance: 2003 Revision.* http://www.cfsan.fda.gov/~ear/pmo03toc.html

Valeeva, N.I., M.P.M. Meuwissen, A.G.J.M. Oude Lansink, and R.B.M. Huirne. 2005. Improving food safety within the dairy chain: an application of conjoint analysis. *J. Dairy Sci.* 88:1601–1612.

Van Gerwen, S.J.C., M.C. te Giffel, K. van 't Riet, R.R. Beumer, and M.H. Zwietering. 2000. Stepwise quantitative risk assessment as a tool for characterization of microbiological food safety. *J. Appl. Micro.* 88(6):938–951.

Van Kessel, J.S., J.S. Karns, L. Gorski, B.J. McCluskey and M.L. Perdue. 2004. Prevalence of Salmonellae, *Listeria monocytogenes*, and fecal coliforms in bulk tank milk on US dairies. *J. Dairy Sci.* 87:2822–2830.

Vasavada, P.C. 1988. Pathogenic bacteria in milk—a review. *J. Dairy Sci.* 71:2809–2816.

Vernozy-Rozand, C., C. Mazuy-Cruchaudet, C. Bavai, M.P. Montet, V. Bonin, A. Dernburg, and Y. Richard. 2005. Growth and survival of *Escherichia coli* O157:H7 during the manufacture and ripening of raw goat milk lactic cheeses. *Int. J. Food Micro.* 105(1):83–88.

Villar, R.G., M.D. Macek, S. Simons, P.S. Hayes, M.J. Goldoft, J.H. Lewis, L.L. Rowan, D. Hursh, M. Patnode, and P.S. Mead. 1999. Investigation of multi-drug-resistant *Salmonella* serotype Typhimurium DT104 infections linked to raw-milk cheese in Washington state. *JAMA* 281(19):1811–1816.

West, H.G. 2008. Food fears and raw-milk cheese. *Appetite* 51(1):25–29.

Weston A. Price Foundation. 2007. Response to the FDA: A point-by-point rebuttal to the anti-raw milk powerpoint presentation, by John F. Sheehan, BSc (Dy), JD, Division of Dairy and Egg Safety. http://www.realmilk.com/documents/SheehanPowerPointResponse.pdf

Weston A. Price Foundation. 2008. A Campaign for Real Milk. http://www.realmilk.com/

Zhou, G., H. Liu, J. He, Y. Yuan, Z. Yuan. 2007. The occurrence of *Bacillus cereus*, *B. thuringiensis* and *B. mycoides* in Chinese pasteurized full fat milk. *Int. J. Food Micro.* 121(2):195–200.

# chapter 4

# Seafood and restructured seafood

*Yi-Cheng Su and Chengchu Liu*

*Contents*

The United States has nearly 13,000 miles of coastline bordering the Pacific and Atlantic Oceans, the Gulf of Mexico, the Gulf of Alaska, the Bering Sea, and the Arctic Ocean, which forms the world's largest exclusive economic zone, consisting of approximately 3.4 million square nautical miles of oceans for fishing and marine resources covered by 200 nautical miles offshore. The U.S. commercial marine fishing industry, including domestic and foreign markets, contributed $35.1 billion to the gross national product in 2006 (NMFS 2007). U.S. consumers spent an estimated $69.5 billion for fishery products in 2006: $46.6 billion at food service establishments, $22.7 billion in retail sales for home consumption, and $318.1 million for industrial fish products (NMFS 2007).

The United States imports about 84% of its seafood and is the third largest consumer of fish and shellfish, behind China and Japan. According to the National Marine Fisheries Service (NMFS) of the National Oceanic and Atmospheric Administration (NOAA), Americans consumed a total of 4.9 billion pounds of seafood in 2007. The average American consumed 16.3 pounds of fish and shellfish (NOAA 2008). Of the total of 16.3 pounds consumed per person in 2007, 12.1 pounds were fresh and frozen finfish and shellfish; shrimp (4.1 pounds per person) remained the number one choice for seafood consumption in the United States.

Seafood is an important source of protein in many diets around the world. Fish and shellfish on average provide 25% of the protein consumed in developing countries and 13% in developed countries (NOAA 2007). Worldwide seafood consumption has doubled since 1973, and global wild-caught fisheries production has peaked at approximately 93 million tons per year (Delgado et al. 2003). Consumer demand for seafood is expected to continue to grow as more and more consumers realize the health benefits of eating seafood containing high levels of omega-3 fatty acids such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Cold water fatty fish, such as herring, mackerel, sardine, salmon, and tuna, is the main source of EPA and DHA. Numerous studies have revealed strong evidence that omega-3 fatty acids reduce blood triglyceride levels, and regular intake of fish oil stimulates blood circulation and reduces the risk of secondary and primary heart attack (Willett et al. 1993; Roche and

Gibney 1996; Stone 1996; Harris 1997; Sanders et al. 1997; Bucher et al. 2002; Davidson et al. 2007).

The U.S. Food and Drug Administration (FDA) announced a qualified health claim about EPA and DHA, stating "Supportive but not conclusive research shows that consumption of EPA and DHA omega-3 fatty acids may reduce the risk of coronary heart disease" (FDA 2004). The FDA recommended that women and young children include up to 12 ounces (two average meals) a week of a variety of fish and shellfish as a regular part of their diet, but avoid eating shark, swordfish, king mackerel, or tilefish because they may contain high levels of mercury (FDA 2006).

## *4.1 Illness associated with seafood consumption*

Seafood is highly perishable because it can harbor microorganisms, including human pathogens, which exist in marine environments. The numbers and types of indigenous microorganisms on freshly harvested seafood depend on the geographical location, harvest season, and on-board handling. While a majority of the indigenous microorganisms associated with seafood present no health threat to human health, human pathogens, such as *Vibrio parahaemolyticus, Listeria monocytogenes*, *Staphylococcus aureus*, and *Clostridium botulinum*, could sometimes be present in seafood and cause foodborne infection. Consumption of improperly cooked or ready-to-eat (RTE) seafood containing these pathogens may lead to development of acute gastroenteritis, sepsis, and, in rare cases, paralysis and death.

Since all the human pathogens associated with seafood can be destroyed by thorough cooking, RTE seafoods are the main vehicles for foodborne illness associated with seafood consumption. RTE seafoods include raw (such as oysters, clams, and sushi) or precooked (such as cooked shrimp, cooked crabmeat, and surimi seafood) products that require no heating or cooking prior to consumption and can be contaminated with human pathogens during handling or post-processing preparation.

The Center for Science in the Public Interest (CSPI) identified 5,778 outbreaks in the United States between 1990 and 2006 involving 168,898 individual illnesses that linked to specific foods (CSPI 2008). The food categories that were most commonly linked to outbreaks were seafood (1,140 outbreaks with 11,809 illnesses), followed by produce, poultry, beef, and eggs (Table 4.1). Among the outbreaks linked to seafood consumption, 694 outbreaks (3,391 illnesses) were associated with consuming finfish (such as tuna and grouper), and 175 outbreaks (3,794 illnesses) were associated with consuming molluscan shellfish including oysters, clams, and mussels. Seafood dishes like crab cakes and tuna burgers were the vehicles of 197 outbreaks (3,715 illnesses). Other seafood, such as shrimp and lobster, were involved in 74 outbreaks (909 illnesses). By comparing

***Table 4.1*** Foods Commonly Linked to Outbreaks that Occurred between 1990 and 2006

| Food Categories | Outbreaks | Cases of Illness |
|---|---|---|
| Seafood | 1,140 | 11,809 |
| Produce | 768 | 35,060 |
| Poultry | 620 | 18,906 |
| Beef | 518 | 14,191 |
| Eggs | 351 | 11,143 |

*Source:* Data adapted from CSPI 2008.

the relative rates of outbreak-related illnesses caused by various foods (seafood, produce, poultry, beef, and eggs), the CSPI reported that fish and shellfish caused more sicknesses per bite than any other food categories.

## *4.2 Outbreaks linked to seafood consumption*

RTE seafoods are usually safe to consume. However, various types of RTE seafoods, including seafood cocktail, seafood salad, shrimp cocktail, smoked fish, and raw shellfish have been linked to outbreaks because of contamination with human pathogens during processing, preparation, and distribution of products (Table 4.2).

### *4.2.1 Seafood salad and seafood cocktail*

Seafood salad and seafood cocktail are common appetizers served at seafood restaurants and on cruise ships. While the seafood ingredients in seafood salad and cocktail are usually cooked before serving, produce (such as lettuce and spinach) used for salads are eaten raw. The produce may come in contact with organic fertilizers, such as manure, that contain human pathogens or viruses. In some cases, produce may be irrigated with contaminated waters or be handled by workers with poor hygiene practices, the latter of which provides an opportunity for post-harvest contamination of foodborne pathogens.

Seafood cocktail was implicated in 8 of 13 documented foodborne outbreaks on cruise ships between 1975 and 1985 (Addiss et al. 1989). Among them, a large outbreak involving 403 passengers who developed gastroenteritis with symptoms clinically compatible with a 27-nm Norwalk-like virus after a 1-week Caribbean cruise was linked to shrimp cocktail consumption (CDC 1988). A large outbreak of 242 cases associated with eating a seafood salad at a Mother's Day brunch in a hotel in New York occurred

***Table 4.2*** Outbreaks Linked to Consumption of Cooked or Smoked Ready-to-Eat Seafood

| Seafood Products | Cases | Year | Location | Reference |
|---|---|---|---|---|
| Shrimp cocktail | 403 | 1985 | Caribbean cruise | CDC 1988 |
| Seafood salad | 242 | 1989 | New York | CDC 1996 |
| Smoked mussels | 2 | 1991 | Australia | Mitchell 1991 |
| Smoked mussels | 2 | 1992 | New Zealand | Brett et al. 1998 |
| Seafood salad | 75 | 1992 | Flight from Peru to Los Angeles | Besser et al. 1994 |
| Gravad, cold-smoked rainbow trout | 9 | 1994–1995 | Sweden | Ericsson et al. 1997 |
| Seafood salad | 114 | 1996 | Texas | Kimura et al. 2006 |
| Surimi crabmeat | 2 | 1996 | Canada | Farber et al. 2000 |
| Seafood salad | 2 | 1999 | Italy | Cavalieri d'Oro et al. 1999 |
| Peel-and-eat shrimp | 51 | 2000 | Palm Beach Princess cruise ship | CDC 2000a |
| Cooked shrimp | 110 | 2000 | *Disney Magic* cruise ship | CDC 2000b |

in 1989 (CDC 1996). The seafood salad was contaminated with *Clostridium perfringens* and had been held at room temperature for hours before being served. An outbreak (75 cases) of cholera, infected by toxigenic *Vibrio cholerae* O1, occurred among more than 100 passengers who ate seafood salad during a flight from Peru to Los Angeles on February 14, 1992 (Besser et al. 1994). A large outbreak of Brainerd diarrhea affecting 114 persons with diarrhea that lasted for more than 4 weeks was linked to seafood salad consumption at a restaurant in Texas in 1996 (Kimura et al. 2006).

In 1999, two patients reported severe diarrhea after eating a precooked ready-to-eat seafood salad including shrimps, scallops, mussels, hen clams, cuttlefishes, and squid in Italy (Cavalieri d'Oro et al. 1999). An epidemiologic case-control investigation involving 454 persons (94 who had eaten the seafood salad and 360 controls who had not eaten the seafood salad in the same canteen) revealed that 37 (39%) of those who had eaten the seafood salad had had at least one episode of diarrhea or other relevant gastrointestinal symptoms, while only one (0.3%) of those who had not eaten the salad reported diarrhea. Toxinogenic *Vibrio cholerae* O1 (biotype El Tor, serotype Ogawa) were isolated from stools of both patients.

### 4.2.2 Smoked fish

The process of smoking foods, including fish, to preserve them has a long history. Two types of processes, cold smoking and hot smoking, can be used to preserve fish. In cold smoking, the fish is cured with smoke at an air temperature not higher than 33°C to avoid significant coagulation of the proteins in the fish flesh. In hot smoking, fish is cured with smoke at a temperature of 70°C to 80°C at some stage in the process in order to cook the flesh (McK. Bannerman 2001). The hot-smoking process is generally sufficient to kill parasites and destroy non-sporeforming bacterial pathogens. Therefore, hot-smoked fish products do not require further heating before consumption. However, the cold-smoking process is not a cooking process and allows human pathogens to survive in the smoked fish products. Since cold-smoked fish is produced without intense thermal treatment and may be consumed without heating, this kind of RTE seafood can serve as a vehicle for foodborne illness. Outbreaks of listeriosis (illness caused by *Listeria monocytogenes* infection) linked to consuming cold-smoked rainbow trout and smoked mussels have been reported in Sweden (Ericsson et al. 1997) and New Zealand (Brett et al. 1998).

### 4.2.3 Shellfish

Shellfish are filter-feeding bivalves that filter water for nutrients and often accumulate bacteria and marine toxins from the surrounding water. Foodborne illnesses linked to seafood consumption are frequently associated with eating shellfish contaminated with pathogens, mainly *Vibrio parahaemolyticus*, and marine toxins.

In the United States, *V. parahaemolyticus* was first identified as an etiological agent of food-related gastroenteritis after three outbreaks involving 425 illnesses associated with consumption of improperly cooked crabs occurred in Maryland in August 1971 (Molenda et al. 1972). Between 1973 and 1998, approximately 40 outbreaks of *V. parahaemolyticus* infections were reported to the Centers for Disease Control and Prevention (CDC) with most of the illnesses resulting from shellfish consumption (Daniels et al. 2000). Since then, several major outbreaks of *V. parahaemolyticus* have been recorded in the United States (Table 4.3). Four large outbreaks of *V. parahaemolyticus* infections resulting in more than 700 cases of illness in the Gulf Coast, Pacific Northwest, and Atlantic Northeast regions of the United States were reported in 1997 and 1998 (CDC 1998, 1999; DePaola et al. 2000). Nearly all the cases were associated with eating raw oysters harvested in the State of Washington and British Columbia of Canada. An outbreak of *V. parahaemolyticus* infection linked to consuming Alaska oysters occurred on board a cruise ship in Alaska in 2004 (McLaughlin

***Table 4.3*** Major Outbreaks of *V. parahaemolyticus* Infection Associated with Oyster Consumption in the United States Since 1997

| Year | Cases | Location | Reference |
|---|---|---|---|
| 1997 | 209 | Oregon, Washington, California, British Columbia of Canada | CDC 1998 |
| 1998 | 43 | Washington | DePaola et al. 2000 |
| 1998 | 416 | Texas | DePaola et al. 2000 |
| 1998 | 8 | Connecticut, New Jersey, New York | CDC 1999 |
| 2004 | 14 | Alaska | McLaughlin et al. 2005 |
| 2006 | 177 | New York, Oregon, Washington | CDC 2006a |

et al. 2005). More recently, in 2006 an outbreak of 177 cases of *V. parahaemolyticus* infection (72 confirmed and 105 probable) in New York, Oregon, and Washington was linked to consumption of raw oysters harvested in Washington and British Columbia (CDC 2006a).

Other than bacterial infection, seafood-borne illness can also be caused by marine toxins, such as saxitoxin, okadaic acid, brevetoxins, and domoic acid, which shellfish accumulate from feeding on toxic algae. These toxins can cause shellfish poisoning, including paralytic shellfish poisoning (PSP), diarrheic shellfish poisoning (DSP), neurotoxic shellfish poisoning (NSP), and amnesic shellfish poisoning (ASP) (FDA 1992). Shellfish contaminated with these chemicals usually looks, smells, and tastes normal. A large outbreak of paralytic shellfish poisoning affected 187 persons, killing 26, in Guatemala in 1987 (Rodrigue et al. 1990).

Between 1973 and 1987, 19 PSP outbreaks, with an average of 8 cases per outbreak, caused by consumption of mussels, clams, oysters, scallops, and cockles were reported to CDC's Foodborne Disease Outbreak Surveillance System (CDC 1991). Four episodes of PSP outbreak with 13 cases reported in Massachusetts and Alaska in 1990 were linked to consumption of boiled blue mussels (*Mytilus edulis*), steamed butter clams, and butter clam broth (CDC 1991). Shellfish, including butter clams, mussels, cockles, steamer clams, sea snails, and razor clams, have been implicated in 42 PSP outbreaks documented in Alaska from 1976 through 1989 (CDC 1991). Seven more PSP outbreaks affecting 43 persons with 13 of them hospitalized occurred in the United States between 1998 and 2002 (Sobel and Painter 2005).

While most cases of PSP have occurred in individuals or small groups who collected shellfish for personal consumption, 10 illnesses caused by PSP toxin were reported in Florida, New Jersey, and Virginia in 2002 associated with consumption of pufferfish caught in waters near Titusville, Florida (CDC 2002). It is estimated that 30 cases of poisoning by marine toxins are reported in the United States each year (CDC 2005). However,

the actual number of poisonings may be much greater because many milder cases are not diagnosed or reported because the healthcare providers are not required to report the illnesses.

### 4.2.4 Other seafoods

In addition to RTE seafoods, other seafoods can also serve as vehicles for foodborne illness. On May 18, 2000, an outbreak of gastroenteritis associated with peel-and-eat shrimp and seafood stew served at a dinner buffet occurred on the cruise ship Palm Beach Princess (CDC 2000a). Investigators of the outbreak could not collect food or patient specimens to identify implicated pathogens because of a delay in receiving reports of the outbreak. However, questionnaire data suggested a statistical association between eating shrimp and seafood stew at the buffet and illness among 51 passengers. Two weeks later, a total of 100 passengers and 10 crew members aboard the *Disney Magic* cruise ship developed gastroenteritis, characterized primarily by diarrhea and vomiting, after eating a lunch buffet containing cooked shrimp (CDC 2000b). Investigation of the outbreak revealed that cooked shrimp, which had been served at the lunch buffet, was contaminated with *Salmonella Newport*. Clinical analysis isolated *Salmonella* (*S. Newport*, *S. Java*, and *S. Lexington*) and enterotoxigenic *E. coli* (ETEC O25:NM and O6:H16) from stool specimens of several passengers and one crew member. Both *Salmonella* and ETEC *E. coli* can cause acute gastroenteritis characterized by nausea, vomiting, abdominal cramps, and diarrhea. Environmental investigations suggest that raw frozen shrimp could have been the source of contamination. Cross-contamination of the cooked shrimp with raw shrimp occurred during food preparation, possibly by mishandling of cooked shrimp by food preparation workers.

Seafoods that have been thoroughly cooked and prepared under sanitary conditions by those practicing good personal hygiene should contain no pathogens. However, illness associated with seafood consumption can be caused by toxic chemicals, such as histamine or other biogenic amines, in fish. Two outbreaks of scombroid food poisoning (illness caused by high levels of histamine) associated with eating tuna steaks from Indonesia and Vietnam were reported in Louisiana and Tennessee in 2006 (CDC 2007a).

## 4.3 Microbial hazards associated with seafood

### 4.3.1 Listeria monocytogenes

*L. monocytogenes* is an important foodborne pathogen that is widely distributed in nature. This organism can be isolated from a variety of

sources, including soil, water, sewage, feces, and food processing environments (Watkins and Sleath 1981; Colburn et al. 1990; Eklund et al. 1995). Consumption of foods contaminated with *L. monocytogenes* can lead to development of serious diseases in humans (listeriosis), particularly in pregnant women, newborns, the elderly, and people with suppressed immune systems. Initial symptoms for listeriosis often include fever, muscle aches, and sometimes gastrointestinal symptoms such as nausea or diarrhea. The illness may be mild but symptoms such as headache, stiff neck, confusion, loss of balance, or convulsions can occur if infection spreads to the nervous system. While most cases of listeriosis occur in adults with weakened immune systems, the elderly, pregnant women, and newborns, infections can occur in healthy persons. Infections during pregnancy can lead to miscarriages, stillbirths, and infection of newborns. The U.S. Department of Agriculture (USDA) estimated that 2,500 cases of listeriosis occur each year in the United States, resulting in nearly 2,300 hospitalizations and 500 deaths (USDA 2000).

*L. monocytogenes* is an environmental contaminant and has been isolated from food processing environments. The cool and wet environment in seafood processing plants is conducive to the presence of *L. monocytogenes* and can be a source for *L. monocytogenes* contamination in seafood (Dillon and Patel 1992; Eklund et al. 1995; Rørvik et al. 1995). This pathogen has been frequently isolated from a variety of frozen and minimally processed seafoods (Wong et al. 1990; Farber 1991; Autio et al. 1999). Incidences of *L. monocytogenes* in frozen seafood, including raw shrimp and lobster tails, scallops, cooked shrimp, and cooked crabmeat have been reported as high as 26% (Weagant et al. 1988).

One important characteristic of *L. monocytogenes* is that it is capable of growing at refrigeration temperatures to a significant number over time (Guyer and Jemmi 1991). Although *L. monocytogenes* can be destroyed by pasteurization and adequate thermal processes, it often enters cooked, RTE products as a post-processing contaminant. Therefore, outbreaks of listeriosis are more frequently associated with consuming RTE foods that are stored in the refrigerator before consumption. Cooked seafoods, particularly shrimp, crab, and lobster that are picked by hand, can be easily contaminated with *L. monocytogenes* during processing through poor manufacturing practices or personal hygiene (McCarthy 1997). Outbreaks of listeriosis linked to consumption of RTE seafood, including cold-smoked fish, smoked mussels, and imitation crabmeat, have been reported in Sweden, New Zealand, and Canada (Ericsson et al. 1997; Brett et al. 1998; Farber et al. 2000).

Surimi seafoods are usually free of human pathogens because of the cooking process involved during production. However, *L. monocytogenes* can enter cooked products as post-processing contamination. Numerous

recalls of surimi seafoods have been recorded due to *L. monocytogenes* contamination since the first reported class I recall of crabsticks manufactured in Japan and distributed in three states in 1988 (Jinneman et al. 1999) (Table 4.4). While no outbreaks of listeriosis linked to surimi seafood consumption have been reported in the United States, a small outbreak of listeriosis linked to the consumption of surimi-based crabmeat occurred in Canada in 1996 (Farber et al. 2000). A husband and wife were hospitalized with symptoms of nausea, fever, vomiting, and diarrhea shortly after consumption of the crabmeat product. *Listeria monocytogenes* serotype 1/2b was isolated from stools of both patients and the surimi-based crabmeat.

Contamination of *L. monocytogenes* in RTE foods usually results in the costly consequences of product recalls, extensive clean-up, investigation of contamination, and loss of consumer confidence. Between October 1993 and September 1998, 813 of 1,328 food product recalls reported to FDA because of microbial contamination were due to *L. monocytogenes* contamination (Wong et al. 2000). The social and economic impact of listeriosis is reported among the highest of foodborne illnesses because of the severity of the disease and its frequent involvement of RTE foods (Roberts and Pinner 1990). A "zero-tolerance" policy was established by the U.S. Food and Drug Administration for *L. monocytogenes* in RTE foods, including RTE seafood (FDA 1999).

### *4.3.2* Vibrio parahaemolyticus

*Vibrio parahaemolyticus* is a human pathogen that occurs naturally in marine environments and a well-documented causative agent of acute gastroenteritis associated with consumption of raw or undercooked seafoods. This organism is recognized as the leading cause of human gastroenteritis associated with seafood consumption in the United States and an important seafood-borne pathogen throughout the world (Ayres and Barrow 1978; Dalsgaard 1998; Kaysner and DePaola 2001). This organism is frequently isolated from a variety of seafoods including finfish (codfish, sardine, mackerel, and flounder) and shellfish (shrimp, crab, lobster, clam, crawfish, and oyster) (Liston 1990). Consumption of raw or undercooked shellfish, particularly raw oysters, contaminated with *V. parahaemolyticus* can lead to development of acute gastroenteritis characterized by diarrhea, vomiting, abdominal cramps and, sometimes, low fever (Rippey 1994). The illness is often self-limited, but the infection may cause septicemia that is life-threatening to people having underlying medical conditions such as liver disease or immune disorders (Bonner et al. 1983).

*Vibrio parahaemolyticus* was first recognized in Japan as a cause of foodborne illness (272 illnesses and 20 deaths) associated with consumption of sardines in 1950 (Fujino et al. 1953). Since then, *V. parahaemolyticus*

***Table 4.4*** Recalls of RTE Seafood Contaminated with *Listeria monocytogenes* in the United States

| Recalled Product | Date | Distribution |
|---|---|---|
| Imitation breaded scallops | 09/19/1990 | New York, New Jersey |
| Imitation crab meat salad | 08/06/1992 | Iowa, Nebraska |
| Cold-smoked sablefish | 11/17/1992 | New York |
| Smoked mussels | 12/28/1992 | California, Colorado, Florida, Georgia, Illinois, Kansas, Maryland, Maine, Michigan, North Carolina, Nevada, Oklahoma, Oregon, Pennsylvania, Texas, Utah |
| Alaska snow crab legs and claws | 01/27/1993 | Northwest United States and Canada |
| Fresh crab meat | 03/15/1993 | Florida |
| Imitation crab meat (chunks) | 03/29/1996 | Arizona, California, Nevada, Oregon, Washington |
| Imitation King crab meat | 06/02/1997 | Washington |
| Surimi (imitation crab) spread | 08/12/1997 | Idaho, Nevada, Oregon, Utah, Wyoming |
| Smoked salmon dip | 04/02/1999 | Indiana, Kentucky, New Jersey, North Carolina, Pennsylvania, Virginia |
| Cold-smoked Atlantic salmon | 06/14/1999 | Massachusetts |
| Imitation crab meat | 07/28/1999 | Canada |
| Shrimp (cooked and peeled) | 05/14/2001 | California, Maryland, Montana, Oregon, Washington |
| Fresh crab claw meat | 04/10/2003 | Georgia, Maryland, New York, Pennsylvania, Texas |
| Smoked salmon | 06/05/2003 | Oregon, Washington |
| Sliced smoked Nova salmon | 12/30/2004 | New York |
| Ready-to-eat seafood dips | 08/16/2007 | Massachusetts, Maryland, New Jersey |
| Dried smoked catfish steaks, smoked seafood products | 05/16/2008 | Nationwide |

*Source:* Data compiled from FDA Enforcement Report (http://www.fda.gov/opacom/Enforce.html) (Accessed April 6, 2009).

has been recognized as a major cause of illness associated with seafood consumption in many Asian countries and has been isolated from a variety of seafood in many countries around the world (Chen et al. 1991; Wong et al. 2000; Deepanjali et al. 2005). In Japan, *V. parahaemolyticus* is a major cause of foodborne illness, and fish is the primary vehicle for the infection (Alam et al. 2002). *V. parahaemolyticus* was the leading cause of 1,710 food poisoning incidents (24,373 cases) reported in Japan between 1996 and 1998 (IDSC 1999) and accounted for 67.5% (1,302 cases) of total bacterial foodborne outbreaks (1,928 cases) reported in Taiwan from 1981 to 2008 (Anonymous 2005).

Since *V. parahaemolyticus* occurs naturally in marine environments, human infection caused by *V. parahaemolyticus* is mainly related to consumption of raw or partially cooked seafood. Outbreaks of *V. parahaemolyticus* infection in the United States have been more frequently associated with raw shellfish consumption. However, illnesses can also occur through consuming products contaminated with *V. parahaemolyticus* after cooking. Cooked foods contaminated with *V. parahaemolyticus* were responsible for 31.1% of the 5,770 foodborne outbreaks that occurred in China from 1991 to 2001 (Liu et al. 2004).

Compared with Asian countries, infections of *V. parahaemolyticus* are less frequently reported in European countries. However, sporadic outbreaks have been reported in countries such as Spain and France. Eight cases of *V. parahaemolyticus* gastroenteritis related to fish or shellfish ingestion occurred in Spain in 1989 (Martínez-Urtaza et al. 2004). An outbreak of 64 illnesses associated with eating raw oysters was reported in Spain in 1999 (Lozano-León et al. 2003). A serious outbreak, affecting 44 patients, linked to consumption of shrimp imported from Asia was reported in France in 1997 (Robert-Pillot et al. 2004). In 2004, 80 guests suffered from *V. parahaemolyticus* infection after attending a wedding at a restaurant in Spain (Martínez-Urtaza et al. 2005). Epidemiologic investigation of the outbreak identified boiled crab, which was processed under unsanitary conditions and held at room temperature for hours before it was served, to be the most possible vehicle of infection.

In the United States, the risk of *V. parahaemolyticus* infection is primarily associated with raw oyster consumption. The United States produces more than 27 million pounds of oysters each year, and most of them are sold live or shucked without further processing (Hardesty 2001; NMFS 2007). It was estimated that 1 in 2,000 meals of raw molluscan shellfish serves as the vehicle for *Vibrio* infection (Ahmed 1991). Raw oyster consumption was reported responsible for about 95% of all deaths associated with seafood consumption in the United States (Oliver 1989). The largest outbreak of *V. parahaemolyticus* gastroenteritis recorded in U.S. history occurred in the summer of 1978, affecting 1,133 of 1,700 persons attending

a dinner in Port Allen, Louisiana (Montville and Matthews 2005). Between 1973 and 1998, 40 outbreaks (>1,000 illnesses) of *V. parahaemolyticus* infections linked to seafood were reported to the CDC with a total of 345 sporadic *V. parahaemolyticus* infections occurring between 1988 and 1997 (Daniels et al. 2000). A majority (88%) of patients with acute gastroenteritis reported eating raw oysters 1 week prior to the illness.

The CDC estimated that 4,500 cases of *V. parahaemolyticus* infection occur each year in the United States (CDC 2008a). *V. parahaemolyticus* was the most frequently reported *Vibrio* species isolated from 232 (42%) of 549 patients with *Vibrio* infection reported to Cholera and Other *Vibrio* Illness Surveillance (COVIS) in 2007 (CDC 2007b). It was also the most frequent (48%) *Vibrio* species reported from non–Gulf Coast states in the United States.

### *4.3.3* Vibrio vulnificus

*V. vulnificus* is more frequently found in the warm water of the Gulf Coast than in areas with cooler water such as New England and the Pacific Northwest. Therefore, outbreaks of *V. vulnificus* infection occur more frequently in the Gulf Coast and the southeast regions. Typical symptoms of *V. vulnificus* infection include wound infection, primary septicemia, and gastroenteritis (Blake et al. 1979; Tacket et al. 1984). Symptoms of infections may include fever, chills, nausea, hypotension, abdominal pain, vomiting, and diarrhea (Janda et al. 1988). This organism is responsible for 20 to 40 cases of primary septicemia each year in the United States (Shapiro et al. 1998). The fatality rate of septicemia developed from the infection is usually high (>50%) and >90% of the suffering patients could become hypotensive within 12 hours of being admitted to hospital (Yamamoto et al. 1990).

One notable clinical finding of *V. vulnificus* infection is the absence of significant diarrhea in a large percentage of patients before their septicemic illnesses. In some cases, the infected patients will develop secondary lesions, typically at the extremities, which often require surgical amputation (Oliver 1989). *V. vulnificus* was the most frequent of the *Vibrio* spp. reported from the Gulf Coast states to COVIS in 2007 (CDC 2007b). Among the 549 patients with *Vibrio* infections, 95 were infected with *V. vulnificus;* 87 hospitalizations (92%) and 30 deaths (32%) resulted.

In addition to infection associated with seafood consumption, *V. vulnificus* can also cause non-foodborne infection through contact with seawater. Analysis of illnesses reported to CDC COVIS from 1997 to 2006 revealed that *V. vulnificus* infections (423 cases) were the most common non-foodborne *Vibrio* infections; 72% of the infections were reported from residents of Gulf Coast states (Dechet et al. 2008). A total of 62 infected

patients died, and others suffered from fever (72%), cellulitis (85%), and amputation (10%). Persons with wounds, particularly those with liver disease, should avoid contact with seawater.

### *4.3.4* Clostridium botulinum

*Clostridium botulinum* is a strict anaerobic, spore-forming bacterium that can produce a series of neurotoxins (type A-G) causing human paralyses and, in some cases, death. This bacterium is widely distributed in the environment, including soil, fresh water, seawater, and ocean sediments. *C. botulinum* type E is mainly associated with the marine environment, and its spores have been isolated from gills and intestinal tracts of a number of marine species (Eklund and Poysky 1965; Nickerson et al. 1967). The classic symptoms of botulism, double vision, blurred vision, drooping eyelids, slurred speech, difficulty in swallowing, dry mouth, and muscle weakness, generally begin 18 to 36 hours after ingestion of food containing botulinal toxins (CDC 2008b). However, the incubation period may be as short as 6 hours or as long as 10 days.

Foodborne botulism is rare, but the mortality rate is high. Outbreaks are usually isolated incidents involving small numbers of people who consumed canned or processed foods that were improperly prepared at home (Smith and Sugiyama 1988). A total of 962 botulism outbreaks involving 2,320 cases and 1,036 deaths were recorded in the United States from 1899 to 1977, with type E toxin involved in 105 outbreaks (CDC 1979). The largest foodborne botulism outbreak ever reported in the United States occurred in 1977 in Michigan when 58 people became ill after eating a hot sauce made with improperly home-canned peppers at a restaurant (Terranova et al. 1978). In the United States, 145 cases of botulism are reported each year. Of those, approximately 15% are foodborne, 65% are infant, and 20% are wound (CDC 2008b). While outbreaks of human botulism caused by the consumption of seafood have rarely been reported, sporadic illnesses have been linked to fishery products such as smoked, salted, or fermented products that were eaten without prior heating (Eklund 1982; CDC 1987; Varma et al. 2004).

*C. botulinum* is classified into two categories (proteolytic and nonproteolytic strains) based on its enzymatic activities. Proteolytic strains of *C. botulinum* include all type A strains and some type B and F strains, which produce putrid odors during growth and form heat-resistant spores. Nonproteolytic strains include all type E strains and some type B and F strains, which do not produce putrid odors from growth and whose spores are less heat-resistant. Growth of proteolytic strains of *C. botulinum* can be inhibited by a water phase salt (WPS) of ≥10%, a water activity ($a_w$) of <0.93, or a temperature <10°C (50°F) (Smelt and Haas 1978). Growth of

nonproteolytic *C. botulinum* can be inhibited by a WPS of ≥5% or an $a_w$ of ≤0.96 (Abrahamsson et al. 1966). One important characteristic of nonproteolytic *C. botulinum* is that it can grow and produce toxins at temperatures as low as 3.3ºC (38ºF), which is a safety concern for vacuum-packed seafoods stored at temperatures higher than 3.3ºC (Solomon et al. 1982).

Despite the physiological characteristics of *C. botulinum*, all the botulinal toxins can be inactivated by heating at 60ºC (140ºF) for 5 min (Sakaguchi 1979). However, *C. botulinum* spores can survive a normal cooking process and germinate in vacuum-packed products under elevated temperatures. Many RTE seafoods that are packed in reduced oxygen or vacuum packs and sold under refrigerated storage may be at risk of growth of *C. botulinum* if products are contaminated with spores from nonproteolytic *C. botulinum*. Vacuum-packed RTE seafood products should be stored at temperatures below 3.3ºC to prevent growth of type E strains of *C. botulinum*.

### *4.3.5 Viruses*

It has been a common perception that most foodborne illnesses are caused by infections of pathogenic bacteria. However, current knowledge reveals that viruses, particularly noroviruses, are the leading cause of foodborne illness in the United States. Many different viruses, including rotaviruses, noroviruses, adenoviruses (type 40 or 41), sapoviruses, and astroviruses, can cause gastroenteritis with symptoms similar to those caused by pathogenic bacteria (CDC 2006b).

Seafood can serve as a vehicle for transmission of noroviruses because of contamination of marine environments by discharge of sewage from plants and fishing boats near harvest area. Noroviruses, previously known as Norwalk or Norwalk-like viruses, are a group of related, single-stranded RNA, nonenveloped viruses that can cause acute gastroenteritis in humans. They can be transmitted via seafood harvested from areas with fecal contamination, or by an infected food handler with poor personal hygiene. Numerous outbreaks of viral gastroenteritis, including one affecting more than 180 persons in 23 clusters in four states (Louisiana, Maryland, Mississippi, and North Carolina) in 1993, have been linked to oyster consumption (Morse et al. 1986; CDC 1993). A review of outbreaks of 50 foodborne diseases associated with passenger ships between January 1970 and June 2003 reported that seafood was the most common food vehicle implicated in the outbreaks (almost one-third) with 866 passengers and crew members infected by noroviruses in several outbreaks (Rooney et al. 2004).

Infection by noroviruses typically produces a sudden illness with main symptoms of vomiting and diarrhea within 12 to 48 hours (CDC 2006b). Infected persons may also develop headache, nausea, fever, and

abdominal pain with symptoms lasting for a few days. Most people can recover from the illness without medications. However, dehydration caused by losing fluids through vomiting or diarrhea is a concern for persons, such as infants and the disabled, who are unable to drink enough fluids during the illness.

In addition to noroviruses, seafood, particularly marine bivalves which filter water for nutrients, harvested from environments contaminated by discharge of sewage or feces can serve as a vehicle for transmission of hepatitis A virus (HAV) (Fiore 2004). Infection with HAV has a longer incubation time (10–50 days) and can cause liver disease with the typical symptom of jaundice.

The severity of the infection varies from a mild illness lasting a few weeks to a severe illness lasting several months. Humans are the main reservoir for hepatitis A. Therefore, HAV can also be spread when an infected person gets in contact with foods during handling or processing.

## *4.4 Toxic chemicals produced by microorganisms*

The majority of seafood outbreaks in the United States are caused by marine toxins, rather than by bacteria or viruses. Among the most common are scombroid fish poisoning, paralytic shellfish poisoning, neurotoxic shellfish poisoning, amnesic shellfish poisoning, and ciguatera poisoning. These seafood poisonings occur mostly in the summer because warmer water temperatures facilitate growth of the dinoflagellates that produce toxic chemicals (CDC 2005). Shellfish can easily become toxic when large numbers of dinoflagellates are present in the marine environment and create algal blooms known as "red tides."

### *4.4.1 Scombroid fish poisoning*

Scombroid fish poisoning is one of the most prevalent illnesses associated with seafood consumption in the United States (FDA 2001). The illness is frequently associated with eating fish containing high levels of histamine or other biogenic amines. Early symptoms of scombroid poisoning include tingling or burning sensations in the mouth with rash, nausea, diarrhea, flushing, sweating, and headache. The illness usually resolves within several hours without medical attention. However, it can be severe enough that respiratory distress, throat swelling, and blurred vision develop and require hospitalization and medical treatment with antihistamines (CDC 2007a). Incidence of scombroid poisoning has been consistently reported in the United States through surveillance and is often underestimated due to mild and transient symptoms. A total of 167 outbreaks with 703 cases

of scombroid poisoning were reported in the United States between 1998 and 2002 (Sobel and Painter 2005).

