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Practical Approaches

Joshua B. Gurtler
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The Microbiological Safety of Low Water Activity Foods and Spices

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The Microbiological Safety of Low Water Activity Foods and Spices

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Part I
Introduction and Overview

The Microbiological Safety of Spices and Low-Water Activity Foods: Correcting Historic Misassumptions

Joshua B. Gurtler, Michael P. Doyle, and Jeffrey L. Kornacki

Abstract Low-water activity (low-a_w) foods (those with a_w<0.70), which were once thought to be microbiologically safe, have, in recent years, been shown to be contaminated with foodborne pathogens, most notably and frequently *Salmonella* spp., leading to numerous food product recalls and foodborne illness outbreaks. Low-a_w food products can no longer be considered inherently safe, simply because *Salmonella* will not grow in such products. Therefore, diligence must be applied to ensure that safe food practices are employed for low-a_w foods. Areas of concern include the sourcing of major and minor ingredients, unsanitary drying or storage conditions, contaminated processing equipment or improper maintenance, faulty sanitary design of manufacturing or processing equipment, sick or infected employees, cross-contamination of ready-to-eat foods, improper sanitation procedures, improper testing methods, inappropriate sampling plans, failure to act on foodborne pathogen-positive samples, and failure to validate and verify antimicrobial intervention treatments. Other areas in need of attention include failure to implement approved Hazard Analysis and Critical Control Points (HACCP) plans in manufacturing facilities, improper supplier or importer standards or failure to monitor or audit suppliers for hygiene and pathogen control, a faulty assumption that a given low-a_w food or food product is innately safe from foodborne pathogen contamination, or, finally, overt criminal negligence on the part of a manufacturer or supplier involving one or more of the items mentioned above. Examples of low-a_w food products that have previously been considered inherently safe from foodborne pathogens are raw flour (responsible for a 2008 outbreak sickening 67 people and hospitalizing 12) and two peanut butter or paste outbreaks in 2007–2009, which sickened over 1,400 people in 48 US states and Canada. It is conceivable that low-a_w food products not yet considered at risk for foodborne pathogens may emerge. *Salmonella* spp., of all common foodborne pathogens, will continue to pose the greatest threat in these foods, due to its uncanny ability to survive desiccation in foods and live for years in the environment of food processing facilities.

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Introduction

Historically, the low-water activity (low a_w) or low moisture content of foods has been considered a significant barrier to the growth of human foodborne pathogenic bacteria; hence, such foods have often been inappropriately assumed to be microbiologically safe. Nevertheless, low-water activity (low-a_w), low moisture content, or dried foods, in recent years, have been increasingly associated with food product recalls and foodborne outbreaks due to contamination by human and zoonotic foodborne pathogens, such as *Salmonella* spp., *Listeria monocytogenes*, *Bacillus cereus*, *Clostridium botulinum*, and enterohemorrhagic *E. coli* (i.e., *E. coli* O157:H7 and other Shiga toxin-producing strains of *Escherichia coli*). Contrary to what was once commonly believed, some foodborne bacterial pathogens are able to survive in a dehydrated/desiccated state for extended periods of time, under what was heretofore believed to be uninhabitable conditions. Some of these conditions are now known to increase the resistance or protect the pathogens from subsequent inactivation interventions or decontamination treatments, such as thermal pasteurization.

Salmonella infections, believed to be mostly foodborne, are estimated at 1.4 million and 80 million in the United States and worldwide per annum, respectively (Lynch and Tauxe 2009; Fig. 1). These numbers equate to 27,000 cases of salmonellosis/week in the United States. Despite major strides in foodborne surveillance in the

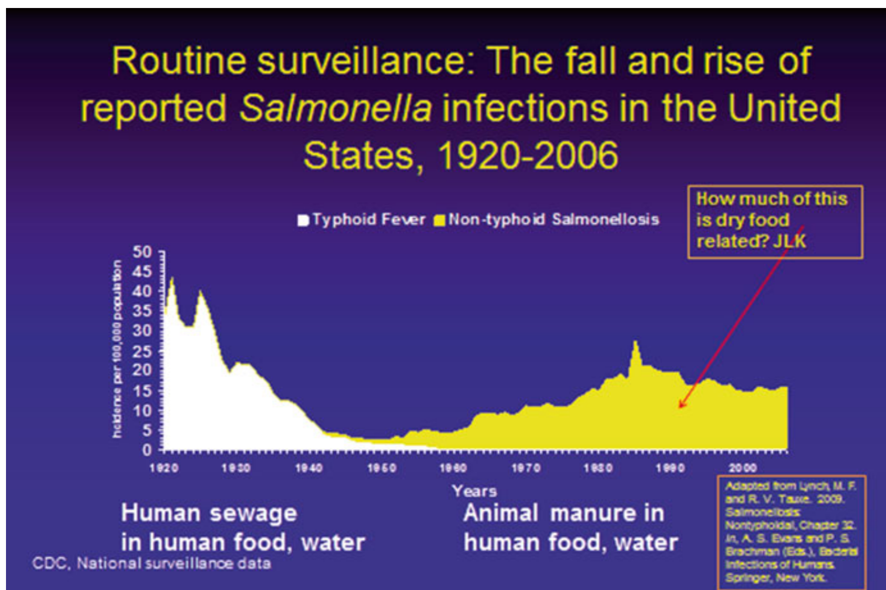

Fig. 1 The Fall and rise of typhoidal Salmonella non-typhoidal Salmonella in the United States between 1920 to 2006

United States in the past 16 years, detection of the 3,800 cases of *Salmonella*/day in the United States is not occurring. This leads to the questions, "*What are the likely foods involved*?" and "*How can this be happening*?" It is likely that foods involved may be those which were previously thought to not cause illness and those foods or ingredients, which may be used in small quantities with low pathogen levels and prevalence, dispersed across a large number of foods or meals. Many dry food ingredients and spices fit into this framework. Consider tiny amounts of spices, garnishes, coatings, and ingredients added to many foods that may have originated from extremely large batches. Consider scenarios wherein these may have been produced in developing nations and shipped to other countries with lot numbers reassigned, thus rendering the originating production lots untraceable. Then consider that these may be further subdivided, repacked, and distributed throughout the consuming country. Consider also a scenario wherein the contaminating pathogen population is sporadically distributed, which could lead to appreciable yet undetected foodborne illness. Scenarios resembling those described above could conceivably occur with a number of dry or low-a_w food spices and ingredients.

Definition of Low-a_w Foods

Low-a_w foods should not necessarily be considered low-moisture foods. In fact, some foods that are not low in moisture may have a low a_w due to water-binding solutes (such as sodium chloride) that bind the available water, subsequently lowering the a_w. In general, a low-a_w food is one in which the majority of the water present in the food is considered to be bound by solutes, making it unavailable for uptake and use by most pathogenic microorganisms. Common examples of water-binding solutes in food products are sodium chloride (salt) and sucrose (sugar), which has six times less water-binding ability than sodium chloride. Water activity (a_w) itself is defined as the vapor pressure of the food being tested divided by the vapor pressure of pure water (i.e., distilled water) at the same temperature. Pure water is prescribed a baseline a_w reading of 1.0 under standardized conditions. Thus, all a_w measurements in foods will be <1.0. A low-a_w food has been considered one with an a_w of <0.70 (Blessington et al. 2013). While some have considered low-a_w foods as those with $a_w < 0.60$, setting the boundary at <0.70 broadens the category so as to include many foods that have historically been regarded as low a_w (e.g., some nuts, jerkies, dried sausages, pepperoni, nougat, and marshmallow).

Implicated Foodborne Pathogens Associated with Low-a_w Foods

Although a number of foodborne pathogens have been associated with contaminated low-a_w foods, a number of serovars of *Salmonella* have been the most commonly implicated bacteria. The disproportionately greater number of *Salmonella*-related recalls and foodborne outbreaks associated with low-a_w foods may be related to the

unusual ability of *Salmonella* to survive desiccated and/or low-a_w conditions, when compared to other members of the bacterial family *Enterobacteriaceae* or when compared to other nonspore-forming foodborne bacteria (e.g., *E. coli* O157:H7 and other Shiga toxin-producing *E. coli*, *Listeria monocytogenes*, *Campylobacter jejuni*, *Vibrio* spp., *Yersinia* spp., *Shigella spp.*, and *Staphylococcus aureus*). One exception is the bacterial genus *Cronobacter* (previously known as *Enterobacter sakazakii*), which has caused rare but often severe instances of foodborne illness in infants and a very small number of severely immune-compromised adults. *Cronobacter* is listed among those pathogens under the category "severe hazard for restricted populations, life threatening or substantial chronic sequelae or long duration" (ICMSF 2001). Nevertheless, the threat of foodborne illness associated with *Cronobacter* spp. in healthy adults and children is exquisitely small; thus, it is not regarded as a major threat to the general human population when compared with the danger of foodborne illness from *Salmonella* spp. Further, although some spore-forming human foodborne pathogens are able to survive in low-a_w foods (e.g., *Bacillus cereus*, *Clostridium perfringens*, and *Clostridium botulinum*), these microorganisms must multiply to relatively high populations before being able to produce illness via toxins or toxico-infections.

Types of Low-a_w Foods Associated with Foodborne Illness Outbreaks or Product Recalls

Low-a_w food products that have been associated with foodborne illness outbreaks or recalls (in no particular order) include dried dairy products (e.g., powdered milks, whey, lactose, cheese), jerky and other dried meat products such as salami, grain-based products, coconut and other dried fruits and vegetables, hydrolyzed vegetable protein, ready-to-eat dried or puffed breakfast cereals, dried teas and herbs, powdered infant formula, dried infant food, peanut and nut pastes, pine nuts, almonds, hazelnuts, sprouting seeds, powdered seeds, dried snack foods and potato chips, tahini, flours, meals, cake mix, chocolate, cocoa, cookie dough, and spices, including black pepper and paprika. Low-a_w food products recalled for foodborne bacterial pathogen contamination from 2010 to 2012 are listed in Table 1.

Foodborne Outbreaks Associated with Consumption of Contaminated Low-a_w Foods

Historically, a few large foodborne outbreaks have been associated with low-a_w foods. In the past two decades, however, the frequency of these outbreaks has increased, possibly due to more sensitive microbiological testing methods, improved epidemiologic traceback investigations, and more proficient sampling plans. Some low-a_w foods that have been implicated in foodborne illness outbreaks include

Table 1 FDA low-a_w food products recalled for foodborne bacterial pathogen contamination from 2010 to June 2014[a]

Year	Specified pathogen and foods recalled
2014	*Listeria monocytogenes*: walnuts, shelled walnuts, dog and cat food, peanut butter *Salmonella*: ground Malabar pepper, sprouted chia seed powder products, chia and flaxseed powder, dried dog food, dog treats, crushed chili powder, chili powder, ground annatto, chile molido puro and chile rojo, sweet basil, organic black peppercorns, cat food, herbal supplement, organic basil *Clostridium botulinum*: salted fish
2013	*Listeria monocytogenes*: sunflower seeds and snack mixes *Salmonella*: bird food (multiple recalls), bird food blends, bird treats, Betta fish food, pet food or treats (multiple recalls), pistachios, dried oregano, dried sweetened breakfast cereal, baking mixes, protein bars
2012	*Listeria monocytogenes*: popcorn *Salmonella*: bird feed, tahini, chocolate, chocolate powder, food-grade limestone, pet food or treats (multiple recalls), peanut butter candy, protein/energy/nutritional snack bars (multiple recalls), peanuts and peanut butter (multiple recalls), multiple peanut-containing foods/candies/desserts/snacks (numerous recalls), almond butter, Thai red pepper, ground hot pepper, crackers, brownie and cookies, nutritional prebiotic formula, powdered milk replacer, children's nutritional powdered dietary supplement, dietary supplement capsules (multiple recalls), starter yeast, cacao nibs, tempeh, rat and hamster feed, flake fish food, salt-free seasoning blend
2011	*Clostridium botulinum*: black bean tortillas *Bacillus cereus*: cookies *E. coli* O157:H7: hazelnuts and mixed nut products *Salmonella*: organic celery seed, peanut butter, pine Nuts, soybean flour and soy meal, pet treats (multiple recalls), cat food, pet pig ear chews (multiple recalls), powdered whey protein mix/supplement (multiple recalls), whey protein isolate, herbal tea (multiple recalls), multiple snack products from one company (viz., wheat snacks with chile, potato chips with chile, and corn churritos with chile)
2010	*Staphylococcus aureus*: gingerbread house *Clostridium botulinum*: dried fish, dried seafood products from one company (viz., dried squid, bream, mackerel, trevally, shrimp, herring, anchovies, scad, mullet) *Salmonella*: walnuts (multiple recalls), nutmeg, dark chocolate candy, dog treats (multiple recalls), cat food (multiple recalls), pistachios (multiple recalls), pet food, vitamin tablets for dogs (multiple recalls), black pepper and black peppercorns, garlic powder, sesame seeds, corn chips, potato chips, potato crisps, nuts (multiple recalls), granola bars (multiple recalls), soy grits and flour, crushed red pepper, multiple food products containing either black pepper, red pepper, or hydrolyzed vegetable protein (e.g., dried soup and sauce mixes, dip mixes, spice packs, snack mixes, bouillon, spice rubs, pretzels, potato chips, corn chips, stuffing, snack mix)[b]

[a]Available online at http://www.fda.gov/Safety/Recalls/ArchiveRecalls/default.htm
[b]Available online at http://www.fda.gov/Safety/Recalls/ArchiveRecalls/2010/ucm202813.htm

almonds, cake mix, puffed and toasted breakfast cereals, chocolate, cocoa, coconut, cookie dough, fishmeal, halva, hazelnuts, dried infant milk product, powered infant formula, dried infant food, venison jerky, peanuts, peanut butter, black pepper, pine nuts, potato chips, powdered milk, salami, a corn snack, a rice-corn snack, a savory snack, tahini, and aniseed tea (Beuchat et al. 2013).