Histamine can be produced by bacteria on fish through enzymatic decarboxylation of histidine. Many bacteria, including *Morganella morganii*, *Proteus vulgaris*, *Klebsiella pneumoniae*, and *Enterobacter aerogenes,* are known to be prolific histamine formers and have been frequently isolated from fish. Among them, *Morganella morganii* is the most prolific histamine former and plays the major role in histamine formation in fish during storage (Kim et al., 2001).

Once histamine is produced in fish, it will not be destroyed by cooking, freezing, or smoking (Bremer et al. 1998; Sobel and Painter 2005). A high histamine level can exist in fish without noticeable changes in appearance or smell of the fish. Therefore, sensory examination commonly used to evaluate freshness of fish cannot be used to predict presence of histamine.

Scombroid poisoning is frequently associated with eating scombroid fish, such as tuna, mackerel, and bonito, which contain high levels of free histidine in muscles. However, non-scombroid fish (such as blue fish, mahi-mahi, sardine, and anchovy), which were not stored at proper temperatures, have also been linked to scombroid outbreaks (Taylor 1986; Rawles et al. 1996). Since formation of histamine in fish is mainly related to bacterial enzymatic activities, keeping fish at low temperatures between catching and consumption has been recognized as the most effective means to prevent scombroid poisoning. The FDA recommends that fish be chilled immediately down to <4.4°C (<40°F) upon death and has established a guidance level of <50 ppm for histamine in fish for consumption (FDA 2001).

## *4.4.2 Shellfish poisoning*

### *4.4.2.1 Paralytic shellfish poisoning*

Paralytic shellfish poisoning (PSP) is caused by saxitoxin produced by planktonic algae (mainly dinoflagellates) accumulated in shellfish, mainly mussels, clams, cockles, and scallops. Symptoms of PSP may develop within 0.5 to 2 hours after ingestion of shellfish containing the toxin. Major symptoms of PSP include tingling, burning, numbness, drowsiness, and incoherent speech. In severe cases, respiratory paralysis and death can occur (FDA 1992). Saxitoxin is heat- and acid-stable and does not alter the odor or taste of food. This toxin cannot be destroyed by cooking or freezing. It is rapidly absorbed through the human gastrointestinal tract and excreted in urine (CDC 2002; Sobel and Painter 2005).

#### 4.4.2.2 *Neurotoxic shellfish poisoning*

Neurotoxic shellfish poisoning (NSP) is the result of exposure to a group of polyethers named brevetoxins. The toxins are produced by a dinoflagellate (*Gymnodinium breve*) commonly found in the Gulf of Mexico, the Caribbean, and New Zealand (Daranas et al. 2001). Illness caused by NSP is characterized by gastrointestinal and neurological symptoms, including tingling and numbness of lips, tongue, and throat, muscular aches, dizziness, nausea, vomiting, diarrhea, and reversal of the sensations of hot and cold (FDA 1992). The symptoms typically occur within a few minutes to a few hours and disappear within 48 hours after onset. Recovery from the illness is usually complete, and most illnesses do not require hospitalization.

#### 4.4.2.3 *Diarrheic shellfish poisoning*

Diarrheic shellfish poisoning (DSP) is caused by a group of high molecular weight polyethers, such as okadaic acid and related toxins, produced by certain dinoflagellates (Sobel and Painter 2005). DSP is more commonly associated with mussel, oyster, and scallop consumption and is primarily observed as a mild gastrointestinal disorder with symptoms of nausea, vomiting, diarrhea, and abdominal pain accompanied by chills, headache, and fever (FDA 1992). The onset of the poisoning is very rapid and may be as little as 30 minutes after toxin ingestion. The duration of the illness may last for 2 to 3 days with symptoms completely disappearing within 3 to 4 days after onset. DSP is generally not life-threatening, and recovery is usually complete with no aftereffects. The illness is more common in Japan and Europe than in the United States (Scoging and Bahl 1998).

#### 4.4.2.4 *Amnesic shellfish poisoning*

Amnesic shellfish poisoning (ASP) is caused by an unusual water-soluble, heat-stable amino acid (domoic acid) that is produced by certain pennate diatoms of the genus *Pseudo-nitzschia* spp. ASP is mainly associated with mussels and was first documented in 1987 after a large outbreak occurred in Canada (Perl et al. 1990). A total of 107 persons developed a distinct illness after consuming contaminated mussels. Among them, 19 persons were hospitalized, and 4 of them died. The first symptoms of ASP are gastrointestinal disorders (vomiting, diarrhea, and abdominal pain) within 24 hours of exposure, followed by neurological problems (confusion, memory loss, disorientation, seizure, and coma) within 48 hours (Jeffery et al. 2004). The illness is particularly serious in elderly patients, with most fatalities involving elderly patients.

### *4.4.3 Ciguatera poisoning*

Ciguatera poisoning is one of the most common seafood-borne illnesses with an estimated 20,000 to 50,000 cases occurring worldwide each year (Whittle and Gallacher 2000). The poisoning is caused by eating tropical reef fish containing ciguatoxins produced by dinoflagellates (*Gambierdiscus toxicus*). Ciguatoxins are lipid soluble, and high concentrations of ciguatoxins can be accumulated in large predatory tropical reef fish (such as barracuda, black grouper, blackfin snapper, cubera snapper, dog snapper, greater amberjack, hogfish, horse-eye jack, king mackerel, and yellowfin grouper) through the food chain as large fish consume contaminated small fish. These fish are commonly caught by fishermen in tropical regions, and illnesses have been reported in the United States (CDC 2006c).

Illness caused by ciguatoxins usually occurs within a few minutes to 6 hours after eating contaminated fish. Common symptoms include nausea, vomiting, diarrhea, cramps, excessive sweating, headache, and muscle aches. People who have ciguatera may also experience neurologic symptoms such as unusual taste sensations, tingling fingers or toes, nightmares, or hallucinations (Sobel and Painter 2005; CDC 2006c). Ciguatera poisoning is a self-limiting illness and rarely fatal. Symptoms usually go away in 1 to 4 weeks but can last for years.

## *4.5 Control measures for ensuring safety of ready-to-eat seafood*

Smoked fish, cooked shrimp and crabmeat, and surimi seafood are commonly consumed without a heating process. These RTE seafoods are potential vehicles for foodborne illness if contamination with human pathogens occurs during production and processing. As mentioned earlier, *L. monocytogenes*, *V. parahaemolyticus,* and *C. botulinum* type E are potential safety concerns for RTE seafood. Thorough cooking, proper storage temperature, avoiding cross-contamination, observing good personal hygiene, and putting a Hazard Analysis and Critical Control Points (HACCP) system into operation are the fundamentals for ensuring safety of RTE seafood for consumption.

### *4.5.1 Controls for* L. monocytogenes

*L. monocytogenes* can grow at low temperatures (>0ºC), over a wide pH range (4.5–9.5), and in up to 10% NaCl (FDA 2001). Many RTE seafoods, such as smoked fish, lightly salted products (caviar and brine-cooked

***Table 4.5*** FDA Survey of *Listeria monocytogenes* in Smoked Fish and Crab (1991–1996)

| | Smoked Fish | | Crab | |
|---|---|---|---|---|
| Year | Sample Analyzed | Sample Positive (%) | Sample Analyzed | Sample Positive (%) |
| 1991 | 133 | 16 (12.0) | 260 | 12 (4.6) |
| 1992 | 207 | 32 (15.5) | 358 | 34 (9.5) |
| 1993 | 233 | 38 (16.3) | 400 | 37 (9.3) |
| 1994 | 264 | 33 (12.5) | 348 | 28 (8.0) |
| 1995 | 177 | 23 (13.0) | 297 | 25 (8.4) |
| 1996 | 198 | 22 (11.1) | 223 | 6 (2.7) |
| Total | 1,212 | 164 (13.5) | 1,886 | 142 (7.5) |

*Source:* Data adapted from Jinneman et al. 1999.

shrimp), and surimi seafood will support growth of *L. monocytogenes* during refrigerated storage. Incidences of *L. monocytogenes* in cold-smoked fish have been reported ranging from 17% to as high as 78% (Eklund et al. 1995; Heinitz and Johnson 1998). Investigation of vacuum-packed cold-smoked and "gravad" fish products (rainbow trout and salmon) has reported that up to 10% of the retail products contained *L. monocytogenes* (Loncarevic et al. 1996). A survey conducted by the FDA on domestic and imported RTE seafoods showed that crabmeat and smoked fish were frequently contaminated with *L. monocytogenes* (Table 4.5).

In addition to raw fish, seafood processing plants have been recognized as a main source of *L. monocytogenes* contamination in RTE seafoods. Effective sanitation programs and good personal hygiene need to be followed in plants producing RTE seafoods in order to minimize possible contamination of *L. monocytogenes* in the final products. Strategies to prevent *L. monocytogenes* contamination in RTE seafoods should include (1) complete inactivation of *L. monocytogenes* in heat-processed products, (2) avoiding cross-contamination of cooked products during post-processing handling, and (3) preventing growth of *L. monocytogenes* in RTE products during storage (Huss et al. 2000).

Since the cool and wet environment in seafood processing plants is conducive to the presence of *L. monocytogenes*, repeated cleaning and sanitizing are required to remove *L. monocytogenes* from the plants. A study conducted by Eklund et al. (1995) reported that *L. monocytogenes* could appear sporadically in a processing plant even after an excellent cleaning and sanitizing process. A thorough cleaning and sanitizing practice, such as heat treatment (80°C by hot steam, hot air, or hot water), of the skinning, slicing, and brining equipment was capable of eliminating

*L. monocytogenes* from the processing lines. No *L. monocytogenes* was isolated from 188 samples taken over a 5-month period after introducing heat treatment in the eradication program.

Freezing a food is generally recognized as an effective process in preventing growth of *L. monocytogenes*. However, freezing cannot be effective in preventing transmission of foodborne listeriosis through RTE products that are thawed and held in a refrigerator before consumption (e.g., frozen crabmeat). Therefore, the FDA (2008) recommends that refrigerated RTE food be formulated with one or more of the following listeristatic control measures:

- pH less than or equal to 4.4
- water activity less than or equal to 0.92
- contain one or more inhibitory substances that, alone or in combination, prevent the growth of *L. monocytogenes*

Other than listeristatic control measures, the FDA also recommends establishing a listericidal measure to control *L. monocytogenes* in RTE food. The control measures may include thermal processing, irradiation (provided that irradiation has been approved and listed in 21 CFR part 179), ultraviolet light, high pressure, pulsed electric field, or listericidal agents. However, such control measures must be proven, in scientific studies, capable of destroying viable cells of *L. monocytogenes* consistently to less than 0.04 CFU/g in the finished product. In the absence of scientific studies, a listericidal control measure that provides a reduction of the number of viable cells of *L. monocytogenes* of 6 orders of magnitude (6 logarithms), also known as a "6D" process, may be acceptable to achieve that goal. Finished products should be transported and stored at an internal temperature ≤4°C (≤40°F). More information can be obtained from the FDA's Guidance for Industry: Control of *Listeria monocytogenes* in Refrigerated or Frozen Ready-To-Eat Foods (FDA 2008).

### 4.5.2 *Controls for* C. botulinum

*C. botulinum* food poisoning associated with seafood consumption is mainly related to vacuum-packed RTE products that are stored at refrigeration temperature. It is not a concern for vacuum-packed products that are frozen immediately after processing, held frozen throughout distribution and storage, and thawed under refrigeration immediately before use. RTE seafood, such as smoked fish, pasteurized surimi-based products, pasteurized crabmeat, and pickled fish, which is packed in a vacuum or reduced oxygen condition, requires special attention to prevent growth of *C. botulinum* and toxin formation.

#### 4.5.2.1 *Guidance for controlling botulinal toxin formation in shelf-stable and refrigerated products*

Several control measures, including water activity ($a_w$), salt concentration, pH, and temperature and thermal processing, can be used to prevent *C. botulinum* toxin formation during storage and distribution of fishery products. The FDA Fish and Fisheries Products Hazards and Controls Guidance (http://www.fda.gov/Food/GuidanceComplianceRegulatoryInformation/GuidanceDocuments/Seafood/FishandFisheriesProductsHazardsandControlsGuide/default.htm) has provided guidance for controlling botulinal toxin formation in shelf-stable and refrigerated products. A summary of controls for botulinal toxin formation in various types of seafood products is provided here for information purposes. Readers should check the Guidance Web site for details.

*Shelf-stable products that do not require refrigeration:*

- Finished product in its final container be heated sufficiently by retorting to destroy the spores of *C. botulinum* types A, B, E, and F (e.g., canned fish).
- Controlling the level of acidity (pH) in the finished product at ≤4.6 to prevent the growth of *C. botulinum* types A, B, E, and F (e.g., shelf-stable acidified products).
- Controlling the water activity ($a_w$) in the product at ≤0.85 to prevent the growth of *C. botulinum* types A, B, E, and F and other pathogens that may be present in the product.
- Controlling the amount of salt in the product at ≥20% salt to prevent the growth of *C. botulinum* types A, B, E, and F and other pathogens that may be present in the product.

*Pasteurized products requiring refrigerated storage:*

- Heating finished product in its final container sufficiently (generally 6D reductions) by pasteurization to destroy the spores of *C. botulinum* type E and nonproteolytic types B and F and then controlling growth of surviving *C. botulinum* type A and proteolytic types B and F in finished product with refrigerated storage at <4.4°C (<40°F) (e.g., pasteurized crabmeat, some pasteurized surimi-based products).
- Heating the product sufficiently (generally 6D reductions) to destroy the spores of *C. botulinum* type E and nonproteolytic types B and F and then minimizing the risk of recontamination by hot filling the product into the final container in a continuous filling system followed by controlling the growth of the surviving *C. botulinum* type A and

proteolytic types B and F and other pathogens that may be present in the finished product with refrigerated storage at <4.4°C (<40°F).

An example of a product that is properly cooked to eliminate nonproteolytic *C. botulinum* is a soup, sauce, or surimi-based product that is pasteurized at an internal temperature of 194°F (90°C) for at least 10 minutes. However, this pasteurization process may not be sufficient for the destruction of nonproteolytic *C. botulinum* in soups or sauces containing dungeness crabmeat, because naturally occurring substances, such as lysozyme, may enable the pathogen to be more easily recovered after damage.

- Controlling the amount of salt in the finished product, in combination with heat damage from pasteurization in the finished product container, sufficient to prevent the growth of *C. botulinum* type E and nonproteolytic types B and F and then controlling the growth of *C. botulinum* type A and proteolytic types B and F and other pathogens that may be present in the finished product with refrigerated storage at <4.4°C (<40°F).

  In some pasteurized surimi-based products, salt in combination with a milder pasteurization process of the finished product in its final package works to prevent growth and toxin formation by *C. botulinum* type E and nonproteolytic types B and F. Surimi-based product containing 2.5% salt (water phase salt) and pasteurized at an internal temperature of 185°F (85°C) for at least 15 minutes is considered properly pasteurized. However, this process may not be suitable for other types of products, because of the unique formulation and processing involved in the manufacture of surimi-based products.

*Products not pasteurized and require refrigerated storage:*

- Controlling the water activity ($a_w$) at <0.97 to inhibit the growth of *C. botulinum* type E and nonproteolytic types B and F by drying and then controlling the growth of *C. botulinum* type A, and proteolytic types B and F, and other pathogens that may be present in the finished product through refrigerated storage at <4.4°C (<40°F).
- Controlling the level of acidity (pH <5.0), salt (>5 %), water activity ($a_w$ <0.97), or some combination of these barriers, in the finished product sufficiently to prevent the growth of *C. botulinum* type E and nonproteolytic types B and F by formulation and then controlling the growth of *C. botulinum* type A and proteolytic types B and F and other pathogens that may be present in the finished product with refrigerated storage at <4.4°C (<40°F).

The minimum temperature for growth and toxin formation by *C. botulinum* type E and nonproteolytic types B and F is 3.3°C (38°F). For type A and proteolytic types B and F, the minimum temperature for growth is 10°C (50°F). Therefore, non-pasteurized vacuum-packed or reduced oxygen packaged RTE seafoods must be kept at <3.3°C (<38°F) from packing to consumption to prevent growth of nonproteolytic *C. botulinum* and their spores.

#### *4.5.2.2 Salting*

"Salting" is a process of using food grade salt to enhance flavor and lower water activity. Salting of fish can be conducted with dry salting, brining, or injecting salt solution into fish flesh. It is commonly used in preparing hot- or cold-smoked fish, which is produced by exposing fish to smoke from smoldering wood or plant materials (FAO 2008a). The safety of smoked fish relies on the amount of water phase salt and the intensity of heat treatment, as evidenced by outbreaks of botulism linked to salted and cold-smoked fish (Eklund 1982; CDC 1987).

The salt content in smoked fish is critical in controlling growth of *C. botulinum* during storage. The salt concentration in the water of a finished product (water phase salt, WPS) should be high enough to inhibit growth of *C. botulinum* but not to make the product unacceptable (too salty for consumption). The WPS in smoked fish can be formulated by dividing the amounts of salt added to a product by the sum of the added salt and the amount of water in the product and expressed as percentage.

$$\text{Water Phase Salt (WPS)} = \frac{\text{Salt added to product}}{\text{Salt added to product} + \text{Water in product}} \times 100$$

WPS in smoked fish after smoking can be validated by taking a sample of flesh from the thickest part of the smoked fish one day after smoking (McK. Bannerman 2001). Cold-smoked fish should contain a WPS of >3.5% to prevent growth of *C. botulinum* (FAO 2008b).

#### *4.5.2.3 Hurdle technology*

Most RTE seafoods, except for a fully salted (>20% salt) or dried product, are not considered shelf-stable products and require time/temperature control during distribution and storage. While keeping RTE seafoods at temperature below 3.3°C (<38°F) can prevent the growth of *C. botulinum*, maintaining products at <3.3°C cannot be ensured once products leave the processors. Other barriers such as reduced water activity, increased salt content, or oxygen-permeable packaging should be considered to provide additional hurdles for *C. botulinum* toxin formation.

An oxygen permeable package provides sufficient exchange of oxygen to allow growth of aerobic spoilage organisms, which will spoil the product before botulinal toxin is produced under moderate abuse temperatures. An oxygen-permeable packaging material allowing an oxygen transmission rate of 10,000 cc/$m^2$/24 hrs (e.g., 1.5 mil polyethylene) can be used for fishery products (FDA 2001). However, oxygen permeable package is not suitable for products packed in oil or in deep containers because the exchange of oxygen in such a product is restricted.

Controlling *C. botulinum* in smoked fish by salt alone is very critical, but adequate salt levels in fish after brining may not always be achievable. Proper drying of the finished products may be required to achieve the minimum WPS level to inhibit growth and toxin formation of *C. botulinum*. Keeping smoked fish at a temperature below 4.4°C (<40°F) provides another barrier for botulinal toxin formation. Information about other hurdle technologies can be obtained from "Hurdle technology to ensure the safety of seafood products" by Leroi et al. (2008).

### *4.5.3 Controls for* V. parahaemolyticus

Several post-harvest treatments including refrigeration, freezing, low-temperature pasteurization, irradiation, and high-pressure processing can be used to control growth or reduce *V. parahaemolyticus* contamination in oysters. In addition, consumer education also plays an important role in reducing *V. parahaemolyticus* infection associated with raw oyster consumption. It is estimated that more than 60% of seafood-associated illness could be avoided if consumers would stop eating raw or undercooked molluscan shellfish (Liston 1990). People in the high-risk groups (those who have liver disease) should be informed of potential risks associated with consumption of raw or undercooked seafood and possible life-threatening disease caused by *Vibrio* infection.

#### *4.5.3.1 Temperature control*

The levels of *V. parahaemolyticus* in oysters at the time of consumption depend on methods of harvesting and post-harvest handling. *V. parahaemolyticus* can multiply quickly in oysters to an infectious dose upon exposure to elevated temperatures before oysters are consumed (Gooch et al. 2002). Nordstrom et al. (2004) reported that levels of *V. parahaemolyticus* in oysters were greatly affected by the intertidal harvest practice in the Pacific Northwest estuaries. Populations of *V. parahaemolyticus* in oysters increased by greater than fourfold after oysters were left in baskets at low tide and exposed to ambient air for several hours before being shipped to plants for processing at high tide. Exposure of oysters to warm air

temperature allows *V. parahaemolyticus* to proliferate in oysters between tides. An overnight submersion for a single tidal cycle reduced *V. parahaemolyticus* in oysters to levels similar to those determined prior to the intertidal exposure. Intertidal harvest of oysters should be performed to avoid harvesting oysters after intertidal exposure to ambient conditions. Oysters should be cooled down to refrigeration temperatures as soon as they are harvested.

The National Shellfish Sanitation Program Guide for the Control of Molluscan Shellfish has established time-to-temperature regulations; these limit the time that oysters can be exposed to elevated temperatures prior to refrigeration to limit growth of *V. parahaemolyticus* post harvest (NSSP 2007). Shellfish harvested for raw consumption must be cooled down to 10°C (50°F) within 10, 12, and 36 hours of harvest when the average monthly maximum air temperature is ≥27°C (≥81°F), 19°C to 27°C (66°F–80°F), and <18°C (<66°F), respectively, by means of ice, mechanical refrigeration, or other approved means capable of lowering temperature of the shellstock to 10°C or lower. However, exposure of products to temperatures higher than 10°C during transportation and storage cannot be totally avoided.

#### *4.5.3.2 Low-temperature storage*

Cold storage, including refrigeration, icing, and freezing, is commonly used to prevent growth of microorganisms in food. Thompson and Vanderzant (1976) found that holding shucked oysters at 3°C for 7 days could reduce *V. parahaemolyticus* in oysters from >11,000 to 0.36 MPN/g. In an early study, Cook and Ruple (1992) reported that *V. vulnificus* and *V. parahaemolyticus* levels decreased to nearly non-detectable levels after oysters were put on ice for 2 weeks. However, a recent study reported that neither on-board or dockside icing followed by refrigeration could reduce the levels of *V. vulnificus* or *V. parahaemolyticus* in the Eastern oyster (*Crassostrea virginica*), and icing negatively impacted oyster survival during subsequent cold storage (Melody et al. 2008).

Frozen storage is the most effective means to preserve product quality and prevent spoilage from growth of bacteria. Several studies have demonstrated that frozen storage was capable of achieving certain degrees of reductions of *V. parahaemolyticus* in oyster meat and half-shell oysters. Muntada-Garriga et al. (1995) reported that *V. parahaemolyticus* in oyster homogenates ($10^{5-7}$ CFU/g) could be completely inactivated after storage at –18 and –24°C for 15 to 28 weeks. Frozen storage of half-shell oysters for up to 4 months at –20°C was capable of reducing low populations of *V. parahaemolyticus* (<1,000 CFU/g) to non-detectable levels (Andrews 2004). A recent study demonstrated that a process of flash freezing (–95.5°C for 12 minutes) followed by storage at –21 ± 2°C for 5 months was capable

of achieving >3.52-log (MPN/g) reductions of *V. parahaemolyticus* in half-shell Pacific oysters (Liu et al. 2009). The reductions were validated with three individual processes (each separated by one week) according to the National Shellfish Sanitation Program's post-harvest processing validation-verification interim guidance for *Vibrio vulnificus* and *Vibrio parahaemolyticus*.

#### *4.5.3.3 Low-temperature pasteurization*

*V. parahaemolyticus* is sensitive to heat. A heat treatment of *V. parahaemolyticus* culture suspension at 70°C for 2 minutes was reported capable of reducing the organism by greater than 7-log units (Johnson and Brown 2002). A low-temperature pasteurization process of placing shellstock oysters in 55°C water for 10 minutes to achieve an internal temperature of 48°C to 50°C for 5 minutes was developed for reducing *V. parahaemolyticus* ($1.2 \times 10^5$ MPN/g) to non-detectable levels (<3 MPN/g) (Andrews et al. 2000). Low-temperature pasteurized oysters had a raw-like quality as long as the internal temperature did not exceed 52.5°C and could be stored in ice for up to 3 weeks. An added benefit of the process is that oysters are likely to be killed during the heat treatment and will be shucked by the process. Therefore, oysters need to be banded before being pasteurized to avoid losing juice during the treatment.

#### *4.5.3.4 Non-thermal processing*

Irradiation and high-pressure processing (HPP) are both non-thermal processes that can be applied to food processing to inactivate bacterial cells. A study of exposing raw oysters to Cobalt-60 gamma radiation found that irradiation with Cobalt-60 gamma radiation at doses of 1.0 kGy to 1.5 kGy reduced the *V. parahaemolyticus* O3:K6 strain from 4 log units to non-detectable levels (<3.0 MPN/g) in artificially inoculated oysters (Andrews et al. 2003). Sensory analysis using difference tests involving 146 volunteers reported that the participants could not differentiate non-irradiated from irradiated oysters. Another study also reported that low dosages (<2 kGy) of irradiation did not significantly affect the sensory characteristics of oysters (Jakabi et al. 2003). However, consumers' concerns of processing food with radiation limits its usage.

Hydrostatic high-pressure processing (HPP) has been reported an effective means of inactivating *V. parahaemolyticus* in oysters. Calik et al. (2002) reported that a HPP treatment of 345 MPa for 90 seconds could reduce *V. parahaemolyticus* cells in oysters ($8.4 \times 10^5$ CFU/g) to non-detectable levels (<10 CFU/g). Cook (2003) reported that a HPP treatment of 300 MPa for 180 seconds could achieve a >5-log reduction of clinical strains (including O3:K6 strain) of *V. parahaemolyticus* in oysters. The efficacy of HPP in inactivating bacteria is dependent on the processing temperature. A recent

study reported that a 5-log reduction of *V. parahaemolyticus* in live oysters could be achieved by HPP treatments of ≥350 MPa for 2 minutes at temperatures between 1°C and 35°C or ≥300 MPa for 2 minutes at 40°C (Kural et al. 2008). The HPP can also be applied for oyster shucking by destroying the adduct muscle under higher pressures. A HPP process of 240 to 275 MPa for 1 to 2 minutes was reported capable of shucking Pacific oysters with minimum changes in appearance (He et al. 2002).

## 4.6 Conclusion

Fish is subjected to rapid deterioration upon harvest because it can be easily contaminated with bacteria in the marine environment. Spoilage bacteria are commonly present on skin and in gills and digestive tracts of fish. Raw fish can also be a source of human pathogens for contamination of RTE seafood during production. Foodborne illnesses associated with seafood consumption usually occur because of inadequate cooking or reheating, improper holding temperatures, improper cooling, or cross-contamination after cooking. Ensuring an efficient cold chain from harvest through distribution to consumers minimizes growth of bacteria in raw or finished products. Good manufacturing practices and good personal hygiene plus a working HACCP system all are critical to ensuring the safety of RTE seafood.

## References

Abrahamsson, K., B. Gullmar, and N. Molin. 1966. The effect of temperature on toxin formation and toxin stability of *Clostridium botulinum* type E in different environments. *Can J Microbiol* 12:385–94.

Addiss, D.G., J.C. Yashuk, D.E. Clapp, and P.A. Blake. 1989. Outbreaks of diarrhoeal illness on passenger cruise ships, 1975–85. *Epidemiol Infect* 103(1):63–72.

Ahmed, F.E. 1991. *Seafood Safety*. Washington, D.C.: National Academy Press.

Alam, M.J., K.I. Tomochika, S.I. Miyoshi, and S. Shinoda. 2002. Environmental investigation of potentially pathogenic *Vibrio parahaemolyticus* in the Seto-Inland Sea, Japan. *FEMS Microbiol Lett* 208: 83–7.

Andrews, L.S., D.L. Park, and Y.P. Chen. 2000. Low temperature pasteurization to reduce the risk of *Vibrio* infections from raw shell-stock oysters. *Food Addit Contam* 17:787–91.

Andrews, L., M. Jahncke, and K. Mallikarjunan. 2003. Low dose gamma irradiation to reduce pathogenic *Vibrio* in live oysters (*Crassostrea virginica*). *J Aquat Food Prod Technol* 12:71–82.

Andrews, L.S. 2004. Strategies to control *Vibrios* in molluscan shellfish. *Food Prot Trends* 24:70–6.

Anonymous. 2005. Food Poisoning in Taiwan, 1981–2008. Department of Health, Taiwan. http://food.doh.gov.tw/foodnew/MenuThird.aspx?SecondMenuID=34&ThirdMenuID=95 (accessed April 4, 2009).

Autio, T., S. Hielm, M. Miettinen, A.-M. Sjöberg, K. Aarnisalo, J. Björkroth, T. Mattila-Sandholm, and H. Korkeala. 1999. Sources of *Listeria monocytogenes* contamination in a cold-smoked rainbow trout processing plant detected by pulsed-field gel electrophoresis typing. *Appl Environ Microbiol* 65:150–5.

Ayres, P.A., and G.I. Barrow. 1978. The distribution of *Vibrio parahaemolyticus* in British coastal waters: report of a collaborative study 1975–6. *J Hyg* 80:281–94.

Besser, R.E., D.R. Feikin, J.E. Eberhart-Phillips, L. Mascola, and P.M. Griffin. 1994. Diagnosis and treatment of cholera in the United States. Are we prepared? *JAMA* 272(15):1203–5.

Blake, P.A., M.H. Merson, R.E. Weaver, D.G. Hollis, and P.C. Heublein. 1979. Disease caused by a marine *Vibrio*: clinical characteristics and epidemiology. *N Engl J Med* 300:1–5.

Bonner, H.R., A.S. Coker, C.R. Berryman, and H.M. Pollock. 1983. Spectrum of *Vibrio* infections in a Gulf Coast community. *Ann Intern Med* 99:464–9.

Bremer, P.J., C.M. Osborne, R.A. Kemp, P. van Veghl, and G.C. Fletcher. 1998. The thermal death times of *Hafnia alvei* in a model suspension and in artificially contaminated hot smoked kahawai (*Arripis trutta*). *J Food Prot* 61:1047–51.

Brett, M.S.Y., P. Short, and J. McLauchlin. 1998. A small outbreak of listeriosis associated with smoked mussels. *Int J Food Microbiol* 43:223–9.

Bucher, H.C., P. Hengstler, C. Schindler, G. Meier. 2002. N–3 polyunsaturated fatty acids in coronary heart disease: a meta-analysis of randomized controlled trials. *Am J Med* 112(4):298–304.

Calik, H., M.T. Morrissey, P.W. Reno, and H. An. 2002. Effect of high-pressure processing on *Vibrio parahaemolyticus* strains in pure culture and Pacific oysters. *J Food Sci* 67:1506–10.

Cavalieri d'Oro, L., E. Merlo, E. Ariano, M.G. Silvestri, A. Ceraminiello, E. Negri, and C. La Vecchia. 1999. *Vibrio cholerae* outbreak in Italy. *Emerg Infect Dis* 5(2):300–1.

CDC [Centers for Disease Control and Prevention]. 1979. Botulism in the United States, 1899–1977. Handbook for epidemiologists, clinicians, and laboratory workers. Issued May 1979.

CDC [Centers for Disease Control and Prevention]. 1987. International outbreak of type E botulism associated with ungutted, salted whitefish. *MMWR* 36(49):812–3.

CDC [Centers for Disease Control and Prevention]. 1988. Water-related disease outbreaks, 1985. *MMWR* 37(SS–2):15–24.

CDC [Centers for Disease Control and Prevention]. 1991. Epidemiologic notes and reports paralytic shellfish poisoning—Massachusetts and Alaska, 1990. *MMWR* 40(10):157–61.

CDC [Centers for Disease Control and Prevention]. 1993. Multistate outbreak of viral gastroenteritis related to consumption of oysters—Louisiana, Maryland, Mississippi, and North Carolina, 1993. *MMWR* 42(49):945–8.

CDC [Centers for Disease Control and Prevention]. 1996. Surveillance for foodborne-disease outbreaks—United States, 1988–1992. *MMWR* 45(SS–5):1–55.

CDC [Centers for Disease Control and Prevention]. 1998. Outbreak of *Vibrio parahaemolyticus* infections associated with eating raw oysters—Pacific Northwest, 1997. *MMWR* 47:457–62.

CDC [Centers for Disease Control and Prevention]. 1999. Outbreak of *Vibrio parahaemolyticus* infection associated with eating raw oysters and clams harvested from Long Island Sound—Connecticut, New Jersey and New York, 1998. *MMWR* 48:48–51.

CDC [Centers for Disease Control and Prevention]. 2000a. Investigation of gastroenteritis aboard Palm Beach Princess. http://www.cdc.gov/nceh/vsp/surv/outbreak/2000/palmbeachprincess.htm (accessed April 3, 2009).

CDC [Centers for Disease Control and Prevention]. 2000b. Investigation summary: outbreak of gastroenteritis illness aboard the Cruise Ship, Disney Magic™. http://www.cdc.gov/nceh/vsp/surv/outbreak/2000/disneymagic.htm (accessed April 3, 2009).

CDC [Centers for Disease Control and Prevention]. 2002. Neurologic illness associated with eating Florida pufferfish, 2002. *MMWR* 51(15):321–3

CDC [Centers for Disease Control and Prevention]. 2005. Marine toxins. http://www.cdc.gov/ncidod/dbmd/diseaseinfo/marinetoxins_g.htm (accessed April 1, 2009).

CDC [Centers for Disease Control and Prevention]. 2006a. *Vibrio parahaemolyticus* infections associated with consumption of raw shellfish—Three States, 2006. *MMWR* 55:854–6.

CDC [Centers for Disease Control and Prevention]. 2006b. Viral gastroenteritis. http://www.cdc.gov/ncidod/dvrd/revb/gastro/faq.htm (accessed April 1, 2009).

CDC [Centers for Disease Control and Prevention]. 2006c. Ciguatera fish poisoning—Texas, 1998, and South Carolina, 2004. *MMWR* 55(34):935–7.

CDC [Centers for Disease Control and Prevention]. 2007a. Scombroid fish poisoning associated with tuna steaks—Louisiana and Tennessee, 2006. *MMWR* 56(32):817–9.

CDC [Centers for Disease Control and Prevention]. 2007b. Summary of human *Vibrio* cases reported to CDC, 2007. http://www.cdc.gov/nationalsurveillance/PDFs/CSTEVibrio2007.pdf (accessed April 5, 2009).

CDC [Centers for Disease Control and Prevention]. 2008a. *Vibrio parahaemolyticus*. http://www.cdc.gov/nczved/dfbmd/disease_listing/vibriop_gi.html (accessed April 2, 2009).

CDC [Centers for Disease Control and Prevention]. 2008b. Botulism. http://www.cdc.gov/nczved/dfbmd/disease_listing/botulism_gi.html (accessed April 6, 2009).

Chen, S., S. Liu, and L. Zhang. 1991. Occurrence of *Vibrio parahaemolyticus* in seawater and some seafoods in the coastal area of Qingdao. *J Ocean Univ Qingdao* 21:43–50.

Colburn, K.G., C.A. Kaysner, C. Abeyta, and M.M. Wekell. 1990. Listeria species in a California coast estuarine environment. *Appl Environ Microbiol* 56:2007–11.

Cook, D.W. 2003. Sensitivity of *Vibrio* species in phosphate-buffered saline and in oysters to high-pressure processing. *J Food Prot* 66:2276–82.

Cook, D.W., and A.D. Ruple. 1992. Cold storage and mild heat treatment as processing aids to reduce the numbers of *Vibrio vulnificus* in raw oysters. *J Food Prot* 55:985–9.

CSPI [Center for Science in the Public Interest]. 2008. Outbreak Alert! 2008. http://www.cspinet.org/new/pdf/outbreak_alert_2008_report_final.pdf (accessed March 26, 2009).

Dalsgaard, A. 1998. The occurrence of human pathogenic *Vibrio* spp. and *Salmonella* in aquaculture. *Int J Food Sci Technol* 33:127–38.

Daniels, N.A., L. MacKinnon, R. Bishop, S. Altekruse, B. Ray, R.M. Hammond, S. Thompson, S. Wilson, N.H. Bean, P.M. Griffin, and L. Slutsker. 2000. *Vibrio parahaemolyticus* infections in the United States, 1973–1998. *J Infect Dis* 181:1661–6.

Daranas, A.H., M. Norte, and J.J. Fernandez. 2001. Toxic marine microalgae. *Toxicon* 39(8):1101–32.

Davidson, M.H., E.A. Stein, H.E. Bays, K.C. Maki, R.T. Doyle, R.A. Shalwitz, C.M. Ballantyne, H.N. Ginsberg, and for the COMBination of prescription Omega-3 with Simvastatin (COMBOS) Investigators. 2007. Efficacy and tolerability of adding prescription Omega-3 fatty acids 4 g/d to Simvastatin 40 mg/d in hypertriglyceridemic patients: an 8–week, randomized, double-blind, placebo-controlled study. *Clin Ther* 29(7):1354–67.

Dechet, A., P.A. Yu, N. Koram, and J. Painter. 2008. Nonfoodborne vibrio infections: an important cause of morbidity and mortality in the United States, 1997–2006. *Clin Infect Dis* 46:970–6.

Deepanjali, A., H.S. Kumar, I. Karunasagar, and I. Karunasagar. 2005. Seasonal variation in abundance of total and pathogenic *Vibrio parahaemolyticus* bacteria in oysters along the southwest coast of India. *Appl Environ Microbiol* 71:3575–80.

DePaola, A., C.A. Kaysner, J. Bowers, and D.W. Cook. 2000. Environmental investigations of *Vibrio parahaemolyticus* in oysters after outbreaks in Washington, Texas, and New York (1997 and 1998). *Appl Environ Microbiol* 66:4649–54.

Delgado, C.L., N. Wada, M.W. Rosegrant, S. Meijer, and M. Ahmed. 2003. *Fish to 2020: Supply and Demand in Changing Global Markets*, International Food Policy Research Institute, Washington, D.C. http://www.ifpri.org/pubs/books/fish2020/oc44front.pdf (accessed March 25, 2009).

Dillon, R.M., and T.R. Patel. 1992. Listeria in seafoods: a review. *J Food Prot* 55:1009–15.

Eklund, M.W. 1982. Significance of *Clostridium botulinum* in fishery products preserved short of sterilization. *Food Technol* 36:107–12, 15.

Eklund, M.W., and F.T. Poysky. 1965. *Clostridium botulinum* type E from marine sediments. *Science* 149:306.

Eklund, M.W., F.T. Poysky, R.N. Paranjpye, L.C. Lashbrook, M.E. Peterson, and G.A. Pelroy. 1995. Incidence and sources of *Listeria monocytogenes* in cold-smoked fishery products and processing plants. *J Food Prot* 58:502–8.

Ericsson, H., A. Eklöw, M.-L. Danielsson-Tham, S. Loncarevic, L.-O. Mentzing, I. Persson, H. Unnerstad, and W. Tham. 1997. An outbreak of listeriosis suspected to have been caused by rainbow trout. *J Clin Microbiol* 35(11):2904–7.

FAO [The Food and Agriculture Organization of the United Nations] 2008a. Report of the twenty-ninth session of the Codex Committee on fish and fishery products. Joint FAO/Who Food Standards Programme Codex Alimentarius Commission. http://www.codexalimentarius.net/download/report/695/al31_18e.pdf (accessed March 31, 2009).

FAO [Food and Agriculture Organization of the United Nations]. 2008b. Proposed draft code of practice for fish and fishery products. Joint FAO/Who Food Standards Programme Codex Committee on Fish and Fishery Products, Twenty-ninth Session, Trondheim, Norway, 18–23 February 2008. ftp://ftp.fao.org/codex/ccffp29/fp29_06e.pdf (accessed April 2, 2009).

Farber, J.M. 1991. *Listeria monocytogenes* in fish products. *J Food Prot* 54:922–4, 34.

Farber, J.M., E.M. Daley, M.T. Mackie, and B. Limerick. 2000. A small outbreak of listeriosis potentially linked to the consumption of imitation crab meat. *Lett Appl Microbiol* 31:100–4.

FDA [Food and Drug Administration]. 1992. Various shellfish-associated toxins. *Foodborne Pathogenic Microorganisms and Natural Toxins Handbook.* http://www.cfsan.fda.gov/~mow/chap37.html (accessed April 1, 2009).

FDA [Food and Drug Administration]. 1999. Risk assessment of the public health impact of foodborne *Listeria monocytogenes*: request for comments and for scientific data and information. *Fed Regist* 64(88):24661–3.

FDA [Food and Drug Administration]. 2001. *Fish and Fisheries Products Hazards and Controls Guidance*, 3rd Ed. http://www.fda.gov/Food/GuidanceComplianceRegulatoryInformation/GuidanceDocuments/Seafood/FishandFisheriesProductsHazardsandControlsGuide/default.htm (accessed October 25, 2009).

FDA [Food and Drug Administration]. 2004. FDA News: FDA announces qualified health claims for omega-3 fatty acids. http://www.fda.gov/bbs/topics/news/2004/NEW01115.html (accessed March 25, 2009).

FDA [Food and Drug Administration]. 2006. FDA/EPA Advisory on seafood consumption still current. http://www.fda.gov/bbs/topics/NEWS/2006/NEW01382.html (accessed March 25, 2009).

FDA [Food and Drug Administration]. 2008. Guidance for Industry: Control of *Listeria monocytogenes* in refrigerated or frozen ready-to-eat foods, Draft Guidance. http://www.fda.gov/Food/GuidanceComplianceRegulatoryInformation/GuidanceDocuments/FoodProcessingHACCP/ucm073110.htm (accessed October 25, 2009).

FDA [Food and Drug Administration]. 2009. Enforcement reports. http://www.fda.gov/opacom/Enforce.html (accessed April 6, 2009.

Fiore, A.E. 2004. Hepatitis A transmitted by food. *Clin Infect Dis* 38(5):705–15.

Fujino, T., Y. Okuno, D. Nakada, A. Aoyama, T. Mukai, and T. Ueho. 1953. On the bacteriological examination of shirasu-food poisoning. *Med J Osaka Univ* 4:299–304.

Gooch, J.A., A. DePaola, J. Bowers, and D.L. Marshall. 2002. Growth and survival of *Vibrio parahaemolyticus* in postharvest American oysters. *J Food Prot* 65:970–4.

Guyer, S., and T. Jemmi. 1991. Behavior of *Listeria monocytogenes* during fabrication and storage of experimentally contaminated smoked salmon. *Appl Environ Microbiol* 57:1523–7.

Hardesty, S. 2001. Marketing opportunities for Pacific coast oysters. Pacific Coast Shellfish Growers Association, Food Marketing and Economics Group, Davis, California.

Harris, William S. 1997. n–3 fatty acids and serum lipoproteins: human studies. *Am J Clin Nutr 65* (suppl):1645S–54S.

He, H., R.M. Adams, D.F. Farkas, and M.T. Morrissey. 2002. Use of high pressure processing for oyster shucking and shelf-life extension. *J Food Sci* 67:640–4.

Heinitz, M.L., and J.M. Johnson. 1998. The incidence of *Listeria* spp., *Salmonella* spp., and *Clostridium botulinum* in smoked fish and shellfish. *J Food Prot* 61:318–23.

Huss, H.H., L.V. Jørgensen, and B.F. Vogel. 2000. Control options for *Listeria monocytogenes* in seafoods. *Int J Food Microbiol* 62:267–74.

IDSC [Infectious Disease Surveillance Center]. 1999. *Vibrio parahaemolyticus*, Japan, 1996–1998. *IASR* 20(7) (No. 233), Ministry of Health, Labour and Welfare, Japan. http://idsc.nih.go.jp/iasr/20/233/tpc233.html (accessed April 5, 2009).

Jakabi, M., D.S. Gelli, J.C.M.D. Torre, M.A.B. Rodas, B.D.G.M. Franco, M.T. Destro, and M.L. Landgraf. 2003. Inactivation by ionizing radiation of *Salmonella enteritidis*, *Salmonella infantis*, and *Vibrio parahaemolyticus* in oyster (*Crassostrea brasiliana*). *J Food Prot* 66:1025–9.

Janda, J.M., C. Powers, R.G. Bryant, and S.L. Abbott. 1988. Current perspectives on the epidemiology and pathogenesis of clinically significant *Vibrio* spp. *Clin Microbiol Rev* 1:245–67.

Jeffery, B., T. Balow, K. Moizer, S. Paul, and C. Boyle. 2004. Amnesic shellfish poisoning. *Food Chem Toxicol* 42:545–57.

Jinneman, K.C., M.M. Wekell, and M.W. Eklund. 1999. Incidence and behavior of *Listeria monocytogenes* in fish and seafood. In *Listeria, Listeriosis and Food Safety*, ed. E.T. Ryser and E.H. Marth, 601–30. New York: Marcel Dekker.

Johnson, M.D., and M.H. Brown. 2002. An investigation into the changed physiological state of *Vibrio* bacteria as a survival mechanism in response to cold temperatures and studies on their sensitivity to heating and freezing. *J Appl Microbiol* 92:1066–77.

Kaysner, C.A., and A. DePaola. 2001. *Vibrio*. In *Compendium of Methods for the Microbiological Examination of Foods*, 4th Ed., ed. F.P. Downes and K. Ito, 405–20. Washington DC: American Public Health Association.

Kim, S.-H., K.G. Field, M.T. Morrissey, R.J. Price, C.-I. Wei, and H. An. 2001. Source and identification of histamine-producing bacteria from fresh and temperature-abused albacore. *J Food Prot* 64:1035–44.

Kimura, A.C., P. Mead, B. Walsh, E. Alfano, S.K. Gray, L. Durso, C. Humphrey, S.S. Monroe, G. Visvesvera, N. Puhr, W.-J. Shieh, M. Eberhard, R.M. Hoekstra, and E.D. Mintz1. 2006. A large outbreak of Brainerd diarrhea associated with a restaurant in the Red River Valley, Texas. *Clin Infect Dis* 43:55–61.

Kural, A.G., A.E.H. Shearer, D.H. Kingsley, and H. Chen. 2008. Conditions for high pressure inactivation of *Vibrio parahaemolyticus* in oysters. *Int J Food Microbiol* 127:1–5.

Leroi, F., J.J. Jofftaud, J.C. Arboleya, F. Amarita, Z. Cruz, E. Izurieta, A. Lasagabaster, I. Martínez de Marañón, I. Miranda, M. Nuin, I. Olabarrieta, H.L. Lauzon, G. Lorentzen, I. Bjørkevoll, R.L. Olsen, M.F. Pilet, H. Prévost, X. Dousset, S. Matamoros, and T. Skjerdal. 2008. Hurdle technology to ensure the safety of seafood products. In *Improving Seafood Products for the Consumer*, ed. Torger Børresen, 399–425. London: CRC Press.

Liston, J. 1990. Microbial hazards of seafood consumption. *Food Tech* 44:56–62.

Liu, C., J. Lu, and Y.-C. Su. 2009. Effects of flash freezing followed by frozen storage on reducing *Vibrio parahaemolyticus* in Pacific raw oysters (*Crassostrea gigas*). *J Food Prot* 72:174–7.

Liu, X., Y. Chen, X. Wang, and R. Ji. 2004. Foodborne disease outbreaks in China from 1992 to 2001—national foodborne disease surveillance system. *J Hygie Res* 33:725–7.

Loncarevic, S., W. Tham, and M.-L. Danielsson-Tham. 1996. Prevalence of *Listeria monocytogenes* and other *Listeria* spp. in smoked and "gravad" fish. *Acta Vet Scand* 37:13–8.

Lozano-León, A., J. Torres, C.R. Osorio, and J. Martínez-Urtaza. 2003. Identification of *tdh*-positive *Vibrio parahaemolyticus* from an outbreak associated with raw oyster consumption in Spain. *FEMS Microbiol Lett* 226:281–4.

Martínez-Urtaza, J., A. Lozano-León, A. DePaola, M. Ishibashi, K. Shimada, M. Nishibuchi, and E. Liebana. 2004. Characterization of pathogenic *Vibrio parahaemolyticus* isolated from clinical sources in Spain and comparison with Asian and North American pandemic isolates. *J Clin Microbiol* 42:4672–8.

Martínez-Urtaza, J., L. Simental, D. Velasco, A. DePaola, M. Ishibashi, Y. Nakaguchi, M. Nishibuchi, D. Carrera-Flores, C. Rey-Alvarez, and A. Pousa. 2005. Pandemic *Vibrio parahaemolyticus* O3:K6, Europe. *Emerg Infect Dis* 11:1319–20.

McCarthy, S.A., 1997. Incidence and survival of *Listeria monocytogenes* in ready-to-eat seafood products. *J Food Prot* 60:372–6.

McK. Bannerman, A. 2001. Hot smoking of fish. Torry Advisory Note No. 82 (Revised), Torry Research Station. http://www.fao.org/wairdocs/tan/x5953e/x5953e00.htm#Contents (accessed March 31, 2009).

McLaughlin, J.B., A. DePaola, C.A. Bopp, K.A. Martinek, and N.P. Napol. 2005. Outbreak of *Vibrio parahaemolyticus* gastroenteritis associated with Alaskan oysters. *N Engl J Med* 353:1463–70.

Melody, K., R. Senevirathne, M. Janes, L.A. Jaykus, and J. Supan. 2008. Effectiveness of icing as a postharvest treatment for control of *Vibrio vulnificus* and *Vibrio parahaemolyticus* in the eastern oyster (*Crassostrea virginica*). *J Food Prot* 71:1475–80.

Mitchell, D.L. 1991. A case cluster of listeriosis in Tasmania. *Comm Dis Intell* 15:427.

Molenda, J.R., W.G. Johnson, M. Fishbein, B. Wentz, I.J. Mehlman, and T.A. Dadisman Jr. 1972. *Vibrio parahaemolyticus* gastroenteritis in Maryland: laboratory aspects. *Appl Microbiol* 24:444–8.

Montville, T.J., and K.R. Matthews. 2005. *Food Microbiology: An Introduction,* 2nd Ed. Washington D.C.: ASM Press.

Morse, D.L., J.J. Guzewich, J.P. Hanrahan, R. Stricof, M. Shayegani, R. Deibel, J.C. Grabau, N.A. Nowak, J.E. Herrmann, G. Cukor, et al. 1986. Widespread outbreaks of clam- and oyster-associated gastroenteritis. Role of Norwalk virus. *N Engl J Med* 314:678–81.

Muntada-Garriga, J.M., J.J. Rodriguez-Jerez, E.I. Lopez-Sabater, and M.T. Mora-Ventura. 1995. Effect of chill and freezing temperatures on survival of *Vibrio parahaemolyticus* inoculated in homogenates of oyster meat. *Lett Appl Microbiol* 20:225–7.

Nickerson, J.T.R., S.A. Goldblith, G. Digioia, and W.W. Bishop. 1967. The presence of *Clostridium botulinum* type E in fish and mud taken from the Gulf of Maine. In *Botulism 1966*, ed. M. Ingram and T.A. Roberts, 25–33. London: Chapman and Hall.

NMFS [National Marine Fisheries Service]. 2007. *Fisheries of the United States 2006.* http://www.st.nmfs.noaa.gov/st1/fus/fus06/fus_2006.pdf (accessed April 3, 2009).

NOAA [The National Oceanic and Atmospheric Administration]. 2007. *Vision 2020: The Future of U.S. Marine Fisheries.* http://www.nmfs.noaa.gov/ocs/documents/Vision_2020_FINAL-1.pdf (accessed March 25, 2009).

NOAA [The National Oceanic and Atmospheric Administration]. 2008. Seafood consumption declines slightly in 2007. http://www.noaanews.noaa.gov/stories2008/20080717_seafood.html (accessed March 25, 2009).

Nordstrom, J.L., C.A. Kaysner, G.M. Blackstone, M.C.L. Vickery, J.C. Bowers, and A. DePaola. 2004. Effect of intertidal exposure on *Vibrio parahaemolyticus* in Pacific Northwest oysters. *J Food Prot* 67:2178–82.

NSSP [National Shellfish Sanitation Program]. 2007. *Guide for the Control of Molluscan Shellfish.* http://www.cfsan.fda.gov/~ear/nss4-toc.html (accessed April 2, 2009).

Oliver, J.D. 1989. *Vibrio vulnificus*. In *Foodborne Bacterial Pathogens*, ed. M.P. Doyle, 569–600. New York: Marcel Dekker.

Perl, T.M., L. Bedard, T. Kosatsky, J.C. Hockin, E.C. Todd, and R.S. Remis. 1990. An outbreak of toxic encephalopathy caused by eating mussels contaminated with domoic acid. *N Engl J Med* 322:1775–80.

Rawles, D.D., G.J. Flick, and R.E. Martin. 1996. Biogenic amines in fish shellfish. *Adv Food Nutr Res* 39:329–64.

Rippey, S.R. 1994. Infectious diseases associated with molluscan shellfish consumption. *Clin Microbiol Rev* 7:419–25.

Robert-Pillot, A., A. Guénolé, J. Lesne, R. Delesmont, J.M. Fournier, and M.L. Quilici. 2004. Occurrence of the *tdh* and *trh* genes in *Vibrio parahaemolyticus* isolates from waters and raw shellfish collected in two French coastal areas and from seafood imported into France. *Int J Food Microbiol* 91:319–25.

Roberts, T., and R. Pinner. 1990. Economic impact of disease caused by *Listeria monocytogenes*. In *Foodborne Listeriosis*, ed. A.J. Miller, J.L. Smith, and G.A. Somkuti, 137–149. Amsterdam: Elsevier Science.

Roche, H.M., and M.J. Gibney. 1996. Postprandial triacylglycerolaemia: the effect of low-fat dietary treatment with and without fish oil supplementation. *Eur J Clin Nutr* 50:617–24.

Rodrigue, D.C., R.A. Etzel, S. Hall, E. de Porras, O.H. Velasquez, R.V. Tauxe, E.M. Kilbourne, and P.A. Blake. 1990. Lethal paralytic shellfish poisoning in Guatemala. *Am J Trop Med Hyg* 42(3):267–71.

Rooney, R.M., E.H. Cramer, S. Mantha, G. Nichols, J.K. Bartram, J.M. Farber, and P.K. Benembarek. 2004. A review of outbreaks of foodborne disease associated with passenger ships: evidence for risk management. *Public Health Rep* 119:427–34.

Rørvik, L.M., D.A. Caugant, and M. Yndestad. 1995. Contamination pattern of *Listeria monocytogenes* and other *Listeria* spp. in a salmon slaughter-house and smoked salmon processing plant. *Int J Food Microbiol* 25:19–27.

Sakaguchi, G. 1979. Botulism. In *Food-Borne Infections and Intoxications*, ed. H. Reimann, 390–433. New York: Academic Press.

Sanders, T.A.B., F.R. Oakley, G.J. Miller, K.A. Mitropoulos, D. Crook, and M.F. Oliver. 1997. Influence of n–6 versus n–3 polyunsaturated fatty acids in diets low in saturated fatty acids on plasma lipoproteins and hemostatic factors. *Arterioscler Thromb Vasc Biol* 17:3449–60.

Scoging, A., and M. Bahl. 1998. Diarrhetic shellfish poisoning in the UK. *Lancet* 352:117.

Shapiro, R.L., S. Alters, L. Hut Wagner, R. Bishop, R. Hammond, S. Wilson, B. Ray, S. Thompson, R.V. Tauxe, and P.M. Griffin. 1998. The role of Gulf Coast oysters harvested in warmer months in *Vibrio vulnificus* infections in the United States, 1988–1996. *J Infect Dis* 178(3):752–9.

Smelt, J.P.P.M., and H. Haas. 1978. Behavior of proteolysis *Clostridium outline* types A and B near the temperature limit of growth. *Eur J Appl Microbiol Biotechnol* 5:143–54.

Smith, L.D.S., and H. Sugiyama. 1988. *Botulism: The Organism, its Toxins, the Disease*. Springfield, Illinois: Charles C. Thomas.

Sobel, J., and J. Painter. 2005. Illnesses caused by marine toxins. *Clin Infect Dis* 41:1290–6.

Solomon, H.M., D.A. Kautter, and R.K. Lynt. 1982. Effect of low temperature on growth on non-proteolysis *Clostridium outline* type B and F and proteolysis type G in crab and broth. *J Food Prot* 45:516–8.

Stone, N.J. 1996. Fish consumption, fish oil, lipids, and coronary heart disease. *Circulation* 94:2337–40.

Tacket, C.O., F. Brenner, and P.A. Blake. 1984. Clinical features and an epidemiological study of *Vibrio vulnificus* infections. *J Infect Dis* 1419:558–61.

Taylor, S.L. 1986. Histamine food poisoning: toxicology and clinical aspects. *Crit Rev Toxicol* 17:91–128.

Terranova, W.A., J.G. Breman, R.P. Locey, and S. Speck. 1978. Botulism type B: epidemiologic aspects of an extensive outbreak. *Am J Epidemiol* 108:150–6.

Thompson, C.A., and C. Vanderzant. 1976. Effect of processing, distribution and storage on *Vibrio parahaemolyticus* and bacterial counts of oysters (*Crassostrea virginica*). *J Food Sci* 41:123–7.

USDA [United States Department of Agriculture]. 2000. Revised action plan for control of *Listeria monocytogenes* for the prevention of foodborne listeriosis. http://www.fsis.usda.gov/oa/topics/lm_action.htm (accessed April 3, 2009).

Varma, J.K., G. Katsitadze, M. Moiscrafishvili, T. Zardiashvili, M. Chokheli, N. Tarkhashvili, E. Jhorjholiani, M. Chubinidze, T. Kukhalashvili, I. Khmaladze, N. Chakvetadze, P. Imnadze, and J. Sobel. 2004. Foodborne botulism in the Republic of Georgia. *Emerg Infect Dis* 10(9):1601–5.

Watkins, J., and K.P. Sleath. 1981. Isolation and enumeration of *Listeria monocytogenes* from sewage, sewage sludge and river water. *J Appl Bacteriol* 50:1–9.

Weagant, S.D., P.N. Sado, K.G. Colburn, J.D. Torkelson, F.A. Stanley, M.H. Krane, S.C. Shields, and C.F. Thayer. 1988. The incidence of *Listeria* species in frozen seafood products. *J Food Prot* 51:655–7.

Whittle, K., and S. Gallacher. 2000. Marine toxins. *Br Med Bull* 56:236–53.

Willett, W.C., M.J. Stampfer, J.E. Manson, G.A. Colditz, F.E. Speizer, B.A. Rosner, C.H. Hennekens, and L.A. Sampson. 1993. Intake of trans fatty acids and risk of coronary heart disease among women. *Lancet* 341(8845):581–5.

Wong, H.-C., W.-L. Chao, and S.-J. Lee. 1990. Incidence and characterization of *Listeria monocytogenes* in foods available in Taiwan. *Appl Environ Microbiol* 56:3101–4.

Wong, H.C., S.H. Liu, L.W. Ku, I.Y. Lee, T.K. Wang, Y.S. Lee, C.L. Lee, L.P. Kuo, and D.Y.C. Shin. 2000. Characterization of *Vibrio parahaemolyticus* isolates obtained from foodborne illness outbreaks during 1992 through 1995 in Taiwan. *J Food Prot* 63:900–6.

Wong, S., D. Street, S.I. Delgado, and K.C. Klontz. 2000. Recalls of foods and cosmetics due to microbial contamination reported to the U.S. Food and Drug Administration. *J Food Prot* 63:1113–6.

Yamamoto, K., Y. Ichinose, H. Shinagawa, K. Makino, A. Nakata, M. Iwanaga, T. Honda, and T. Miwatani. 1990. Two-step processing for activation of the cytosine/hemolysin of *Vibrio cholerae* 01 biotype E1 Tor: Nucleotide sequence of the structural gene (*hly*A) and characterization of the processed products. *Infect Immun* 58:2706–9.

# chapter 5

# *Modified atmosphere packaging for fruits and vegetables*

*John S. Novak*

*Contents*



## *5.1 Introduction*

A market need for minimal processing solutions to add quality and safety during storage shelf-life for fresh fruits and vegetables has created and developed many prospective technologies and options including high-pressure processing (HPP), pulsed electric field (PEF), radiowave frequency, ultrasound, hypobaric storage, irradiation, electrolyzed water, ozone, natural and synthetic antimicrobials, organic acids, chlorine dioxide, hydrogen peroxide, and modified atmosphere packaging (MAP) (Rico et al. 2007). A brief review of available control measures and their benefits with respect to the microbial safety of minimally processed fruits and vegetables, as detailed in other chapters, is necessary as a background introduction to MAP.

Traditionally, chlorine solutions have been widely accepted for the sanitation of minimally processed fruits and vegetables. It is known that organic compounds also react with chlorine in the unfavorable formation of carcinogenic compounds such as chloramines and trihalomethanes (Wei et al. 1999). Typically, the most common sanitizers used for decontaminating fresh produce include chlorine solutions at levels of 50 to 200 ppm free chlorine for contact times from several seconds to several minutes (Watada and Qui 1999; Francis and O'Beirne 2002). The optimum contact time for 70 ppm chlorine solution has been determined to be 12 to 13 sec (Kabir 1994).

Fresh-cut fruits and vegetables are minimally processed today through methods that mechanically cut and peel using sharp knives and rotating carborundum drums, chemical peels, or high-pressure peelers (Ahvenainen 1996). Dull knives can rupture cells and release tissue fluids impairing the quality of the produce (Ahvenainen 1996). Cold rinse water (<5°C) is recommended as well as 5 to 10 liters of water per kg of product before cutting and an additional 3 liters of water per kg of product after peeling or cutting (Ahvenainen 1996). In immersion therapy, the newest technology, fruit is cut while it is submerged in water to control turgor and prevent the movement of fruit fluids while the product is being cut and to flush potentially damaging enzymes away from plant tissues (Allende et al. 2006). Water-jet cutting using a concentrated stream of high-pressure water has also shown promise in cutting produce (Allende et al. 2006).

Chemical sanitizers are added to rinse waters to prevent recontamination of washed products. Chlorine dioxide has a high oxidation capacity, 2.5 times greater than chlorine, and it does not react with nitrogen-containing compounds or ammonia to form dangerous chloramine compounds (Benarde et al. 1965; White 1992). Unfortunately, the microbial reductions were not expected to be more than 90% or 99% (Sapers 2003). Chlorine and chlorine dioxide at >50 ppm were equivalent in killing *Enterobacter sakazakii* on apples (Kim et al. 2006).

Although sulphites had been used to prevent browning in fruits and vegetables, the U.S. Food and Drug Administration (FDA) restricted the use of sulphites in 1990 due to side effects with asthma (Ahvenainen 1996). Alternatives have included combinations of citric acid, ascorbic acid, potassium sorbate, or 4-hexylresorcinol (Ahvenainen 1996). There has also been a trend to use natural browning inhibitors including salad ingredients or pineapple juice (Lozano-de-Gonzales et al. 1993). Organic acids including lactic acid, citric acid, acetic acid, and tartaric acid have been shown to be strong antimicrobial agents for fresh-cut fruits and vegetables (Bari et al. 2005). Citric acid and ascorbic acid have been used to reduce microbial counts on vegetables (Priepke et al. 1976). Calcium propionate, due to its ability to uncouple microbial transport processes,

has been used for antimicrobial treatments of honeydew melons (Saftner et al. 2003). Hydrogen peroxide is useful as an oxidant and antimicrobial, but also may result in browning of shredded lettuce (Juven and Pierson 1996; Parish et al. 2003).

Recently, warm water washes have served a need to remove produce contaminants. Blanching consists of heating produce at high temperatures (85°C –100°C) for short periods of time in water or steam as inactivation of microorganisms is temperature dependent whereas fruit and vegetable degradation is exposure time dependent (Rico et al. 2007). Heat shock is a method that combines a washing step at 45°C to 70°C for less than 5 minutes combined with an alternative antimicrobial agent or chlorine (Loaiza-Velarde et al. 1997; Hisaminato et al. 2001). For certain vegetables, such as potatoes, browning could be reduced by a heat treatment of 15°C for 2 weeks prior to peeling as reducing sugars were decreased by the storage conditions (Mattila et al. 1995). Unfortunately, quality and microbial safety quite frequently need to be balanced against each other.

Ozone is a strong oxidizing agent with antimicrobial properties that has been mainly used with whole or un-cut commodities such as apples, grapes, oranges, pears, broccoli, cucumbers, raspberries, and strawberries to reduce microorganisms (Beltran et al. 2005). Another positive attribute of ozone is the ability to convert many non-biodegradable organic materials into biodegradable forms, minimizing the accumulation of organic waste in the environment (Kim et al. 1999). Despite effectiveness against microorganisms in solution, the effectiveness of ozone in the presence of organic matter or microorganisms that are attached to food products is significantly reduced (Guzel-Seydim et al. 2004). Containment of ozone gas at 5 ppm in dried fruit storage for 60 min has been shown to be very effective against *E. coli* and *Staphylococcus aureus* (Najafi and Khodaparast 2009).

Electrolyzed water, generated by electrolysis of aqueous sodium chloride to produce an electrolyzed basic solution at the cathode and an electrolyzed acidic solution at the anode, results in the production of oxygen gas, chlorine gas, hypochlorite ion, hypochlorous acid, and hydrochloric acid at the anode and hydrogen gas and sodium hydroxide at the cathode (Kim et al. 2000; Hsu 2003). It has been shown to have a higher efficiency against aerobic microorganisms than ozone, but at the expense of produce quality when the commodities are cut (Wang et al. 2004).

Microbial reductions of up to 2.0 logs have been reported using light pulses in the ultraviolet (UV) light spectrum (Marquenie et al. 2002). Ultraviolet light acts as an antimicrobial agent due to DNA damage, but high UV doses can also cause increased stress of the produce, respiration rates, and induction of lignification-like processes that affect produce appearance negatively (Rame et al. 1997; Allende and Artes 2003). Low-dose gamma irradiation has been approved by the U.S. FDA for use

on fruit and vegetables at a maximum level of 1.0 kGy and is an effective control measure for eliminating pathogenic bacteria and parasites from the surfaces of fruits and vegetables (Molins et al. 2001; Rico et al. 2007). However, low-dose irradiation does not destroy toxins, is ineffective against spore-forming microorganisms, and suffers from poor consumer perceptions (Crawford and Ruff, 1996).

Other physical or non-chemical treatments for fruits and vegetables include HPP, PEF, and ultrasound technologies (Cortes et al. 2008; Mizrach 2008; Oey et al. 2008). High-pressure processing (3,000–8,000 bars) inactivates microorganisms and enzymes without harming fruit and vegetable flavors or nutrients, but expansion during pressurization and decompression also damages vegetable and fruit tissues (Palou et al. 2000). Alone HPP is not very effective against spore-formers. PEF can inactivate microorganisms by causing cell membrane permeability changes (Barbosa et al. 1997). Bacteria in aqueous fruit and vegetable juices could be reduced 3 to 5 logs, depending on the specific strain of bacteria, using 200 pulses separated by a 1 sec pulse period and an intensity of 25 kV (Wesierska and Trziszka 2007). Although using PEF only reduced the spoilage bacterium, *Enterobacter aerogenes*, by 1.1 logs in horchata, a Spanish low-acid vegetable beverage, the lag phase of surviving cells was significantly increased delaying continued growth (Selma et al. 2003). Sonication with ultrasound pressure waves of frequencies from 20 kHz to 10 MHz causes cavitation, thinning of cell membranes, localized heating, and production of free radicals in food processing to inactivate microorganisms (Butz and Tauscher 2002; Piyasena et al. 2003). Sonication alone has not been effective in inactivation of foodborne bacteria, but still holds promise when coupled with other technologies such as HPP, chlorine, heat, or ozone (Piyasena et al. 2003).

With respect to recent advancements in technology, computers and electronic monitoring systems are making a significant impact on the minimally processed fruit and vegetable industry. Electronic data acquisition loggers that are placed inside shipments or storage containers of produce are providing a means to certification and verification that temperature-abused conditions have not occurred during long periods of shipment or storage (Studman 2001). In addition, preventative safety measures practiced through hazard analysis critical control point (HACCP) programs can play important roles in reducing foodborne illness (Kvenberg et al. 2000; Meng and Doyle 2002). Better surveillance of fruit and vegetable conditions and pre-planned measures of control may provide better solutions to increased safety concerns.

Biological control of foodborne pathogens on fruits and vegetables using competition for growth and survival among natural microorganisms or their products is an area of potential safety control for

the future. Antagonistic strains of *Bacillus* have proven effective for the postharvest control of gray mold on pears (Mari et al. 1996). A number of microbiological antagonists exist for the control of postharvest diseases including bacteria, yeasts, and yeast-like fungi (Ippolito and Nigro 2000; Chanchaichaovivat et al. 2007). Bacteriocins such as nisin and pediocin have been used in an attempt to replace chemical disinfection of fresh-cut lettuce against contamination with *Listeria monocytogenes* decreasing viability by 1.2 to 1.6 logs, but only controlling growth to a minimal extent (Allende et al. 2007). The combined effectiveness of chemical disinfectants such as EDTA, citric acid, sodium lactate, potassium sorbate, or phytic acid have shown more promise against *L. monocytogenes* in combination with pediocin and nisin by causing a significant reduction of native microflora and inoculating populations on fresh produce (Bari et al. 2005).

In terms of natural plant extracts, flavonoids produced by plant cells possess antifungal, antiviral, and antibacterial properties with a diverse range of pharmacological properties (Cushnie and Lamb 2005). Many plant extracts have been shown to possess antimicrobial activities with regard to food spoilage and safety (Friedman et al. 2002). Vanillin, an essential oil extract of the vanilla bean, at 12 mM concentrations was found to inhibit total aerobic microbial growth by 37% and 66% in two varieties of apples (Rupasinghe et al. 2006). Other essential oils from clove and cinnamon were found to be effective against *Candida albicans*, *Eurotium repens*, and *Aspergillus flavus* (Rodriguez et al. 2007). Although not a plant-derived antimicrobial compound, chitosan represents a natural deacetylated form of chitin obtained from crustacean shells or certain fungi that has been used as an antimicrobial film to cover fruits and vegetables (Du et al. 1997).

Recently, the FDA has approved a spray mixture of bacteriophage viruses for meat and poultry products to protect consumers from *L. monocytogenes* (Bren 2007). Similar strategies should be applicable for improving fruit and vegetable safety. For *L. monocytogenes*, phage-mediated reductions have been reported on contaminated melons (Leverentz et al. 2003). Phages have also been used to control growth of *Campylobacter jejuni*, *L. monocytogenes*, *Salmonella* Typhimurium, and *E. coli* O157:H7 (Bigwood et al. 2008).

Unfortunately, none of the aforementioned alternative methods, including chlorine, are capable of providing microbial sterility with respect to foodborne pathogens without a corresponding loss of product quality. Consequently, it has been recommended that by combining different preservation techniques or hurdles, the damaging effects of higher levels of the processing technologies could be minimized with respect to fruit and vegetable quality while ensuring a reduction in microbial growth (Gorris and Tauscher 1999). As MAP is already in use by the produce industry, it holds significant advantages for acceptance over other

strategies for ensuring safety of fruits and vegetables that maintain fresh-picked, minimally processed qualities. MAP is perceived by most consumers as a mild preservation method and therefore may be more acceptable to consumers than any of the other technologies (Bruhn 1995). The main aspects of MAP follow.

## 5.2 *Modified atmosphere packaging*

MAP is defined as the displacement of air (78.08% $N_2$, 20.95% $O_2$, 0.93% Ar, 0.03% $CO_2$) in a contained area in order to obtain an atmosphere significantly different from air required to envelope a product (Kader and Saltveit 2003). The modified atmosphere is achieved by either of two ways: (1) passively, when a package is sealed under normal air conditions and allowed to change autonomously without external influence or (2) actively, when a package is flushed with a specific gas mixture prior to closure and sealing (Rico et al. 2007). Controlled atmosphere packaging (CAP) implies precise monitoring and control of the gaseous mixture (Floros 1990). The strategy used to create the desired atmosphere is less important than providing an atmosphere that can enable the product to maintain quality, freshness, and be safe to consume after an extended period of storage.

Fruits and vegetables continue to respire even after harvest. This results in a constantly changing environment when they are closed within a sealed container. Figure 5.1 depicts the change in the air composition of sealed barrier pouches containing a sliced apple or cut lettuce due to respiration of the produce stored at 4°C. Over time, $O_2$ is consumed and $CO_2$ is produced as a consequence of aerobic respiration (Figure 5.1). The variations in respiration rates are product specific and temperature dependent (Tano et al. 2007). The respiration rates can be measured by monitoring the composition of package headspaces (Del Nobile et al. 2006; Figure 5.1). It is known that damaged, cut, sliced, or bruised plant tissues exhibit increased respiratory rates (Laties 1978). Tissues with high respiratory rates have shorter postharvest storage lives (Eskin 1990). Additionally, metabolic rates increase by a factor of 2 or 3 for each 10°C increase in temperature (Beaudry et al. 1992). Therefore, low temperatures can reduce respiration rates, and increase shelf-life, but very low temperatures (<4°C) can also result in chill injury to the produce (Rico et al. 2007). MAP can therefore be beneficial to fruits and vegetables that possess high respiratory rates and are sensitive to chilling injury (Lee et al. 1996). Low temperature sensitive products include avocado, banana, cherimoya, grapefruit, lemon, lime, mango, papaya, pineapple, beans, cucumber, okra, pepper, and tomato (Kader, 1997; Saltveit, 1997). MAP is often used

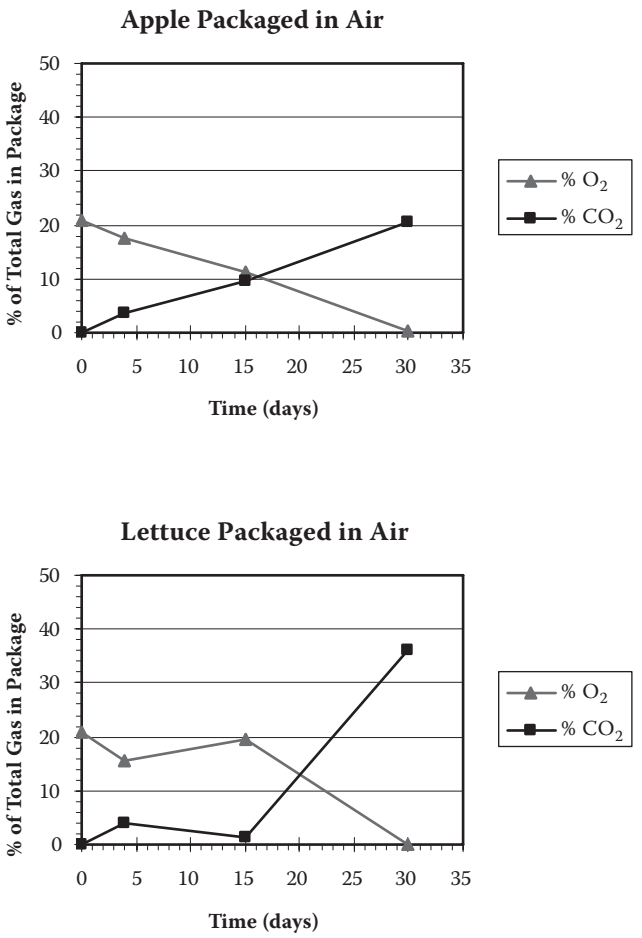


***Figure 5.1*** Effects of the respiration rate of fruits and vegetables on the gaseous headspace composition of a sealed barrier pouch initially packaged under air and stored at 4°C.

to slow down respiration rates resulting in reduced product metabolism and maturation (Kader et al. 1989).

The gases traditionally used in MAP include $CO_2$, $O_2$, and $N_2$. Carbon dioxide is bacteriostatic with inhibitory effects increasing with decreasing temperature (Jay 1992). Oxygen is used to sustain respiration while decreasing the negative effects of anaerobic atmosphere genesis such as product degeneration, low pH, and an increased risk of botulism (Jayas and Jeyamkondan 2002). Nitrogen is often used as an inert filler gas for

displacement of air in a package (Jayas and Jeyamkondan 2002). The literature provides only sparse evidence for the replacement of $N_2$ by other noble gases such as argon or helium (Kader and Watkins 2000).

## 5.3 Quality attributes

MAP can be used to inhibit or decrease many of the plant physiological effects caused by wounding fruit or vegetable tissues such as increased respiration rates, ethylene production leading to increased ripening rates, membrane deterioration, water loss, susceptibility to microbial spoilage, chlorophyll loss, pigment formation, decreased acidity, increased sweetness caused by interconversion of sugars, volatilization of flavors, tissue softening, enzymatic browning, lipolysis, and lipid oxidation (Rico et al. 2007). High $CO_2$ environments inhibit ethylene biosynthesis and action (Kader and Saltveit 2003). In addition to preserving chlorophyll, MAP can enhance the production of other pigments such as carotenoids, anthocyanins, and phenolic compounds (Kader and Saltveit 2003). MAP can decrease enzymatic cell wall degradation causing fruit softening or enzymatic lignification resulting in vegetable toughening (Kader and Saltveit 2003).