The bacterial foodborne pathogens that were implicated in the aforementioned list of foods associated with foodborne illness outbreaks were *E. coli* O157:H7, *Clostridium botulinum*, *Cronobacter* spp., *Staphylococcus aureus*, as well as the following *Salmonella enterica* serovars (the number of outbreaks greater than 1 associated with each serovar is listed in parentheses): Agona (4), Agona PT15, Anatum, Derby, Durham, Ealing, Eastbourne, Enteritidis (2), Enteritidis PT30 and 9C, Give, Java PT Dundee, Javiana, Manchester, Mbandaka, Montevideo, Napoli, Newport, Nima, Oranienburg, Orion, Potsdam, Rubislaw, Seftenburg (2), St. Paul, Stanley, Tennessee (2), Typhi, Typhimurium (4), Typhimurium DT104, and Wandsworth (Beuchat et al. 2013). These outbreaks were reported to have occurred in the following countries: Australia, Austria, Belgium, Canada, China, Denmark, Finland, France, Germany, Israel, Norway, the Netherlands, New Zealand, Sweden, Trinidad, the United Kingdom, and the United States (Beuchat et al. 2013). The fact that most outbreaks are reported in developed nations reflects the improved surveillance systems and epidemiological advancements.

One example of a food commodity that has the potential to be contaminated with *Salmonella* is peanut butter. An outbreak of fifteen cases of salmonellosis occurred in Australia in 1996, which was traced back to *Salmonella* serovar Mbandaka-contaminated peanut butter containing populations of less than 4 CFU/g. Ten years later, a 2006–2007 foodborne outbreak of salmonellosis caused by *Salmonella* Tennessee was also traced to peanut butter in 48 US states, manufactured over a 6-month time span, with over 700 reported cases of human illness. One year later, another salmonellosis outbreak in 46 US states as well as in Canada (sickening more than 700, including 9 deaths) was traced to peanut butter. In this outbreak, the manufacturer periodically shipped peanut butter that had tested positive for *Salmonella* and is currently facing trial for criminal negligence. During this outbreak investigation, it was discovered that the manufacturing plant had identified at least 12 *Salmonella*-positive samples in the years 2007 and 2008, yet the CEO was documented as telling employees, "*No salmonella has been found anywhere else in our products or in our plants*, *or in any unopened containers of our product*" (PCA 2009).

Another example of a foodborne outbreak associated with a low-a_w food product that was previously thought to be microbiologically safe occurred in raw flour in New Zealand in 2008–2009. *Salmonella* Typhimurium PT 42 was implicated in the outbreak, which sickened 67 people, 19 of which were reported with bloody diarrhea and 12 were hospitalized (McCallum et al. 2013).

Pet Food as a Human Foodborne Illness Risk

Pet foods (most notably dog foods) and pet treats, in recent years, have been recalled on numerous occasions due to contamination with *Salmonella* spp. Foodborne illnesses have also been associated with pet foods, including a 20-state outbreak of salmonellosis involving 49 reported cases and 10 hospitalizations in 2012, from *Salmonella* Infantis-contaminated dried dog food. An important finding during this

outbreak was that not only does contaminated pet food pose a risk to humans on the basis of cross-contamination but some elderly and low-income individuals actually used pet food as a source of human nourishment, in the absence of other adequate food sources. Other pet-related foods that have been recalled for *Salmonella* contamination include bird food (multiple recalls), bird food blends, bird treats, Betta fish food, cat food (multiple recalls), pet pig ear chews (multiple recalls), and vitamin tablets for dogs (multiple recalls).

Spices as Contaminated Low-a_w Foods

Spices are defined by the International Standard Organization (ISO) as "*vegetable products or mixtures thereof, free from extraneous matter, used for flavoring, seasoning, and imparting aroma in foods.*" Recent foodborne outbreaks and product recalls related to *Salmonella*-contaminated spices have raised the level of public health concern for spices as agents of foodborne illnesses. Presently, most spices are grown outside the United States, with 75 % of US spice imports coming from the following eight countries: India, Indonesia, China, Brazil, Peru, Madagascar, Mexico, and Vietnam. Many of these countries are underdeveloped, and spices are harvested and stored with little heed to sanitation. The US Food and Drug Administration has regulatory oversight of spices in the United States; however, the agency's control is largely limited to enforcing regulatory compliance through sampling and testing, once imported foodstuffs have crossed the US border. Thus, food safety and hygiene practices occurring prior to importation may be considered a "black box." Testing by industry is generally done for indicator organisms such as aerobic plate counts or *Enterobacteriaceae*. These tests are conducted to provide information on the extent that suppliers have adhered to or deviated from good hygienic practices. The FDA Food Safety Modernization Act will likely impose stricter preventive controls and testing requirements on producers and importers.

Sampling for Pathogens in Spices

Statistical sampling plans are inefficient but necessary tools for ensuring food safety. Furthermore, testing for the presence of pathogens in spices and dried food products has been challenging, due to methodological limitations, sometimes including small sample sizes. Antibacterial compounds are present in some in spices (e.g., cloves, cinnamon, oregano, nutmeg, garlic, anise seed, ginger, allspice, thyme, tarragon, cumin, lemongrass, bay leaf, rosemary, marjoram, caraway seed, mint, sage, fennel, coriander, cardamom, and onions). These antimicrobials may hinder the recovery of injured microorganisms. Thus, specialized culturing methods or neutralizing techniques are necessary, and unless caution is exercised, the prevalence and levels of pathogens will be underestimated. Hence, establishment of microbiological specifications, depending on final usage of spices, is key.

Antimicrobial Interventions for Spices

To date, ethylene oxide (EtO) has been the most widely used intervention by the US spice industry to control human microbial contaminants such as *Salmonella* and *E. coli*. High-pressure steam has also been used, as well as irradiation. Recently, other decontamination strategies have been explored, including treatments with hot ethanol vapor, hydrochloric acid, microwave treatments, and cold plasma.

Factors Associated with Foodborne Pathogen-Contaminated Low-a_w Foods

Numerous factors have been identified as contributing to the problem of low-a_w foods contaminated with foodborne pathogens. Some of these include:

- Contaminated major ingredients (e.g., peanuts, nuts or seeds, soy and grain flours and meals, dried fish or meats, cocoa, dried milk, dried fruits or vegetable, dried tea leaves, infant foods, etc.).
- Contaminated minor ingredients or premixes (herbs, spices and seasonings, vitamin or mineral premixes, protein mixes, prebiotic mixes, dietary supplements, starter yeast, etc.).
- Insanitary drying or storage conditions.
- Contaminated processing equipment or improper maintenance.
- Faulty sanitary design of manufacturing or processing equipment.
- Sick or infected food production employees.
- Cross-contamination of ready-to-eat food products.
- Improper sanitation procedures.
- Improper microbiological testing methods, inappropriate sampling plans, or failure to act on foodborne pathogen-positive samples.
- Failure to validate and verify an antimicrobial intervention treatment in order to ensure an effective level of pathogen inactivation.
- Failure to implement an approved HACCP plan in a manufacturing facility.
- Improper supplier or importer criteria or failure to monitor or audit suppliers for hygiene and pathogen control.
- A faulty assumption that a given low-a_w food or food product is innately safe from foodborne pathogen contamination.
- Overt negligence on the part of a manufacturer or supplier.

Validation of Intervention Processes and Use of Surrogate Microorganisms

Antimicrobial intervention processes used to treat low-a_w foods should, ideally, be tested in microbiological challenge studies with the pathogens of interest, to achieve the targeted level of bacterial reduction (e.g., 2, 3, or 5 log CFU/g inactivation).

However, due to biosafety constraints, the pathogenic foodborne bacteria of interest may not be able to be used in factory settings, or a suitable biosafety level 2 laboratory may not be available. In such cases, challenge studies are often conducted with non-pathogenic (surrogate) microorganisms used to model the inactivation characteristics of a pathogen of interest. The National Advisory Committee on the Microbiological Criteria for Foods (2009) recommends the use of a surrogate microorganism in lieu of pathogens for in-plant inactivation studies. Commenting on the utility of surrogate microorganisms, the US Food and Drug Administration (FDA) in their *Glossary of Kinetics of Microbial Inactivation for Alternative Food Processing Technologies* stated that surrogates enable biological verification of a given antimicrobial process or treatment without introducing pathogens into a food (FDA 2000).

One example of a validated surrogate bacterium is *Clostridium sporogenes* PA 3679, which is used in lieu of *Clostridium botulinum* in thermal process validation. PA 3679 is an extensively tested and validated surrogate bacterium that provides reasonable assurance and a margin of safety when modeled for *C. botulinum* in thermal challenge studies (FDA 2000; NACMCF 2010). The FDA (2000) has also stated that a microorganism described as a surrogate bacterium must be "*a non-pathogenic species and strain responding to a particular treatment in a manner equivalent to a pathogenic species and strain*." Therefore, microbial intervention challenge studies performed with a surrogate bacterium that has not been validated, or one that is demonstrably more sensitive than the pathogen to a particular treatment, may discredit results that are intended to model the reduction of a targeted pathogen. One surrogate bacterium that has been used for modeling foodborne pathogen thermal destruction in low-a_w foods is *Enterococcus faecium* NRRL-B2354 (Almond Board of California 2007; Kornacki 2012). Even in this instance, validation of its appropriateness for use in a particular dry food matrix in place of a given pathogen may be necessary.

Outlook

Although contaminated raw meats, as well as fresh produce, by foodborne pathogens will continue to pose significant food safety issues, the future may reveal further problems associated with low-a_w, low-moisture, or dried foods. It is conceivable that low-a_w food products not yet considered at risk for foodborne illnesses may emerge. *Salmonella* will likely continue to pose the greatest threat in these foods, due to its uncanny ability to survive desiccation.

Conclusions

This book will provide an understanding of the microbial challenges to the safety of low-a_w foods, including spices, especially in light of recent outbreaks and recalls, as well as providing insights into regulatory issues. Industrially used decontamination

methods, and other potential interventions, as well as appropriate product sampling and testing methods will also be addressed.