It is also important to keep precise control of MAP as the nutritional quality of produce with respect to ascorbic acid (vitamin C) can be decreased in atmospheres containing $O_2$ levels below 5% and $CO_2$ levels above 5% (Kader and Saltveit 2003). Additionally, $O_2$ levels below 1% and $CO_2$ levels above 20% result in the production of off-flavors due to the accumulation of ethanol, acetaldehyde, and ethyl acetate (Kader and Saltveit 2003). Figures 5.2 and 5.3 give a representative summary of the variations in recommended storage conditions with respect to preferred concentrations of $CO_2$ and $O_2$ for select products of fruits and vegetables, respectively. Changes in prescribed gas mixtures are also influenced by wounding or cutting (Figures 5.2 and 5.3).

Quality attributes of fruits and vegetables are the major visual parameters influencing consumers. To that extent, browning enzymes such as polyphenol oxidases play a central role. Browning in apples is more rapid than in lettuce due to a greater synthesis of polyphenol oxidases (Murata et al. 1995, 2004). If MAP is passive (Figure 5.1), the browning of produce has already occurred before package conditions become favorable for browning inhibition after 15 days or more (Figure 5.1). Active MAP, where the package conditions are instantaneously reached due to a gas flush, would provide better results. The control of browning has focused on phenyl-alanine ammonialyase (PAL), an enzyme in the phenylpropanoid pathway, induced by cutting or wounding (Hisaminato et al. 2001). Other enzymes involved in browning include peroxidase and polyphenol oxidase (Zawistowski et al. 1995). As these enzymes are $O_2$-dependent catalysts, it

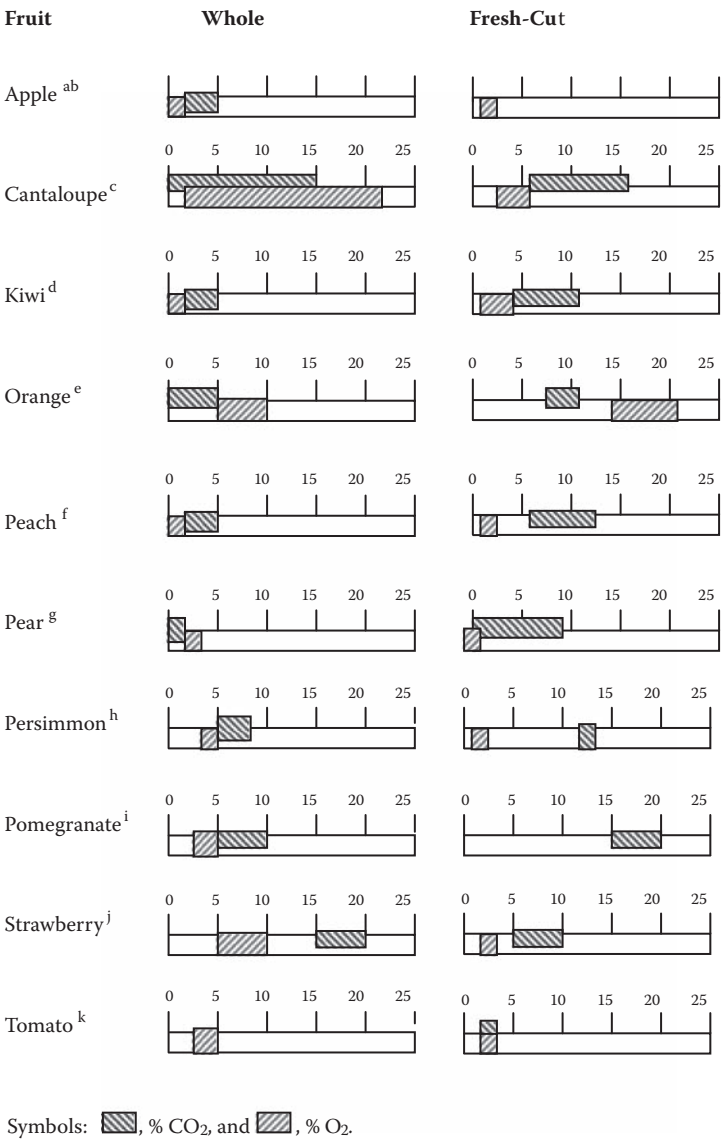


***Figure 5.2*** The literature recommended storage conditions at 5°C for various fruits. Legend: [a] Irving 1984; [b] Kader 1985; [c] de Arruda et al.; 2004; [d] Rocculi et al. 2005; [e] Porat et al. 2004; [f] Yang et al. 2005; [g] Ning et al. 1997; [h] Cia et al. 2006; [i] Artes et al. 2000; [j] Zhang et al. 2006; [k] Lei and Xu 2002.

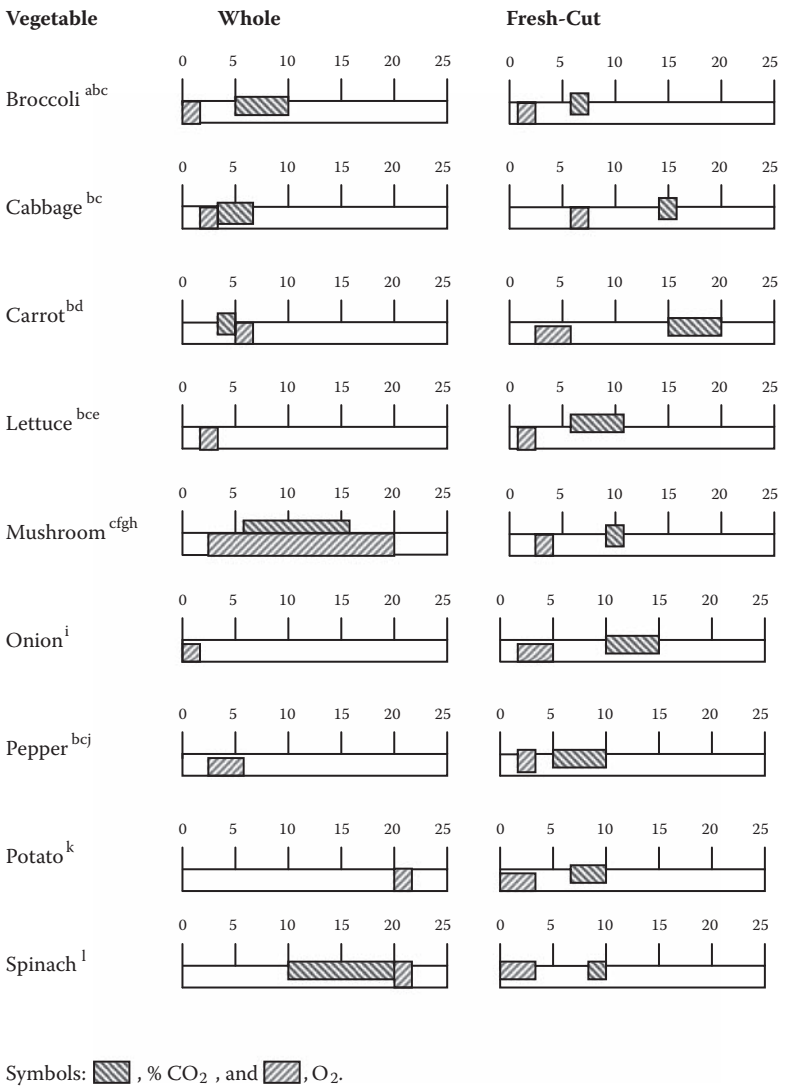


***Figure 5.3*** The literature recommended storage conditions at 5°C for various vegetables. Legend: [a] Ishikawa et al. 1998; [b] Irving 1984; [c] Kader 1985; [d] Alasalvar et al. 2004; [e] Beltran et al. 2005; [f] Nichols 1985; [g] Dong and Choe 2006; [h] Kidder et al. 2007; [i] Ibaraki 2002.; [j] Howard and Hernandez-Brenes 1998; [k] Tudela et al. 2002; [l] Park et al. 2006.

is believed that reducing $O_2$ availability can be used to inhibit browning. Produce loss of firmness is caused by the demethylation of pectins by pectin methylesterase followed by depolymerization to polygalacturonic acid by polygalacturonase (Vu et al. 2004). Likewise, the improvement of fruit texture can be enhanced by the increase of cross-linking between pectin chains and cations caused by a lower level of $O_2$ availability (Roy et al. 2001). This is a clear example where CAP is favored over passive MAP.

## *5.4 Packaging film characteristics*

There are numerous types of films available for MAP. However, in order to achieve a favorable atmosphere specific to a particular fruit or vegetable, there are multiple criteria that require attention including product respiration rate, product mass, film surface area, film thickness, and film gas transmission rates (Zagory 1997). Most films do not result in optimal $O_2$ and $CO_2$ atmospheres, especially when the produce has a high respiration rate (Exama et al. 1993). To complicate matters, the respiration rates of fruits and vegetables are known to increase more with temperature than with film permeabilities, leading to anoxia and safety risks (Exama et al. 1993). The benefits of film packaging include maintenance of high humidity and reduced water loss, improved sanitation, minimized produce abrasions and bruises, reduced contamination among produce, and provision of a medium for transport of active ingredients such as fungicides, ethylene absorbers, $O_2$ scavengers, $CO_2$ emitters, and other chemicals (Kader and Watkins 2000).

With respect to film selection, precedence is given to $O_2$ transmission as the limiting factor in successful MAP (Kader 1986). All films except those with barrier properties such as Saran, Mylar, and Nylon were reported to be capable of satisfying $O_2$ transfer rates for foods with low respiration rates including carrots, celery, cabbage, and green peppers (Exama et al. 1993). The diffusion of $O_2$ and $CO_2$ through air is 8.5 and 1.5 million times greater, respectively, than through low density polyethylene films (Mannapperuma et al. 1989). Rubber type films such as natural rubber and polybutadiene were selected for produce with a moderate respiration such as cauliflower (Exama et al. 1993). For produce with high respiration rates such as strawberry and mushroom, silicone rubber films would be expected (Exama et al. 1993). An additional counterbalance for films not meeting $O_2$ transmission requirements could include the use of microperforations or microporous membranes (Exama et al. 1993). Films are needed that match the film permeability to $O_2$ and $CO_2$ with fruit and vegetable consumption of $O_2$ and production of $CO_2$ in order to maintain equilibrium of the atmosphere in the package.

An empirical equation was developed to predict the dependence of $O_2$ and $CO_2$ transmission rates with respect to the size and number of microperforations in films for achieving desired gaseous compositions of MAP for fresh-cut produce (Gonzalez et al. 2008). The microperforations were small holes in film from 50 to 200 μm in diameter (Ghosh and Anantheswaran 2001). The $O_2$ and $CO_2$ transmission rates obtained using the equation for films 29 to 57 μm in thickness with pore dimensions of 40 × 30 μm to 350 × 110 μm were in agreement with other models where the total diffusive pass length of a perforation was considered as the sum of the perforation length and a correction factor (Gonzalez et al. 2008). Landec Corporation of Menlo Park, CA, has developed a polymeric membrane with an $O_2$ transmission rate that increases with increasing temperature thus avoiding anaerobic conditions after temperature abusive conditions (Zagory 1997). Numerous models are available for measuring gas transfer in packages as well as for predicting permeability of films for use with specific fruits and vegetables (Cameron et al. 1995).

## 5.5 *Predictive modeling of MAP*

Attention to the respiration rate variations of fruits and vegetables is crucial to the successful design of MAP systems, and models must incorporate the changing $O_2$ and $CO_2$ concentrations within a package as well as the effects of temperature and storage time (Fonseca et al. 2002). Due to the complexity of the respiration process, empirical models were developed for each product as a function of controlled variables such as temperature or gas composition (Fonseca et al. 2002). The dynamic process can be represented using a respiratory quotient (RQ) as a measure of the ratio of $CO_2$ produced to $O_2$ consumed for a given product (Fonseca et al. 2002). RQ values range from 0.7 to 1.3, lower if metabolic substrates are lipids, 1.0 if substrates are carbohydrates, and higher if substrates are acidic (Kader 1987). A high RQ value above 1.0 is usually an indication of fermentation and anaerobic metabolism (Fonseca et al. 2002). Respiration rates are measured in one of three systems: a closed system, a flowing or flushed system, or a permeable system (Fonseca et al. 2002).

In the closed system, $O_2$ consumption and $CO_2$ production are measured following containment of the introduced gas mixtures (Fonseca et al. 2002). A problem encountered with this measurement system involves the effect of $O_2$ depletion and $CO_2$ production on the respiration rates themselves (Fonseca et al. 2002). A method was developed for the determination of $O_2$ consumption as a function of $O_2$ concentration by mathematical characterization of $O_2$ depletion by tomato fruit in a closed system with

time (Cameron et al. 1989). The flow-through system involves a constant gas flow rate, but it is very difficult to measure that rate (Fonseca et al. 2002). Further complications include the need for an expected respiration rate estimation (Fonseca et al. 2002). However, the least accurate method for respiration rate measurement is the permeable system as it requires determination of additional variables including the package dimensions, thickness of the gas exchange material, and permeability characteristics (Fonseca et al. 2002). Other factors affecting respiration rates include fruit or vegetable maturity stage, increased respiration due to wounding, as well as the most important external factor influencing respiration rate, temperature (Fonseca et al. 2002). There has been difficulty comparing reported respiration rates for produce in the literature due to a lack of standardization among studies and non-uniformity of units used in existing respiration rate models (Fonseca et al. 2002).

Perforation-mediated MAP relies on the use of macroperforations in the film to control $O_2$ and $CO_2$ exchange (Fonseca et al. 2000). Empirical and additive models with several parameters relating $O_2$ and $CO_2$ mass transfer coefficients with temperature, diameter, and length of perforations in the films have been developed previously (Emond et al. 1991). A suitable estimation of gas transfer for $O_2$ and $CO_2$ exchange through a single perforation was obtained using different temperatures (5–20°C) and tube dimensions (length from 9 to 17 mm and diameter from 6 to 30 mm) (Fonseca et al. 2000). The ratio between $CO_2$ and $O_2$ mass transfer coefficients for a single film perforation was found to be independent of temperature and perforation dimensions (Fonseca et al. 2000).

Software has been recently developed by Mahajan et al. (2007) (PACK in MAP) to select suitable packaging materials while defining the amount of product to be packed or the area of film that needs to be available for gas exchange taking into account respiration rate, temperature, packaging film and film area, produce weight, package geometry, and package volume. The software database includes information from 38 products, 75 respiration rate models, and permeability data for 27 polymeric films (Mahajan et al. 2007). Much of the film permeability data comes from the earlier study by Exama et al. (1993). The major benefit of such software is the avoidance of time-consuming experiments to determine the best film to be used in packaging a specific type of product (Mahajan et al. 2008). An added benefit is the ability of the PACK in MAP software to accurately simulate the evolution of a gas composition with respect to temperature variations as would be expected during the unavoidable temperature abuse possibilities that exist during the normal distribution chain process for any given product (Mahajan et al. 2008).

## 5.6 Microbiological safety challenges

Aside from the beneficial quality attributes, a common argument against MAP for produce commodities with high potential microbial contamination levels is the potential vulnerability from a safety standpoint as spoilage organisms may be inhibited while promoting the growth of pathogens (Farber et al. 2003). The primary MAP gas that has been shown to have inhibitory effects on microorganisms is $CO_2$ through interference with cellular membrane function, enzyme inhibition, acidic pH, and effects on the physico-chemical properties of proteins (Farber 1991).

The main microflora present on fresh leafy vegetables include predominant soil bacteria such as *Pseudomonas* and *Erwinia* spp. (Ahvenainen 1996). Levels of $CO_2$ (10%–20%) that inhibit spoilage microorganisms such as the aerobic soil bacteria also enhance other spoilage bacteria that are common to food processes, such as lactic acid bacteria, and there may be a shift in the predominant microflora present (Amanatidou et al. 1999). Harmful pathogens such as *Clostridium perfringens*, *C. botulinum*, and *Listeria monocytogenes* that can withstand anaerobic conditions as well as concentrations of $CO_2$ above 50% may proliferate at the expense of innocuous food-spoilage indicator microorganisms (Phillips 1996; Zagory 1999). Combinations of gases used in MAP need to be carefully evaluated along with film permeabilities to ensure safety from anomalous but opportunistic pathogens that may contaminate fruits and vegetables.

### 5.6.1 Clostridia

*Clostridium botulinum* is a strict anaerobic spore-forming bacterium that is found in the soil or associated with produce contacting soil and is capable of producing a very potent neurotoxin. The strains of this microorganism responsible for most cases of foodborne botulism can be distinguished as proteolytic, with minimal growth temperature requirements (12°C), minimal pH (4.6), minimal water activity (0.95), and maximum NaCl concentration (10%) as compared to nonproteolytic, with minimal growth temperature requirements (3°C), minimal pH (5.0), minimal water activity (0.97), and maximum NaCl concentration (4%) (Lund and Peck 2000; Novak et al. 2005). The microorganism can thrive and produce dangerous neurotoxins under conditions of low atmospheric oxygen. Therefore, low $O_2$ packages (<1%) have been determined to provide a health risk potential from this pathogen as well as contributing to product off-flavors and odors due to anaerobic respiration (Zagory 1995). Fortunately, despite the potential for *C. botulinum* growth, MAP packages of produce were found to be grossly spoiled beyond consumer acceptability levels before the detection of toxins (Petran et al. 1995; Larson et al. 1997; Hao et al. 1998; Larson and

Johnson 1999). There are occasional lapses in proper temperature storage that could produce contrary results with fatal consequences, but these are often of low probability (Solomon et al. 1990). Research studies have shown that toxin production could also vary with the vegetable variety serving as the growth substrate for *C. botulinum* (Austin et al. 1998). For these reasons, it is important to combine proper film characteristics with the right combination of gases.

Although of lessened concern with fresh fruits and vegetables as compared with cooked meat products, another anaerobic spore-former, *C. perfringens,* was found to be quite resistant to $CO_2$ atmospheres especially under temperature abuse conditions (Novak and Yuan 2004). Low temperature in combination with 75% $CO_2$ was shown to be effective in preventing the outgrowth of *C. perfringens* spores (Hintlian and Hotchkiss 1987). $CO_2$ at a lower concentration of 5% was able to stimulate germination of *C. perfringens* spores and influenced outgrowth and toxin formation (Enfors and Molin 1978). Additionally, temperature (>25°C) decreased the effectiveness of MAP, and enabled uninhibited growth of *C. perfringens* (Hintlian and Hotchkiss 1987). Conditions limiting growth of *C. perfringens* are often considered measures to limiting the growth of *C. botulinum* as well. As the primary reason for *C. botulinum* growth and toxin production is low $O_2$ concentration, the food industry practice of using perforated films with mushrooms reduces the risk of foodborne illnesses (Sugiyama and Yang 1975). Pursuant to such logic is the use of high $O_2$ MAP.

### 5.6.2 *High oxygen map*

At low concentrations of $O_2$, *Staphylococcus aureus* can grow and produce enterotoxin A if mushroom packages are temperature-abused (Martin and Beelman 1996; Gonzalez-Fandos et al. 2000). A novel approach to counteract risks of low $O_2$ include the use of high $O_2$ levels (70%–100%) combined with $CO_2$ for MAP (Amanatidou et al. 1999). This strategy does not need to be used exclusively for concerns with anaerobes. High $O_2$ (80%) MAP was found to inhibit the growth of several generic groups of bacteria, yeasts and molds, and a range of specific food-associated pathogenic and spoilage microorganisms such as *Aeromonas hydrophila*, *Salmonella enteritidis*, *Pseudomonas putida*, *Rhizopus stolonifer*, *Botrytis cinerea*, *Penicillium roqueforti*, *P. digitatum*, and *Aspergillus niger* (Day 2001). High $O_2$ MAP was able to inhibit or stimulate the growth of *Pseudomonas fragi*, *Bacillus cereus*, *Lactobacillus sake*, *Yersinia enterocolitica*, and *Listeria monocytogenes* alone, but with the addition of 10% to 30% $CO_2$ all were inhibited (Day 2001). In another study, *Aeromonas caviae* was growth inhibited in 70% to 95% $O_2$, yeast were also growth inhibited in high $O_2$ MAP, as was *L. monocytogenes* (extended lag phase using 95%

$O_2$), whereas *Erwinia carotova* was growth stimulated by high $O_2$ MAP (Jacxsens et al. 2001).

The use of $N_2$ for MAP serves the displacement of $O_2$, inhibition of aerobic growth, and as a package filler to replace gases such as $CO_2$ that can be absorbed by the product (Parry 1993). Argon-containing and $N_2O$-containing MAP were found to have little to no antimicrobial effects on microorganisms, when compared with $N_2$-containing MAP (Day 2001). As expected 10% $CO_2$ reduced respiration rates of produce, but high $O_2$ or Ar did not influence respiration rates significantly (Day 2001). Whether for safety concerns, economics, or lack of applied effectiveness, currently high $O_2$ MAP has not found commercial validation and is not practiced in the fruit and vegetable industry.

### *5.6.3* Listeria monocytogenes

*L. monocytogenes* is a very hardy Gram-positive bacilli that is isolated from the environment, capable of growth under refrigeration (4°C), invades the gastrointestinal epithelium, and can grow intracellularly within the host's phagocytic cells. It also has a low infective dose (1,000 cells) that can lead to lethality for the host. The ability of *L. monocytogenes* to grow at low temperatures increased concerns over select MAP conditions that could delay growth of competitive lactic acid bacteria with respect to *L. monocytogenes*, which could proliferate over extended periods of low temperature storage under MAP (Farber et al. 2003). *L. monocytogenes* has been shown to be capable of growth on lettuce under 97% $N_2$ + 3% $O_2$ or 100% $N_2$ at either 4°C or 8°C (Beuchat and Brackett 1990; Francis and O'Beirne 1997). Increased $CO_2$ levels (20%) have been shown to increase growth of lactic acid bacteria, producing antilisterial agents such as nisin, leading to the inhibition of *L. monocytogenes* (Francis and O'Beirne 1998). However, other studies have shown that the growth of *L. monocytogenes* on produce under MAP conditions was influenced more by vegetable type than by the gas atmosphere used (Carlin and Nguyen-The 1994; Jacxsens et al. 1999). There have even been reports of *L. monocytogenes* growth on sliced apples under controlled atmospheres and in peeled potatoes under vacuum (Conway et al. 1998; Juneja et al. 1998). Certainly, more studies are required to fully understand the requirements for MAP control of *L. monocytogenes*.

### *5.6.4* Aeromonas hydrophila

*Aeromonas hydrophila* is a species frequently associated with human disease obtained from contaminated ready-to-eat salads (Ahvenainen 1996). Similar to *L. monocytogenes*, *A. hydrophila* is a psychrotroph that can grow at refrigerated temperatures and is not affected by $O_2$ (1.5%) or $CO_2$ (up to

50%) (Francis et al. 1999). The shelf-life of broccoli, asparagus, and cauliflower was extended under 11% to 18% $O_2$, 3% to 10% $CO_2$, and balance $N_2$ without affecting the growth of *A. hydrophila* (Berrang et al. 1989). Although similar to *L. monocytogenes*, $CO_2$ levels above 50% that inhibited growth of the microorganism also resulted in damage to the produce (Bennik et al. 1995). The high levels of $CO_2$ might actually increase competitive microflora such as lactic acid bacteria, reducing the produce pH, but also creating damage to the produce as a result (Garcia-Gimeno et al. 1996).

### 5.6.5 *Other pathogens of concern:* Salmonella *and* E. coli

Although *E. coli* is commonly regarded as a facultative anaerobe harmlessly associated with the gastrointestinal tract, there are a number of divergent pathogenic forms that can cause a number of illnesses ranging from mild diarrhea to cholera-like and invasive dysentery (McClure 2005). *Salmonella* are also common inhabitants of the gastrointestinal tract that under opportunistic conditions rank as leading causes of foodborne outbreaks worldwide (Maurer and Lee 2005). Taken together, *Salmonella* and *E. coli* contaminate fruits and vegetables primarily through the applications of fertilizers to growing crops or irrigation and processing water tainted with human or animal waste (Maurer and Lee 2005). With regard to *Salmonella* spp. and *E. coli*, 10% to 20% $CO_2$ inhibited *S.* Enteritidis, but *S. Typhimurium* and *E. coli* were unaffected or even stimulated to increase growth (Amanatidou et al. 1999). Combining high $O_2$ (90%) with the 10% to 20% $CO_2$ was able to inhibit both *S.* Enteritidis and *E. coli* (Amanatidou et al. 1999). Produce effects also entered into the equation as *S.* Enteritidis growth decreased on carrots and lettuce when packaged under a low $CO_2$ mix of 5% $CO_2$, 5.2% $O_2$, and 89.8% $N_2$ (Kakiomenou et al. 1998). Unfortunately, *E. coli* O157:H7 and *S. Typhimurium* could only be inhibited by high concentrations of $CO_2$ alone, which also resulted in damage to the produce (Amanatidou et al. 1999; Francis et al. 1999).

### 5.6.6 *Other pathogens of concern:* Campylobacter *and others*

Although the potential exists for microaerophilic *Campylobacter* spp. to exist on packaged produce with low $O_2$ environments following contamination from manure, irrigation water, or wild animal feces, there are few reports of its association with products other than meats (Wallace 1997). Lettuce, spinach, radish, green onions, parsley, and potatoes were examined for *Campylobacter* spp. without any positive findings (Park and Sanders 1992). Another study confirmed the absence of *Campylobacter* spp. on lettuce, carrot, cauliflower, celery, broccoli, and sliced green peppers (Odumeru et al. 1997). Nevertheless, the potential risks cannot be

overlooked as studies have shown the potential for *Campylobacter jejuni* to survive on cilantro, green pepper, and romaine lettuce packaged under air, 5% $O_2$, 10% $CO_2$, 85% $N_2$, and vacuum for 15 days at 4°C when purposefully added (Tran et al. 2000).

Typically viruses, such as hepatitis A, have been involved in illnesses involving cold vegetable salads, but mostly these have been transmitted by infected food handlers as opposed to proliferation under MAP conditions (Beuchat 1996). There have only been a few MAP produce products that have resulted in U.S. outbreaks in the last few decades and these have included coleslaw (*C. botulinum*), ready-to-eat salad vegetables (*Salmonella* Newport), and bagged spinach (*E. coli* O157:H7) (Farber et al. 2003; CDC 2006). As consumer options for fresh produce have increased, so have foodborne illnesses linked to fresh-cut produce; however, the same increases in illnesses due to produce packaged under MAP have not occurred (Farber et al. 2003). To that extent MAP has been proven to be a very safe option for shelf-life extension of fresh fruits and vegetables.

## 5.7 Summary/conclusions

In general, MAP of fruits and vegetables is an acceptable but complex combination of contained storage conditions that maintain quality and freshness under minimal processing in order to extend shelf-life and avoid spoilage and foodborne illness caused by microorganisms. The proportions of common MAP gases such as $CO_2$, $O_2$, and $N_2$ need to be tailored and optimized to a specific product. Variations in respiration rates of fruits and vegetables ensure constantly changing conditions within a package and as such must be constantly adapted to maintain an optimal equilibrium atmosphere specific to the product and its current state of quality: whole, cut, or bruised, and premature, ripe, or over-ripe. In that regard, individual commodity respiration rates are matched with film permeabilities that can provide a suitable equilibrium atmosphere. Temperature becomes the major parameter of control for decreasing respiration, slowing physiological changes due to ripening in the produce, and for minimizing growth of microorganisms causing either spoilage or foodborne illness. Numerous software models exist for measuring gas transfer in packages as well as for predicting permeability of films for use with specific produce.

Although the organoleptic quality attributes of fruits and vegetables are the determining primary factors for consumers' purchase preferences, products can still harbor vulnerability from a safety standpoint at the microscopic level. Foodborne illness concerns from pathogens such as

*C. botulinum*, *L. monocytogenes*, or *E. coli* O157:H7 are sometimes lowered due to competition from natural and harmless spoilage microorganisms such as pseudomonads or lactobacilli. It is important that MAP conditions do not sway this competitive balance to favor the illness-causing foodborne pathogens. However, that concept only implies greater precision in control of MAP conditions and not the elimination of MAP as a useful strategy to enhance safety, quality, and shelf-life of fruits and vegetables. MAP technologies have withstood decades of challenges with extremely few anomalous safety-related outbreaks. As control measures and technologies for maintaining optimized MAP conditions continue to improve, it can be expected that MAP will continue to provide solutions to rather than causes for food safety concerns.

## *Acknowledgment*

The author is grateful to Christine Boisrobert, Food Applications Manager, Air Liquide Industrial U.S. LP, for her critical assessment and helpful peer review of this chapter.

## *References*

Ahvenainen, R. 1996. New approaches in improving the shelf life of minimally processed fruit and vegetables. *Trends Food Sci. Technol.* 7:179–187.

Alasalvar, C., Al-Farsi, M., Quantick, P. C., Shahidi, F., and Wiktorowicz, R. 2004. Effect of chill storage and modified atmosphere packaging (MAP) on antioxidant activity, anthocyanins, carotenoids, phenolics and sensory quality of ready-to-eat shredded orange and purple carrots. *Food Chem.* 89(1):69–76.

Allende, A., Martinez, B., Selma, V., Gil, M. I., Suarez, J. E., and Rodriguez, A. 2007. Growth and bacteriocin production by lactic acid bacteria in vegetable broth and their effectiveness at reducing *Listeria monocytogenes in vitro* and in fresh-cut lettuce. *Food Microbiol.* 24:759–766.

Allende, A., Tomas-Barberan, F. A., and Maria, I. G. 2006. Minimal processing for healthy traditional foods. *Food Sci. Technol.* 17:513–519.

Amanatidou, A., Smid, E. J., and Gorris, L. G. M. 1999. Effect of elevated oxygen and carbon dioxide on the surface growth of vegetable-associated microorganisms. *J. Appl. Microbiol.* 86:429–438.

Artes, F., Villaescusa, R., and Tudela, J. A. 2000. Modified atmosphere packaging of pomegranate. *J. Food Sci.* 65(7):1112–1116.

Austin, J. W., Dodds, K. L., Blanchfield, B., and Farber, J. M. 1998. Growth and toxin production by *Clostridium botulinum* on inoculated fresh-cut packaged vegetables. *J. Food Prot.* 61(3):324–328.

Barbosa, G. V., Pothakamury, U., Palou, E., and Swanson, B. G. 1997. In *Non-Thermal Preservation of Foods*, Vol. 3, 53–110. New York: Marcel Dekker.

Bari, M. L., Ukuku, D. O., Kawasaki, T., Inatsu, Y., Isshiki, K., and Kawamoto, S. 2005. Combined efficacy of nisin and pediocin with sodium lactate, citric acid, phytic acid, and potassium sorbate and EDTA in reducing the *Listeria monocytogenes* population of inoculated fresh-cut produce. *J. Food Prot.* 68:1381–1387.

Beaudry, R. M., Cameron, A. C., Shirazi, A., and Dostal-Lange, D. L. 1992. Modified atmosphere packaging of blueberry fruit: Effect of temperature on package $O_2$ and $CO_2$. *J. Am. Soc. Hort. Sci.* 117:436–441.

Beltran, D., Selma, M. V., Marin, A., and Gil, M. I. 2005. Ozonated water extends the shelf-life of fresh-cut lettuce. *J. Agric. Food Chem.* 53(14):5654–5663.

Benarde, M. A., Israel, B. M., Oliveri, V. P., and Granstrom, M. L. 1965. Efficiency of chlorine dioxide as a bactericide. *J. Appl. Microbiol.* 13:776.

Bennik, M. H. J., Smid, E. J., Rombouts, F. M., and Gorris, L. G. M. 1995. Growth of psychrotrophic foodborne pathogens in a solid surface model system under the influence of carbon dioxide and oxygen. *Food Microbiol.* 12:509–519.

Berrang, M. E., Brackett, R. E., and Beuchat, L. R. 1989. Growth of *Aeromonas hydrophila* on fresh vegetables stored under a controlled atmosphere. *Appl. Environ. Microbiol.* 55(9):2167–2171.

Beuchat, L. R., and Brackett, R. E. 1990. Survival and growth of *Listeria monocytogenes* on lettuce as influenced by shredding, chlorine treatment, modified atmosphere packaging, and temperature. *J. Food Sci.* 55(3):755–758.

Beuchat, L. R. 1996. Pathogenic microorganisms associated with fresh produce. *J. Food Prot.* 59(2):204–216.

Bigwood, T., Hudson, J. A., Billington, C., Carey-Smith, G. V., and Heinemann, J. A. 2008. Phage inactivation of foodborne pathogens on cooked and raw meat. *Food Microbiol.* 25:400–406.

Bren, L. 2007. Bacteria-eating virus approved as food additive. *FDA Consumer Magazine.* January–February 2007.

Bruhn, C. M. 1995. Consumer attitudes and market response to irradiated food. *J. Food Prot.* 58:175.

Butz, P., and Tauscher, B. 2002. Emerging technologies: Chemical aspects. *Food Res. Int.* 35(2/3):279–284.

Cameron, A. C., Boylan-Pett, W., and Lee, J. 1989. Design of modified atmosphere packaging systems: Modeling oxygen concentrations within sealed packages of tomato fruits. *J. Food Sci.* 54:1413–1421.

Cameron, A. C., Talasila, P. C., and Jones, D. W. 1995. Predicting film permeability needs for modified atmosphere packaging of lightly processed fruits and vegetables. *Hort. Sci.* 30(1):25–34.

Carlin, F., and Nguyen-The, C. 1994. Fate of *Listeria monocytogenes* on four types of minimally processed green salads. *Lett. Appl. Microbiol.* 18:222–226.

CDC 2006. *E. coli* sickens more than 35 in New Jersey and Long Island. *New York Times* December 4, 2006. http://www.cdc.gov/foodborne/ecolispinach/100606.htm. Last accessed 05–23–08.

Chanchaichaovivat, A., Ruenwongsa, P., and Panijpan, B. 2007. Screening and identification of yeast strains from fruits and vegetables: Potential for biological control of postharvest chilli anthracnose (*Colletotrichum capsici*). *Biological Control*. 42:326–335.

Cia, P., Benato, E. A., Sigrist, J. M. M., Sarantopoulos, C., Olivieira, L. M., and Padula, M. 2006. Modified atmosphere packaging for extending the storage life of "Fuyu" persimmon. *Postharvest Biol. Technol.* 42(3):228–234.

Conway, W. S., Janisiewicz, W. J., Watada, A. E., and Sams, C. E. 1998. Survival and growth of *Listeria monocytogenes* on fresh-cut apple slices. *Phytopathol.* 88:S18.

Cortes, C., Esteve, M. J., and Frigola, A. 2008. Color of orange juice treated by high intensity pulsed electric fields during refrigerated storage and comparison with pasteurized juice. *Food Control.* 19:151–158.

Crawford, L. M., and Ruff, E. H. 1996. A review of the safety of cold pasteurization through irradiation. *Food Control.* 7(2):87–97.

Cushnie, T. P. T., and Lamb, A. J. 2005. Antimicrobial activity of flavonoids. *Int. J. Antimicrob. Agents* 26:343–356.

Day, B. P. F. 2001. Modified atmosphere packaging of fresh fruit and vegetables: An overview. *Acta Hort.* 553:585–590.

De Arruda, M. C., Jacomino, A. P., Spoto, M. H. F., Gallo, C. R., and Moretti, C. L. 2004. Preservation of minimally processed net melon under active modified atmospheres. *Cienc. e Tecnol. de Aliment.* 24(1):53–58.

Del Nobile, M. A., Baiano, A., Benedetto, A., and Massignan, L. 2006. Respiration rate of minimally processed lettuce as affected by packaging. *J. Food Eng.* 74:60–69.

Dong, M. S., and Choe, S. Y. 2006. Modified atmosphere packaging for mushrooms to delay pH increase rate, thus to reduce weight loss, decolorization and PPO activity of mushroom during storage, and to maintain freshness and quality for increased period by substituting air inside the package with gas containing nitrogen, oxygen, and carbon dioxide. Korean Patent Application KR 2006067520 A 20060620 20041215.

Du, J. M., Gemma, H., and Iwahori, S. 1997. Effect of chitosan coating on the storage of peach, Japanese pear, and kiwifruit. *J. Jpn. Soc. Hortic. Sci.* 66:15–22.

Emond, J. P., Castaigne, F., Toupin, C. J., and Desilets, D. 1991. Mathematical modeling of gas exchange in modified atmosphere packaging. *Trans. Am. Soc. Agri. Eng.* 34(1):239–245.

Enfors, S.-O., and Molin, G. 1978. The influence of high concentrations of carbon dioxide on the germination of bacterial spores. *J. Appl. Bacteriol.* 45:279–285.

Eskin, M. N. A. 1990. Biochemical changes in raw foods: Fruits and vegetables. In *Biochemistry of Foods,* ed. M. N. A. Eskin, 70–78. Toronto, ON: Academic Press.

Exama, A., Arul, J., Lencki, R. W., Lee, L. Z., and Toupin, C. 1993. Suitability of plastic films for modified atmosphere packaging of fruits and vegetables. *J. Food Sci.* 58(6):1365–1370.

Farber, J. M. 1991. Microbiological aspects of modified-atmosphere packaging technology—a review. *J. Food Prot.* 54(1):58–70.

Farber, J. N., Harris, L. J., Parish, M. E., Beuchat, L. R., Suslow, T. V., Gorney, J. R., Garrett, E. H., and Busta, F. F. 2003. Ch. IV. Microbiological safety of controlled and modified atmosphere packaging of fresh and fresh-cut produce. *Comp. Rev. Food Sci. & Food Safety.* 2(supplement):142–160.

Floros, J. D. 1990. Controlled and modified atmospheres in food packaging and storage. *Chem. Eng. Prog.* 86(6):25–32.

Fonseca, S. C., Oliveira, F. A. R., Lino, I. B. M., Brecht, J. K., and Chau, K. V. 2000. Modeling $O_2$ and $CO_2$ exchange for development of perforation-mediated modified atmosphere packaging. *J. Food Eng.* 43:9–15.

Fonseca, S. C., Oliveira, F. A. R., and Brecht, J. K. 2002. Modelling respiration rate of fresh fruits and vegetables for modified atmosphere packages: A review. *J. Food Eng.* 52:99–119.

Francis, G. A., and O'Beirne, D. 1997. Effects of gas atmosphere, antimicrobial dip, and temperature on the fate of *Listeria innocua* and *Listeria monocytogenes* on minimally processed lettuce. *Int. J. Food Sci. Technol.* 32:141–151.

Francis, G. A., and O'Beirne, D. 1998. Effects of storage atmosphere on *Listeria monocytogenes* and competing microflora using a surface model system. *Int. J. Food Sci. Technol.* 33:465–476.

Francis, G. A., and O'Beirne, D. 2002. Effects of vegetable type and antimicrobial dipping on survival and growth of *Listeria innocua* and *E. coli*. *Int. J. Food Sci. Technol.* 37(6):711.

Francis, G. A., Thomas, C., and O'Beirne, D. 1999. The microbiological safety of minimally processed vegetables. *Int. J. Food Sci. Technol.* 34:1–22.

Friedman, M., Henika, P. R., and Mandrell, R. E. 2002. Bactericidal activities of plant essential oils and some of their isolated constituents against *Campylobacter jejuni*, *Escherichia coli*, *Listeria monocytogenes*, and *Salmonella enterica*. *J. Food Prot.* 65:1545–1560.

Garcia-Gimeno, R. M., Sanchez-Pozo, M. D., Amaro-Lopez, M. A., and Zurera-Cosano, G. 1996. Behavior of *Aeromonas hydrophila* in vegetable salads stored under modified atmosphere at 4 and 15°C. *Food Microbiol.* 13:369–374.

Ghosh, V., and Anantheswaran, R. C. 2001. Oxygen transmission rate through micro-perforated films: Measurement and model comparison. *J. Food Proc. Eng.* 24:113–133.

Gonzalez, J., Ferrer, A., Oria, R., and Salvador, M. L. 2008. Determination of $O_2$ and $CO_2$ transmission rates through microperforated films for modified atmosphere packaging of fresh fruits and vegetables. *J. Food Eng.* 86:194–201.

Gonzalez-Fandos, E., Gimenez, M., Olarte, C., and Simon, A. 2000. Effect of packaging conditions on the growth of micro-organisms and the quality characteristics of fresh mushrooms (*Agaricus bisporus*) stored at inadequate temperatures. *J. Appl. Microbiol.* 89:624–632.

Gorris, L. G. M., and Tauscher, B. 1999. Quality and safety aspects of novel minimal processing technologies. In *Processing Foods: Quality Optimization and Process Assessment*, ed. F. A. R. Oliveira, and J. C. Oliveira, 325–339. Boca Raton, FL: CRC Press.

Guzel-Seydim, Z. B., Greene, A. K., and Seydim, A. C. 2004. Use of ozone in the food industry. *Lebesm.-Wiss. U.-Technol.* 37:453–460.

Hao, Y. Y., Brackett, R. E., Beuchat, L. R., and Doyle, M. P. 1998. Microbiological quality and the inability of proteolytic *Clostridium botulinum* to produce toxin in film-packaged fresh-cut cabbage and lettuce. *J. Food Prot.* 61:1148–1153.

Hintlian, C. B., and Hotchkiss, J. H. 1987. Comparative growth of spoilage and pathogenic organisms on modified atmosphere-packaged cooked beef. *J. Food Prot.* 50:218–223.

Hisaminato, H., Murata, M., and Homma, S. 2001. Relationship between enzymatic browning of cut lettuce and phenylalanine ammonia-lyase activity, and prevention of browning by inhibitors of polyphenol biosynthesis. *Biosci. Biotechnol. Biochem.* 65:1016–1021.

Howard, L. R., and Hernandez-Brenes, C. 1998. Antioxidant content and market quality of jalapeno pepper rings as affected by minimal processing and modified atmosphere packaging. *J. Food Quality* 21(4):317–327.

Hsu, S. Y. 2003. Effects of water flow rate, salt concentration and water temperature on efficiency of an electrolyzed oxidizing water generator. *J. Food Eng.* 60:469–473.

Ibaraki, T. 2002. Studies on modified atmosphere packaging on quality of intact or pre-cut welsh onion. *Fukuoka-ken Nogyo Sogo Shikenjo Tokubetsu Hokoku.* 18:1–83.

Ippolito, A., and Nigro, F. 2000. Impact of preharvest application of biological control agents on postharvest diseases of fresh fruits and vegetables. *Crop Protection* 19:715–723.

Irving, A. R. 1984. Transport of fresh horticultural produce under modified atmospheres. *CSIRO Food Res. Q. P.* 44(2):25–33.

Ishikawa, Y., Wessling, C., Hirata, T., and Hasegawa, Y. 1998. Optimum broccoli packaging conditions to preserve glutathione, ascorbic acid, and pigments. *J. Jap. Soc. Hort. Sci.* 67(3):367–371.

Jacxsens, L., Devlieghere, F., Van der Steen, C., and Debevere, J. 2001. Effect of high oxygen and sensorial qualities of fresh-cut produce. *Int. J. Food Microbiol.* 71:197–210.

Jacxsens, L., Devlieghere, F., Falcato, P., and Debevere, J. 1999. Behavior of *Listeria monocytogenes* and *Aeromonas* spp. on fresh-cut produce packaged under equilibrium-modified atmosphere. *J. Food Prot.* 62(10):1128–1135.

Jay, J. M. 1992. Modern food microbiology. In *Modern Food Microbiology*, ed. J. M. Jay, 1–701. New York: Chapman & Hall.

Jayas, D. S., and Jeyamkondan, S. 2002. Modified atmosphere storage of grains, meats, fruits, and vegetables. *Biosys. Eng.* 82(3):235–251.

Juneja, V. K., Martin, S. T., and Sapers, G. M. 1998. Control of *Listeria monocytogenes* in vacuum-packaged pre-peeled potatoes. *J. Food Sci.* 63(5):911–914.

Juven, B., and Pierson, M. D. 1996. Antibacterial effects of hydrogen peroxide and methods for its detection and quantitation. *J. Food Prot.* 59(11):1233–1241.

Kabir, H. 1994. Fresh-cut vegetables. In *Modified Atmosphere Food Packaging*, ed. A. L. Brods, and V. A. Herndon, 155–160. Naperville, IL: Institute of Packaging Professionals.

Kader, A. A. 1985. Modified atmosphere and low pressure systems during transport and storage. In *Post Harvest Technology of Horticultural Crops*, ed. A. A. Kader, R. F. Kashmire, F. G. Mitchell, M. S. Reid, N. F. Sommer, and J. F. Thompson, 58–64. Oakland, CA: University of California, Division of Agriculture and Natural Resources.

Kader, A. A. 1986. Biochemical and physiological basis for effects of controlled and modified atmospheres on fruits and vegetables. *Food Technol.* 40(5):99–104.

Kader, A. A. 1987. Respiration and gas exchange of vegetables. In *Postharvest Physiology of Vegetables,* ed. J. Weichmann, 25–43. New York: Marcel Dekker.

Kader, A. A. 1997. A summary of CA requirements and recommendations for fruits other than apples and pears. In *Proceedings of the 7th International Controlled Atmosphere Research Conference: Vol. 3,* ed. A. A. Kader, 1–34. Davis, CA.

Kader, A. A., and Saltveit, M. E. 2003. Atmosphere modification. *Food Sci. Technol.* 123:229–246.

Kader, A. A., and Watkins, C. B. 2000. Modified atmosphere packaging—toward 2000 and beyond. *Hortechnol.* 10(3):483–486.

Kader, A. A., Zagory, D., and Kerbel, E. L. 1989. Modified atmosphere packaging of fruits and vegetables. *CRC Crit. Rev. Food Sci. Nutr.* 28:1–30.

Kakiomenou, K., Tassou, C., and Nychas, G.-J. 1998. Survival of *Salmonella enteritidis* and *Listeria monocytogenes* on salad vegetables. *World J. Microbiol. Biotechnol.* 14:383–387.

Kidder, J. W., Charlick, G. B., Romig, W. R., and Lobato, A. 2007. Apparatus, system, and method for storage of mushrooms. Patent Application WO 2007044835 A2 20070419.

Kim, C., Hung, Y. C., and Brackett, R. E. 2000. Roles of oxidation-reduction potential in electrolyzed oxidizing and chemically modified water for the inactivation of food-related pathogens. *J. Food Prot.* 63:19–24.

Kim, H., Ryu, J.-H., and Beuchat, L. R. 2006. Survival of *Enterobacter sakazakii* on fresh produce as affected by temperature, and effectiveness of sanitizers for its elimination. *Int. J. Food Microbiol.* 111:134–143.

Kim, J. G., Yousef, A. E., and Dave, S. 1999. Application of ozone for enhancing the microbiological safety and quality of foods: A review. *J. Food Prot.* 62:1071–1087.

Kvenberg, J., Stolfa, P., Stringfellow, D., and Garrett, E. S. 2000. HACCP development and regulatory assessment in the United States of America. *Food Control*. 11:387–401.

Larson, A. E., Johnson, E. A., Barmore, C. R., and Hughes, M. D. 1997. Evaluation of the botulism hazard from vegetables in modified atmosphere packaging. *J. Food Prot.* 60(10):1208–1214.

Larson, A. E., and Johnson, E. A. 1999. Evaluation of botulinal toxin production in packaged fresh-cut cantaloupe and honeydew melons. *J. Food Prot.* 62(8):948–952.

Laties, G. G. 1978. The development and control of respiratory pathways in slices of plant storage tissues. In *Biochemistry of Wounded Plant Tissues,* ed. G. Kahl, 421–466. Berlin: Walter de Gruyter.

Lee, L., Arul, J., Lencki, R., and Castaigne, F. 1996. Review on modified atmosphere packaging and preservation of fresh fruits and vegetables: Physiological basis and practical aspects—part II. *Packaging Technol. Sci.* 9(1):1–17.

Lei, Q., and Xu, W. 2002. Active modified atmosphere packaging for fresh tomato fruits. *Shanghai Shuichan Daxue Xuebao.* 11(4):357–361.

Leverentz, B., Conway, W. S., Camp, M. J., Janisiewicz, W. J., Abuladze, T., Yang, M., Saftner, R., and Sulakvelidze, A. 2003. Biocontrol of *Listeria monocytogenes* on fresh-cut produce by treatment with lytic bacteriophages and a bacteriocin. *Appl. Environ. Microbiol.* 69:4519–4526.

Loaiza-Velarde, J. G., Tomas-Barberan, F. A., and Saltveit, M. E. 1997. Effect of intensity and duration of heat-shock treatments on wound-induced phenolic metabolism in iceberg lettuce. *J. Am. Soc. Hort. Sci.* 122:873–877.

Lozano-de-Gonzales, P. G., Barrett, D. M., Wrolstad, R. E., and Durst, R. W. 1993. Enzymatic browning inhibited in fresh and dried apple rings by pineapple juice. *J. Food Sci.* 58:399–404.

Lund, B. M., and Peck, M. W. 2000. *Clostridium botulinum*. In *The Microbiological Safety and Quality of Food*, ed. B. M. Lund, T. C. Baird-Parker, and G. W. Gould, 1057–1109. Gaithersburg, MD: Aspen.

Mahajan, P. V., Oliveira, F. A. R., Montanez, J. C., and Frias, J. 2007. Development of user-friendly software for design of modified atmosphere packaging for fresh and fresh-cut produce. *Inn. Food Sci. Emerg. Technol.* 8:84–92.

Mahajan, P. V., Oliveira, F. A. R., Montanez, J. C., and Iqbal, T. 2008. Packaging design for fresh produce. *New Food* 1:35–36.

Mannapperuma, J. D., Zagory, D., Singh, R. P., and Kader, A. A. 1989. Design of polymeric packages for modified atmosphere storage of fresh produce. In *Proceedings of the 5th International Controlled Atmosphere Research Conference: Vol. I,* ed. J. K. Fellman, 225–233. Washington: Wenatchee.

Mari, M., Guizzardi, M., and Pratella, G. C. 1996. Biological control of gray mold in pears by antagonistic bacteria. *Biological Control* 7:30–37.

Marquenie, D., Michiels, C. W., Geeraerd, A. H., Schenk, A., Soontjens, C., Van Impe, J. F. 2002. Using survival analysis to investigate the effect of UV-C and heat treatment on storage rot of strawberry and sweet cherry. *Int. J. Food Microbiol.* 73:187–196.

Martin, S. T., and Beelman, R. B. 1996. Growth and enterotoxin production of *Staphylococcus aureus* in fresh packaged mushrooms (*Agaricus bisporus*). *J. Food Prot.* 59:819–826.

Mattila, M., Ahvenainen, R., and Hurme, E. 1995. Prevention of browning of prepeeled potato. In *Proceedings of Workshop on Systems and Operations for Post-Harvest Quality,* ed. J. De Baerdemaeker, 225–234. Brussels, Belgium: COST 94 Commission of the European Community.

Maurer, J., and Lee, M. 2005. *Salmonella*: Virulence, stress response, and resistance. In *Understanding Pathogen Behaviour,* ed. M. Griffiths, 215–239. Cambridge, U.K.: Woodhead Publishing.

McClure, P. 2005. *Escherichia coli*: Virulence, stress response, and resistance. In *Understanding Pathogen Behaviour,* ed. M. Griffiths, 240–278. Cambridge, U.K.: Woodhead Publishing.

Meng, J., and Doyle, M. P. 2002. Introduction. Microbiological food safety. *Microbes and Infection* 4:395–397.

Mizrach, A. 2008. Ultrasonic technology for quality evaluation of fresh fruit and vegetables in pre- and postharvest processes. *Postharv. Biol. Technol.* 48:315–330.

Molins, R. A., Motarjemi, Y., and Kaferstein, F. K. 2001. Irradiation: A critical control point in ensuring the microbiological safety of raw foods. *Food Control.* 12:347–356.

Murata, M., Noda, I., and Homma, S. 1995. Enzymatic browning of apples on the market: Relationship between browning, polyphenol content and polyphenol oxidase. *Nippon Shokuhin Kagaku Kogaku Kaishi.* 42:820–826.

Murata, M., Tanaka, E., Minoura, E., and Homma, S. 2004. Quality of cut lettuce treated by heat shock: Prevention of enzymatic browning, repression of phenylalanine ammonia-lyase activity, and improvement on sensory evaluation during storage. *Biosci. Biotechnol. Biochem.* 68(3):501–507.

Najafi, M. B. H., and Khodaparast, M. H. H. 2009. Efficacy of ozone to reduce microbial populations in date fruits. *Food Control* 20:27–30.

Nichols, R. 1985. Postharvest physiology and storage. In *The Biology and Technology of the Cultivated Mushroom*, ed. P. B. Flegg, D. M. Spencer, and D. A. Wood, 209. New York: Wiley-Interscience.

Ning, B., Kubo, Y., Inaba, A., and Nakamura, R. 1997. Physiological responses of Chinese pear "Yali" fruit to $CO_2$-enriched and/or $O_2$-reduced atmospheres. *Engie Gakkai Zasshi.* 66(3/4):613–620.

Novak, J. S., and Yuan, J. T. C. 2004. The fate of *Clostridium perfringens* spores exposed to ozone and/or mild heat pretreatment on beef surfaces followed by modified atmosphere packaging. *Food Microbiol.* 21:667–673.

Novak, J. S., Peck, M. J., Juneja, V. K., and Johnson, E. A. 2005. *Clostridium botulinum* and *Clostridium perfringens*. In *Foodborne Pathogens: Microbiology and Molecular Biology,* ed. P. M. Fratamico, A. K. Bhunia, and J. L. Smith, 383–407. Wymondham, U.K.: Caister.

Odumeru, J. A., Mitchell, S. J., Alves, D. M., Lynch, J. A., Yee, A. J., Wang, S. L., Styliadis, S., Farber, J. M. 1997. Assessment of the microbiological quality of ready-to-use vegetables for health-care food services. *J. Food Prot.* 60(8):954–960.

Oey, I., Lille, M., Loey, A. V., and Hendrickx, M. 2008. Effect of high-pressure processing on colour, texture and flavour of fruit- and vegetable-based food products: A review. *Trends Food Sci. Technol.* 19:320–328.

Palou, E., Lopez-Malo, A., Barbosa-Canovas, G. V., and Welti-Chanes, J. 2000. High hydrostatic pressure and minimal processing. In *Minimally Processed Fruits and Vegetables,* ed. S. M. Alzamora, M. S. Tapia, and A. Lopez-Malo, 205–222. Gaithersburg, MD: Aspen.

Parish, M. E., Beuchat, L. R., Suslow, T. V., Harris, L. J., Garret, E. H., Farber, J. N., and Busta, F. F. 2003. Methods to reduce/eliminate pathogens from fresh and fresh-cut produce. *Comp. Rev. Food Sci. Food Safety* 2:161–173.

Park, C. E., and Sanders, G. W. 1992. Occurrence of thermotolerant campylobacters in fresh vegetables sold at farmers' outdoor markets and supermarkets. *Can. J. Microbiol.* 38(4):313–316.

Park, J. N., Kyoung, J. H., and Jeong, C. S. 2006. Effect of active modified atmosphere packaging and storage temperature on the quality of spinach. *Horticult. Environ. Biotechnol.* 47(1):5–9.

Parry, R. T. 1993. Introduction. In *Principles and Applications of MAP of Foods,* ed. R. T. Parry, 1–18. New York: Blackie Academic and Professional.

Petran, R. L., Sperber, W. H., and Davis, A. R. 1995. *Clostridium botulinum* toxin formation in romaine lettuce and shredded cabbage: Effect of storage and packaging conditions. *J. Food Prot.* 58:624–627.

Phillips, C. A. 1996. Review: Modified atmosphere packaging and its effects on the microbiological quality and safety of produce. *Int. J. Food Sci. Technol.* 31:463–479.

Piyasena, P., Mohareb, E., and McKellar, R. C. 2003. Inactivation of microbes using ultrasound: A review. *Int. J. Food Microbiol.* 87:207–216.

Porat, R., Weiss, B., Cohen, L., Daus, A., and Aharoni, N. 2004. Reduction of postharvest rind disorders in citrus fruit by modified atmosphere packaging. *Postharvest Biol. Technol.* 33(1):35–43.

Priepke, P. E., Wei, L. S., and Nelso, A. I. 1976. Refrigerated storage of prepackaged salad vegetables. *J. Food Sci.* 41:379–382.

Rame, J., Chaloupecky, V., Sojkova, N., and Bencko, V. 1997. An attempt to demonstrate the increased resistance of selected bacterial strains during repeated exposure to UV radiation at 254 nm. *Central Eur. J. Pub. Health* 4:30–31.

Rico, D., Martin-Diana, A. B., Barat, J. M., and Barry-Ryan, C. 2007. Extending and measuring the quality of fresh-cut fruit and vegetables: A review. *Trends Food Sci. & Technol.* 18:373–386.

Rocculi, P., Romani, S., and Dalla Rosa, M. 2005. Effect of MAP with argon and nitrous oxide on quality maintenance of minimally processed kiwifruit. *Postharvest Biol. Technol.* 35(3):319–328.

Rodriguez, A., Batlle, R., and Nerin, C. 2007. The use of natural essential oils as antimicrobial solutions in paper packaging. Part II. *Prog. Org. Coatings* 60:33–38.

Roy, S., Taylor, T., and Kramer, H. 2001. Textural and ultrastructural changes in carrot tissue as affected by blanching and freezing. *J. Food Sci.* 66:176–808.

Rupasinghe, H. P. V., Bouter-Bitzer, J., Ahn, T., and Odumeru, J. A. 2006. Vanillin inhibits pathogenic and spoilage microorganisms *in vitro* and aerobic microbial growth in fresh-cut apples. *Food Res. Int.* 39:575–580.

Saftner, R. A., Baj, J., Abbott, J. A., and Lee, Y. S. 2003. Sanitary dips with calcium propionate, calcium chloride, or calcium amino acid chelates maintain quality and shelf stability of fresh-cut honeydew chunks. *Postharvest Biol. Technol.* 29:257–269.

Saltveit, M. E., Jr. 1997. A summary of CA and MA requirements and recommendations for harvested vegetables. In *Proceedings of the 7th International Controlled Atmosphere Research Conference: Vol. 4,* ed. M. E. Saltveit, 98–117. Davis, CA.

Sapers, G. M. 2003. Washing and sanitizing raw materials for minimally processed fruit and vegetable products. In *Microbial Safety of Minimally Processed Fruit and Vegetable Products,* ed. J. S. Novak, G. M. Sapers, and V. K. Juneja, 222. Boca Raton, FL: CRC Press.

Selma, M. V., Fernandez, P. S., Valero, M., and Salmeron, M. C. 2003. Control of *Enterobacter aerogenes* by high-intensity, pulsed electric fields in horchata, a Spanish low-acid vegetable beverage. *Food Microbiol.* 20:105–110.

Solomon, H. M., Kautter, D. A., Lilly, T., and Rhodehamel, E. J. 1990. Outgrowth of *Clostridium botulinum* in shredded cabbage at room temperature under modified atmosphere. *J. Food Prot.* 53(10):831–833.

Studman, C. J. 2001. Computers and electronics in postharvest technology—a review. *Computers and Electronics in Agriculture*. 30:109–124.

Sugiyama, H., and Yang, K. H. 1975. Growth potential of *Clostridium botulinum* in fresh mushrooms packaged in semipermeable plastic film. *Appl. Microbiol.* 30:964–969.

Tano, K., Oule, M. K., Doyon, G., Lencki, R. W., and Arul, J. 2007. Comparative evaluation of the effect of storage temperature fluctuation on modified atmosphere packages of selected fruit and vegetables. *Postharvest Biol. Technol.* 46:212–221.

Tran, T. T., Thunberg, R. I., Bennett, R. W., and Matthews, R. N. 2000. Fate of *Campylobacter jejuni* in normal air, vacuum, and modified atmosphere packaged fresh-cut vegetables. In *114th Annual Meeting, Association of Official Analytical Chemists; 2000 Sept. 10–14; Philadelphia, PA,* p. 86. Gaithersburg, MD: AOAC.

Tudela, J. A., Espin, J. C., and Gil, M. I. 2002. Vitamin C retention in fresh-cut potatoes. *Postharv. Biol. Technol.* 26(1):75–84.

Vu, T. S., Smout, C., Sila, D. N., LyNguyen, B., Van Loey, A. M. L., and Hendrickx, M. E. 2004. Effect of preheating on thermal degradation kinetics of carrot texture. *Innov. Food Sci. Emerg.Technol.* 5:37–44.

Wallace, R. B. 1997. *Camplyobacter.* In *Foodborne Microorganisms of Public Health Significance,* ed. A. D. Hocking, G. Arnold, I. Jenson, K. Newton, and P. Sutherland, 265–284. Tempe, Australia: Trenear Printing Service.

Wang, H., Feng, H., and Luo, Y. 2004. Microbial reduction and storage quality of fresh-cut cilantro washed with acidic electrolyzed water and aqueous ozone. *Food Res. Int.* 37:949–956.

Watada, A. E., and Qui, L. 1999. Quality of fresh-cut produce. *Postharvest Biol. Technol.* 15:201–205.

Wei, C. I., Huang, T. S., Kim, J. M., Lin, W. F., Tamplim, M. L., and Bartz, J. A. 1999. Growth and survival of *Salmonella montevideo* on tomatoes and disinfection with chlorinated water. *J. Food Prot.* 58:829–836.

Wesierska, E., and Trziszka, T. 2007. Evaluation of the use of pulsed electrical field as a factor with antimicrobial activity. *J. Food Eng.* 78:1320–1325.

White, G. C. 1992. Ozone. In *Handbook of Chlorination and Alternative Disinfectants (3rd ed.)*, 1046–1110. New York: Van Nostrand Reinhold.

Yang, H.-S., Feng, G.-P., An, H.-J., and Li, Y.-F. 2005. Microstructure changes of sodium carbonate-soluble pectin of peach by AFM during controlled atmosphere storage. *Food Chem.* 94(2):179–192.

Zagory, D. 1995. Principles and practice of modified atmosphere packaging of horticultural commodities. In *Principles of Modified-Atmosphere Packaging and Sous-Vide Product Packaging,* ed. J. M. Farber, and K. L. Dodds, 175–204. Lancaster, PA: Technomic Publishing.

Zagory, D. 1997. Advances in modified atmosphere packaging (MAP) of fresh produce. *Perishables Handling Newsletter* 90:2–4.

Zagory, D. 1999. Effects of post-processing handling and packaging on microbial populations. *Postharvest Biol. Technol.* 15:313–321.

Zawistowski, J., Biliaderis, C. G., and Eskin, N. A. M. 1995. In *Oxidative Enzymes in Foods*, ed. D. S. Robinson and N. A. M. Eskin, 1–217. New York: Elsevier.

Zhang, M., Xiao, G. Salokhe, V. M. 2006. Preservation of strawberries by modified atmosphere packages with other treatments. *Packaging Technol. Sci.* 19(4):183–191.

# chapter 6

# Cooling of cooked ready-to-eat meats and computer simulation

*Lihan Huang and Shiowshuh Sheen*

*Contents*

## 6.1 Introduction

*Clostridium perfringens* is a spore-forming foodborne human pathogen that frequently complicates the safety of ready-to-eat (RTE) meat and poultry products. This microorganism is ubiquitously distributed in the environment, including soil, water, air, and intestinal tracts of many warm-blooded animals and humans (Brynestad and Granum, 2002; Juneja et al., 2001). As a spore former, this organism can survive relatively harsh environmental conditions and enter raw foods in the form of spores. As a result, raw materials used in preparing RTE meat and poultry products may be natural carriers of *C. perfringens* spores.

*C. perfringens* is a typical Gram-positive, anaerobic bacterium that thrives particularly well under conditions where oxygen is absent or the level is low. Cooking of meat and poultry products may create a condition that allows this microorganism to grow and multiply. Under normal cooking conditions, a thermal treatment process is usually designed to induce certain physical and chemical changes in RTE meats. Vegetative cells of common foodborne pathogens, such as *Listeria monocytogenes, Salmonella* spp., and *Escherichia coli* O157:H7, along with other spoilage microorganisms, are generally eliminated in the cooking step. However, the temperature conditions used in the cooking step are often insufficient to kill spores of *C. perfringens*. Instead, the spores may be activated during cooking. Since cooking expels oxygen from the food and eliminates background flora, the spores may germinate, outgrow, and multiply during cooling.

Under anaerobic conditions, germinated *C. perfringens* can grow rapidly at temperatures between 30°C and 47°C, a range of temperature that spores are exposed to during cooling (Craven, 1980). According to the literature, the optimum growth temperature for this microorganism is between 43°C and 47°C (Hall and Angelotti, 1965).

Cooling of cooked products is a critical step to prevent the germination, outgrowth, and multiplication of *C. perfringens* in cooked meats. As cooked products are cooled from their final cooking temperature to their final storage temperature, they are exposed to temperatures between 50°C and 10°C, a range suitable for the growth of this organism. At the optimum temperatures, the generation time can be as short as 7.1 min in cooked ground beef (Willardsen et al., 1979). Due to the high growth rate of *C. perfringens* at optimum temperatures, rapid cooling is essential to prevent outbreaks caused by cooked meats. Because of the importance of cooling in preventing the growth of *C. perfringens*, the U.S. Department of Agriculture (USDA) Food Safety and Inspection Service (FSIS) has issued

guidelines that require the internal temperature of cooked meats during a cooling process be reduced from 54.4°C (130°F) to 26.7°C (80°F) in less than 1.5 h, and from 26.7°C to 4.4°C (40°F) in less than 5 h. If a meat processor cannot meet this "safe harbor," it is necessary to show that the custom cooling regimen (or in the case of a process deviation) would result in less than a 1-log increase in *C. perfringens* and no growth of *Clostridium botulinum*. In the event of process deviation, mathematical growth models can be used to assess the extent of *C. perfringens* growth and evaluate the safety of the implicated meat product. To estimate the potential growth of Clostridia in cooked or partially cooked meat products, it is necessary to understand the growth kinetics of microorganisms and develop mathematical models that accurately describe the microbial growth behaviors.

## 6.2 *Models and methods for microbial growth under isothermal conditions*

The growth of bacteria in foods usually follows a sigmoidal trend and is affected by both intrinsic and extrinsic conditions. Examples of extrinsic conditions include temperature and time, which defines the temperature history of a food. Intrinsic conditions are the physical and chemical properties of a food, which may include pH, salt level, and the existence and concentration of antimicrobial agents. Under normal conditions, bacterial growth typically exhibits three sequentially progressing phases. The first phase is the lag phase, during which no apparent change in the counts of bacteria can be observed. The second phase is the exponential phase, during which the number of bacteria increases exponentially. The last phase is the stationary phase, in which the number of bacteria reaches the maximum. The duration of the lag phase ($\lambda$) and the bacterial growth rate ($K$) in the exponential phase are affected primarily by temperature, but they also are influenced by many intrinsic conditions.

To estimate bacterial growth, a model must be able to describe these three distinct phases. A primary model is a mathematical model capable of describing the three-phase time-dependent growth curve under constant temperature conditions. Since $\lambda$ and $K$ are affected by temperature, a secondary model is developed to describe the effect of temperature on these two parameters. A tertiary model is a mathematical model that correlates kinetic parameters ($\lambda$ and $K$) to both intrinsic and extrinsic factors, such as temperature, pH, salt concentration, water activity, and other relevant ingredients. The combined application of the models of different levels makes it possible to estimate the growth of microorganisms under static or dynamic conditions.

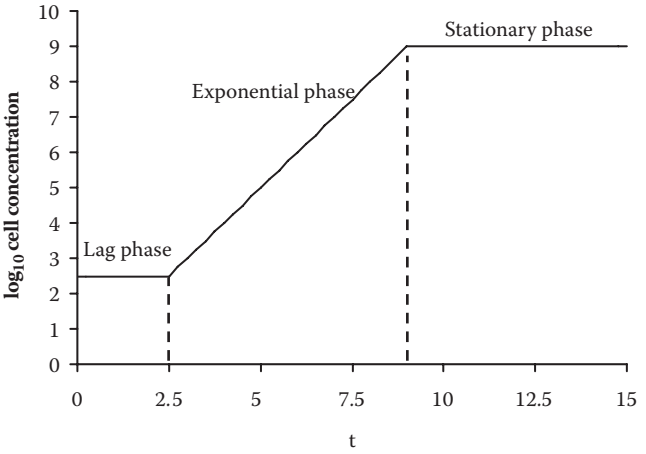


***Figure 6.1*** A hypothetical isothermal growth curve with three distinctive growth phases (initial cell concentration = 2.5 logs, lag phase = 2.5, specific growth rate = 1.0 log per unit time, final cell concentration = 9.0 logs).

## *6.2.1 Primary models*

The primary models are the basic block for predictive microbiology. Models at the primary level are designed to describe the growth of the microorganism as a function of time under isothermal conditions. Figure 6.1 illustrates a hypothetical growth curve with the three-phase growth phenomenon of microorganisms under isothermal conditions. A primary model must be a smooth curve that gradually progresses from the lag phase, through the exponential phase, and to the final stationary phase.

### *6.2.1.1 Empirical models*

Primary models of different complexity have been developed and used in estimating the microbial growth. The simplest models are empirical models such as those modified from the original Gompertz and logistic equations. Both modified Gompertz and logistic models use a transition function that allows the curves to gradually progress from the lag phase to the stationary phase. For both modified Gompertz and logistic models, the general mathematical equation is expressed as

$$L(t) = L_{\max} + (L_{\max} - L_0)S(t) \qquad \text{Eq. 6.1}$$

In Eq. 6.1, $L(t)$ is the logarithm (base 10) of the counts of microorganisms at any given time $t$; $L_{max}$ is the logarithm of the maximum counts of microorganisms; $L_0$ is the logarithm of the initial counts; and $S(t)$ is the sigmoidal function, which is written as

$$S(t) = \begin{cases} \exp\left\{-\exp\left[-\mu\left(t-M\right)\right]\right\} & \textit{Gompertz} \\ \dfrac{1}{1+\exp\left[-\mu\left(t-M\right)\right]} & \textit{Logistic} \end{cases} \qquad \text{Eq. 6.2}$$

In Eq. 6.2, $M$ is the inflection point of a growth curve, and μ is the relative rate constant at $t = M$, which is the point of the curve where the maximum slope is located. From Eqs. 6.1 and 6.2, two of the most important growth parameters can be derived. The first parameter is the duration of a lag phase (λ) under an isothermal condition, which can be calculated from

$$\lambda = \begin{cases} M - \dfrac{1}{\mu} & \textit{Gompertz} \\ M - \dfrac{2}{\mu} & \textit{Logistics} \end{cases} \qquad \text{Eq. 6.3}$$

The other parameter is the specific or exponential growth rate, $K$, which is located at the point where $t = M$, and can be calculated from

$$K = \begin{cases} \dfrac{L_{max} - L_0}{e}\mu & \textit{Gompertz} \\ \dfrac{L_{max} - L_0}{4}\mu & \textit{Logistic} \end{cases} \qquad \text{Eq. 6.4}$$

Although slightly different in mathematical characteristics, both modified Gompertz and logistic models can adequately describe microbial growth under isothermal conditions. Figure 6.2 illustrates the comparison between the modified Gompertz and logistic models used to fit the same hypothetical growth curve shown in Figure 6.1. Table 6.1 lists the growth parameters estimated using these two models. It is evident that the specific growth rates and the lag phases are slightly overestimated by the modified Gompertz and logistics models. The major difference between the modified Gompertz model and the modified logistic model

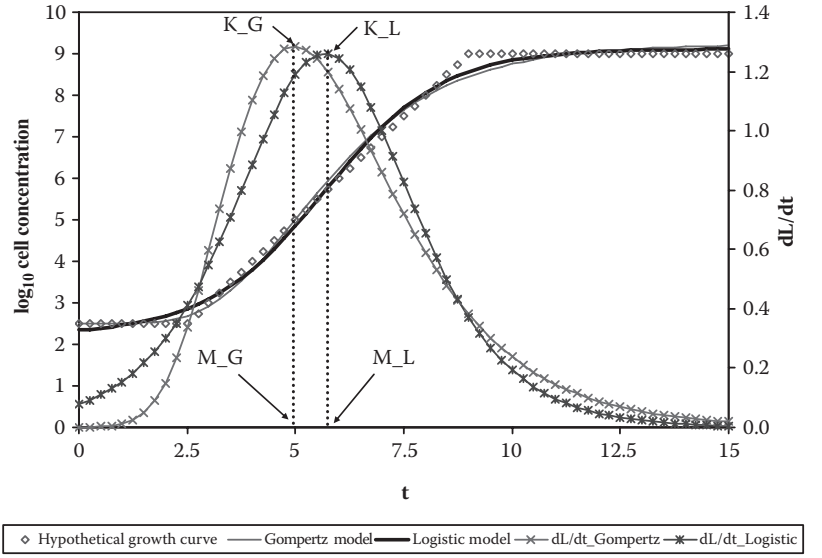


***Figure 6.2*** Comparison between Gompertz and logistic models used to fit the hypothetical growth curve shown in Figure 6.1.

***Table 6.1*** Growth Parameters Calculated from the Modified Gompertz and Logistic Models Used to Fit the Growth Curve Shown in Figure 6.1

| Parameters | Target Value | Gompertz | Logistic |
|---|---|---|---|
| $L_0$ | 2.5 | 2.51 | 2.35 |
| $L_{max}$ | 9.0 | 9.23 | 9.11 |
| K | 1.0 | 1.29 | 1.26 |
| λ | 2.5 | 3.06 | 2.94 |

is the shapes of the first derivatives of Eq. 6.2 (Figure 6.2). The peak of each curve of the first derivative is actually the specific growth rate for each model. For the modified logistic model, the first derivative curve is symmetric with respect to its inflection point M, which is not the case for the modified Gompertz model.

The empirical mathematical models shown in Eq. 6.1 are suitable for describing isothermal growth of microorganisms in foods. It can derive important and sufficient information about the growth parameters (*K* and λ) to estimate the extent of microbial growth if the foods are held under constant temperature conditions.

*6.2.1.2 Biologically based growth models*

*6.2.1.2.1 Baranyi model.* The major criticisms of the empirical models are that these models are just curves that closely resemble microbial growth curves. The empirical models lack biological meaning, although two parameters ($K$ and $\lambda$) derived from the modified Gompertz and logistic models, namely the lag and exponential phases, can be used to describe two of the most important phenomena of microbial growth.

The Baranyi model is probably one of the earliest models that attempted to describe the fundamental mechanism that drives microbial growth. Baranyi (1995) hypothesized that the growth of microorganisms was controlled by their physiological state, which was affected by the prior history, and the duration of the lag phase was controlled by the formation and accumulation of critical substances. Based on these assumptions, the differential equation for microbial growth can be expressed as

$$\frac{d}{dt}C = \alpha(t)\mu(C)C \qquad \text{Eq. 6.5}$$

In Eq. 6.5, $C$ is the actual concentration of microorganisms; $\alpha(t)$ is called the adjustment function, which is a function of time and varies between 0 and 1; and $\mu(C)$ is the potential specific growth rate. The product of $\alpha(t)$ and $\mu(C)$ represents the actual specific rate (Baranyi, McClure, et al., 1993; Baranyi and Roberts, 1994; Baranyi, Roberts, and McClure, 1993). In Baranyi and Roberts (1994) and Baranyi et al. (1995), the definitions of $\alpha(t)$ and $\mu(C)$ were further clarified, and a new term, $\mu(t)$, was used to represent the product of these two terms:

$$\mu(t) = \alpha(t)\mu(C) = \mu_{\max}\frac{q(t)}{1+q(t)}\left(1-\frac{C}{C_{\max}}\right), \qquad \text{Eq. 6.6}$$

where $q(t)$ is related to the formation and accumulation of critical substances, which are governed by Michaelis-Menten kinetics (Baranyi et al., 1995), and $C_{\max}$ is the maximum cell concentration. With this definition, the differential equation for microbial growth becomes

$$\begin{aligned}\frac{dq}{dt} &= \nu q \\ \frac{dC}{dt} &= \mu_{\max}\frac{q}{1+q}\left(1-\frac{C}{C_{\max}}\right)C\end{aligned} \qquad \text{Eq. 6.7}$$

Using $y$ to denote the natural logarithm of $C$, and assuming that $\nu$ is a constant, Eq. 6.7 can be solved analytically, and the most recent Baranyi model takes the form of

$$y(t) = y_0 + \mu_{max} A(t) - \ln\left[1 + \frac{e^{\mu_{max} A(t)} - 1}{e^{y_{max} - y_0}}\right], \quad \text{Eq. 6.8}$$

where

$$A(t) = t + \frac{1}{\nu}\ln\left(e^{-\nu t} + e^{-h_0} - e^{-\nu t - h_0}\right). \quad \text{Eq. 6.9}$$

In this equation, $\mu_{max}$ is termed as the maximum growth rate to differentiate it from specific growth rate $K$ used in other equations. The value of $\mu_{max}$ is equal to 2.303 × $K$, the specific growth rate expressed as the $\log_{10}$ of the microbial count per unit time. To simplify the equation, it is further assumed that $\nu = \mu_{max}$, and $A(t)$ is then actually defined by

$$A(t) = t + \frac{1}{\mu_{max}}\ln\left(e^{-\mu_{max} t} + e^{-h_0} - e^{-\mu_{max} t - h_0}\right) \quad \text{Eq. 6.10}$$

The final Baranyi growth model becomes

$$y(t) = y_0 + \mu_{max} t + \ln\left(e^{-\mu_{max} t} + e^{-h_0} - e^{-\mu_{max} t - h_0}\right) - \ln\left(1 + \frac{e^{\mu_{max} t - h_0} - e^{h_0}}{e^{y_{max} - y_0}}\right). \quad \text{Eq. 6.11}$$

In Eq. 6.11, $h_0$ is a transformation of q at the time of inoculation, which is calculated from

$$h_0 = -\ln\left(\frac{q_0}{q_0 + 1}\right) = -\ln\left(\alpha_0\right). \quad \text{Eq. 6.12}$$

According to Baranyi and Roberts (1994), the lag phase of a growth curve under an isothermal condition can be calculated from

$$\lambda = \frac{h_0}{\mu_{max}}. \quad \text{Eq. 6.13}$$

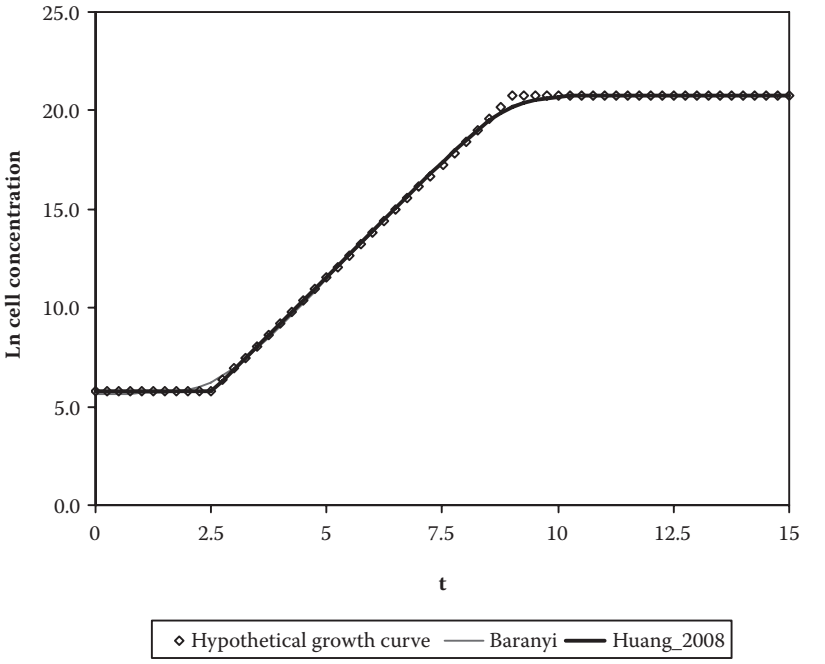


***Figure 6.3*** Comparison between Baranyi and Huang models used to fit the hypothetical growth curve shown in Figure 6.1.