Low-a_w foods (those with $a_w < 0.70$) or low moisture content or dried foods, which were previously thought to be microbiologically safe, have, in recent years been shown to be contaminated with foodborne pathogens, most notably and frequently *Salmonella* spp., leading to numerous foodborne illness outbreaks and food product recalls. Low-a_w food products can no longer be produced and distributed under the assumption that they are inherently safe from foodborne pathogens; hence, food processors must be diligent in ensuring that appropriate food safety controls are in place to prevent pathogen contamination. Areas of concern include the safe sourcing of major and minor ingredients, insanitary drying or storage conditions, contaminated processing equipment or improper maintenance, faulty sanitary design of manufacturing or processing equipment, sick or infected employees, cross-contamination of ready-to-eat foods, improper sanitation procedures, improper testing methods, inappropriate sampling plans, failure to act on foodborne pathogen-positive samples, failure to validate and verify antimicrobial intervention treatments, failure to implement an approved HACCP plan in a manufacturing facility, improper supplier or importer criteria or failure to monitor or audit suppliers for hygiene and pathogen control, and a faulty assumption that a given low-a_w food or food product is innately safe from foodborne pathogen contamination. Examples of low-a_w food products that have previously been considered safe are raw flour (responsible for a 2008 outbreak sickening 67 and hospitalizing 12) and two peanut butter outbreaks in 2007–2009, which sickened more than 1,400 in 48 US states and Canada, including nine deaths. It is likely that new or overlooked low-a_w food products may be considered at risk for contamination in the future. Based on the published literature, the most likely pathogen of concern will continue to be *Salmonella*.

References

Almond Board of California (2007) Guidelines for process validation using *Enterococcus faecium* NRRL B-2354. http://www.almondboard.com/Handlers/Documents/Enterococcus-Validation-Guidelines.pdf. Accessed 20 Jan 2014

Beuchat LR, Komitopoulou E, Beckers H, Betts RP, Bourdichon F, Fanning S, Jousten MH, Ter Kuile BH (2013) Low-water activity foods: increased concern as vehicles of foodborne pathogens. J Food Prot 76:150–172

Blessington T, Theofel CG, Harris LJ (2013) A dry-inoculation method for nut kernels. Food Microbiol 33:292–297

Food and Drug Administration (2000) Kinetics of microbial inactivation for alternative food processing technologies. http://www.fda.gov/food/foodscienceresearch/safepracticesforfood-processes/ucm100158.htm. Accessed 20 Jan 2014

ICMSF (2001) Microorganisms in foods, volume &: microbiological testing in food safety management. In: Appendix 8-A. Springer, New York, pp 166–172

Kornacki JL (2012) *Enterococcus faecium* NRRL B-2354: tempest in a teapot or serious food pathogen? Food Safety Magazine, April/May 18(2):38–45, 69

Lynch MF, Tauxe RV (2009) Salmonellosis: nontyphoidal, chapter 32. In: Evans AS, Brachman PS (eds) Bacterial infections of humans. Springer, New York

McCallum L, Paine S, Sexton K, Dufour M, Dyet K, Wilson M, Campbell D, Bandaranayake D, Hope V (2013) Title? Foodborne Pathogens Dis 10:159–164

National Advisory Committee on the Microbiological Criteria for Foods (2010) Parameters for determining pack/challenge study protocols. J Food Prot 73:140–202

PCA Letter to Employees (2009) 12 January 2009 http://www.marlerblog.com/files/2013/01/pcaemailtolightsey.1.12.2009.pdf. Accessed 20 Jan 2014

Challenges in the Control of Foodborne Pathogens in Low-Water Activity Foods and Spices

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Abstract Vegetative cells of some foodborne pathogens, as well as bacterial spores, are capable of surviving within dry processing environments and low-water activity foods for lengthy periods of time. Between 2007 and 2012, there were 7,315 cases of bacterial infection and 63 deaths due to contaminated low-water activity foods worldwide. *Salmonella* is the pathogen of greatest concern, responsible for 94 % of US low-water activity food recalls and 53 % of outbreaks worldwide from 2007 to 2012. This chapter provides an overview of the challenges of controlling foodborne microorganisms in low-water activity foods, including a description of practices for preventing pre- and post-process contamination. Potential sources of bacterial contamination in the food supply chain include incoming raw materials, the external environment (e.g., pests, water, and air), inadequate hygienic facility and equipment design, inadequate sanitation practices, and lack of process control. Primary control points include the sourcing of raw commodities and ingredients, controlling cross-contamination from harvest to post-process, controlling the entry of water into dry processing areas, employing effective dry cleaning and sanitation processes, and implementing validated lethal processes.

Keywords Low-water activity foods • Spices • Dry food processing

Introduction

Low-water activity foods are those with a water activity (a_w) level less than 0.70 (Blessington et al. 2013), and they do not support the growth of either vegetative or spore-forming bacteria. However, in the United States, 5,141 low-water activity food products were recalled from 2007 to 2012 because of bacterial pathogen

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contamination (FDA 2012). In 2009, three major recalls in the USA, involving pistachio nuts (662 products), dry milk (286 products), and peanut butter (3,918 products), were examples of serious health and economic burdens due to pathogen contamination of low-water activity foods. In 2012, peanut butter contaminated with *Salmonella* Bredeney resulted in 42 cases of infection and a large number of recalled products (CDC 2012a; FDA 2012). From 2007 to 2012, a total of 41 outbreaks (worldwide reports) involving low-water activity foods resulted in 7,315 cases leading to 536 hospitalizations and 63 deaths (CDC 2012a; EFSA 2009, 2010, 2011; Harris et al. 2012; Rodriguez-Urrego et al. 2008; SFI 2012).

The presence, survival, and heat resistance of bacterial pathogens in low-water activity foods provide a continuing challenge to the food industry (Podolak et al. 2010). Low-water activity foods include products which may or may not be subjected to a pathogen inactivation step. These foods may also contain ingredients that are added after the inactivation step (Scott et al. 2009). Reduction of risk for human illness can be achieved by controlling points of potential contamination in the field, during harvesting, processing, and distribution, as well as in retail markets, food-service facilities, or the home. The effective application of such controls requires a coordinated effort between production, processing, and distribution. This chapter presents an overview of the challenges in controlling bacterial foodborne pathogens in low-water activity foods, with particular emphasis on available practices to prevent pre- and post-process contamination. Mycotoxigenic molds and their toxins will not be addressed.

Pathogens of Concern in Low-Water Activity Foods and Spices

Vegetative cells and endospores of many foodborne pathogens can survive in dry processing environments and low-water activity foods for lengthy periods of time (Beuchat et al. 2013). Disease outbreaks reported by various countries that were associated with low-water activity foods from 2007 to 2012 are presented in Table 1. Recalls of low-water activity foods in the USA from 2007 to 2012 are listed in Tables 2 and 3. Table 2 includes recalls of food products at the preprocessing stage (pre- and postharvest). Table 3 lists recalls of processed foods, some of which may not have undergone a lethal processing step. Bacterial pathogens which have been associated with outbreaks and/or recalls involving low-water activity foods during 2007 to 2012 include *Bacillus cereus*, *Clostridium botulinum*, *Clostridium perfringens*, enterohemorrhagic *Escherichia coli*, *Listeria monocytogenes*, *Salmonella enterica*, and *Staphylococcus aureus* (Tables 1, 2, and 3). *Cronobacter* spp. (formerly, *Enterobacter sakazakii*) has rarely been the cause of reported outbreaks (Beuchat et al. 2013). However, *Cronobacter* may be present and survive for long periods of time in powdered infant formula; hence, it is a pathogen of concern in these foods.

Table 1 Foodborne disease outbreaks associated with low-water activity foods between 2007 and 2012, worldwide

Stage[a]	Year	Microorganism	Product	Cases	Location	Reference
NA[b]	2008	*Salmonella* spp.	Rice, seeds, nuts, and almonds	2	EU	(EFSA 2010)
	2007 2007	*B. cereus*	Rice, seeds, nuts, and almonds	62		(EFSA 2009)
		E. coli STEC		45		
		Salmonella spp.		34		
		S. aureus		26		
		Staphylococcus spp.		120		
		B. cereus	Herbs and spices	149		
		C. perfringens		19		
		Salmonella spp.		3		
Preprocessing (pre- and postharvest)	2011	*E. coli* O104:H4	Fenugreek seeds	4321	Germany	(EFSA 2011)
	2009	*E. coli* O157:H7	Raw cookie dough	77	USA (30 states)	(CDC 2012a)
	2007	*S.* Weltevreden	Alfalfa seeds	25	EU (Scandinavia)	(SFI 2012)
No lethality	2011	*E. coli* O157:H7	Raw shelled walnuts	11	Canada	(CDC 2012a)
		E. coli O157:H7	In-shell hazelnuts	8	USA (3 states)	
	2008	*S.* Rissen	White ground pepper	87	USA (Multistate)	
Ingredient in processed food	2011	*S.* Enteritidis	Turkish pine nuts	43	USA (5 states)	(CDC 2012a)
	2010	*S.* Montevideo	Black pepper in salami	272	USA (44 states)	
Processed food	2012	*S.* Bredeney	Peanut butter	42	USA (20 states)	(CDC 2012a)
		S. Infantis	Dry dog food	49	USA (20 states)	
		S. Oranienburg	Dry milk	16	Russia	(SFI 2012)
	2010	*B. cereus*	Rice	103	USA (Florida)	(CDC 2012a)
				6	USA (Pennsylvania)	
				22	USA (Tennessee)	
		C. botulinum	Dried tofu	NA	Taiwan	(SFI 2012)

(continued)

Table 1 (continued)

Stage[a]	Year	Microorganism	Product	Cases	Location	Reference
	2009	*B. cereus*	Rice	13	USA (Alabama)	(CDC 2012a)
				15	USA (Washington)	
		S. Montevideo	Pistachios roasted	NA	USA	
		S. Enteritidis	Fried rice	11	USA (Virginia)	
	2008	*B. cereus*	Rice	10	USA (Georgia)	(CDC 2012a)
		C. perfringens	Spanish rice	2	USA (Colorado)	
		S. Kedougou	Infant formula	42	Spain	(Rodriguez-Urrego et al. 2008)
		S. Agona	Rice cereals	28	USA (15 states)	(CDC 2012a)
		S. Enteritidis	Rice	82	USA (Washington)	
		Salmonella spp.	Sweets and chocolate	4	EU	(EFSA 2010)
		Salmonella spp.	Infant formula	8	France	(SFI 2012)
		S. Typhimurium	Peanut butter	714	USA (46 states)	(CDC 2012a)
		C. botulinum	Peanut butter	5	Canada	(Harris et al. 2012)
	2007	*B. cereus*	Fried rice	8	USA (Washington)	(CDC 2012a)
		S. Schwarzengrund	Dry pet food	62	USA (18 states)	
		Salmonella spp.	Sweets and chocolate	242	EU	(EFSA 2009)
		Salmonella spp.	Imperial rice	27	USA (Florida)	(CDC 2012a)
		S. Tennessee	Peanut butter	425	USA (44 states)	
		S. Wandsworth	Rice and corn snack	75	USA (20 states)	

[a]Stage of the food chain where contamination took place
[b]Information was not specified regarding the source

Table 2 Recalls associated with low-water activity foods not receiving a lethality treatment or at a preprocessing (pre- and postharvest) stage in the USA between 2007 and 2012 (FDA 2012)

Stage[a]	Year	Microorganism	Product implicated
Preprocessing	2010	*Salmonella* spp.	Black pepper
			Garlic powder
	2009		Hazelnut
No lethality treatment	2012	*E. coli* O157:H7	Organic cacao nibs
	2011	*E. coli* O157:H7	Hazelnut and mixed nut products
		Salmonella spp.	Bulk Turkish pine nuts
			Organic celery seed
			Pine nuts
			Peppermint organic tea
			Soybean flour and soy meal
	2010	*Salmonella* spp.	Black pepper
			Black peppercorns
			Nutmeg
			Nuts
			Seasonings
			Sesame seeds
			Shelled walnuts
			Spice packages
			Pistachio kernel products
			Seasoning salt
			Gravy mix
			Onion dip mix
			Seasoning mixes
			Dip mix
			Soup mix
			Sauce mix
			Soy grits and flour
	2009	*Salmonella* spp.	Hazelnut
			Hazelnut kernels
			Raw shelled hazelnut kernels
			Red pepper
			Shelled hazelnuts and shelled organic hazelnuts
			White and black pepper
			Curry spice
	2007	*Salmonella* spp.	Parsley powder dehydrated bulk
			Four-cheese risotto mix
Preprocessing/no lethality treatment[b]	2010	*Salmonella* spp.	Spices, spice blends, rub, seasoning
			Walnuts
			Whole raw pistachios and kernels
	2009		Shelled hazelnuts
			Raw hazelnut kernels