Nonlinear regression is used to obtain the parameters included in the Baranyi model (Eq. 6.11). Since $h_0$ defines the initial physiological state of the microorganism, this value may vary from curve to curve. As a result, a mean of $h_0$ values of different curves (either at the same temperature or under different temperature conditions) is calculated after the first round of curve fitting. A second round of curve fitting is performed again to obtain $\mu_{max}$ and $\lambda$ using the mean $h_0$ value for each curve. Figure 6.3 shows the fitting of the hypothetical growth (Figure 6.1) using the Baranyi model.

*6.2.1.2.2 Huang Model*

Based on the three-phase growth phenomenon, Huang (2008) developed a new model that includes a transition function and logistic kinetics to directly define the lag, exponential, and stationary phases. The derivative form of the model takes the form of

$$\frac{dC}{dt} = kC\left(C_{max} - C\right) f\left(\lambda\right), \qquad \text{Eq. 6.14}$$

where

$$f(\lambda) = \frac{1}{1+\exp\left[-\alpha\left(t-\lambda\right)\right]}.$$

In Eq. 6.14, the term $f(\lambda)$ is a transition function that defines the duration of the lag phase. This function has a unique mathematical property, with its value ranging from 0 to 1. Within the lag phase, $f(\lambda)$ is zero. Outside the lag phase, $f(\lambda)$ is 1.0. This function helps define the fact that no growth should be observed within the lag phase. After the lag phase, the microbial growth would follow the simple logistic rule with $C_{max}$ as the carrying capacity. The constant $\alpha$, which is different from $\alpha(t)$ in the Baranyi model, defines the rate of transition from the lag phase to the exponential phase. This value was fixed at 25 in Huang (2008) to ensure a smooth and rapid transition from the lag phase to exponential phase. This differential equation can be solved analytically, and the resulting equation is

$$y(t) = y_0 + y_{max} - \ln\left\{\exp\left(y_0\right) + \left[\exp\left(y_{max}\right) - \exp\left(y_0\right)\right]\exp\left[-k\exp\left(y_{max}\right)B(t)\right]\right\}, \quad \text{Eq. 6.15}$$

where

$$B(t) = t + \frac{1}{\alpha}\ln\frac{1+\exp\left(-\alpha\left(t-\lambda\right)\right)}{1+\exp\left(\alpha\lambda\right)}.$$

The definition of $y(t)$ is the same as it is in the Baranyi model. The maximum growth rate is defined by

$$\mu_{max} = kC_{max}. \quad \text{Eq. 6.16}$$

With this definition, Eq. 6.15 becomes

$$y(t) = y_0 + y_{max} - \ln\left\{\exp\left(y_0\right) + \left[\exp\left(y_{max}\right) - \exp\left(y_0\right)\right]\exp\left[-\mu_{max}B(t)\right]\right\}. \quad \text{Eq. 6.17}$$

Figure 6.3 also shows the fitting of the Figure 6.1 hypothetical growth curve. The performance of the Huang model is almost identical to the

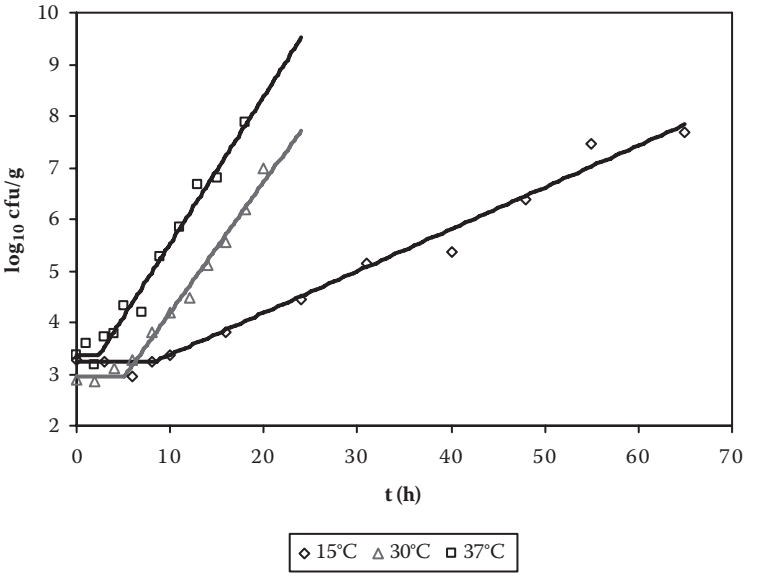


***Figure 6.4*** Using the reduced Huang model to describe growth curves without stationary phases (Huang 2008).

Baranyi model in the exponential and stationary phases since both models employ the competitive logistic rule for these two phases of microbial growth. However, the Huang model clearly has a more distinctive and mathematically identifiable lag phase than the Baranyi model.

The experimental data reported in Huang (2008) using the Huang model suggested that the duration of a lag phase is not affected by the initial concentration of bacteria. Under an isothermal condition, bacteria remain dormant in the lag phase, after which they enter the exponential phase of growth.

Due to the unique definition of the transitional function used in the Huang (2008) model, it is possible to use the same model to fit growth curves without the stationary phase (Figure 6.4). For this special case, the Huang model can be reduced to

$$y(t) = y_0 + k_{\max}\left\{t + \frac{1}{\alpha}\ln\frac{1+\exp\left[-\alpha\left(t-\lambda\right)\right]}{1+\exp\left(\alpha\lambda\right)}\right\}. \qquad \text{Eq. 6.18}$$

*6.2.1.2.3 Huang 2004 approach*

Since *C. perfringens* may exist as spores in RTE meats, the spores must germinate and outgrow before an increase in the number of the *C. perfringens*

can be observed. It is assumed that not all spores germinate at the same time and some spores may germinate faster than others. The germinated spores begin to outgrow and actively divide until the number of cells increases to the maximum capacity, which is also governed by the logistic rule. The number of cells does not begin to increase during the germination and outgrowth of *C. perfringens* spores, which corresponds to the lag phase of the growth process. Mathematically, the cells are either in the state of dormancy or in the state of active dividing. According to Juneja et al. (2001), Juneja and Marks (2003), and Huang (2004), the germination, outgrowth, and growth of *C. perfringens* spores can be described by a set of two differential equations:

$$\frac{dC_L}{dt} = -K_L C_L$$
$$\frac{dC_D}{dt} = -\frac{dC_L}{dt} + K_D C_D \left(1 - \frac{C_D}{C_{Max}}\right) \qquad \text{Eq. 6.19}$$

In Eq. 6.19, $C_L$ represents the concentration of *C. perfringens* cells in the state of dormancy, and $C_D$ is the concentration of cells that are actively dividing. This equation also suggests that the dormant cells leave the state of dormancy following the first-order kinetics. After the cells leave the state of dormancy, they begin to actively divide. Apparently, $C_L$ is equal to the initial concentration of the inoculums. For *C. perfringens* spores, $C_D$ is equal to zero at the time of inoculation. At any given time, the number of cells recoverable from the food includes the cells in the state of dormancy and the cells that are actively dividing. Therefore, the total concentration of cells ($C$) recoverable from the food can be calculated from

$$C = C_L + C_D \qquad \text{Eq. 6.20}$$

According to Eq. 6.19, the increase in the number of actively dividing cells is affected by both the rate at which the cells leave the state of dormancy and the rate at which the cells actively divide. To solve this equation for fitting an isothermal growth curve, it is necessary to determine both $K_L$ and $K_D$. To simplify the problem, it is assumed that $K_L$ is a fraction of $K_D$ (Huang, 2004), and the relationship between $K_L$ and $K_D$ becomes

$$K_L = \alpha K_D \qquad \text{Eq. 6.21}$$

With Eq. 6.21, the differential growth equation can be written as

$$\frac{dC_L}{dt} = -\alpha K_D C_L$$
$$\frac{dC_D}{dt} = -\frac{dC_L}{dt} + K_D C_D \left(1 - \frac{C_D}{C_{Max}}\right) \qquad \text{Eq. 6.22}$$

Within the limits of temperatures suitable for microbial growth, α ranges between 0 and 1; that is, $0 \le \alpha \le 1$. If $\alpha = 0$, all the cells would remain in the state of dormancy and never enter the state of active division. The cell concentration would remain constant and is equal to the concentration of cells initially inoculated into the food. If $\alpha = 1$, then all the initially inoculated cells would immediately enter the state of active division. In Huang (2004) and Juneja et al. (2006), α was fixed at 0.01 when this model was used to fit the growth curves of *C. perfringens* in cooked meats. It is necessary to mention that $K_D$ in Eq. 6.19 or 6.22 is equivalent to $\mu_{max}$ in the Baranyi model or Huang model, which is equal to $2.303 \times K$.

The duration of the lag phase of an isothermal growth process is not explicitly expressed in Eq. 6.22. However, the duration of the lag phase during an isothermal process can be calculated from (Huang, 2004; Juenja and Marks, 2002):

$$\lambda = \frac{\ln\left(1 + \frac{K_D}{K_L}\right)}{K_D} = \frac{\ln\left(1 + \alpha^{-1}\right)}{K_D} \qquad \text{Eq. 6.23}$$

Eq. 6.22 is an initial value problem that cannot be solved analytically, but can be easily solved using a numerical method (Huang, 2004); this will be discussed in more detail in Section 6.3 of this chapter. Figure 6.5 shows the results of numerical analysis to solve Eq. 6.22 used to fit the hypothetical growth curve shown in Figure 6.1. The specific growth rate determined from solving the different equations (Eq. 6.22) is 1.06 $\log_{10}$ per unit time, and the duration of the lag phase is 2.48. Both the specific growth rate and lag phase are almost identical to the values specified in Figure 6.1. Another advantage of the Huang 2004 approach is that it also can be used to fit growth curves without stationary phase (Juneja et al., 2006).

### *6.2.2 Secondary models and the effect of temperature*

The effect of temperature on microbial growth is primarily manifested in the changes in the growth rate and the lag phase duration as the growth temperature varies. Generally, microorganisms would grow within the lower limit ($T_{min}$) and the upper limit ($T_{max}$) of the temperature. At the

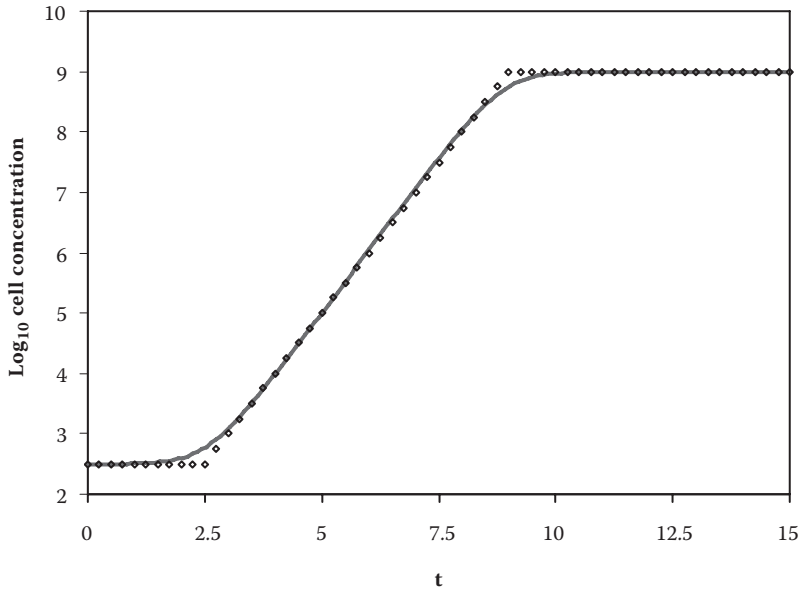


***Figure 6.5*** Using the two-stage differential growth model to fit the hypothetical growth curve shown in Figure 6.1 (Huang, 2004).

optimum temperature ($T_{opt}$), the microbial growth would exhibit the highest growth rate and the lowest lag phase duration. Microorganisms cannot grow at temperatures below $T_{min}$ or above $T_{max}$. At temperatures between $T_{min}$ and $T_{opt}$, the growth rate would generally increase and the duration of lag phase would decrease with temperature. As the temperature increases above $T_{opt}$, the microbial growth would generally slow down. Although many secondary models have been developed to describe the effect of temperature on microbial growth, the most widely used and the most practical secondary model is probably the Ratkowsky model, which is written as

$$K_{max}(T)^{\delta} = \beta_1 (T - T_{min})^{2\delta} \left\{1 - \exp\left[\beta_2 (T - T_{max})\right]\right\} \qquad \text{Eq. 6.24}$$

The δ value in Eq. 6.24 can be 0.5 or 1.0. If δ is 0.5, then Eq. 6.24 is the traditional Ratkowsky model, more commonly known as the square-root model (Ratkowsky et al., 1983). If δ is 1.0, then Eq. 6.24 becomes a modified Ratkowsky model (Zwietering et al., 1991), which is a variant of the original square-root model. The coefficients $\beta_1$ and $\beta_2$ define the rate at which $K_{max}$ responds to temperature.

The lag phase duration usually changes inversely with $K_{max}$. Therefore, the Ratkowsky model can also be used to describe the changes in λ as a function of temperature (Eq. 6.26; Juneja and Marks, 1999; Juneja et al., 1999; Zwietering et al., 1991).

$$\lambda^{-\delta} = \beta_1 \left(T - T_{min}\right)^{2\delta} \left\{1 - \exp\left[\beta_2 \left(T - T_{max}\right)\right]\right\} \qquad \text{Eq. 6.25}$$

Although Eq. 6.24 and Eq. 6.25 are empirical in nature, it is one of the best models for describing the effect of temperature on the growth of microorganisms in foods. The $T_{min}$ and $T_{max}$ estimated by the Ratkowsky model can be very close to the biological limits of temperature for microorganisms in foods (Ratkowsky et al., 1982; Ratkowsky et al., 1983).

### 6.2.2 *Tertiary model*

A tertiary model is a mode that relates both intrinsic and extrinsic conditions to the growth parameters. Common intrinsic factors include pH, fat content, water activity, and concentrations of certain ingredients that affect the growth of microorganisms. For *C. perfringens,* Juneja et al. (1996) developed a comprehensive model to describe the effect of temperature, pH, sodium chloride, and sodium pyrophosphate on the generation time and growth rate of *C. perfringens* in a model food system.

## 6.3 *Methods and models for growth under dynamic conditions*

Under a dynamic condition, temperature changes with time, and so does the rate constant. Therefore, it is necessary to combine a primary model with a secondary model to estimate the growth of microorganisms under dynamic temperature conditions. The differential forms of the Baranyi model (Eq. 6.7), Huang model (Eq. 6.14), and Huang 2004 model (Eq. 6.22) can be directly used to estimate the dynamic growth of microorganisms. The empirical primary models, however, cannot be directly used in a dynamic temperature condition. A transformation of the modified Gompertz and logistic equations is needed. To transform the empirical models, a first derivative of Eq. 6.1 must be taken, resulting in

$$\frac{dL}{dt} = \begin{cases} \mu\left(L - L_0\right) \ln\left(\dfrac{L_{max} - L_0}{L - L_0}\right) & \textit{Gompertz} \\ \mu\left(L - L_0\right) \dfrac{\left(L_{max} - L\right)}{L_{max} - L_0} & \textit{Logistic} \end{cases} \qquad \text{Eq. 6.26}$$

Since the rate constant changes with temperature, all differential forms of the growth models cannot be solved directly by analytical methods, but they can be easily solved using proper numerical methods. Although many numerical methods can be used to solve differential equations, the Runge-Kutta method remains a method of choice for solving differential growth equations to estimate the growth of microorganisms under dynamic temperature conditions. This method is a standard method for solving differential equations and is discussed in more detail in many textbooks; however, a brief introduction of the fourth-order Runge-Kutta method is given here (Chandra and Singh, 1995).

Denoting $f(x, t)$ as a general differential equation for the growth model:

$$\frac{dx}{dt} = f\left(x, t\right), \qquad \text{Eq. 6.27}$$

where $x$ is $L$ or $C$ in Eqs. 6.7, 6.14, 6.22, or 6.26, and $t$ is the growth time.

The Runge-Kutta method is based on the previous value and a small increment in the independent variable to solve an initial value problem. For growth models, the independent variable is time. To use the Runge-Kutta method, the time domain is first divided into small segments of equal length, with each segment equal to $h = t/n$, where $n$ is the number of segments. To estimate the value of $x$, the Runge-Kutta method first calculates four coefficients based on the previous value:

$$\begin{aligned} k_1 &= hf\left(t_{i-1}, x_{i-1}\right) \\ k_2 &= hf\left(t_{i-1} + \frac{h}{2}, x_{i-1} + \frac{k_1}{2}\right) \\ k_3 &= hf\left(t_{i-1} + \frac{h}{2}, x_{i-1} + \frac{k_2}{2}\right) \\ k_4 &= hf\left(t_{i-1} + h, x_{i-1} + k_3\right) \end{aligned} \qquad \text{Eq. 6.28}$$

where $i$ is the $i^{\text{th}}$ point of time ($i = 1$ to $n$). These four coefficients are then used to calculate the value of $x_i$ at the $t = t_i$:

$$x_i = x_{i-1} + \frac{1}{6}\left(k_1 + 2k_2 + 2k_3 + k_4\right) \qquad \text{Eq. 6.29}$$

The Runge-Kutta method can be implemented using any computing language, even in a spreadsheet such as Microsoft Excel. In each growth

model, there is a rate constant. For the modified Gompertz and logistic model, the relative growth rate μ should be used.

In a dynamic process, the temperature changes with time. Therefore, the rate constant is an implicit function of time. Let $T = g(t)$, then the rate constant can be expressed as a function of time, which is

$$K_{\max}\left(g\left(t\right)\right)=\beta_1\left(g\left(t\right)-T_{\min}\right)^2\left\{1-\exp\left[\beta_2\left(g\left(t\right)-T_{\max}\right)\right]\right\} \quad \text{Eq. 6.30}$$

With a known initial value of microbial concentration, the dynamic growth equation can be successfully solved (Huang, 2003, 2004; Juneja et al., 2006).

## 6.4 *Heat transfer and transient temperature history*

As a product cools after cooking, the temperature of the food changes continuously. This is an unsteady state heat transfer process. To estimate the potential growth of *C. perfringens* in cooked meats during cooling, it is necessary to have an accurate temperature history at the slowest cooling point, which is usually the geometric center of the RTE meats. Several methods can be used to obtain the temperature history at the geometric center of a solid food. The most accurate method is to directly measure the temperature history at the geometric center. The second method is to estimate the temperature history using the starting and ending temperatures at the geometric center. Another method is to estimate the temperature history by computer simulation based on the physical properties of the food and environmental conditions. Due to the limitations of this book, it is impossible to discuss all these methods in detail, but a brief introduction to each of these methods is given in this chapter.

### 6.4.1 *Direct measurement of temperature*

With the advancement of measurement and data acquisition technologies, it is now relatively easy to measure the temperature of meats during cooling. Many types of data-loggers are commercially available. Modern data-loggers are sufficiently rugged, miniaturized, and can be attached and moved with cooked meats. Table 6.2 lists some of the websites of the manufacturers of data-loggers.

The most widely used transducers for measuring temperature are probably thermocouples that can be directly inserted into cooked meats. For the temperature range experienced during cooling of cooked meats,

***Table 6.2*** Lists of Models and Manufacturers of Data-Loggers

| Model | Manufacturer/Distributor |
|---|---|
| OM-DAQPRO-5300 | Omega Engineering (www.omega.com) |
| iTCX-W | |
| OM-CP-BRIDGE110-10 | |
| OM-CP-EVENT101 | |
| OM-CP-HITEMP-150 | |
| OM-CP-HITEMP-150FP | |
| OM-CP-LEVEL101-SS | |
| OM-CP-OCTTEMP | |
| OM-CP-QUADTEMP | |
| OM-CP-TCTEMP1000 | |
| OM-CP-THERMOVAULT | |
| HOBO U12 Stainless Steel Temperature **(U12-015)** | MicroDAQ.com, Ltd (www.microdaq.com) |
| HOBO U12 Temp Logger w/5-in Stainless Probe (**U12-015-02**) | |
| Advantech Data Acquisition I/O Module | Cole-Parmer (www.coleparmer.com) |
| Model K-18808-06, K-18808-08 | |
| Cole-Parmer Temperature Datalogger | |
| Model K-38010-15 | |

Type-T thermocouple probes are probably the most suitable and accurate for measuring temperature changes. To accurately measure the temperature of cooked meats at the geometric center, it is necessary to choose a thermocouple probe that is small but strong enough to penetrate into the center of the meat. However, it is also important to ensure that the section of the probe inserted into the meat is 10 to 15 times longer than the diameter of the probe to prevent the conduction of the heat through the metal sheath to the tip of the probe.

### *6.4.2 Estimation by start and end points of temperature*

When a direct measurement of temperature is technically infeasible, one may try to use the starting and end-point temperatures to estimate the temperature history of cooked meats. To use this method, one assumes that the temperature of the product changes exponentially with time. However, this method is the least accurate method for thermally conductive foods such as cooked meats, and it only works under certain physical conditions. In general, this method is also known as the lumped-capacitance method

when the internal resistance to heat transfer is negligible (Inpcropera and DeWitt, 1996; Singh, 1992) and is based on the balance of heat energy during cooling. Assuming that the temperature of the product is uniformly distributed at the start of cooling, the transfer of heat to the food is governed by (Inpcropera and DeWitt, 1996; Juneja et al., 1994)

$$-h_s A\left(T - T_\infty\right) = \rho V C_p \frac{dT}{dt} \qquad \text{Eq. 6.31}$$

In Eq. 6.31, $h_s$ is the surface heat transfer coefficient (W/m²·°C), $A$ is the surface area (m²), $T_\infty$ is the ambient temperature (°C), ρ is density (kg/m³), $V$ is the volume (m³), and $C_p$ is the heat capacity (J/kg·°C). Denoting $T_0$ as the initial temperature, this equation can be integrated to produce

$$\frac{T - T_\infty}{T_0 - T_\infty} = \exp\left[-\frac{h_s A}{\rho V C_p} t\right] \qquad \text{Eq. 6.32}$$

Eq. 6.32 is more conveniently expressed as two important dimensionless numbers in engineering—the Biot number and the Fourier number:

$$Bi = \frac{h_s L_c}{k}$$
$$Fo = \frac{\alpha t}{L_c^{\,2}} \qquad \text{Eq. 6.33}$$

In Eq. 6.33, $L_c$ is the characteristic length of an object, which is half of the thickness of a plane, half of the radius of a long cylinder, or one-third of the radius of a sphere, and α is the thermal diffusivity of the product (W/m °C). The accuracy of the lump-capacitance model is highly dependent on the Biot number. In general, the Biot number must be < 0.1 for the lamp-capacitance model (Inpcropera and DeWitt, 1996).

The coefficient terms in Eq. 6.32 also can be used to calculate the thermal time constant, τ, which is equal to $(\rho V C_p)/(h_s A)$. Therefore, the lump-capacitance model also can be expressed as

$$\frac{T - T_\infty}{T_0 - T_\infty} = \exp\left(-\frac{t}{\tau}\right). \qquad \text{Eq. 6.34}$$

According to Eqs. 6.33 and 6.34, only under a special condition can the starting and end-point temperatures be used to estimate the temper-

ature history of cooked meats during cooling. The first condition is that the temperature is uniform at the initiation of the cooling process. The second condition is that the Biot number is < 0.1. The third condition is that the thermal time constant is equal to the total cooling time (*t_cool*). Under these strict physical conditions, the starting and end-point temperatures can be used to estimate the cooling history with reasonable accuracy:

$$T = T_\infty + \left(T_0 - T_\infty\right) \exp\left(-\frac{t}{t\_cool}\right) \qquad \text{Eq. 6.35}$$

### 6.4.3 *Computer simulation of the cooling process*

The entire history of cooking and cooling of meat products can be described by the physical laws governing the process of heat transfer. The transient heat transfer process during cooking and cooling of meats is basically a heat conduction problem with convective boundary conditions. Computer simulation can be used to solve this type of problem with relative ease. Including the cooking (heating) process in the simulation is actually beneficial to solving the heat transfer problem and obtaining a more accurate temperature history at the geometric center of cooked meats during cooling. The reason is that the transient heat transfer process during cooling is also an initial value problem. The accuracy of a computer simulation depends on the accurate definition of the initial conditions of a heat transfer process. At the end of cooking and the starting of cooling, it is very difficult to obtain the temperature distribution within the cooked products, and it is highly unlikely in the real world that the temperature would be evenly distributed. By simulating the heating process together with the cooling of cooked products, it is possible to obtain a more accurate temperature history during cooling. The transient heat transfer during cooking and cooling of meats can be described by a general partial differential equation in Cartesian coordinates (Incropera and DeWitt, 1996):

$$\frac{\partial T}{\partial t} = \frac{k}{\rho C_p}\left(\frac{\partial^2 T}{\partial x^2} + \frac{\partial^2 T}{\partial y^2} + \frac{\partial^2 T}{\partial z^2}\right) \qquad \text{Eq. 6.36}$$

In Eq. 6.36, $k$ is the thermal conductivity of the product (W/m °C); ρ and $C_p$ are density and heat capacity; and $x$, $y$, and $z$ are the coordinates of any location in the product. For most meat products, the changes in the physical properties can be considered negligible during cooking or cooling. The initial condition of this partial differential equation is $T(x, y, z) = T_0(x, y, z)$, which is the temperature profile before cooking or cooling starts. The boundary conditions for this equation are

$$-k\frac{\partial T}{\partial \vec{n}} = h\left(T - T_{\infty}\right) + w_e L_v \qquad \text{Eq. 6.37}$$

Eq. 6.37 describes the convection of heat to the surface of meats and the flux of thermal energy caused by evaporative loss. The terms $w_e$ and $L_v$ are rate of moisture evaporation (kg/m²s) and latent heat (J/kg). Eq. 6.36 can be used to describe heat transfer in a three-dimensional system of any geometrical shapes. Many real foods have a much simpler geometry. If heat is conducted along one direction, the heat transfer equation can be simplified to

$$\frac{dT}{dt} = \frac{k}{\rho C_p}\frac{d^2T}{dx^2} \qquad \text{Eq. 6.38}$$

For cylindrically shaped foods, the heat transfer equation becomes

$$\frac{dT}{dt} = \frac{k}{\rho C_p}\left(\frac{d^2T}{dr^2} + \frac{1}{r}\frac{dT}{dr}\right) \qquad \text{Eq. 6.39}$$

For a spherical food, the heat transfer equation becomes

$$\frac{dT}{dt} = \frac{k}{\rho C_p}\left(\frac{d^2T}{dr^2} + \frac{2}{r}\frac{dT}{dr}\right) \qquad \text{Eq. 6.40}$$

## 6.5 *Numerical analysis of heat transfer*

With the advancement of modern computing technology, the heat transfer equation can be routinely solved by numerical methods. The most frequently used numerical methods for solving the unsteady state heat transfer equations are finite difference methods and finite element methods (Chandra and Singh, 1995; Sheen and Hayakawa, 1991; Sheen et al., 1993). Huang (2007) developed an approach to simultaneously determine the surface heat transfer coefficient and thermal diffusivity using an implicit finite difference method. This method can be directly used to simulate the process of heating and cooling of meat products. Many commercial packages for solving heat transfer partial differential equations are also available. One example of such products is FlexPDE (http://www.pdesolutions.com), a finite element package capable of solving 3-D partial differential equations.

Solving transient heat transfer equations requires accurate data of physical properties. It is desirable to directly measure the physical

properties of meat products. However, for most meat products, the thermal diffusivity is around 1.2 ~ 1.4 × $10^{-7}$ m²/s. The density of meats is very close to water, and the thermal conductivity ranges between 0.4 and 0.5 W/m °C. The heat capacity is around 3.56 to 3.77 kJ/kg °C for beef meat and 3.62 kJ/kg °C for broilers (Rahman, 1996).

## 6.6 Practical applications of growth models and dynamic simulation

### 6.6.1 Primary and secondary models

All primary models, except the more recently developed Huang model, have been used to describe the growth of *C. perfringens* in cooked meats under temperature conditions applicable to cooling. Juneja et al. (1999) investigated the growth of *C. perfringens* in a broth system (trypticase-peptone-glucose-yeast extract) at different temperatures and developed a secondary model to describe the exponential growth rate ($K$) and lag phase duration ($\lambda$) as a function of temperature.

$$K^{\frac{1}{2}} = 0.044\left(T - 10.13\right)\left\{1 - \exp\left[0.419\left(T - 51.02\right)\right]\right\}^{\frac{1}{2}} \qquad \text{Eq. 6.41}$$

$$\lambda^{\frac{1}{2}} = 0.020\left(T - 10.13\right)\left\{1 - \exp\left[0.190\left(T - 51.02\right)\right]\right\}^{\frac{1}{2}} \qquad \text{Eq. 6.42}$$

Juneja et al. (2001) studied the growth of *C. perfringens* in irradiated ground beef (15% fat) and used Eq. 6.19 to solve growth curves without the stationary phase; they subsequently developed a secondary model for the exponential growth rate:

$$K^{\frac{1}{2}} = 0.035\left(T - 11.18\right)\left\{1 - \exp\left[0.231\left(T - 51\right)\right]\right\}^{\frac{1}{2}} \qquad \text{Eq. 6.43}$$

Using the same approach, Juneja and Marks (2002) continued to investigate the growth of *C. perfringens* in cured chicken, and developed a model to estimate the growth rate as a function of temperature:

$$K^{\frac{1}{2}} = 0.0358\left(T - 12.3\right)\left\{1 - \exp\left[0.201\left(T - 51\right)\right]\right\}^{\frac{1}{2}} \qquad \text{Eq. 6.44}$$

The growth differential equation (Eq. 6.19) also was solved using the Runge-Kutta method (Huang 2004 approach) to fit the complete growth curves (including all three phases) of *C. perfringens* in 93% lean cooked ground beef (Huang 2004), and the resulting secondary equation is

$$K_D = 0.003187\left(T - 9.11\right)^2 \left\{1 - \exp\left[0.5446\left(T - 51.21\right)\right]\right\} \qquad \text{Eq. 6.45}$$

The Huang 2004 approach also was used to fit the growth curves obtained from cooked cured pork (Juneja et al., 2006). The secondary model for *C. perfringens* in cooked cured pork is

$$K = 0.00243\left(T - 13.5\right)^2 \left\{1 - \exp\left[0.41\left(T - 50.6\right)\right]\right\} \qquad \text{Eq. 6.46}$$

The Baranyi model was used by Amezquita et al. (2005) to fit the growth curves of *C. perfringens* in cooked boneless ham. The original Ratkowsky square-root model was used to describe the growth of *C. perfringens* as a function of the absolute temperature, which is expressed as

$$\sqrt{k_{\max}} = 0.0599\left(T_K - 283.9\right) \qquad \text{Eq. 6.47}$$

Juneja et al. (2008) also used the Baranyi model to fit the growth curves in *C. perfringens* in cooked uncured beef, resulting in

$$K^{\frac{1}{2}} = 0.0504\left(T - 10.15\right)\left\{1 - \exp\left[0.238\left(T - 52.98\right)\right]\right\}^{\frac{1}{2}} \qquad \text{Eq. 6.48}$$

All the secondary models for exponential growth rate K suggested that the minimum growth temperature for *C. perfringens* in cooked meats is around 10°C to 14°C, which is the minimal temperature range for growth of *C. perfringens*. All secondary models, except Eq. 6.47, suggest that the maximum growth temperature for *C. perfringens* in cooked meats is between 51°C and 53°C. The optimum growth temperature is located between 45°C and 48°C, according to Figure 6.6, which is also agreeable with the data reported in the literature (Craven, 1980). In general, the growth rate of *C. perfringens* in cured meats is about one-third to one-half of the growth rate found in uncured meats (Figure 6.6).

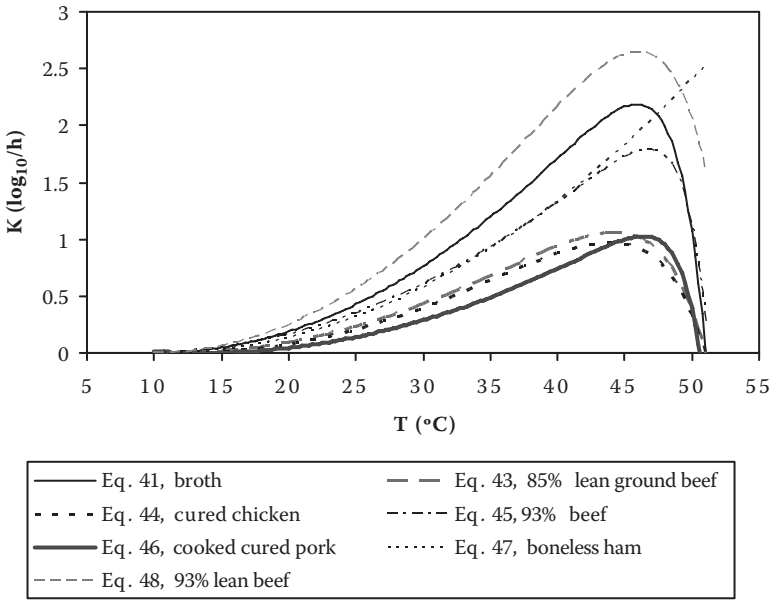


***Figure 6.6*** The exponential growth rate (K) as a function of temperature and described by the Ratkowski models.

## 6.6.2 *Dynamic simulation of* C. perfringens *growth*

### 6.6.2.1 *FlexPDE as a simulation package*

Although a few papers have been published on dynamic simulation of growth of *C. perfringens* in cooked meats (e.g., Amezquita, Wang, and Weller, 2005; Huang, 2004), computer codes used for computation were specially developed. The computer program developed by Huang (2004) was based on Visual Basic Professional Version 6 (Microsoft, Redmond, WA). The program can simulate the growth of *C. perfringens* in cooked meats under both dynamic and isothermal conditions, but it does not have the capability to simulate the heat transfer process during cooking or cooling. The computational codes developed by Amezquita et al. (2005) were an integrated model capable of simulating the temperature history of cooked meats and the growth of *C. perfringens*. A finite element method was used by heat transfer during heating and cooling of boneless ham (Amezquita, Wang, and Weller, 2005). The computer program was based on a computational platform Matlab Version 6.5 (The Mathworks, Inc., Natick, MA), which requires special programming knowledge and is not a user-friendly computing package.

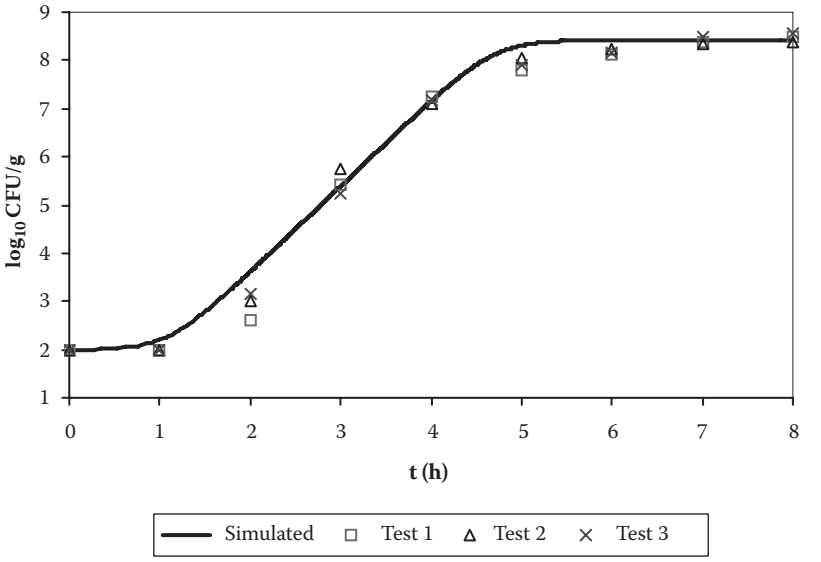


***Figure 6.7*** Simulation of *C. perfringens* growth in cooked beef at 47°C using Eq. 6.45. The raw data were taken from Huang (2004).