[a]Stage of the food chain where contamination occurred
[b]Recall included products which received no lethality treatment and products that were contaminated at preprocessing (pre- and postharvest)

Table 3 Recalls associated with processed low-water activity foods in the USA between 2007 and 2012 (FDA 2012)

Microorganism	Year	Product implicated
C. botulinum	2008	Chai concentrate
L. monocytogenes	2012	Popcorn with select flavors
	2010	Peanut butter, dips, spreads, cheese, salsa
	2007	Italian sausage hoagie
Salmonella spp.	2012	Crush roasted Thai red pepper
		Crackers
		Prebiotic powder formula
		Dog food
		Flake fish food
	2011	Dry cat food
		Natural peanut butter chunky
		Peanut butter
		Powdered protein products
		Snack products with chili
	2010	Cat food
		Corn chips
		Pet food
		Egg noodle
		Pancake, cake, and cookie mix
		Potato chips
		Potato crisps
		Pretzels
		Spreads
		Batter mix
		Cheese ball mix
		Mixes containing pretzels
		Snack mix
		Snack mix and cashews
		Barbecue potato chips
	2009	Dry roasted hazelnut kernels
		Chocolate-covered peanuts
		Instant oatmeal variety pack
		Maple and brown sugar instant oatmeal
		Variety pack instant oatmeal
		Dry roasted hazelnut kernels
		Instant nonfat dried milk, whey protein (286 products)
		Peanut butter and paste (3,918 products)
	2008	Pancake and waffle mix
		Pet food
		Puffed rice wheat cereals
		Dry pet food
	2007	Peanut butter
		Roasted sesame tahini
		Topping, chocolate and peanut butter flavor
		Corn sticks
		Sweet dairy whey powder
		White chocolate baking squares

Bacillus cereus is widespread in nature (Granum 2007) and has been involved in four outbreaks between 2007 and 2012 that were associated with consumption of cooked rice served in restaurants, banquets, prison, or the home (Table 1). *B. cereus* was also responsible for two outbreaks in the EU involving cereals, as well as herbs and spices (Table 1). The presence of *B. cereus* has not been the cause of low-water activity product recalls between the years 2007 and 2012 in the USA (Tables 2 and 3). *B. cereus* is frequently found in raw materials such as vegetables, starch, and spices, due to its presence in soil and on growing plants, in food ingredients such as flours, and in dry foods such as milk powders and cereals. However, *B. cereus* must grow in a food to large numbers (>10^5 CFU/g) to cause an outbreak. Spores of *B. cereus* can survive in dry foods and dry processing environments for long periods of time. These spores can then germinate and grow in reconstituted products that are not properly processed and/or stored (Beuchat et al. 2013). The temperature and moisture conditions in which processed food is stored are critical factors to preventing spore germination and outgrowth.

Similarly, *C. botulinum* spores are commonly present in soil and the intestinal tract of animals (Johnson 2007). Botulism can result from the ingestion of botulinum neurotoxin preformed in foods. *C. botulinum* spores can also grow and produce the toxin in the gastrointestinal (GI) tract under certain conditions. Outbreaks of botulinum intoxication in the last five years were associated with the consumption of peanut butter (Canada, 2008) and dried tofu (Taiwan, 2010) (Table 1). In 2003, the presence of *C. botulinum* spores in honey resulted in a severe case of infant botulism in the USA (Barash et al. 2005). Isolates from the honey reflected those found in local soil (Beuchat et al. 2013).

Like *C. botulinum*, *C. perfringens* is also present throughout the natural environment, including soil, foods, dust, and the intestinal tract of animals (McClane 2007). Spores of *C. perfringens* survive well in dust and on surfaces and are resistant to routine cooking temperatures (McClane 2007). *C. perfringens* produces toxins and exhibits rapid growth in many foods (McClane 2007). *C. perfringens* was responsible for an outbreak involving cooked rice in the USA (2008) and one involving herbs and spices in the EU (2007) (Table 1). Spores of *C. perfringens* have also been detected in powdered infant formula (Beuchat et al. 2013), where the bacterium would be of concern if rehydrated formula was subject to temperature abuse for growth of clostridia to occur.

Enteric pathogens, such as enterohemorrhagic *E. coli* and *Salmonella*, can contaminate raw agricultural commodities through vehicles such as raw or improperly composted manure, irrigation water containing untreated sewage, or contaminated wash water (Beuchat and Ryu 1997). Contact with animals and unpasteurized products of animal origin are additional routes of contamination (Beuchat and Ryu 1997). Contact surfaces, including human hands, are potential points of contamination (Beuchat and Ryu 1997). *Cronobacter* species such as *C. sakazakii* are also members of the *Enterobacteriaceae* family and have been isolated from dry milk. However, the natural habitat of this pathogen remains unknown (Pagotto et al. 2007). Growth of enteric pathogens in food is not necessary for them to cause illness.

Shiga toxin-producing *E. coli* has been associated with several foodborne illness outbreaks involving low-water activity foods (Table 1). A major outbreak occurred

in Germany in 2011, due to fenugreek sprouts from *E. coli* O104:H4-contaminated seeds imported from Egypt. Also, in 2011, raw shelled walnuts and hazelnuts (which do not undergo a lethal processing treatment) were the vehicle of two *E. coli* O157:H7 outbreaks in North America. *E. coli* O157:H7-contaminated raw cookie dough, not intended to be consumed raw, caused a major outbreak in the USA in 2009. The specific ingredient in the cookie dough that was contaminated was not definitively identified (Beuchat et al. 2013); however, wheat flour (a low-a_w ingredient) was suspected, influencing the manufacturer to now use heat-treated flour in its cookie dough product. Enterohemorrhagic *E. coli* is widely present in and able to colonize farm environments, but it does not survive well in low-water activity environments (Beuchat et al. 2013). This is likely why its involvement in outbreaks and recalls of low-water activity foods is more limited than that of *Salmonella*.

Salmonella is responsible for the vast majority of foodborne illness outbreaks (Table 1) and US recalls (Tables 2 and 3) associated with low-water activity foods in the past 6 years (2007–2012). *Salmonella* is widespread in nature and survives in dry foods for weeks, months, or even years (Chang et al. 2013; Beuchat and Mann 2010a; Komitopoulou and Peñaloza 2009; Beuchat and Scouten 2002; Burnett et al. 2000; Archer et al. 1998). Food composition, water activity, and temperature collectively influence its survival in foods (He et al. 2011; Mattick et al. 2001; Corry 1974; Dega et al. 1972; Moats et al. 1971). The pathogen exhibits increasing resistance at decreasing water activity during heat treatment (Beuchat and Scouten 2002; Doyle and Mazzotta 2000; Archer et al. 1998). The presence of fat in the food offers additional protection against inactivation by heat (Ma et al. 2009; Shachar and Yaron 2006; van Asselt and Zwietering 2006; Juneja and Eblen 2000). In an extensive review, conducted by Podolak et al. (2010) on the sources and risk factors of *Salmonella* in low-water activity foods, its presence was linked to cross-contamination through poor sanitation practices, substandard facility and equipment design, improper maintenance, poor operational and manufacturing practices (GMPs), as well as inadequate ingredient control and pest control. Carrasco et al. (2012) provided an in-depth review of the role that cross-contamination plays in the contamination of low-water activity foods with *Salmonella*.

Similar to *Salmonella*, *Cronobacter* species including *C. sakazakii* is able to survive in low-water activity foods for long periods of time (Caubilla Barron and Forsythe 2007; Gurtler and Beuchat 2007; Lin and Beuchat 2007). Neither *Salmonella* nor *Cronobacter* will survive typical pasteurization treatments in foods having high water activity. Hence, as with *Salmonella*, contamination of processed, low-water activity foods with *Cronobacter* usually occurs as a result of poor process control or by post-process contamination (Breeuwer et al. 2003).

Although not involved in as many low-water activity food outbreaks and recalls as *Salmonella*, *Staphylococcus aureus* is also well adapted to survival in low-water activity environments. Humans and animals are the main reservoirs of this toxin producer, which is able to survive for months after contaminating a dry food (Beuchat et al. 2013; Scott 1958). Contamination of food can occur by direct human contact, indirectly by skin fragments, or through respiratory tract droplet nuclei (Seok Seo and Bohach 2007). Sanitation and hygienic manufacturing practices and

appropriately designed facilities and equipment play a crucial role in preventing food contamination.

L. monocytogenes has not been involved in low-water activity food-associated outbreaks reported between 2007 and 2012 but has been the causative agent of several US product recalls of these foods (Table 3). *L. monocytogenes* is a well-documented contaminant of soil, water, and avian and mammalian feces (Swaminathan et al. 2007) and, because of its widespread distribution, can contaminate foods by many routes.

Origin of Pathogen Contamination in Low-Water Activity Foods

The origin of pathogen contamination in low-water activity foods depends on the history and use of the food. A number of raw materials and ingredients of primary agricultural origin are consumed without processing or are included as an added ingredient in a previously processed food (e.g., nuts, herbs, and spices). Other low-water activity raw agricultural products are used in the manufacture of processed low-water activity foods (e.g., peanuts in peanut butter), and others are intended to be cooked or heat processed before consumption (e.g., wheat flour, oats). A discussion of potential sources of contamination in the supply chain during preharvest, postharvest, processing, and post-processing follows.

Pre- and Postharvest Contamination (Preprocessing)

The likelihood of bacterial pathogens being present in a raw food at the preprocessing stage (pre- and postharvest) of the food chain is associated with the pathogens being present in the production environment. The type and population of microorganisms on nuts, seeds, cereal grains, herbs, and spices are mainly determined by the soil and plant microflora. Nuts come in contact with the soil during harvest (e.g., hazelnuts, almonds, pecans, walnuts) or growth (e.g., peanuts). Seeds (e.g., cacao beans), herbs, and spices (ICMSF 2005) may also be in contact with the soil during harvest and/or growth. A variety of bacteria can grow on cereal crops (e.g., bacilli, enteric bacteria), and cereal grains are subject to microbiological contamination while growing in the field (Gilbert et al. 2010). For example, a *Salmonella* Agona outbreak in 2002 involving herbal tea in Germany was traced back to contaminated aniseeds imported from Turkey. The source of contamination was attributed to the use of manure as a natural fertilizer (Koch et al. 2005). In 2011, in Germany, an *E. coli* O104:H4 outbreak involving 4,321 cases (Table 1) was traced back to contaminated seeds imported from Egypt. The imported fenugreek seeds were processed by a single German producer into sprouts (EFSA 2011). The European Food Safety Authority (EFSA) found no evidence of environmental contamination at the

sprout producer or signs of employee cross-contamination. Hence, the contamination was attributed to the importer, and the source of the contamination speculated to be the use of natural fertilizer or contaminated irrigation water during seed production (EFSA 2011).

Although soils are partially removed from nuts, plants, cereals, and seeds during harvest, significant amounts can still remain and thus be brought into storage and processing facilities (Danyluk et al. 2007). Kokal and Thorpe (1969) observed *E. coli* contamination on almonds obtained from both the trees and from the ground where they were left to dry. In another study by Marcus and Amling (1973), the proportion of pecan samples contaminated with *E. coli* collected from cattle-grazed orchards was sixfold higher than that of samples from non-cattle-grazed orchards. Sources of soil contamination in the field include bacteria in the farm environment, animal waste, contaminated manure/compost, biological solids from human waste water, and contaminated irrigation water (Jacobsen and Bech 2012; Critzer and Doyle 2010; Beuchat and Mann 2010b; Isaacs et al. 2005).

Water is, in general, a major source of microbial contamination in food commodities that are eventually marketed as low-water activity foods. Possible preharvest sources are runoff from nearby animal pastures and irrigation from a contaminated source (FDA 1998). The type of irrigation is also a contributing factor. There is a lower likelihood of transmitting pathogens from contaminated water through drip irrigation than an overhead sprinkler system (Berger et al. 2010; FDA 1998). The use of water in postharvest processing is also a risk factor. The addition of water to dust or dried raw materials can result in rapid outgrowth of the bacteria present (Danyluk et al. 2007). Control of water in storage and processing environments is one of the most critical factors in reducing the risk of pathogen contamination of dry foods. Water can also present suitable conditions for production of heat-stable microbial toxins by, for example, *B. cereus* (Beuchat et al. 2013).

Dust also increases the risk of cross-contamination by accumulating microorganisms that can be spread by airflow (Podolak et al. 2010; Doan and Davidson 2000). For example, when hulls and shells of almonds and macadamia nuts are mechanically removed from the kernel under dry conditions, large volumes of fine particulate matter are generated. This dust, comprised of soil, hulls, and shells, is difficult to eliminate from the processing environment and can contribute significantly to cross-contamination of the kernels (Danyluk et al. 2007). The first US reported outbreak of salmonellosis traced to chocolate occurred in 1973, where 119 cases of infection with *S.* Eastbourne were linked to contaminated raw cocoa beans (Craven et al. 1975). *Salmonella* was also detected in accumulated dust at the bean-processing areas (Craven et al. 1975). Pathogen-contaminated dust is often associated with product contamination, and the unexpected addition of water to this dust creates exceptionally high-risk conditions due to the logarithmic increase in pathogen numbers that may occur.

In addition to natural microflora, soil, water, and dust, birds, rodents, and insects all influence the risk of pathogen contamination of nuts, seeds, herbs, cereals, and spices (Danyluk et al. 2007; Doan and Davidson 2000). Worker hygiene and sanitation can also be important (FDA 1998; Podolak et al. 2010).

Sources of microbial contamination on raw potatoes and on low-water activity products derived from them include their natural microflora, soil, dust, water, and contaminants from human handling and animals (Doan and Davidson 2000). Contamination of raw milk and dairy products also results from direct contact with contaminated sources in the farm environment (e.g., soil, water, and equipment) and excretion from the udder of an infected ruminant (Oliver et al. 2005). Potatoes, however, are rarely consumed raw, and milk sold in interstate commerce is required, by law, to undergo a pasteurization process prior to commercial distribution (CFR 21 1240.61 2013). Hence, the presence of pathogenic microorganisms in low-water activity dairy as well as potato products is usually associated with either the lack of process control or post-process contamination.

Processing and Post-process Contamination

Contamination of low-water activity foods with pathogenic bacteria during processing and post-process is mainly due to cross-contamination (Carrasco et al. 2012; Podolak et al. 2010). Processed low-water activity foods receiving a pathogen inactivation treatment were responsible for 54 % of US recalls and 63 % of outbreaks (reported worldwide) between 2007 and 2012 (Tables 1 and 3). Minimally processed foods (which do not receive a lethal step) have been responsible for fewer US recalls (35 %) and outbreaks globally (12 %). Of the pathogens involved, *Salmonella* was responsible for 94 % of all low-water activity food product recalls and 53 % of total outbreaks between 2007 and 2012. Therefore, *Salmonella* is the pathogen of greatest concern in processed and minimally processed low-water activity food.

Various sources and manufacturing practices contribute to cross-contamination in the processing facility. Raw materials (Scott et al. 2009) are a major source of pathogens. Lack of effective prerequisite programs at the supplier can result in pathogens being introduced into the facility through raw materials. In some cases, the presence of pathogens in raw materials cannot be prevented by currently acceptable growing, harvesting, and preprocessing practices. In addition to directly contaminating the end product, pathogen-containing raw materials can contaminate equipment, employees, and other product lines. Raw materials which are not further processed can also contaminate finished products if they are as an ingredient subsequent to the final lethality treatment in a process. For example, in 2007, an outbreak in the USA involving *S.* Wandsworth, *S.* Typhimurium, *S.* Mbandaka, and *S.* Haifa resulted in 75 cases of infection associated with consumption of a contaminated corn and puffed rice snack (Table 1). The seasoning mix, which was used as an added ingredient after a lethal process was applied to the heat-treated cereals, was the source of the contamination. The seasonings were obtained from domestic as well as international suppliers (Sotir et al. 2009). A 2010 outbreak of 272 *S.* Montevideo infections in the USA was associated with consumption of contaminated salami (Table 1). The source of contamination was red and black pepper applied to the surface of salamis after the lethal process. The pepper was produced

in Asia, distributed by a US spice company, and finally delivered to the salami processing facility (CDC 2012b). *S.* Montevideo was isolated from samples of the crushed red and black pepper. These results underline the risk of cross-contamination from raw materials, highlighting the need for strict supplier control when raw materials are used as an added ingredient to processed food.

A second major source of pathogen cross-contamination in dry foods is the environment, which includes personnel, equipment, pests, dust, water, and air (Beuchat et al. 2013). Employees carrying pathogens via shoes or clothing worn outside the plant can lead to cross-contamination if the plant and its personnel are not adhering to good manufacturing practices (GMPs) as described in 21 CFR110 (Podolak et al. 2010; Scott et al. 2009). Pests (e.g., birds, insects, rodents) may also carry pathogens through openings in buildings that require repair (including the roofs, floors, and doors). For example, birds' nests can shed pathogens into the facility through a roof leak, unfiltered air intakes, etc. (Scott et al. 2009). Contaminated equipment entering the facility can also introduce pathogens (Scott et al. 2009). Ventilation units connected to outside air without proper filtration, as well as unsanitary vents, are an additional source of contamination from the external environment (Scott et al. 2009). A 2008–2009 salmonellosis illness outbreak associated with processed peanuts (Tables 1 and 3) resulted in one of the largest food product recalls in US history (Wittenberger and Dohlman 2010). A joint FDA and Georgia Department of Agriculture investigation of the production facility revealed multiple possibilities for sources of *Salmonella* contamination, including evidence of rain and other water leakage into areas used to store roasted peanuts, as well as practices that allowed for cross-contamination between raw and roasted peanuts (Cavallaro et al. 2011). The FDA investigation of the 2012 peanut butter outbreak (*S.* Bredeney) found raw in-shell peanuts outside the plant in uncovered trailers and exposed to rain (FDA 2013a). Birds were also observed landing on these trailers (FDA 2013a). Inside the warehouse, facility doors were open to the outside, allowing pests to enter the premises (FDA 2013a). The combination of pathogens in the processing environment and lack of water control has often led to product contamination because of the high populations of pathogens that can result if growth occurs.

A third major source of cross-contamination is the lack of proper facility and equipment design, including deficiencies in GMPs (Beuchat et al. 2013; Carrasco et al. 2012; Podolak et al. 2010). An inspection of the US dry dog food production facility associated with a 2012 outbreak of *S.* Infantis infection revealed that the processing facility did not have hygienic equipment and facility design (CDC 2012c). There were no hand washing and sanitizing facilities where needed. Equipment had gouges and cuts, with attempted repairs using duct tape and cardboard (CDC 2012c). Equipment had accumulated food residue and dust in areas with poor access to cleaning (CDC 2012c). Similarly, the investigation of the peanut butter processing facility associated with a 2012 outbreak of salmonellosis revealed that there were no hand-washing sinks in the peanut production or packaging areas (FDA 2013a). There were also no documented cleaning records (FDA 2013a). There was a leaking sink in a washroom, which resulted in water accumulation on the floor (FDA 2013a). In addition, employees had bare-hand contact

with ready-to-package peanuts and were found to improperly handle equipment and tools (FDA 2013a).

Separate hygiene zones are generally established by compartmentalization of a food processing facility. Zones should be established based on an evaluation of risk (Beuchat et al. 2013; Chen et al. 2009a). The lack of proper control measures in each zone, including the transition areas, could result in cross-contamination from the low-hygiene to high-hygiene zones (Chen et al. 2009a). This includes a lack of adequate physical separation, control of traffic and airflow, employee attire, as well as cleaning and the use of water (Beuchat et al. 2013; Chen et al. 2009a). Indeed, the use of water in the processing of low-water activity foods is one of the major risk factors for pathogen contamination (Beuchat et al. 2013; Chen et al. 2009a). Different areas of a processing facility have different water requirements. Hence, much of the microbiological control in a dry processing facility is focused on keeping areas dry to avoid resuscitation and growth of dormant microorganisms (Beuchat et al. 2013; Chen et al. 2009a). One of the most difficult challenges is to design and operate a processing facility that can be properly cleaned and sanitized, while maintaining separation of dry and wet areas of the facility. Lack of proper hygiene practices and controls can ultimately lead to pathogens finding a growth niche in the facility (Chen et al. 2009a). Bacteria attached to surfaces in the presence of water are less sensitive to disinfection, especially when present in a biofilm (Aviles et al. 2013; Møretrø et al. 2012). Hence, an environment that may appear marginally dustier after dry cleaning may be much lower risk than a wet-cleaned environment without visual dust. This is because wet cleaning introduces moisture into cracks and areas that are difficult to clean and may not fully dry before production start-up (Chen et al. 2009a). This can ultimately lead to the development of large concentrations of microorganisms in hidden growth niches.

Finally, for low-water activity foods that are subjected to a lethal process, a major source of pathogen contamination is a lack of adequate process control (Podolak et al. 2010; Chen et al. 2009b). This may be the result of using unvalidated or inadequately controlled processing operations. Generic lethality processing standards for high-moisture foods should not be used for low-water activity foods, as they may result in inadequate pathogen inactivation (Podolak et al. 2010). For example, in the *Salmonella* outbreak investigation of Georgia processed peanuts in 2008–2009, there was uncertainty as to whether the peanut roaster routinely reached a temperature sufficient to kill *Salmonella* (Cavallaro et al. 2011).

Current Challenges in the Control of Foodborne Pathogens in Low-Water Activity Foods

Controlling foodborne pathogens in low-water activity foods is an ongoing challenge. Raw materials may be produced under conditions in which the entry of pathogens into the food is not or cannot be controlled. Dry foods may be contaminated with pathogens during harvest and storage and through preprocessing environments.

Control of pests, dust, and water is essential for pathogen control in these foods, yet facility and equipment design may be inadequate to control these factors and conventional wet cleaning/sanitation practices may promote pathogen survival and growth by not controlling water. Many low-water activity food products are considered ready to eat and are not cooked before consumption as well as have a long shelf life; hence, the production process must comply with high standards of hygiene. Parameters for inactivation of pathogens in processing dry foods are often not well defined or validated. As such, many products have no history of being subject to validated pathogen-killing processes. The fact that pathogens survive during storage of these foods for long periods of time and that these foods often have a long shelf life substantially increases their risk to public health.

Processing Practices to Reduce Pathogen Contamination in Low-Water Activity Foods

Potential sources of pathogen contamination in low-water activity foods and ingredients are numerous and varied; hence, multiple processing practices specific for the product are usually necessary for reducing the risk of contamination. The Grocery Manufacturers Association (GMA) has developed guidelines to control *Salmonella* in processing facilities and enhance the microbial safety of low-water activity foods (GMA 2009). An industry handbook for safe processing of nuts was also developed (GMA 2010a) as well as equipment (GMA 2010b) and facility (GMA 2010c) design checklists to self-evaluate compliance with GMA sanitary design principles. Additionally, in light of the burden of foodborne illnesses in the USA, the FDA has issued the Food Safety Modernization Act (FSMA) rule, which was signed into law in January 2011. This law shifts the focus of regulators from responding to an event to preventing one from occurring, using a risk-based approach (FDA 2013b). Guidance for industry and rules related to the FDA FSMA include produce food safety standards, preventive controls for human food, a foreign supplier verification program, preventive controls for animal food, and accredited third-party certification (FDA 2013b). Guidance documents advise food processors to assess the risk that ingredients may contain pathogens. For this, a series of questions involving the nature of the product, the type of treatment used on the product, the use of the product, whether the process to kill pathogens was validated, and the cooking instructions for consumers must be addressed (Beuchat et al. 2013). Good agricultural, manufacturing, and hygienic practices need to be employed at every step in the processing/manufacturing chain. In the processing facility, segregated hygiene areas must be established based on the need for moisture control and exposure of product to the environment, and an environmental monitoring program must be established for the pathogen of greatest risk. Hygienic principles of equipment design and installation need to address the need for water control and dry and wet cleaning. An overview of available practices during preharvest, postharvest, processing, and post-processing follows.