The FlexPDE software is a user-friendly and multi-purpose finite element analysis package capable of simulating complex physical processes. It can also solve ordinary differential equations (ODEs) for bacterial growth. For this reason, this finite element computing package is used in this chapter to demonstrate the dynamic simulation of *C. perfringens* growth in cooked meats.

Figure 6.7 illustrates the simulation of *C. perfringens* growth in cooked beef at 47°C using FlexPDE Version 4.2. The raw data in this figure were taken from Huang (2004), and the growth rate was calculated from Eq. 6.45. Figure 6.8 demonstrates the simulation of the growth of *C. perfringens* in cooked beef cooled exponentially from 51°C to 10°C in 18 h. The simulation results shown in Figures 6.7 and 6.8 duplicated the results previously published by Huang (2004), indicating the FlexPDE is suitable for numerical analysis of growth of *C. perfringens* in cooked meats under both isothermal and dynamic temperature conditions.

#### 6.6.2.2 *Computer simulation of* C. perfringens *growth during dynamic cooling*

As mentioned previously, the accuracy of estimating the growth of *C. perfringens* in cooked meats during dynamic cooling is affected by the secondary growth models and the temperature history at the geometric

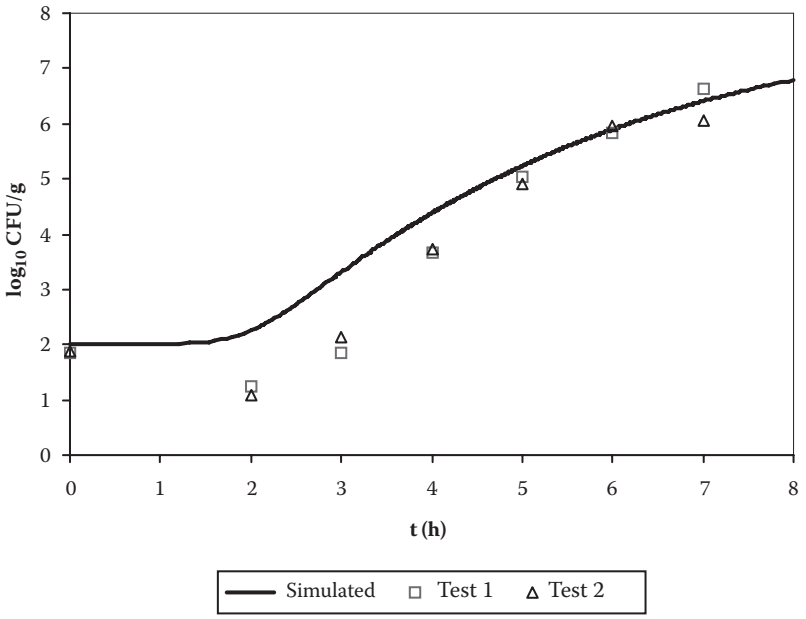


***Figure 6.8*** Simulation of growth of *C. perfringens* in cooked beef with temperature changing exponentially from 51°C to 10°C in 18 h. The raw data were taken from Huang (2004).

center of the product. If it is possible to physically measure the temperature history of the product, the data can be directly used to simulate the growth of *C. perfringens*. If the temperature history cannot be physically obtained, it is possible to use a computer simulation to simulate the temperature of cooked meats during cooling. If the temperature of a product is not uniformly distributed at the end of cooking, it is necessary to simulate the entire temperature history during both heating and cooling. This is the approach used in this chapter to demonstrate the application of computer simulation to estimate the growth of *C. perfringens* in cooked beef.

FlexPDE is used to simulate the growth of *C. perfringens* in cooked beef using physical examples taken from the literature. The objective of computer simulation was to demonstrate the effect of cooling temperature and heat transfer coefficient during cooling on *C. perfringens* growth in cooked meats. The physical example of cooking is taken from Obuz et al. (2002). Conditions for the cooling process are taken from Amezquita, Wang, and Weller (2005). Physical properties and conditions of the simulated product are listed in Table 6.3. The product is a cylindrically shaped beef roast (0.09 m in diameter and 0.19 m in height). The thermal properties

***Table 6.3*** Physical Conditions and Thermal Properties Used in Computer Simulation of *C. perfringens* Growth during Cooling

| Parameters | Value |
|---|---|
| Material and Shape[a] | Cylindrical beef roast |
| Diameter (m)[a] | 0.09 |
| Height (m)[a] | 0.19 |
| Thermal conductivity (W/m °C)[b] | 0.5 |
| Thermal diffusivity ($W/s^2$)[b] | $1.3 \times 10^{-7}$ |
| Surface heat transfer coefficient ($W/m^2$ °C) | |
| Heating[a] | 75 |
| Cooling[c] | 10 |
| Rate of evaporation ($kg/m^2s$) | $1.8 \times 10^{-3}$ |
| Heating temperature (°C)[a] | 163[a] |
| Cooling ambient temperature ($T_a$, °C) | 1, 5, 10, 15 |
| Heating time (h)[a] | 1.5 |
| Cooling time (h) | 10 |

[a] Obuz et al. (2002)
[b] Rahman (1996)
[c] Amezquita, Wang, and Weller (2005)

of the product are taken from Rahman (1996). It was assumed that heat loss through moisture evaporation was negligible during cooling. During cooking, however, it was assumed that moisture evaporated at a rate of $1.8 \times 10^{-3}$ $kg/m^2s$. Moisture evaporation was needed to compensate the heating through latent heat, which is calculated from Amezquita, Wang, and Weller (2005).

$$L_v = -2.5\times10^3 T + 2.5\times10^6 \qquad \text{Eq. 6.49}$$

Both the Baranyi model (Eq. 6.7) and Huang 2004 approach (Eq. 6.19) were used to estimate the growth of *C. perfringens* during cooling. The secondary model was based on Eq. 6.47 (Amezquita et al., 2005) for the Baranyi model and Eq. 6.45 for the Huang 2004 approach. To use Eq. 6.47 during cooling, the upper temperature was set at 51.21°C, the same as the highest temperature used in Eq. 6.45. These two secondary models were chosen because they are very similar. The curves are almost identical at temperatures below 42°C.

To validate the suitability of using FlexPDE to simulate the heat transfer process, it was used to duplicate the heating process described in Figure 6.2 of Obuz et al. (2002). Figure 6.9 shows the temperature histories on the surface and at the geometric center of a cylindrically shaped beef roast. The temperature histories simulated by FlexPDE are similar

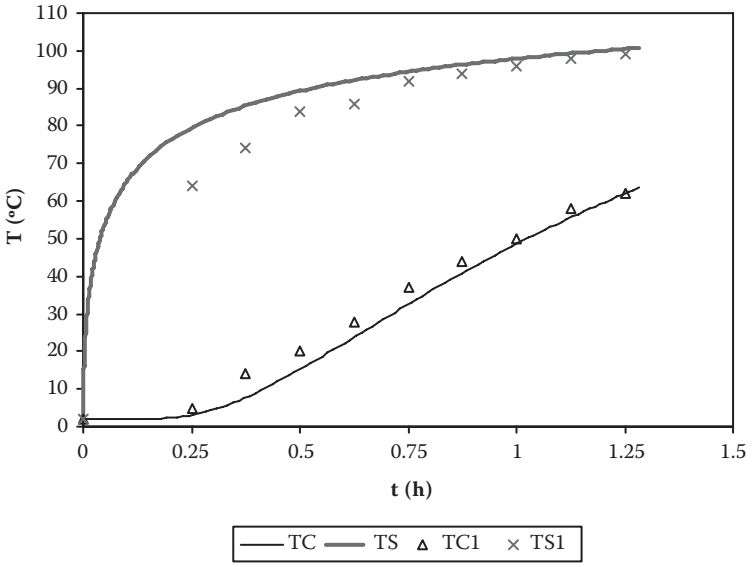


***Figure 6.9*** Computer simulation of the temperature profiles during cooking a cylindrically shaped beef roast (Diameter = 0.09 m, Height = 0.19 m). TC and TS are temperatures at the geometric center and on the surface of beef roast. TC1 and TS1 are temperature histories at the geometric center and on the surface of beef roast, taken from Figure 6.2 of Obuz et al. (2002). The initial temperature is 2°C. Other physical conditions and properties are listed in Table 6.3.

to the results reported in Figure 6.2 of Obuz et al. (2002), indicating that FlexPDE can be used as a tool to simulate the heat transfer process during the cooking of processed meats, considering the fact that the thermal property data were not directly measured but taken from a reference book (Rahman, 1996).

#### 6.6.2.3 *Scenario 1—Effect of cooling temperature on growth of* C. perfringens

In this example, FlexPDE was used to simulate the temperature histories of a cylindrically shaped beef roast during cooking and cooling. The objective was to evaluate the effect of cooling temperature on the growth of *C. perfringens*. FlexPDE was used to simulate both heating and cooling of the beef roast. The physical conditions for heating and cooling are listed in Table 6.3. In sum, all the beef roasts simulated using FlexPDE underwent the same heating conditions during cooking, and the same heat transfer coefficient (10 W/m²s) was applied during the cooling. The only difference

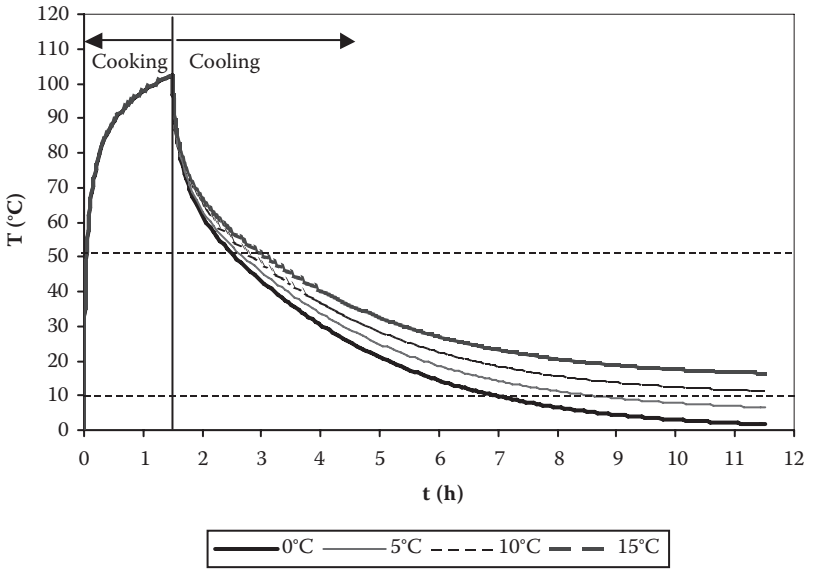


***Figure 6.10*** Simulated surface temperature histories during cooking and cooling of beef roasts. The legends represent cooling air temperatures. The dotted horizontal lines represent the upper and lower temperature limits. The heat transfer coefficient during cooling was 10 W/m²s.

was the cooling temperature. The initial cell concentration was 2 $\log_{10}$ CFU/g. Figure 6.10 shows the simulated surface temperature histories of beef roast during cooking and cooling. Figure 6.11 shows the temperature histories at the geometric center of the beef roasts. The temperature histories shown in these two figures illustrate that the heating process was indeed the same among all these simulated processes. However, the temperature histories were significantly affected by the cooling air temperature. In general, the surface temperature of the beef roasts began to decrease immediately after cooling started and gradually equilibrated to the cooling air temperatures (Figure 6.10). The center temperatures, however, did not begin to decrease immediately after cooling started. Instead, the center temperatures continued to increase because of the residual heat in the beef roasts at the initial stage of cooling. After the center temperature peaked, it also began to decrease gradually (Figure 6.12). With cooling temperatures of 0°C and 5°C, the center temperatures passed through the "growth zone (between $T_{max}$ and $T_{min}$)" in 4.3 h and 5.6 h, respectively. With cooling temperatures of 10°C and 15°C, the center temperatures of beef roasts were above the minimum growth temperatures during the

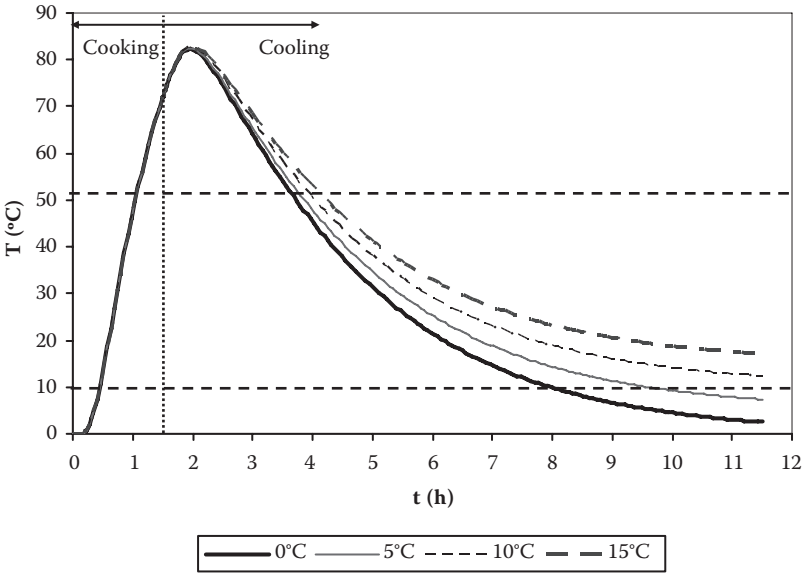


***Figure 6.11*** Simulated center temperature histories during cooking and cooling of beef roasts. The legends represent center temperatures. The dotted horizontal lines represent the upper and lower temperature limits. The heat transfer coefficient during cooling was 10 W/m²s.

entire 10 h process of cooling. The time needed to pass through the temperature range between 51°C and 27°C was approximately 1.4, 2.0, 2.3, and 2.9 h with cooling air temperatures of 0, 5, 10, and 15°C, respectively.

Figure 6.13 shows the estimated growth of *C. perfringens* in beef roasts during cooling using the Huang 2004 approach. Using this method, the estimated overall growth during the entire 10 h cooling process was 0.03, 0.05, 0.19, and 0.63 logs, corresponding to the cooling temperature maintained at 0, 5, 10, and 15°C, respectively. With the Baranyi model (Figure 6.14), the estimated overall growth of *C. perfringens* in cooked beef roasts was 0.09, 0.19, 0.54, and 1.09 logs, corresponding to cooling at 0, 5, 10, and 15°C. The relative growth estimated by the Huang 2004 approach and the Baranyi model was basically identical at lower cooling temperatures (0°C and 5°C). At higher cooling temperatures (10°C and 15°C), the relative growth estimated by the Baranyi model was slightly higher than that estimated by the Huang 2004 approach. The overestimation by the Baranyi model in the latter cases can be attributed to the incompleteness of the secondary model (Eq. 6.47). Since the secondary model used for the Baranyi model (Eq. 6.47) does not have a term to define the upper growth

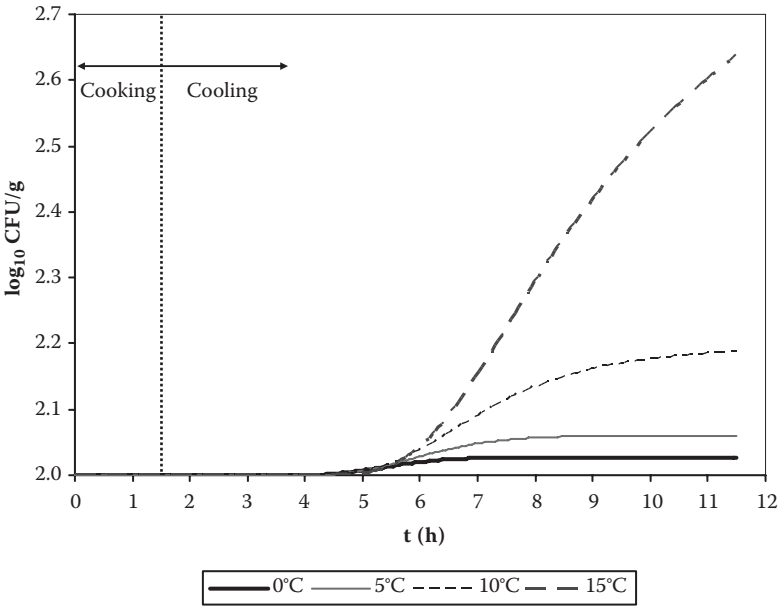


***Figure 6.12*** Estimation of growth of *C. perfringens* in cooked beef during cooling using the Huang 2004 approach. Shown in the figure is the estimated growth during a 10 h period with cooling air maintained at 0, 5, 10, and 15°C, respectively. The heat transfer coefficient during cooling was 10 W/m²s.

temperature limit, the growth rates estimated by this model were higher than those calculated by Eq. 6.45. Although an upper temperature was imposed during numerical analysis for the Baranyi model, the growth at temperatures above 42°C was overestimated, which led to slightly higher estimations than the Huang 2004 approach.

#### *6.6.2.4 Scenario 2—Effect of heat transfer coefficient on growth during cooling*

This example demonstrates the effect of reduced surface heat transfer coefficients during cooling on the growth of *C. perfringens* in cooked beef. The surface heat transfer coefficient measures the rate at which thermal energy is transferred from a solid surface to the ambient medium during cooling. A lower surface heat transfer coefficient suggests that the thermal energy is removed from cooked beef roasts in a less efficient manner. In the examples shown in the previous section, the surface heat transfer coefficient was set at 10 W/m²s. In this section, the surface heat transfer coefficient was set at 5 W/m²s during cooling.

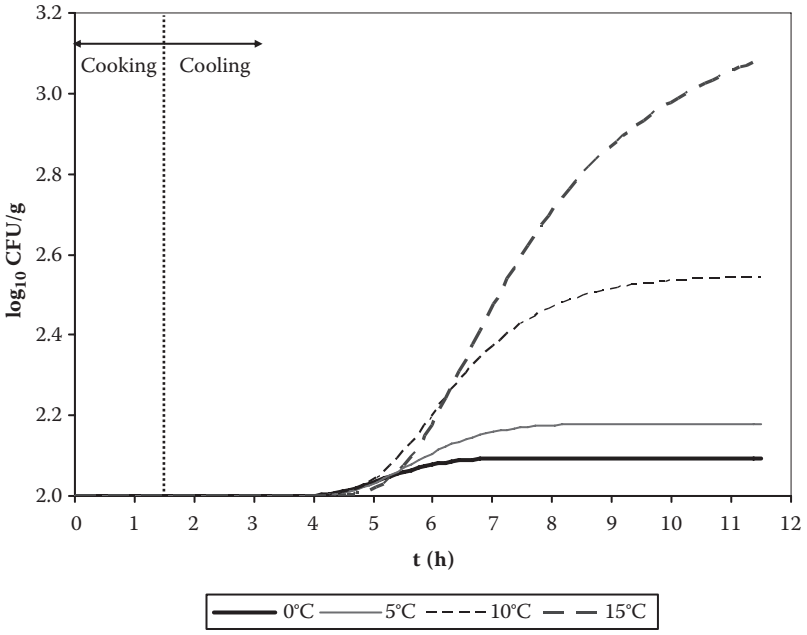


***Figure 6.13*** Estimation of growth of *C. perfringens* in cooked beef during cooling using the Baranyi model. Shown in the figure is the estimated growth during a 10 h period with cooling air maintained at 0, 5, 10, and 15°C, respectively. The heat transfer coefficient during cooling was 10 W/m²s.

With a reduced surface heat transfer coefficient, heat was removed at a much slower rate than the examples shown in the previous section. Figure 6.14 illustrates the effect of cooling temperature on the surface temperature of cooked beef roasts. It is apparent that the surface temperature is above the minimum growth temperature, except for the process with 0°C ambient temperature. At the geometric center, the time needed to pass through the temperature range of 51°C and 27°C was 3, 3.4, 4, and 5 h for cooling temperatures of 0, 5, 10, and 15°C, respectively, at the reduced heat transfer coefficient (Figure 6.15). The relative growth estimated by the Huang 2004 approach was 0.54, 0.97, 1.54, and 2.12 logs, as compared to 1.18, 1.71, 2.50, and 3.15 logs estimated by the Baranyi model. The relative growth estimated by the Baranyi model was slightly higher than the results estimated using the Huang 2004 approach. The difference in the estimation of relative growth is not caused by the primary model, but the difference in the secondary models.

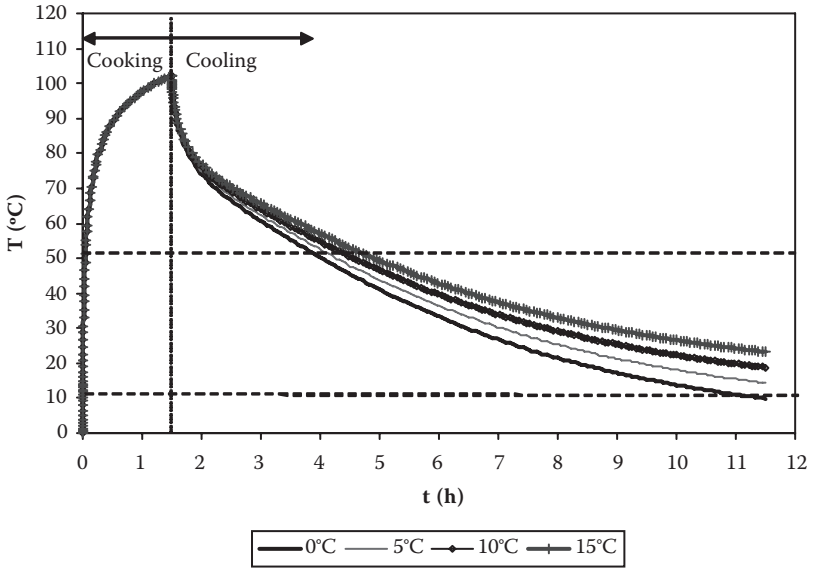


***Figure 6.14*** Simulated surface temperature histories during cooking and cooling of beef roasts. The legends represent cooling air temperatures. The dotted horizontal lines represent the upper and lower temperature limits. The heat transfer coefficient during cooling was 5 W/m²s.

The computer simulation shown in these two sections clearly demonstrates the effects of cooling temperature and the surface heat transfer coefficient on the growth of *C. perfringens* during cooling. It also demonstrates that computer simulations can be used to estimate and evaluate the growth of *C. perfringens* in cooked meats during cooling in the event of process deviation.

## *6.7 Conclusions*

In general, both empirical and mechanistic models can be used to describe the growth of *C. perfringens* in cooked meats under isothermal and dynamic conditions and achieve a reasonable degree of accuracy. Computer simulation can become a viable tool for evaluating the safety of cooked meat products exposed to temperature abuse at the stage of industrial production and commercial distribution. It suggested, however, that any model and computer simulation methodology be experimentally validated before being used in the real-world applications.

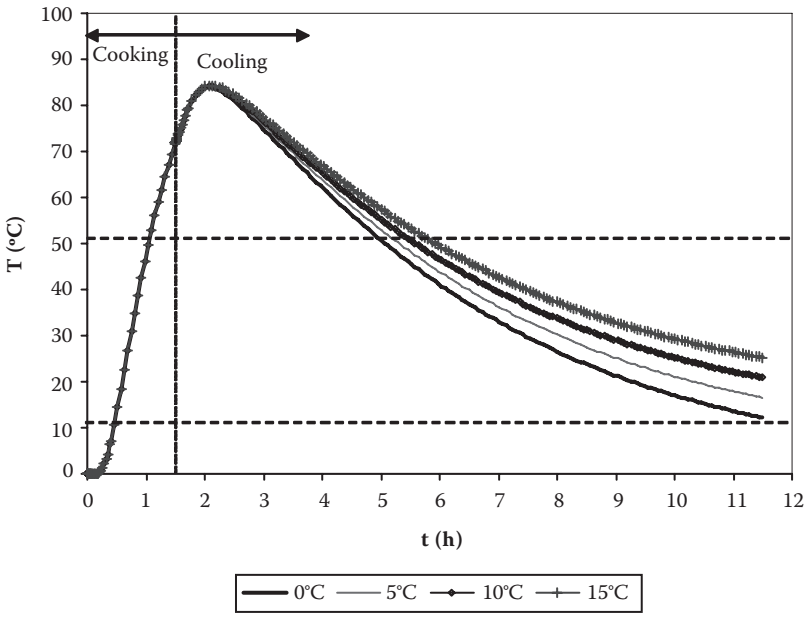


***Figure 6.15*** Simulated center temperature histories during cooking and cooling of beef roasts. The legends represent center temperatures. The dotted horizontal lines represent the upper and lower temperature limits. The heat transfer coefficient during cooling was 5 W/m²s.

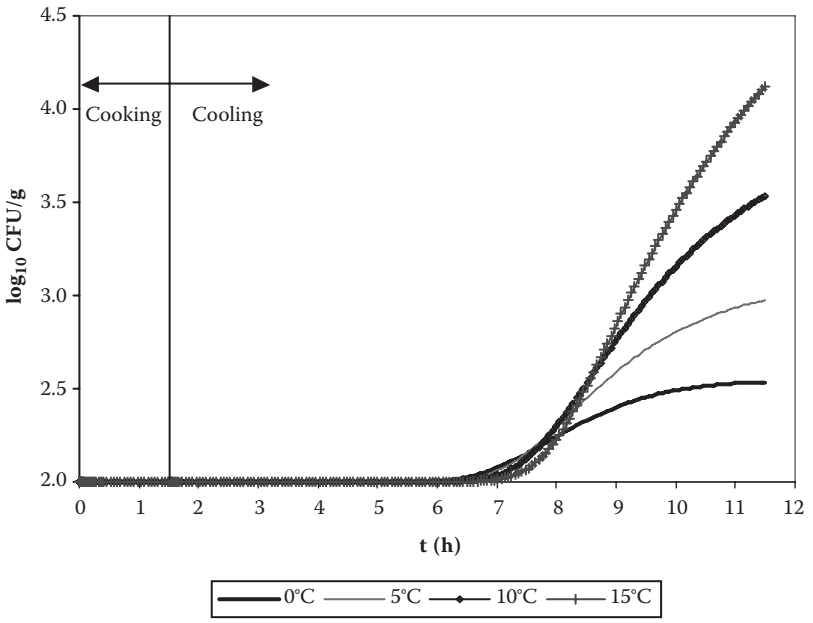


***Figure 6.16*** Estimation of growth of *C. perfringens* in cooked beef during cooling using the Huang 2004 approach. Shown in the figure is the estimated growth during a 10 h period with cooling air maintained at 0, 5, 10, and 15°C, respectively. The heat transfer coefficient during cooling was 5 W/m²s.

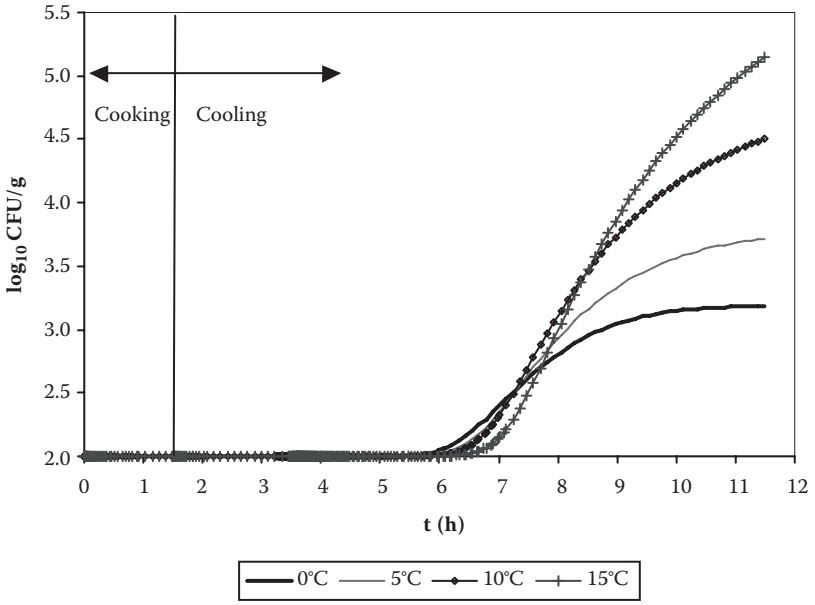


***Figure 6.17*** Estimation of growth of *C. perfringens* in cooked beef during cooling using the Baranyi model. Shown in the figure are the estimated growth during a 10 h period with cooling air maintained at 0, 5, 10, and 15°C, respectively. The heat transfer coefficient during cooling was 5 W/m²s.

## *References*

Amezquita, A., Wang, L., and Weller, C. L. 2005. Finite element modeling and experimental validation of cooling rates of large ready-to-eat meat products in small meat-processing facilities. *Trans. ASAE.* 48(1):287–303.

Amezquita, A., Weller, C. L., Wang, L., Thippareddi, H., and Burton, D. E. 2005. Development of an integrated model for heat transfer and dynamic growth of *Clostridium perfringens* during the cooling of cooked boneless ham. *Int. J. Food Microbiol*. 101:123–44.

Baranyi, J., McClure, P. J., Sutherland, J. P., and Roberts, T. A. 1993. Modeling bacterial growth responses. *J. Ind. Microbiol.* 12:190–4.

Baranyi, J., and Roberts, T. A. 1994. A dynamic approach to predicting bacterial growth in food. *Int. J. Food Microbiol.* 23:277–94.

Baranyi, J., Roberts, T. A., and McClure, P. 1993. A non-autonomous differential equation to model bacterial growth. *Food Microbiol.* 10:43–59.

Baranyi, J., Robinson, T. P., Kaloti, A., and Mackey, B. M. 1995. Predicting growth of *Brochothrix thermosphacta* at changing temperature. *Int. J. Food Microbiol.* 27:61–75.

Brynestad, S., and Granum P. E. 2002. *Clostridium perfringens* and foodborne infections. *Int. J. Food Microbiol*. 74:195–202.

Chandra, P. K., and Singh, R. P. 1995. *Applied Numerical Methods for Food and Agricultural Engineers*. CRC Press, Boca Raton, FL.

Craven, D. E. 1980. Growth and sporulation of *Clostridium perfringens* in foods. *Food Tech.* 34(4):80–7, 95.

Hall, R. A., and Angelotti, R. 1965. *Clostridium perfringens* in meat and meat products. *Appl. Microbiol*. 13:352–6.

Huang, L. 2003. Dynamic computer simulation of *Clostridium perfringens* growth in cooked ground beef. *Int. J. Food Microbiol*. 87: 217–227.

Huang, L. 2004. Numerical analysis of the growth of *Clostridium perfringens* in cooked beef under isothermal and dynamic conditions. *J. Food Safety.* 24:53–70.

Huang, L. 2007. Numerical analysis of survival of *Listeria monocytogenes* during in-package pasteurization of frankfurters by hot water immersion. *J. Food Sci*. 72: E285–292.

Huang, L. 2008. Growth kinetics of *Listeria monocytogenes* in broth and beef frankfurters—determination of lag phase duration and exponential growth rate under isothermal conditions. *J. Food Sci.* 73:E235–42.

Incropera, F. P., and DeWitt, D. P. 1996. *Fundamentals of Heat and Mass Transfer.* 4th Edition. John Wiley & Sons, New York, NY.

Juneja, V. K., Huang, L., and Thippareddi, H. H. 2006. Predictive model for growth of *Clostridium perfringens* in cooked cured pork. *Int. J. Food Microbiol*. 110:85–92.

Juenja, V. K., and Marks, H. M. 1999. Proteolytic *Clostridium botulinum* growth at 12–48°C simulating the cooling of cooked meat: development of a predictive model. *Food Microbiol.* 16:583–92.

Juneja, V. K., and Marks, H. M. 2002. Predictive model for growth of *Clostridium perfringens* during cooling of cooked cured chicken. *Food Microbiol*. 19:313–27.

Juenja, V. K., Marmer, B. S., Phillips, J. G., and Palumbo, S. A. 1996. Interactive effects of temperature, initial pH, sodium chloride, and sodium pyrophosphate on the growth kinetics of *Clostridium perfringens. J. Food Prot.* 59:963–8.

Juneja, V. K., Novak, J. S., Marks, H. M., and Combas, D. E. 2001. Growth of *Clostridium perfringens* from spores inocula in cooked cured beef: development of a predictive model. *Inov. Food Sci. & Emerging Tech.* 2:289–301.

Juneja, V. K., Snyder, O. P., and Cygnarowicz-Provost, M. 1994. Influence of cooling rate on outgrowth of *Clostridium perfringens* spores in cooked ground beef. *J. Food Prot.* 57:1063–7.

Juneja, V. K., Whiting, R. C., Marks, H. M., and Snyder, O. P. 1999. Predictive model for growth of *Clostridium perfringens* at temperatures applicable to cooling of cooked meat. *Food Microbiol*. 15:335–49.

Obuz, E., Powell, T. H., and Dikeman, M. E. 2002. Simulation of cooking cylindrical beef roasts. *Leb. Wiss. Tech.* 35:637–44.

Rahman, S. 1996. *Food Properties Handbook.* CRC Press, Boca Raton, FL.

Ratkowsky, D. A., Lowry, R. K., McKeekin, T. A., Stokes, A. N., and Chandler, R. E. 1983. Model for bacterial culture growth rate through the entire biokinetic temperature range. *J. Bacteriol.* 154:1222–6.

Ratkowsky, D. A., Olley, J., McKeekin, T. A., and Ball, A. 1982. Relationship between temperature and growth rate of bacterial cultures. *J. Bacteriol*. 149:1–5.

Sheen, S., and Hayakawa, K. 1991. Finite difference simulation for heat conduction with phase change in an irregular food domain with volumetric change. *Int. J. Heat Mass Trans.* 34:1337–46.

Sheen, S., Tong, C. H., Fu, Y. C., and Lund, D. B. 1993. Lethality of thermal processes for food in anomalous-shaped plastic containers. *J. Food Eng.* 20:199–213.

Singh, R. P. 1992. Heating and cooling processes of foods. In *Handbook of Food Engineering*, ed. D. B. Lund and D. B. Heldman, 247–276. Marcel Dekker, New York, NY.

Willardsen, R. R., Busta, F. F., and Allen, C. E. 1979. Growth of *Clostridium perfringens* in three different beef media and fluid thioglycollate medium at static and constantly rising temperatures. *J. Food Prot*. 42:144–148.

Zwietering, M. H., De Koos, J. T., Hasenack, B. E., De Wit, J. C., and van't Riet, K. 1991. Modeling of bacterial growth as a function of temperature. *Appl. Environ. Microbiol.* 57:1094–101.

*chapter 7*

# *Risk assessment and HACCP for ready-to-eat foods*

*Greg M. Burnham*

*Contents*

## 7.1 Introduction

In the last decade of the twentieth century the tragic deaths of four young children during a highly publicized foodborne illness outbreak of *Escherichia coli* O157:H7 in the Pacific Northwest helped to redirect our national food safety initiatives (Woteki 1998). This event, like the impact Upton Sinclair's *The Jungle* had on the passage of the Pure Food and Drugs and Meat Inspection Acts in the early twentieth century, brought together government, public health groups, consumers, and industry to make lasting change in our overarching food safety goal of reducing the incidence of foodborne illness. Declaring *E. coli* O157:H7 as an adulterant in ground beef (regulatory control), requiring "safe handling" labels on packages of raw meat and poultry products (consumer education), as well as mandated Hazard Analysis Critical Control Point (HACCP) systems for the meat and poultry industries (shared responsibility for safety) were all a direct result of this collective stakeholder response to address root causes attributable to the outbreak (Woteki 1998).

Today, in the first decade of the twenty-first century, a large foodborne illness outbreak involving jalapeño and serrano peppers (and maybe certain varieties of tomatoes) contaminated with *Salmonella* Saintpaul (CDC 2008) is just the latest in a long line of food safety risks that have strained our response resources. Likely contributing factors to the scale of this outbreak were that foods prepared with these items were generally ready-to-eat (RTE) and once prepared may have been held at inappropriate temperatures. When this chapter was written this outbreak and its causes were still under investigation. Other notable outbreaks associated with RTE foods in the first decade of the twenty-first century include *Salmonella* contaminated peanut butter in 2006 and 2007 (CDC 2007) as well as *E. coli* O157:H7 contaminated RTE spinach in 2006 (CDC 2006a). Unfortunately, as a result of these outbreaks and many others, we are not currently meeting our "Healthy People 2010" national health objectives. So it is necessary, as we move forward in our efforts to reduce the incidence of foodborne illness, to apply a systematic approach in allocating limited resources. This systematic approach is a risk analysis framework anchored firmly with a comprehensive risk assessment. Risk assessment not only provides a scientific base to the risk analysis framework but also

provides vital information which can be used in the development and execution of an effective HACCP system.

## *7.2 Hazard Analysis Critical Control Point (HACCP)*

HACCP arrived on the scene in the early 1960s during a collaboration of the Pillsbury Company, U.S. Army Natick Research Laboratories, and the U.S. Air Force Space Laboratory Project Group in cooperation with the National Aeronautic and Space Administration to develop rations for the U.S. space program (FDA 2001). HACCP, as a widely accepted food safety system, really gained momentum when the National Academy of Sciences published "An evaluation of the role of microbiological criteria for foods and food ingredients" in 1985 (NAS 1985). Later, in 1992, the National Advisory Committee on Microbiological Criteria for Foods published their HACCP principles (FDA 2001). These principles include:

Conducting a hazard analysis
Establishing critical control points
Setting critical limits
Monitoring to ensure limits are met
Establishing corrective actions for deviations
System verification
Record keeping

The first regulatory mandates for HACCP were by the Food and Drug Administration (FDA) in their low-acid canned food regulations and seafood HACCP regulations. However, HACCP had truly arrived in the food industry in 1996 when the USDA adopted the "Pathogen Reduction: HACCP Systems: Final Rule," requiring all meat and poultry processors to have HACCP as their main food safety system (USDA 1996).

After the initial hazard analysis of an item specific HACCP system, individual process steps where identified hazards can be either prevented, eliminated, or reduced to a safe level are classified as critical control points (CCPs). It is on these individual process steps classified as CCPs that the remainder of the HACCP system is focused. However, as pointed out by Whiting and Buchanan (2008), these individual CCPs are not linked to each other to offer a comprehensive measure of control or that they have any direct impact on public health by reducing the incidence of foodborne illness. Also for a HACCP system to be successful, effective prerequisite programs that address agricultural practices, cleaning and sanitization, personal hygiene, etcetera, must be in place. As such the HACCP system is a limited tool with which food safety managers incorporate specific

process controls aimed at a specific hazard. In order to relate the potential for a certain food under an HACCP system and the system's ability to result in a reduction of the incidence of foodborne illness associated with that food, a careful integration of the HACCP system into a comprehensive risk analysis framework is needed.