Practices to Reduce Contamination at Pre- and Postharvest (Preprocessing)

Preharvest and harvest control of pathogen contamination is critical for low-water activity foods that do not undergo a lethal processing treatment. To mitigate the risk of pathogens in the soil, growers should adopt measures to ensure that animal waste from adjacent fields or waste storage facilities does not contaminate production sites (FDA 1998; Simonne and Treadwell 2008; Gilbert et al. 2010). This includes providing barriers to secure manure storage and treatment areas and implementing good agricultural practices to minimize leachate (FDA 1998; Simonne and Treadwell 2008). Wildlife, farm, and other domestic animals should be excluded from the fields (FDA 1998; Simonne and Treadwell 2008). Additionally, the sources, distribution, and quality of water should be identified, and the risk of pathogen contamination from such sources should be assessed (FDA 1998). Wells should be maintained in proper working condition, water should be tested for microbiological quality periodically, and good agricultural practices (GAPs) should be followed (FDA 1998; Gilbert et al. 2010). GAPs with regard to fertilization and irrigation should be in place (Simonne and Treadwell 2008; Gilbert et al. 2010). Best practices for each product should be individually assessed, and training programs in worker health and hygiene must be established (FDA 1998; Simonne and Treadwell 2008). Toilet facilities should be accessible, properly located, and clean (with handwashing stations and proper sewage disposal) (FDA 1998; Simonne and Treadwell 2008). Storage facilities should be cleaned prior to use (FDA 1998; Gilbert et al. 2010). Harvesting and packaging equipment should be appropriately designed and used and kept clean (FDA 1998; Gilbert et al. 2010). As much dirt and mud as possible should be removed from the product before it leaves the field (FDA 1998). Ensuring the raw agricultural product is quickly and thoroughly dried is crucial (Beuchat et al. 2011).

Reducing Contamination During Processing and Post-process (Including Minimally Processed Low-Water Activity Food)

The first step in reducing contamination during processing is to control the quality of incoming raw materials and ingredients. Knowledge about ingredient suppliers and verifying the effectiveness of their control programs is important. For this, a supplier approval program should be in place (Scott et al. 2009). The supplier control program is of particular importance for raw or processed ingredients, which are added to the final product after the latter has gone through an inactivation step (e.g., spices and nuts). Following a sampling and testing plan such as that established by The International Commission on Microbiological Specifications for Foods (ICMSF) case 15 ($n=60$, $c=0$, $m=0$) wherein each of the 60 samples is 25 g is useful, realizing that sampling and testing alone does not ensure the absence of pathogens (Scott et al. 2009).

Ensuring that there is no post-process recontamination of the product is the next step to mitigating pathogen contamination (Beuchat et al. 2011). For this, compartmentalization of the facility into processing lines, wet and dry zones, and hygiene zones should be established by physical means (Beuchat et al. 2013). Wet processing lines should be constructed according to the same criteria as ultrahigh temperature (UHT) processing lines, with cleaning being conducted in a separate room from the main line (Beuchat et al. 2011). Within the dry zones, different hygiene zones must be established, where appropriate and traffic within different hygiene zones must be controlled (Beuchat et al. 2011). Dry zones should be kept dry and cleaned using a dry cleaning/sanitation process (Beuchat et al. 2011; Gilbert et al. 2010). Monitoring programs targeting the external environment, employee sanitation, operational and manufacturing practices, and presence of pests must also be established, as well as hygienic design of buildings and equipment (Chen et al. 2009a; Gilbert et al. 2010).

Another important aspect of pathogen control efforts is process control and validation (Beuchat et al. 2011; Chen et al. 2009b). Food processors should determine the process parameters that provide an appropriate level of protection (ALOP) for each product. Validation of the selected process parameters is critical (Beuchat et al. 2013; Chen et al. 2009b).

Finally, verification that the validated control measures are properly functioning, that standard operating procedures as well as hygienic practices are in place (verified by environmental monitoring), and that overall the final product adheres to predefined safety criteria (including finished product microbiological testing) assures the effectiveness of the food safety management system that is in place and drives continuous improvement (Chen et al. 2009b).

Conclusions

Major challenges for pathogen control in low-water activity foods include sourcing of low-risk raw commodities and ingredients, controlling cross-contamination from harvest to post-process, and implementing and monitoring validated lethal processes. The ability of pathogens to survive for long periods of time in dry foods, ingredients, and processing environments greatly increases the challenge of controlling pathogens in low-water activity foods. Food processors should assess the risk of pathogen contamination of the product and ingredients. Effective controls for incoming raw materials and ingredients should be in place as well as monitoring programs addressing the external environment, employee sanitation, operational and manufacturing practices, and presence of pests, as well as appropriate hygienic design of building and equipment. Process control, which includes validation and verification of control measures, is essential to increase the effectiveness of food safety management systems and effectively reduce risks to public health.

References

Archer J, Jervis ET, Bird J, Gaze JE (1998) Heat resistance of *Salmonella* Weltevreden in low-water activity environments. J Food Prot 61:969–973

Aviles B, Klotz C, Eifert J, Williams R, Ponder M (2013) Biofilms promote survival and virulence of *Salmonella enterica* sv. Tennessee during prolonged dry storage and after passage through an in vitro digestion system. Int J Food Microbiol 162(3):252–259

Barash JR, Tang TW, Arnon SS (2005) First case of infant botulism caused by *Clostridium baratii* type F in California. J Clin Microbiol 43:4280–4282

Berger CN, Sodha SV, Shaw RK, Griffin PM, Pink D, Hand P, Frankel G (2010) Fresh fruit and vegetables as vehicles for the transmission of human pathogens. Environ Microbiol 12:2385–2397

Beuchat LR, Mann DA (2010a) Factors affecting infiltration and survival of *Salmonella* on in-shell pecans and pecan nutmeats. J Food Prot 73:1257–1268

Beuchat LR, Mann DA (2010b) Survival and growth of *Salmonella* in high-moisture pecan nutmeats, in-shell pecans, inedible nut components, and orchard soil. J Food Prot 73:1975–1985

Beuchat LR, Ryu JH (1997) Produce handling and processing practices. Emerg Infect Dis 3:459–465

Beuchat LR, Scouten A (2002) Combined effects of water activity, temperature and chemical treatments on the survival of *Salmonella* and *Escherichia coli* O157:H7 on alfalfa seeds. J Appl Microbiol 92:382–395

Beuchat LR, Komitopoulou E, Beckers H, Betts RP, Bourdichon F, Fanning S, Jousten MH, Ter Kuile BH (2013) Low-water activity foods: Increased concern as vehicles of foodborne pathogens. J Food Protect 76:150–172

Beuchat L, Komitopoulou E, Betts R, Beckers H, Bourdichon F, Fanning S, Ter Kuile BH (2011) Persistence and survival of pathogens in dry foods and dry food processing environments. International Life Sciences Institute – Europe Report Series 2011:1–48. http://www.ilsi.org/Europe/Documents/Persistence%20and%20survival%20report.pdf. Accessed Aug 2012

Blessington T, Theofel CG, Harris LJ (2013) A dry-inoculation method for nut kernels. Food Microbiol 33:292–297

Breeuwer P, Lardeau A, Peterz M, Joosten H (2003) Desiccation and heat tolerance of *Enterobacter sakazakii*. J Appl Microbiol 95:967–973

Burnett S, Gehm E, Weissinger W, Beuchat L (2000) Survival of *Salmonella* in peanut butter and peanut butter spread. J Appl Microbiol 89:472–477

Carrasco E, Morales-Rueda A, Garcia-Gimeno RM (2012) Cross-contamination and recontamination by *Salmonella* in foods: a review. Food Res Int 45:545–556

Caubilla Barron J, Forsythe SJ (2007) Dry stress and survival time of *Enterobacter sakazakii* and other *Enterobacteriaceae* in dehydrated powdered infant formula. J Food Protect 70:2111–2117

Cavallaro E, Date K, Medus C, Meyer S, Miller B, Kim C, Nowicki S, Cosgrove S, Sweat D, Phan Q, Flint J, Daly ER, Adams J, Hyytia-Trees E, Gerner-Smidt PG, Hoekstra RM, Schwensohn C, Langer A, Sodha SV, Rogers MC, Angulo FJ, Tauxe RV, Williams IT, Barton Behravesh C (2011) *Salmonella* Typhimurium infections associated with peanut products. N Engl J Med 365:601–610

CDC (Centers for Disease Control and Prevention) (2012a) Foodborne outbreak online database. http://wwwn.cdc.gov/foodborneoutbreaks/. Accessed Aug 2012

CDC (Centers for Disease Control and Prevention) (2012b) *Salmonella* Montevideo infections associated with salami products made with contaminated imported black and red pepper – United States, July 2009–April 2010 Morbid Mortal Weekly Rep (MMWR) http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5950a3.htm?s_cid=mm5950a3_w. Accessed Jan 2013

CDC (Centers for Disease Control and Prevention) (2012c) Multistate outbreak of human *Salmonella* infantis infections linked to dry dog food. http://www.cdc.gov/salmonella/dog-food-05-12/index.html. Accessed Jan 2013

CFR (Code of Federal Regulation) 21 140.61 (2013) http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=1240.61. Accessed Aug 2013
Chang AS, Sreedharan A, Schneider KR (2013) Peanut and peanut products: a food safety perspective. Food Control 32:296–303
Chen YH, Scott VN, Freier TA, Kuehm J, Moorman M, Meyer J, Morille-Hinds T, Post L, Smoot L, Hood S, Shebuski J, Banks J (2009a) Control of *Salmonella* in low-water activity foods II: hygiene practices to minimize *Salmonella* contamination and growth. Food Prot Trends 29:435–445
Chen YH, Scott VN, Freier TA, Kuehm J, Moorman M, Meyer J, Morille-Hinds T, Post L, Smoot L, Hood S, Shebuski J, Banks J (2009b) Control of *Salmonella* in low-water activity foods III: process validation and environmental monitoring – part three of a three-part series. Food Prot Trends 29:493–508
Corry JEL (1974) The effect of sugars and polyols on the heat resistance of Salmonellae. J Appl Microbiol 37:31–43
Craven P, Baine W, Mackel D, Barker W, Gangarosa E, Goldfield M, Rosenfeld H, Altman R, Lachapelle G, Davies JW, Swanson RC (1975) International outbreak of *Salmonella eastbourne* infection traced to contaminated chocolate. Lancet 305:788–792
Critzer FJ, Doyle MP (2010) Microbial ecology of foodborne pathogens associated with produce. Curr Opin Biotech 21:125–130
Danyluk MD, Harris LJ, Sperber WH (2007) Nuts and cereals. In: Doyle MP, Beuchat L (eds) Food microbiology: fundamentals and frontiers, 3rd edn. ASM Press, Washington, DC, pp 171–183
Dega CA, Goepfert JM, Amundson CH (1972) Heat resistance of *Salmonellae* in concentrated milk. Appl Microbiol 23:415–420
Doan CH, Davidson PM (2000) Microbiology of potatoes and potato products: a review. J Food Protect 63:668–683
Doyle ME, Mazzotta AS (2000) Review of studies on the thermal resistance of *salmonellae*. J Food Protect 63:779–795
EFSA (European Food Safety Authority) (2011) Shiga toxin-producing *E. coli* (STEC) O104:H4 2011 outbreaks in Europe: taking stock. EFSA J 9:2390. http://www.efsa.europa.eu/en/efsajournal/doc/2390.pdf. Accessed Aug 2012
EFSA (European Food Safety Authority) (2010) The Community Summary Report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in the European Union in 2008. EFSA J 8:1496. http://www.efsa.europa.eu/en/efsajournal/pub/1496.htm. Accessed Aug 2012
EFSA (European Food Safety Authority) (2009) The community summary report on food-borne outbreaks in The European Union in 2007. http://www.efsa.europa.eu/en/efsajournal/pub/271r.htm. Accessed Aug 2012
Food and Drug Administration (FDA) (1998) Guide to minimize microbial food safety hazards for fresh fruits and vegetables. http://www.fda.gov/downloads/Food/GuidanceComplianceRegulatoryInformation/GuidanceDocuments/ProduceandPlanProducts/UCM169112.pdf. Accessed Aug 2012
Food and Drug Administration (FDA) (2012) Archive for recalls, market withdrawals & safety alerts. http://www.fda.gov/Safety/Recalls/ArchiveRecalls/default.htm. Accessed Dec 2012
Food and Drug Administration (FDA) (2013a) FDA investigation summary: multistate outbreak of *Salmonella* Bredeney infections linked to peanut butter made by Sunland Inc. http://www.fda.gov/food/foodsafety/corenetwork/ucm320413.htm#problem. Accessed Feb 2013
Food and Drug Administration (FDA) (2013b) The new FDA Food Safety Modernization Act (FSMA). http://www.fda.gov/Food/FoodSafety/FSMA/default.htm. Accessed Feb 2013
Gilbert S, Lake R, Cressey P, King N (2010) Risk profile: *Salmonella* (non-typhoidal) in cereal grains. Prepared for New Zealand Food Safety Authority under project MRP/09/01. http://www.foodsafety.govt.nz/elibrary/industry/salmonella-in-cereals.pdf. Accessed Dec 2012
Granum E (2007) *Bacillus cereus*. In: Doyle MP, Beuchat L (eds) Food microbiology: fundamentals and frontiers, 3rd edn. ASM Press, Washington, DC, pp 445–455

Grocery Manufacturers Association (GMA) (2009) Control of *Salmonella* in low-water activity foods. http://www.gmaonline.org/downloads/technical-guidance-and-tools/SalmonellaControlGuidance.pdf. Accessed Aug 2012

Grocery Manufacturers Association (GMA) (2010a) Industry handbook for safe processing of nuts. http://www.gmaonline.org/downloads/technical-guidance-and-tools/Industry_Handbook_for_Safe_Processing_of_Nuts_1st_Edition_22Feb10.pdf. Accessed Aug 2012

Grocery Manufacturers Association (GMA) (2010b) Equipment design checklist for low-water activity foods. http://www.gmaonline.org/resources/research-tools/technical-guidance-and-tools. Accessed Aug 2012

Grocery Manufacturers Association (GMA) (2010c) Facility design checklist. http://www.gmaonline.org/resources/research-tools/technical-guidance-and-tools. Accessed Aug 2012

Gurtler JB, Beuchat LR (2007) Survival of *Enterobacter sakazakii* in powdered infant formula as affected by composition, water activity, and temperature. J Food Protect 70:1579–1586

Harris LJ, Beuchat LR, Danyluk MD, Palumbo M (2012) USDA NIFSI, 2009-01951. http://ucfoodsafety.ucdavis.edu/files/132896.pdf. Accessed Dec 2012

He YS, Guo DJ, Yang JY, Tortorello ML, Zhang W (2011) Survival and heat resistance of *Salmonella enterica* and *Escherichia coli* O157:H7 in peanut butter. Appl Environ Microbiol 77:8434–8438

International Commission on Microbiological Specifications for Foods (ICMSF) (2005) Microorganisms in foods 6: microbial ecology of food commodities, Kluwer, NY

Isaacs S, Aramini J, Ciebin B, Farrar JA, Ahmed R, Middleton D, Chandran AU, Harris LJ, Howes M, Chan E, Pichette AS, Campbell K, Gupta A, Lior LY, Pearce M, Clark C, Rodgers F, Jamieson F, Brophy I, Ellis A (2005) An international outbreak of salmonellosis associated with raw almonds contaminated with a rare phage type of *Salmonella* enteritidis. J Food Protect 68:191–198

Jacobsen CS, Bech TB (2012) Soil survival of *Salmonella* and transfer to fresh water and fresh produce. Food Res Int 45:557–566

Johnson E (2007) *Clostridium botulinum*. In: Doyle MP, Beuchat L (eds) Food microbiology: fundamentals and frontiers, 3rd edn. ASM Press, Washington, DC, pp 401–422

Juneja VK, Eblen BS (2000) Heat inactivation of *Salmonella typhimurium* DT104 in beef as affected by fat content. Lett Appl Microbiol 30:461–467

Koch J, Schrauder A, Alpers K, Werber D, Frank C, Prager R, Rabsch W, Broll S, Feil F, Roggentin P, Bockemühl J, Tschäpe H, Ammon A, Stark K (2005) *Salmonella* Agona outbreak from contaminated aniseed, Germany. Emerg Infect Dis 11:1124–1127

Kokal D, Thorpe DW (1969) Occurrence of *Escherichia coli* in almonds of nonpareil variety. Food Technol 23:93–98

Komitopoulou E, Peñaloza W (2009) Fate of *Salmonella* in dry confectionery raw materials. J Appl Microbiol 106:1892–1900

Lin L-C, Beuchat LR (2007) Survival of *Enterobacter sakazakii* in infant cereal as affected by composition, water activity, and temperature. Food Microbiol 24:767–777

Ma L, Zhang G, Gerner-Smidt P, Mantripragada V, Ezeoke I, Doyle MP (2009) Thermal inactivation of *Salmonella* in peanut butter. J Food Protect 72:1596–1601

McClane B (2007) *Clostridium perfringens*. In: Doyle MP, Beuchat L (eds) Food microbiology: fundamentals and frontiers, 3rd edn. ASM Press, Washington, DC, pp 423–444

Marcus KA, Amling HJ (1973) *Escherichia coli* field contamination of pecan nuts. Appl Microbiol 26:279–281

Mattick KL, Jorgensen F, Wang P, Pound J, Vandeven MH, Ward LR, Legan JD, Lappin-Scott HM, Humphrey TJ (2001) Effect of challenge temperature and solute type on heat tolerance of *Salmonella* serovars at low water activity. Appl Environ Microbiol 67:4128–4136

Moats WA, Dabbah R, Edwards VM (1971) Survival of *Salmonella anatum* heated in various media. App Microbiol 21:476–481

Møretrø T, Heir E, Nesse LL, Vestby LK, Langsrud S (2012) Control of *Salmonella* in food related environments by chemical disinfection. Food Res Int 45:532–544

Oliver SP, Jayarao BM, Almeida RA (2005) Foodborne pathogens in milk and the dairy farm environment: food safety and public health implications. Foodborne Pathog Dis 2:115–129

Pagotto FJ, Lenati RF, Farber JM (2007) *Enterobacter sakazakii*. In: Doyle MP, Beuchat L (eds) Food microbiology: fundamentals and frontiers, 3rd edn. ASM Press, Washington, DC, pp 271–291

Podolak R, Enache E, Stone W, Black DG, Elliott PH (2010) Sources and risk factors for contamination, survival, persistence, and heat resistance of *Salmonella* in low-water activity foods. J Food Protect 73:1919–1936

Rodriguez-Urrego J, Herrera-León S, Echeita-Sarriondia A, Soler P, Simon F, Mateo S (2008) Nationwide outbreak of *Salmonella* serotype Kedougou associated with infant formula, Spain. Euro Surveill 15:19582

Safe Food International (SFI) (2012) Food/water borne illness outbreaks. http://regionalnews.safefoodinternational.org/page/Food%2FWater+Borne+Illness+Outbreaks. Accessed Aug 2012

Shachar D, Yaron S (2006) Heat tolerance of *Salmonella enterica* serovars Agona, Enteritidis, and Typhimurium in peanut butter. J Food Protect 69:2687–2691

Scott WJ (1958) The effect of residual water on the survival of dried bacteria during storage. J Gen Microbiol 19:624–633

Scott V, Chen Y, Freier T, Kuehm J, Moorman M, Meyer J, Morille-Hinds T, Post L, Smoot L, Hood S, Shebuski J, Banks J (2009) Control of *Salmonella* in low-water activity foods I: minimizing entry of *Salmonella* into a processing facility. Food Protect Trends 29:342–353

Seok Seo K, Bohach GA (2007) *Staphylococcus aureus*. In: Doyle MP, Beuchat L (eds) Food microbiology: fundamentals and frontiers, 3rd edn. ASM Press, Washington, DC, pp 493–518

Simonne A, Treadwell D (2008) Minimizing food safety hazards for organic growers. Department of Family, Youth, and Community Sciences, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida. http://edis.ifas.ufl.edu/pdffiles/FY/FY106200.pdf. Accessed Aug 2012

Sotir MJ, Ewald G, Kimura AC, Higa JI, Sheth A, Troppy S, Meyer S, Hoekstra RM, Austin J, Archer J, Spayne M, Daly ER, Griffin PM (2009) Outbreak of *Salmonella* Wandsworth and Typhimurium infections in infants and toddlers traced to a commercial vegetable-coated snack food. Pediatr Infect Dis J 28:1041–1046

Swaminathan B, Cabanes D, Zhang W, Cossart P (2007) *Listeria monocytogenes*. In: Doyle MP, Beuchat L (eds) Food microbiology: fundamentals and frontiers, 3rd edn. ASM Press, Washington, DC, pp 457–492

van Asselt ED, Zwietering MH (2006) A systematic approach to determine global thermal inactivation parameters for various food pathogens. Int J Food Microbiol 107:73–82

Wittenberger K, Dohlman E (2010) Peanut outlook: impacts of the 2008–09 foodborne illness outbreak linked to *Salmonella* in peanuts. Outlook No. (OCS-10a-01). http://www.ers.usda.gov/media/146487/ocs10a01_1_.pdf. Accessed Jan 2013

Part II
Pathogen Persistence and Control in Low a_w Foods and Processing Plants

Adaptation of Pathogenic Microorganisms to Dry Conditions

Pieter Breeuwer

Abstract Pathogenic microorganisms surviving in dry products are responsible for numerous foodborne disease outbreaks, but a complete understanding of physiological survival mechanisms is still lacking. The response of pathogens to low water activity is very complex and involves immediate physiological actions of the cell as well as a coordinated genetic response. Compatible solutes, such as trehalose, may play an important role in bacterial survival, but, as has been shown for *Cronobacter* spp., trehalose-negative strains survived dry stress as well as the wild-type strains, suggesting that other mechanisms may play a role. Another contributing factor thought to be associated with resistance to drying is the production of extracellular polysaccharides (EPS); nevertheless, it has also been shown that the role of EPS in drying tolerance is not clear-cut and that additional research is needed to understand its role in resistance against drying. The coordinated genetic response of bacteria to stress involves multiple regulons or specific genes (O'Byrne and Booth, Int J Food Microbiol 74:203–216, 2002; Pichereau et al., Int J Food Microbiol 55:19–25, 2000; Riedel and Lehner, Proteomics 7:1217–1231, 2007). However, to elucidate the exact role of the various genes in bacterial dry stress resistance will likely keep researchers busy for a very long time. In the last decade some sectors within the food industry have responded by significantly improving the hygiene in dry areas of food processing facilities by, for example, introducing strict dry cleaning procedures and better attention to hygienic zoning. A comprehensive approach has been developed and published by microbiologists through the Grocery Manufacturers Association (Scott et al., Food Prot Trends 342–353, 2009a, Scott et al., Food Prot Trends 435–445, 2009b; Chen et al., Food Prot Trends 493–434, 2009).

Keywords Desiccation • Low moisture • Foodborne pathogens • *Salmonella* • *Cronobacter*

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Introduction

Microorganisms need available water to grow; therefore, removal of water or drying is one of the most efficient microbiological preservation methods known. Microorganism growth can be restricted or prevented under desiccated conditions; however, they may still survive dry conditions in a metabolically dormant state and can be present and alive for extended periods of time in low-water-activity foods, including powders. This capacity to survive is not very surprising, given selective pressures including changing weather conditions on earth, in particular frequent transitions from wet to dry conditions, and the propensity of microorganisms to adapt to such conditions by utilizing sophisticated genetic, metabolic, and physiological mechanisms enabling their evolution and survival. Nevertheless, there is a common misconception by the general public that products like powdered infant formula are sterile, and it remains a challenge to educate people that a reconstituted product should be consumed within a relatively short time span to avoid growth of contaminating microorganisms.

The survival of pathogens, such as *Salmonella* spp. and *Bacillus cereus*, in low-water-activity foods such as chocolate, nuts, and other foods is well documented. It is perhaps best illustrated by the multitude of outbreaks with pathogens such as *Salmonella* (Scott et al. 2009a). For bacteria such as *Salmonella* and Shiga toxin-producing *E. coli* (STEC), it was determined that, depending on the temperature, these bacteria could survive up to 35 days at 25 °C and much longer at 4 °C after being dried on paper disks (Hiramatsu et al. 2005). *Salmonella* can also survive for prolonged periods of time in chocolate (Tamminga et al. 1976), peanut butter (Burnett et al. 2000), and nuts (Komitopoulou and Peñaloza 2009).