## 7.3 Food safety risk analysis

Many excellent texts provide details of numerous specific aspects of risk analysis and its application in the food industry. For the purposes of this chapter a more generalized overview in the sections on food safety risk analysis and specifically risk assessment are excerpted, with permission, from Food Safety Risk Analysis, a Guide for National Food Safety Authorities, published by the Food and Agriculture Organization and the World Health Organization of the United Nations (FAO/WHO 2006).

### 7.3.1 Introduction

Changing global patterns of food production, international trade, technology, public expectations for health protection, and many other factors have created an increasingly demanding environment in which food safety systems operate. An array of foodborne hazards, both familiar and new, pose risks to health. These risks must be assessed and managed to meet growing and increasingly complex sets of public health objectives. Risk analysis, a systematic, disciplined approach for making food safety decisions developed primarily in the last two decades, includes three major components: risk management, risk assessment (which is discussed in detail in the following section), and risk communication.

It has been the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) who have played leading roles in the development of food safety risk analysis. In 1991, the Joint FAO/WHO Conference on Food Standards, Chemicals in Food, and Food Trade recommended that the Codex Alimentarius Commission (CAC) incorporate risk assessment principles into its decision-making process. The 19th and 20th sessions of the CAC, in 1991 and 1993, endorsed the recommendation of the Conference to base its food safety decisions and standards on risk assessment and encouraged the relevant Codex Committees to harmonize their standard-setting methodologies. As part of the body of work being carried out by FAO/WHO and the CAC, considerable progress has been made in developing a systematic framework for applying principles and guidelines for food safety risk analysis.

Food safety risk analysis has now gained wide acceptance as the preferred way to assess possible links between hazards in the food

production/distribution chain and actual risks to human health, and takes into account a wide range of inputs to decision-making on appropriate control measures. When used to establish public health goals relative to food standards and other food control measures, risk analysis fosters comprehensive scientific evaluation, wide stakeholder participation, transparency of process, consistent treatment of different hazards, and systematic decision-making by risk managers.

To use food safety risk analysis in establishing public health goals to reduce disease incidence/burden, there must be an agreed upon acceptable level of protection (ALOP), a concept established by the Sanitary and Phytosanitary Agreement of the World Trade Organization (WTO 1995). Food production/distribution chain controls are then designed using risk-based microbiological targets defined by CAC to achieve the ALOP and include the following:

Food Safety Objective (FSO)—maximum frequency and/or concentration of a hazard at the time of consumption which provides or contributes to the ALOP.

Performance Objective (PO)—maximum frequency and/or concentration of a hazard at a specified step in the food chain which provides or contributes to an FSO or ALOP.

Performance Criteria (PC)—effect in frequency and/or concentration of a hazard by the application of one or more control measures to provide or contribute to a PO or an FSO.

These intermediate targets communicate in an explicit way to the affected food industry the limits required at specific points in the food production/distribution chain to achieve a specified public health goal or ALOP. While an FSO can be used as a metrics for translating control measures into public health outcomes, POs and PC are more likely the metrics to be used because they can be applied at points in the food production chain where controls can be implemented, monitored, and verified (INFOSAN 2007). This can be expressed using the following equation, introduced by the International Commission on Microbiological Specifications for Foods (ICMSF 2002):

$$H_0 - \Sigma R + \Sigma I \leq FSO$$

where

$H_0$ = initial level of the hazard
$\Sigma R$ = cumulative reduction of the hazard by application of POs and PC
$\Sigma I$ = cumulative increase of the hazard
FSO = food safety objective

### 7.3.2 Risk management

Fundamentally, food safety risk analysis is used to develop an estimate of the risks to human health and safety, to identify and implement appropriate measures to control the risks, and to communicate with stakeholders about the risks and measures applied. In a typical instance, a food safety problem or issue is identified, and risk managers initiate a risk management process, which they then see through to completion. This is best accomplished within a systematic, consistent, and readily understood framework in which scientific knowledge of risk and evaluations of other factors relevant to public health protection are used to select and implement appropriate control measures. There are four distinct risk management activities within this risk management process: (1) Preliminary risk management activities, (2) Identification and selection of risk management options, (3) Implementation of the risk management decision, and (4) Monitoring and review. The responsibilities of risk managers during this process also include commissioning a risk assessment when one is needed, and making sure that risk communication occurs wherever necessary.

### 7.3.3 Risk assessment

Risk assessment is a scientific evaluation, either qualitative or quantitative, of the risk associated with an identified hazard. It provides risk managers information in order to make food safety risk analysis decisions. It should be detailed enough to adequately answer questions, but not so meticulous that it delays information gathering or confuses risk managers (Buchanan 2004). This topic is covered at length in the following section.

### 7.3.4 Risk communication

Risk communication is an integral part of food safety risk analysis. Risk communication helps to provide timely, relevant, and accurate information to, and to obtain information from, members of the risk analysis team and external stakeholders, in order to improve knowledge about the nature and effects of a specific food safety risk. Successful risk communication is a prerequisite for effective risk management and risk assessment. It contributes to transparency of the risk analysis process and promotes broader understanding and acceptance of risk management decisions. Good risk communication requires thoughtful planning and some commitment of resources; risk managers may find that establishing an infrastructure for communication and building a climate in which communication is encouraged, expected, and natural are among the most important steps they can take to achieve a successful outcome for a risk management process.

## *7.4 Risk assessment*

### *7.4.1 Introduction*

Risk assessment is the central scientific component of food safety risk analysis and has evolved primarily because of the need to make decisions to protect health in the face of scientific uncertainty. Risk assessment can be generally described as characterizing the potential adverse effects to life and health resulting from exposure to hazards over a specified time period. Risk management and risk assessment are separate but closely linked activities, and ongoing, effective communication between those carrying out the separate functions is essential. Individual risk assessments should be "fit-for-purpose" and can generate estimates of risks in various forms. Although risk managers commission and guide the production of a risk assessment and evaluate its outputs, the risk assessment itself is generally an external product, independently produced by scientists.

This section takes the broad view that several approaches to risk assessment can be used to establish an association of sufficient strength between foodborne hazards, control measures, and risks to consumers, such that controls can be genuinely described as "risk-based," that is, based on a scientific assessment of risk. Risk assessment incorporating, in one way or another, the four analytical steps described by Codex is its main focus. These steps include:

1. *Hazard Identification*—the identification of biological, chemical, and physical agents capable of causing adverse health effects and which may be present in a particular food or group of foods.
2. *Hazard Characterization*—the qualitative and/or quantitative evaluation of the nature of the adverse health effects associated with biological, chemical, and physical agents that may be present in food. For chemical agents, a dose-response assessment is performed. For biological or physical agents, a dose-response assessment should be performed if the data are obtainable.
3. *Exposure Assessment*—the qualitative and/or quantitative evaluation of the likely intake of biological, chemical, and physical agents via food, as well as exposures from other sources if relevant.
4. *Risk Characterization*—the qualitative and/or quantitative estimation, including attendant uncertainties, of the probability of occurrence and severity of known or potential adverse health effects in a given population based on hazard identification, hazard characterization, and exposure assessment.

For microbiological hazards, the occurrence and transmission of the hazard at various stages from food production to consumption is evaluated,

thus moving "forward" through the various stages of the food chain to arrive at an estimate of risk. While the accuracy of estimated risks is often limited by uncertain dose-response information, the greatest strength of such risk assessments arguably lies in their ability to model the relative impacts of different food control measures on risk estimates.

### *7.4.2 Responsibilities of risk managers*

The decision to proceed with a risk assessment depends on factors such as the health risk priority ranking, urgency, regulatory needs, and availability of resources and data. It is likely that a risk assessment will be commissioned when: (1) The hazard exposure pathway is complex, (2) Data on the hazard(s) and/or health impacts are incomplete, (3) The issue is of significant regulatory and/or stakeholder concern, (4) There is a mandatory regulatory requirement for a risk assessment, or (5) There is a need to verify that an interim (or precautionary) regulatory response to an urgent food safety problem is scientifically justified.

Risk managers, in consultation with risk assessors, should fulfill several tasks when commissioning a risk assessment and seeing it through to completion. While risk managers do not need to know all the details of how a risk assessment is carried out, they do need a general understanding of risk assessment methodologies and what the outcomes mean. This understanding is both acquired through, and contributes to, successful risk communication.

### *7.4.3 Forming the risk assessment team*

A risk assessment team should be appropriate to the circumstances. When strategic and large-scale risk assessments are undertaken, the general criteria described below relating to risk assessment teams apply. However, small-scale and straightforward risk assessments may be undertaken by very small teams or even by individuals, especially where a primary risk assessment is already available and the scientific work involves mostly adaptation using local data. A large-scale risk assessment generally requires a multidisciplinary team that may include experts with biological, chemical, food technology, epidemiological, medical, statistical, and modeling skills, among others. Finding scientists with the required knowledge and expertise can be a challenging task for risk managers. Risk managers need to take care to ensure that the assembled team is objective, balanced in terms of scientific perspectives, and free from undue biases and conflicts of interest. It is also crucial to elicit information about potential financial or personal conflicts of interest that could bias an individual's scientific judgment. Typically, this information is solicited by a

questionnaire before appointments are made to a risk assessment team. Exceptions are sometimes made if an individual has essential, unique expertise; transparency is essential when any such decisions on inclusion are made.

### *7.4.4 Specification of purpose and scope*

Risk managers should prepare a "purpose statement" for a risk assessment, which should identify the specific risk or risks to be estimated and the broad risk management goal(s). For example, a risk assessment might be designed to provide quantitative estimates of foodborne risks due to *Campylobacter* in broiler chickens on an annual basis for the national population, and the risk assessment might be primarily used to evaluate risk management options at various points from production to consumption of broiler chickens, to maximize reduction in risk. The purpose statement generally flows directly from the risk management goal(s) agreed on when the risk assessment is commissioned.

In some situations, an initial exercise may be to set up a risk assessment framework model to identify data gaps and establish the research program required to generate the scientific inputs needed to complete a risk assessment at a later date. Where a risk assessment can be completed using currently available scientific knowledge, the model can still identify further research that will allow later refinement of the outputs. The "scope" portion of the risk assessment description should identify the parts of the food production chain that are to be evaluated and should establish boundaries for risk assessors with regard to the nature and extent of scientific information to be considered. By considering existing risk assessments in consultation with their risk assessors, risk managers may be able to substantially narrow the scope of the work and the data needed.

### *7.4.5 Questions to be addressed by risk assessors*

Risk managers, in consultation with risk assessors, should formulate the specific questions that need to be answered by the risk assessment. Depending on the scope of the risk assessment needed and the resources available, considerable discussion may be required to arrive at clear and realizable questions that will yield answers to guide risk management decisions. As with the statement on purpose and scope, questions to be addressed by the risk assessment often flow from the broad risk management goal(s) agreed on when the risk assessment is commissioned. The questions asked by the risk managers can have an important influence on the choice of risk assessment methodologies used to answer them.

### 7.4.6 Establishing risk assessment policy

While risk assessment is fundamentally an objective, scientific activity, it inevitably contains some elements of policy and subjective scientific judgment. For example, when scientific uncertainty is encountered in the risk assessment, inferential bridges are needed to allow the process to continue. The judgments made by the scientists or risk assessors often entail a choice among several scientifically plausible options, and policy considerations inevitably affect, and perhaps determine, some of the choices. Thus gaps in scientific knowledge are bridged through a set of inferences and "default assumptions." At other points in a risk assessment, assumptions driven by values-based, social consensus, often developed through long experience with how such issues should be handled, may be required. Documentation of all such default assumptions contributes to the consistency and transparency of risk assessments. These policy decisions are spelled out in a risk assessment policy, which should be developed by risk managers and risk assessors in active collaboration in advance of the risk assessment. Policies governing values-based choices and judgments should be decided primarily by risk managers, whereas policies governing science-based choices and judgments should be decided primarily by risk assessors, with active communication between the two functional groups in each case.

### 7.4.7 Specifying outputs

Outputs of a risk assessment may be sought in non-numerical (qualitative) or numerical (quantitative) form. Non-numerical risk estimates provide a less definitive basis for decisions but are adequate for several purposes, such as establishing relative risks or evaluating relative impacts on risk reduction of different control measures. Numeric estimates of risk can take one of two formats: (1) point estimate, which is a single numerical value representing, for example, the risk in a worst case scenario, or (2) probabilistic risk estimates, which include variability and uncertainty and are presented as a distribution reflecting more real-life situations. To date, point estimates have been more common outputs of chemical risk assessments while probabilistic outputs are the usual product of microbiological risk assessments.

### 7.4.8 Time and resources

While it is desirable to maximize scientific inputs and commission specific research to fill data gaps when conducting a risk assessment, all risk assessments are inevitably constrained in some ways. In commissioning

a risk assessment, risk managers must ensure that sufficient resources (e.g., time, money, personnel, and expertise) are available relative to the purpose and scope, and establish a realistic timetable for completion of the work.

### *7.4.9 General characteristics of risk assessment*

Irrespective of the context, risk assessments generally share a number of basic characteristics. While these attributes are described comprehensively in the sections that follow, in some situations a specific risk assessment is a relatively simple and straightforward exercise. In such cases, the general characteristics can be substantially modified; for instance, it may sometimes be possible for food safety experts to conduct an adequate risk assessment quickly and efficiently, without the need to assemble a multidisciplinary risk assessment team.

### *7.4.10 Objectivity and transparency*

A risk assessment should be objective and unbiased. Opinions or value judgments on issues other than science (for instance on economic, political, legal, or environmental aspects of the risk) should not be allowed to influence the outcome, and risk assessors should explicitly identify and discuss any judgments on the sufficiency of the science that was relied on. A participatory process should be used in initiating, performing, and finalizing a risk assessment, and reporting should be in a style that allows risk managers and other stakeholders to properly understand the process. Above all, a risk assessment must be transparent, and in documenting the process the risk managers should, for example, reveal any biases, identify scientific inputs, and clearly state assumptions.

### *7.4.11 Functional separation of risk assessment and risk management*

In general, the functions of risk assessment and risk management should be carried out separately to the extent practicable, so that the science remains independent from regulatory policy and values. However, delineating the functional boundaries between risk assessors, risk managers, and risk communicators in all situations is a significant challenge. Functional separation may be more obvious when different bodies or officials are responsible for risk assessment and risk management tasks. However, functional separation can also be achieved with limited resources and

personnel where risk assessments are undertaken by people who act as both risk assessors and risk managers. What is important in these cases is to have conditions in place which ensure that risk assessment tasks are carried out separately from risk management tasks. Whatever the functional separation arrangements, a highly interactive, iterative process is essential for risk analysis as a whole to be effective. Communication between risk assessors and risk managers is also a critical element in the process.

### *7.4.12 Basis in science*

It is a primary tenet that risk assessment be soundly based on scientific data. Representative data of sufficient quality and detail must be located from appropriate sources and assembled in a systematic manner. Descriptive and computational elements should be supported with scientific references and accepted scientific methodologies, as appropriate.

When a risk assessment is commissioned, there often are insufficient data available to complete the assignment. Scientific information to support many food safety risk assessments is available from a variety of sources. Risk assessments carried out at the national level are rapidly increasing in number, and many of them can be accessed through web-based portals. For instance, microbiological risk assessments carried out by the U.S. Food Safety and Inspection Service are available at www.fsis.usda.gov/Science/Risk_Assessments/index.asp. FAO and WHO administer international panels of experts on chemical and microbiological hazards to provide risk assessments as the basis for Codex standards.

While risk assessors conducting a given risk assessment may try to fill data gaps and to obtain adequate input data, inevitably default assumptions will need to be made at some steps during risk assessment. These assumptions must remain as objective, biologically realistic, and consistent as possible. Risk assessment policy provides broad guidelines, but default assumptions specific to a particular problem may have to be made on a case-by-case basis. It is essential that any such assumptions are transparently documented.

Sometimes when data are lacking, expert opinions can be used to address important questions and uncertainties. A variety of knowledge elicitation techniques have been developed for this purpose. Experts may be unaccustomed to describing what they know or how they know it; knowledge elicitation techniques reveal expert knowledge and help to make expert opinions as evidence-based as possible. Approaches that can be used include interviews, the Delphi method, surveys and questionnaires, among others.

### *7.4.13 Uncertainty and variability*

Definitive data needed to derive quantitative risk estimates are often lacking, and sometimes there are significant uncertainties inherent in biological or other models used to represent the processes that contribute to risk. Uncertainty about the available scientific information is often addressed in a risk assessment by using a range of possible data values.

Two distinct characteristics of scientific information are relevant in this context. *Variability* is a characteristic of phenomena that differ from one observation to the next; for example, people eat different amounts of a food, and the level of a particular hazard present in a food also can vary widely from one serving of food to another. *Uncertainty* is the quality of being unknown, for example because inadequate data exist or because the biological phenomena involved are not well understood. For instance, in assessing a chemical hazard scientists may need to rely on data from toxicity tests in rodents because insufficient human epidemiological data exist.

Risk assessors must ensure that risk managers understand the impacts of limitations of available data on the results of the risk assessment. Risk assessors should provide an explicit description of uncertainties in the risk estimate and their origins. The risk assessment should also describe how default assumptions may have influenced the degree of uncertainty in the outputs. As necessary or appropriate, the degree of uncertainty in the results of a risk assessment should be described separately from the effects of variability inherent in any biological system.

### *7.4.14 Peer review*

Peer review reinforces transparency and allows wider scientific opinion to be canvassed in relation to a specific food safety issue. External review is especially important where new scientific approaches are being applied. Open comparison of the outcomes of similar risk assessments where different scientific defaults and other judgments have been used can yield useful insights.

## *7.5 Risk assessment methodology*

Different risk assessment methods are used to assess different kinds of food safety problems. Methods vary according to the class of hazard (i.e., chemical, biological, or physical hazard), the food safety scenario (e.g., concerning known hazards, emerging hazards, new technologies such as biotechnology, complex hazard pathways such as for antimicrobial resistance), and the time and resources available.

Differences in risk assessment methodology are most apparent for chemical compared with microbiological hazards. This is partly due to intrinsic differences between the two classes of hazards. The differences also reflect the fact that for many chemical hazards, a choice can be made as to how much of the chemical may enter the food supply, for example, for food additives and for residues of veterinary drugs and pesticides used on crops. Use of these chemicals can be regulated or restricted so that residues at the point of consumption do not result in risks to human health. Microbial hazards, in contrast, are ubiquitous in the food chain, they grow and die, and despite control efforts, they often can exist at the point of consumption at levels that do present obvious risks to human health.

Risk assessment outputs can range from qualitative to quantitative with various intermediate formats. The characteristics of risk assessments presented above apply to all types. In qualitative risk assessments, outputs are expressed in descriptive terms such as high, medium, or low. In quantitative risk assessments, the outputs are expressed numerically and may include a numerical description of uncertainty. In some cases, intermediate formats are referred to as semi-quantitative risk assessments. For instance, one semi-quantitative approach may be to assign scores at each step in the pathway and express outputs as risk rankings.

Deterministic (point estimate) approaches describe an approach in which numerical point values are used at each step in the risk assessment; for example, the mean or the 95th percentile value of measured data (such as food intake or residue levels) may be used to generate a single risk estimate. Deterministic approaches are the norm in chemical risk assessment.

In stochastic (probabilistic) approaches to risk assessment, scientific evidence is used to generate statements of probabilities of individual events, which are combined to determine the probability of an adverse health outcome. This requires mathematical modeling of the variability of the phenomena involved, and the final risk estimate is a probability distribution. Stochastic (probabilistic) models are then used to create and analyze different scenarios of risk. This approach is generally viewed as being most reflective of the real world, but stochastic models are often complex and difficult to generate.

Stochastic models are only now beginning to be used to complement the "safety evaluation" approaches traditionally used in managing chemical foodborne hazards, in particular for contaminants. On the other hand, probabilistic approaches are the norm in the newer discipline of microbial risk assessment and provide a mathematical description of the dynamics of hazard transmission from production to consumption. Exposure data are combined with dose-response information to generate probabilistic estimates of risk. Even one colony-forming unit of the pathogen in

an edible portion of food is assumed to have some probability of causing infection; in this respect, such risk models resemble risk assessment methodology for chemical carcinogens.

Model validation is the process of evaluating a simulation model used in a risk assessment for its accuracy in representing a food safety system, for example, by comparing model predictions of foodborne disease with human surveillance data, or by comparing model predictions on hazard levels at intermediate steps in the food production chain with actual monitoring data. While validation of the outputs of a risk assessment is desirable, this activity is not always practical. This is especially true for chemical risk assessments, where chronic adverse health effects in humans may be predicted from animal tests but can rarely be validated with human data.

### *7.5.1 Risk assessment for biological hazards*

Biological risk assessments typically use a quantitative model to describe the baseline food safety situation and estimate the level of consumer protection currently afforded. Then, some of the inputs into the model are changed, such as the level of the hazard in the raw food at the time of primary production, the conditions of processing, and the temperature at which packaged material is held during retail and in the home. Changing inputs in a series of simulations enables the risk assessors to predict the impacts of the various control measures on the level of risk compared to that estimated in the baseline model.

### *7.5.2 Hazard identification*

A wide range of biological hazards can cause foodborne illness. Long-familiar hazards include microbes, viruses, parasites, and toxins of biological origin, but new hazards are continually being identified, such as the prion agent of BSE and multi-antibiotic resistant strains of *Salmonella*. In a given case, a risk profile may have identified specific strains or genotypes of pathogens that pose risks in a particular situation, and assessment may focus on these.

### *7.5.3 Hazard characterization*

A wide range of hazard factors (e.g., infectivity, virulence, antibiotic resistance) and host factors (e.g., physiological susceptibility, immune status, previous exposure history, concurrent illness) affect hazard characterization and its associated variability. Epidemiological information is essential for full hazard characterization.

While dose-response data are essential for quantitative biological risk assessment, such data are often difficult to obtain for specific hazards. Relatively little human data is available to model dose-response curves for specific populations of interest, and assumptions often have to be made in this area, for example, by using surrogate dose-response data from a different pathogen. However, data from outbreak investigations can be a useful source in establishing the dose-response relationship. Dose-response relationships can be developed for a range of human responses, such as infection, morbidity, hospitalization, and death rates associated with different doses.

### *7.5.4 Exposure assessment*

A food-chain exposure pathway model up to the point of consumption is developed for the hazard so that a human dose-response curve can be used to generate estimates of risk. Consideration of the whole food chain, while not always necessary, should be encouraged to the extent required to answer the risk managers' questions. The level of human exposure depends on many factors, including the extent of initial contamination of the raw food, characteristics of the food and the food processes in terms of the hazard organism's survival, multiplication, or death, and storage and preparation conditions before eating. Some transmission pathways, for instance those for *Campylobacter* in poultry, may involve cross-contamination at retail or in the home.

### *7.5.5 Risk characterization*

Risk estimates can be qualitative (high, medium, or low rankings for a pathogen) or presented in quantitative terms (cumulative frequency distributions of risk per serving(s), annual risks for targeted populations, or relative risks for different foods or different pathogens).

Considerable challenges lie ahead in carrying out quantitative microbial risk assessments for hazard-food combinations that pose significant risks to human health. Codex has stated in its guidelines for microbiological risk assessment that "a microbiological risk assessment should explicitly consider the dynamics of microbiological growth, survival, and death in foods and the complexity of the interaction (including sequelae) between human and agent following consumption as well as the potential for further spread." However, biological characteristics of the pathogen/host relationship are often uncertain. and modeling the exposure pathway from production to consumption often suffers from substantial data gaps.

Bearing these challenges in mind, risk characterization for microbial hazards may be somewhat inaccurate, but the greater strength of

microbial risk assessment lies in its ability to model different food control measures and their impact on estimates of relative risks. Modeling "what-if" scenarios, such as changing the assumed prevalence of infection in the live animal population from which the food is derived, is also an essential part of economic analysis.

### *7.5.6 Sensitivity analysis*

Sensitivity analysis is a tool that can help risk managers select those controls that best achieve risk management goals. Sensitivity analysis, as a scientific process, shows the effects of changes in various inputs (data or assumptions) on the outcomes of a risk assessment. One of the most useful insights gained from a sensitivity analysis is estimating how much the uncertainty or variability associated with each input factor contributes to the overall uncertainty and variability in the risk estimate. Input distributions where uncertainty has the greatest impact on the outcome can be identified, and this process also can help set priorities for research to reduce uncertainty.

### *7.5.7 Establishment of "targets" in the food chain as regulatory standards*

The concept of setting food safety "targets" at various points in the food production chain to achieve a defined FSO was described in the introduction to food safety risk analysis above. Risk assessors are involved in developing risk-based microbiological targets by simulating their impacts in risk models. In most cases, the goal of such simulations is to develop practical risk-based metrics that can be directly incorporated (and monitored) in HACCP plans, such as process criteria, product criteria, and microbiological criteria to achieve a performance objective. However, considerable methodological challenges remain in this area.

## *7.6 Risk assessment and RTE foods*

With the increased application of food safety risk analysis to enhance food safety systems like HACCP and establish food safety policy, there has been a deluge of recent research information. This information ranges from hazard identification and simple modeling tools that could be used in conducting a risk assessment addressing a specific food/hazard combination to comprehensive risk assessments which address broad food groups and multiple hazards. Some recent reports on microbiological hazards associated with various RTE foods or environments in which

they are processed have shown many specific organisms pose a potential risk to human heath (Atanassova et al. 2008; Bassett and McClure 2008; Cabedo et al. 2008; Christison et al. 2007; Gombas et al. 2003; and Meldrum et al. 2005). More detailed information on specific microbiological hazards associated with RTE foods can be found in Chapter 1 of this book.

Although many microbiological hazards associated with RTE foods have been identified, *Listeria monocytogenes* has been, by far, the subject of most risk assessment research. Despite its low incidence of three cases per million people (CDC 2006b), Listeriosis (infection caused by *L. monocytogenes*) can cause severe illness and often death in immunodeficient population subgroups, which is likely why it remains an important research topic, especially for RTE foods. Other reasons this organism is a target for study in RTE foods is that it is ubiquitous in nature, can enter the food production chain at many stages before consumption, and can grow at refrigeration temperatures given sufficient time. Further, there have been alarming prevalence rates reported for this organism in many RTE foods: for example, 72% of vacuum packaged corned beef samples obtained from retail stores in Australia, and ~8% (71% of one brand) of hot dog samples obtained from supermarkets in the United States among others (Lianou and Sofos 2007).

To provide sound scientific advice for mitigation strategies and respond to the needs of its member countries for adaptable risk assessments the Codex Committee on Food Hygiene requested a risk assessment of *L. monocytogenes* in RTE foods to specifically address three questions (WHO/FAO 2004). First, what is the estimated risk of serious illness caused by *L. monocytogenes* based on level of contamination? Second, what is the estimated risk of serious illness to different immunodeficient population subgroups (very young, very old, pregnant, or otherwise immunocompromised) compared to the general population? And third, what is the estimated risk of serious illness caused by *L. monocytogenes* contamination of foods that support its growth compared to those foods which do not? The risk assessment was carried out according to the four analytical steps described by Codex, in that there was a hazard identification, hazard characterization, exposure assessment, and risk characterization. The risk assessment is published in a WHO/FAO Technical Report entitled Risk Assessment of *Listeria monocytogenes* in Ready-to-Eat Foods as part of their Microbiological Risk Assessment Series (WHO/FAO 2004). The report indicates that estimating the likelihood of serious illness based on the level of contamination is complicated by the type of food, susceptibility of the individual, and virulence factors of the specific strain of the pathogen. Models developed to predict this relationship indicate that most all cases of Listeriosis are a result of consuming high levels of this

pathogen, but risk still remains even at very low levels (1 CFU/g) (WHO/FAO 2004). Estimating serious illness by comparing the general population to population subgroups based on available information indicates equal risk among similar groups from one country to another (United States and France) with certain immunodeficient population subgroups having greater relative susceptibility than the general population. For example, there is nearly a 10-fold increase in dose-response for persons over 65 years of age compared to the reference population (<65 years of age with no other condition) in France and about a 1000-fold increase in dose-response for the perinatal subgroup compared to the intermediate-age population (<60 years of age) in the United States (WHO/FAO 2004). Because of the varying properties of foods, defining a general value of increased risk of Listeriosis in RTE foods is not possible. However, findings detailed in the report show that there is a 100- to 1000-fold increase in risk, on a per-serving basis, associated with foods which support the growth of *L. monocytogenes* compared to those which do not support its growth (WHO/FAO 2004).

The main strategies in achieving continuous improvement in reducing Listeriosis rates as identified by the ILSI Research Foundation/Risk Science Institute expert panel (ILSI 2005) include preventing *L. monocytogenes* contamination of food and preventing the growth of *L. monocytogenes* to high numbers (the latter having the greater impact). The expert panel also identified science-based education targeted at immunodeficient population subgroups and their caregivers on the risk of Listeriosis associated with certain (RTE) foods as an important effort. Production and process controls could be achieved by implementation of effective food safety measures which are consistently met. These include (1) programs to minimize/prevent contamination, including good manufacturing practices, sanitation standard operating procedures, and HACCP, (2) intensive environmental sampling, (3) time and temperature controls throughout distribution, (4) reformulating foods so that they do not support the growth of *L. monocytogenes*, and (5) post-packaging treatments to destroy *L. monocytogenes* (ILSI 2005).

HACCP as a food safety program to control contamination and prevent growth is an effective tool, especially at the manufacturing level of industry. However, a great deal of risk associated with Listeriosis from RTE foods can be linked to practices at retail and food service. HACCP at this level in industry, though encouraged by the Food Code, is not mandatory. This is likely due, in part, to the intrinsic characteristics of retail and food service operations making mandated HACCP difficult to implement and regulate. To increase the application of HACCP, more appropriately HACCP-based food safety systems which incorporate HACCP principles,

at retail and food service requires simplicity and flexibility (Lianou and Sofos 2007). Without mandated HACCP at retail and food service but with an increase in the application of food safety systems based on HACCP principles, clarification of the role of regulators in compliance enforcement is required. A manual developed by the FDA (FDA 2006) provides guidance in applying HACCP principles to risk-based inspections which can help regulators evaluate and improve HACCP-based food safety systems and help strengthen regulator/manager partnerships (Lianou and Sofos 2007).

## 7.7 Conclusion

It must be remembered that a risk assessment provides information not solutions. It is still necessary for the risk manager or policy maker to determine if the risk is acceptable or if controls are necessary (Wooldridge 2008). If the risk is acceptable it can then be said that the risk is within an established FSO which will result in an ALOP. If controls are necessary, POs or PC must be implemented at appropriate steps in the food production/distribution chain to achieve an established FSO and ALOP. A means to communicate these POs and PC to all levels of industry (manufacture, retail, food service, etc.) is through HACCP or other food safety system based on HACCP principles. Because HACCP is not mandated for all categories of RTE food production or at retail and food service, other means of communicating information to all levels of the food production/distribution chain, including consumers, are necessary to achieve public health goals related to the incidence of foodborne illness. In addition to enhancing communications, further development of risk assessment tools to enhance precision and reduce uncertainty, conducting additional risk assessments as needs are identified, and determining how to best make risk management decisions based on risk assessments are all key to our food safety strategy (Woteki 1998).

## References

Atanassova, V., F. Reich, and G. Klein. 2008. Microbiological Quality of Sushi from Sushi Bars and Retailers. *J. Food Prot*., 71:860–864.

Bassett, J., and P. McClure. 2008. A Risk Assessment Approach for Fresh Fruits. *J. Appl. Microb*., 104:925–943.

Buchanan, R.L. 2004. Principles of Risk Analysis as Applied to Microbial Food Safety Concerns. *Mitt. Lebensm. Hyg*., 95:6–12.

Cabedo, L., L. Picart Barrot, and A. Teixidó Canelles. 2008. Prevalence of *Listeria monocytogenes* and *Salmonella* in Ready-to-Eat Food in Catalonia, Spain. *J. Food Prot*., 71:855–859.

Christison, C.A., D. Lindsay, and A. von Holy. 2007. Cleaning and Handling Implements as Potential Reservoirs for Bacterial Contamination of Some Ready-to-Eat Foods in Retail Delicatessen Environments. *J. Food Prot*., 70:2878–2883.

Centers for Disease Control and Prevention (CDC). 2006a. Update on Multi-State Outbreak of *E. coli* O157:H7 Infections from Fresh Spinach, October 6, 2006. Available at: http://www.cdc.gov/ecoli/2006/september/updates/100606.htm. Accessed: 26 September 2008.

Centers for Disease Control and Prevention (CDC). 2006b. Preliminary FoodNet Data on the Incidence of Infection with Pathogens Transmitted Commonly Through Foods—10 States, United States. *Morbidity and Mortality Weekly Report*, 55:392–395.

Centers for Disease Control and Prevention (CDC). 2007. Multistate Outbreak of *Salmonella* Serotype Tennessee Infections Associated with Peanut Butter—United States 2006–2007. *Mortality and Morbidity Weekly Report*, 56(21):521–524.

Centers for Disease Control and Prevention (CDC). 2008. Investigation of Outbreak of Infections Caused by *Salmonella* Saintpaul. Available at: http://www.cdc.gov/salmonella/saintpaul. Accessed: 26 September 2008.

Food and Agriculture Organization and the World Health Organization of the United Nations (FAO/WHO). 2006. Food Safety Risk Analysis, A Guide for National Food Safety Authorities. FAO/WHO Rome, Italy. Available at: http://www.who.int/foodsafety/publications/micro/riskanalysis06.pdf. Accessed: 18 September 2008.

Food and Drug Administration (FDA). 2001. Food Code. Available at: http://www.cfsan.fda.gov/~dms/fc01-toc.html. Accessed: 26 September 2008.

Food and Drug Administration (FDA). 2006. Managing Food Safety: A Regulator's Manual for Applying HACCP Principles to Risk-Based Retail and Food Service Inspections and Evaluating Voluntary Food Safety Management Systems. Available at: http://www.cfsan.fda.gov/~acrobat/hret3.pdf. Accessed: 28 September 2008.

Gombas, D.E., Y. Chen, R.S. Clavero, and V.A. Scott. 2003. Survey of *Listeria monocytogenes* in Ready-to-Eat Foods. *J. Food Prot*., 66:559–569.

ILSI Research Foundation/Risk Science Institute (ILSI), Expert Panel on *Listeria monocytogenes* in Foods. 2005. Achieving Continuous Improvement in Reductions in Foodborne Listeriosis—A Risk-Based Approach. *J. Food Prot*., 68:1932–1994.

International Commission on Microbiological Specifications for Foods (ICMSF). 2002. Microorganisms in Foods, vol. 7. *Microbiological Testing in Food Safety Management.* Kluwer Academic/Plenum Publishers, New York, NY.

International Food Safety Authorities Network (INFOSAN). 2007. Use of Microbiological Risk Assessments in Risk Management. Available at: http://www.who.int/foodsafety/fs_management/No_05_riskmanagement_Sept07_en.pdf. Accessed: 18 September 2008.

Lianou, A., and J.N. Sofos. 2007. A Review of the Incidence and Transmission of *Listeria monocytogenes* in Ready-to-Eat Products in Retail and Food Service Environments. *J. Food Prot*., 70:2172–2198.

Meldrum, R.J., C.D. Ribeiro, R.M.M. Smith, A.M. Walker, M. Simmons, D. Worthington, and C. Edwards. 2005. Microbiological Quality of Ready-to-Eat Foods: Results from a Long-Term Surveillance Program (1995 through 2003). *J. Food Prot*., 68:1654–1658.

National Academy of Sciences (NAS). 1985. *An Evaluation of the Role of Microbiological Criteria for Foods and Food Ingredients.* National Academy Press, Washington, DC.

U.S. Department of Agriculture (USDA). 1996. Pathogen Reduction; Hazard Analysis and Critical Control Point (HACCP) Systems. Final Rule. *Federal Register*, 61(144):38805–38855.

Whiting, R.C., and R.L. Buchanan. 2008. Using Risk Assessment Principles in an Emerging Paradigm for Controlling the Microbial Safety of Foods, p. 29–50. In D. Schaffner (ed.), *Microbial Risk Analysis of Foods*. ASM Press, Washington, DC.

Wooldridge, M. 2008. Qualitative Risk Assessment, p. 1–28. In D. Schaffner (ed.), *Microbial Risk Analysis of Foods*. ASM Press, Washington, DC.

World Health Organization and the Food and Agriculture Organization of the United Nations (WHO/FAO). 2004. Risk Assessment of *Listeria monocytogenes* in Ready-to-Eat Foods. WHO/FAO Geneva, Switzerland. Available at: http://www.fao.org/docrep/010/y5394e/y5394e00.htm. Accessed: 28 September 2008.

World Trade Organization (WTO). 1995. The WTO Agreement on the Application of Sanitary and Phytosanitary Measures (SPS agreement). Available at: http://www.wto.org/English/tratop_e/sps_e/spsagr_e.htm. Accessed: 26 September 2008.

Woteki, C. 1998. The Importance of Risk Analysis in Meeting Food Safety Goals. Available at: http://www.fsis.usda.gov/oa/speeches/1998/cw_umd.htm. Accessed: 18 September 2008.

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Food Science & Technology

# READY-TO-EAT FOODS

## Microbial Concerns and Control Measures

With growing consumer demand for ready-to-eat (RTE) foods that are wholesome and require less handling and preparation, the production of RTE foods has increased and their variety has expanded considerably, spanning from bagged spinach to pre-packaged school lunches. But since RTE foods are normally consumed directly without cooking—a step that kills pathogenic microorganisms that may be present in the food products—concerns exist with regard to their safety.

Several severe and high-profile outbreaks of foodborne illness linked to the consumption of RTE foods have prompted the USDA and FDA to issue stringent rules and regulations governing the manufacturing of RTE foods. **Ready-to-Eat Foods: Microbial Concerns and Control Measures** comprehensively reviews individual common RTE foods and their specific safety-related aspects. This text explores the extensive research conducted by the food industry, academia, and research institutes that examines the potential health risks of contaminated RTE foods, investigates the growth behavior of common contaminating foodborne pathogens, and develops intervention technologies and control measures.

The book supplies an overview of food safety of RTE foods and various categories into which they fall. It also addresses the microorganisms of concern, the effect of processing on the survival of pathogenic and spoilage microorganisms, food safety, practical control measures, and intervention strategies. **Ready-to-Eat Foods: Microbial Concerns and Control Measures** is a critical reference for scientists and professionals working on the forefront of food safety and RTE food manufacturing.

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CRC Press
Taylor & Francis Group
an informa business
www.crcpress.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

ISBN: 978-1-4200-6862-7
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