Although not common, there have been outbreaks with *Cronobacter* spp. related to the consumption of infant formula (FAO/WHO Report 2004). But the question, "why specifically were *Cronobacter* spp. implicated?" is one that is not so easily answered. Preliminary data suggest that the infectious dose of *Cronobacter* spp. is high (Pagotto et al. 2003), and it was not clear as to why *Cronobacter* should be more associated with infant formula than other genera of bacteria within the family *Enterobacteriaceae*. The first clues came from research revealing that *Cronobacter* spp. are ubiquitous in dry environments (Kandhai et al. 2004) and that the strains have greater resistance to drying compared to other *Enterobacteriaceae* (EB) bacteria (Breeuwer et al. 2003), allowing the bacteria to survive for long periods in dry environments. For example, at 25 °C only one log of *Cronobacter* was inactivated after >40 days upon air-drying and reconstitution, whereas other EB species were reduced by 3 or more log units. Later work revealed that *Cronobacter* strains could survive spray drying (Arku et al. 2008) and could survive up to 30 months in desiccated foods (Barron and Forsythe 2007).

A recurring question is how can these contaminating bacteria survive in these dry environments? Even after decades of research, the survival mechanisms of bacteria upon dry stress are only partially understood. This chapter attempts to provide details of mechanisms used by microorganisms to survive in dry environments and address what this means for managing the production of dry foods such as powdered infant formula, nonfat dry milk, and other dried food products.

Survival Mechanisms

The greatest challenge to the survival of bacteria exposed to drying is in managing the water content within their cells, as well as avoiding structural damage to the essential components in the cell, including the protein structures and DNA. In general, the survival process can be divided into three phases: (1) initial drying, (2) desiccation, and (3) rehydration. The initial drying is arguably the most critical step in which water is removed and cells must respond rapidly to avoid loss of turgor and damage to the cell membrane. Upon complete cellular desiccation, cells must react by preventing irreversible damage to essential cellular components. An overview of the various bacterial mechanisms for survival to initial osmotic shock is provided in Fig. 1 (Reproduced from Pichereau et al. 2000). In the second stage, the challenge for the cells is to withstand oxidative damage and keep their DNA intact over extended periods of time. In the last stage, cells are revived, but at the same time, they also have to cope with the stress of rapid changes following the reintroduction of water to desiccated cells. For details on these mechanisms, the reader is referred to several excellent reviews on the subject (Potts 1994; Potts 2001; Sleator and Hill 2001; Pichereau et al. 2000; O'Byrne and Booth 2002).

One of the mechanisms proposed that may protect cells from desiccation damage is the "water-replacement hypothesis." The concept is that solutes such as sucrose

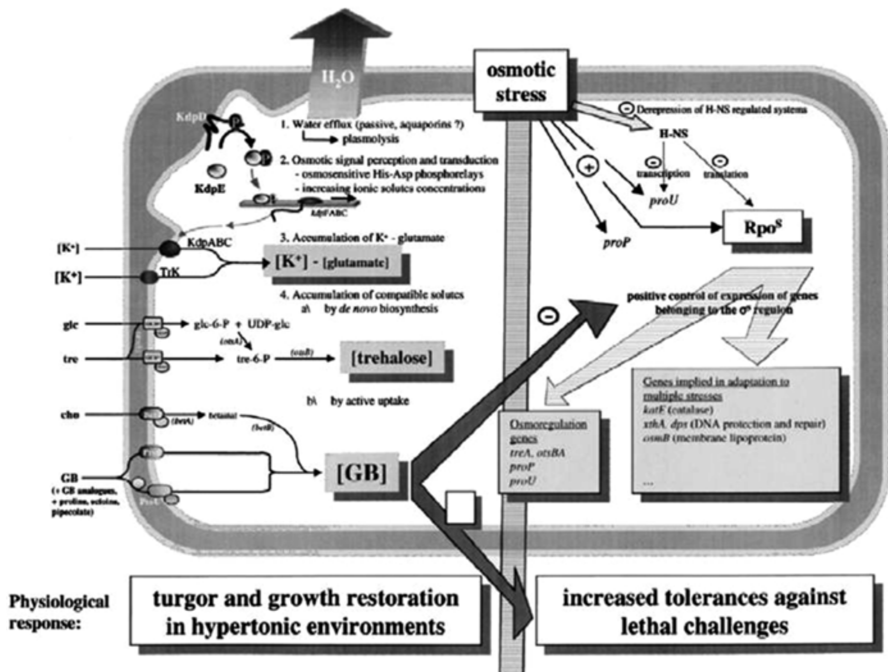
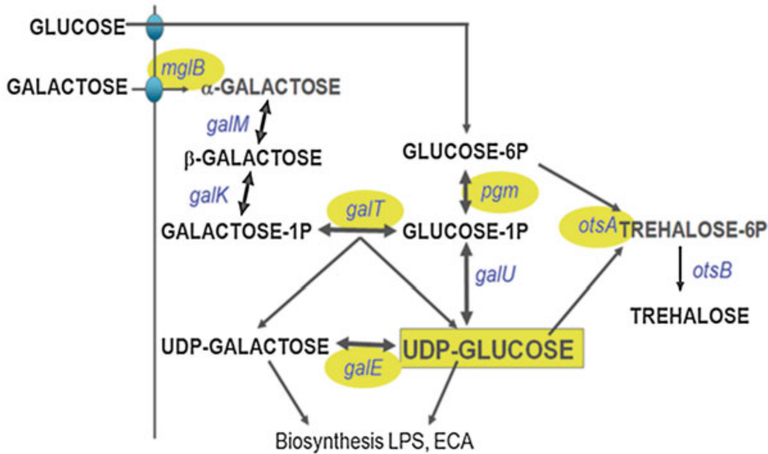


Fig. 1 Schematic representation of the bacterial response to hyperosmolarity (Reproduced with permission Pichereau et al. 2000)



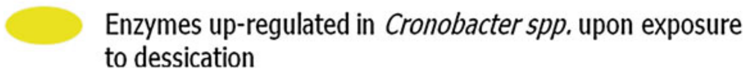


Fig. 2 Overview of enzymes involved in the synthesis of trehalose. In yellow are the enzymes upregulated in *Cronobacter* upon exposure to desiccation as determined by cDNA-AFLP (see also Table 1)

and trehalose replace water to conserve the membrane and protein structure to protect the cell from dehydration damage (Crowe et al. 1992). In recent years, the important role of these solutes in drying resistance has been confirmed (Morgan et al. 2006; Gomez Zavaglia et al. 2003; Welsh and Herbert 1999). In *Cronobacter*, the role of trehalose was studied in the Nestlé Research Center by creating *otsA* mutants (Diep 2006). The *otsA* gene codes for an enzyme used for the synthesis of trehalose (Fig. 2). Experimental studies confirmed that, in contrast to the wild-type strain, the *otsA*-negative strains were not able to accumulate trehalose in their cells when stressed with salt. However, the survival of 2 out of 3 *otsA*-negative mutants was not affected by drying for 7 days and subsequent dehydration (Fig. 3a). This was in contrast to the same mutation in an *E. coli* strain, for which a 3-log reduction upon rehydration was observed. This led to the hypothesis that *Cronobacter* may have (an) alternative mechanism(s) to protect itself from desiccation.

Interestingly, one of the three *otsA* mutants was sensitive to drying, with about a 4-log reduction after 7 days of drying and no survival after 46 days (Fig. 3b). This particular strain had an additional phenotype in that it did not produce EPS. The role that EPS may have in drying resistance has been determined with rock-inhabiting phototrophic microorganisms (Knowles and Castenholz 2008) and cyanobacterium *Nostoc commune* (Tamaru et al. 2005). In *E. coli* and other bacteria producing EPS compounds, such as colanic acid, it was determined that mucoid strains were more resistant to drying than non-mucoid strains (Ophir and Gutnick 1994). Also, in *Salmonella*, it was determined that strains lacking the O-polysaccharide element of

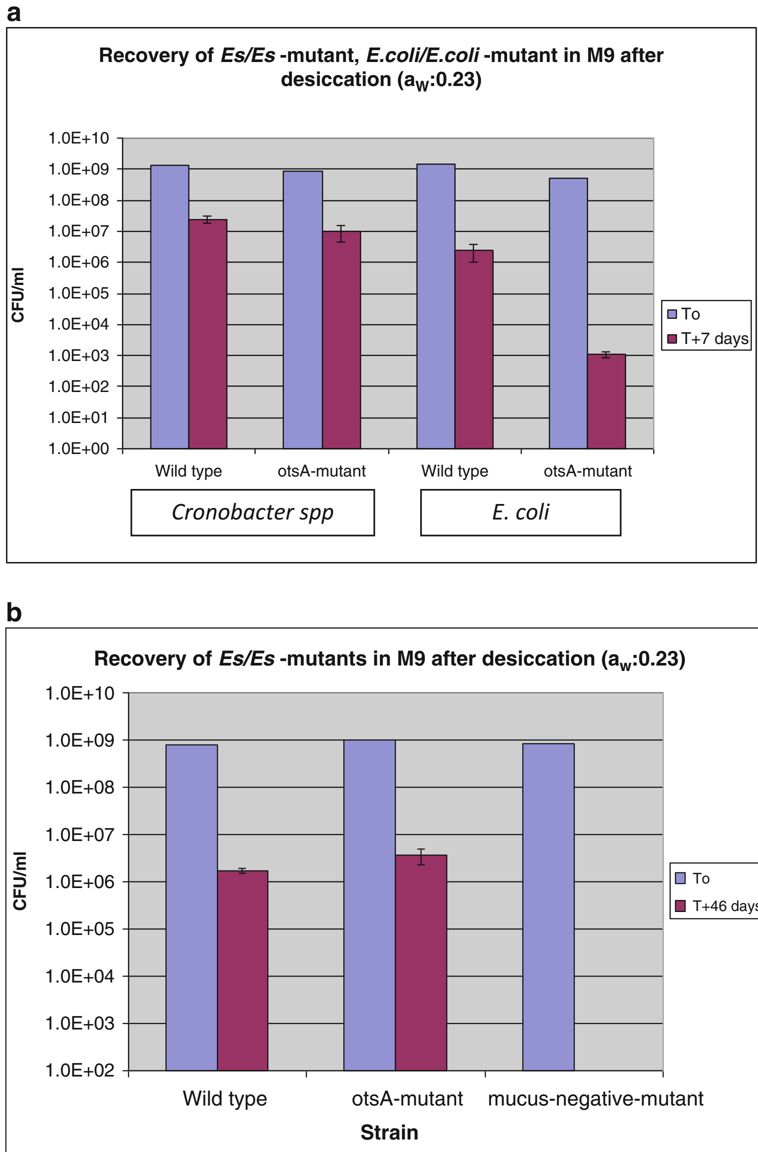


Fig. 3 (**a**) Recovery of *Cronobacter* (Es) and *E. coli otsA* mutants after desiccation compared to wild-type strain; (**b**) recovery of mucoid negative *otsA* mutant of *Cronobacter* after 46 days desiccation compared to wild-type strain

the outer membrane LPS were more sensitive to desiccation (Garmiri et al. 2008). *Cronobacter* spp. have been reported to produce EPS that has a structure similar to that of colanic acid (Lehner et al. 2005). Nevertheless, further experiments with this sensitive *Cronobacter* strain could not clearly elucidate the involvement of EPS in

the cells' tolerance to drying (Diep 2006). Addition of EPS to the drying-sensitive mutant strain did not enhance its survival, and other *Cronobacter* spp. naturally devoid of EPS did not exhibit decreased survival after drying. The involvement of LPS, in particular the ECA (enterobacterial common antigen), characteristic for *Enterobacteriaceae* was also addressed, but addition of tunicamycin, a drug that putatively inhibits the ECA LPS, had no effect on survival after drying. Also, *wecA Salmonella* mutants, which were depleted of ECA LPS and O-polysaccharide, had similar survival profiles as the wild-type strain. More research is needed to elucidate the role of these polysaccharides in the tolerance of *Cronobacter* spp. to drying.

Genetic Response of Bacteria to Drying Conditions

In addition to the rapid physiological response of bacteria under drying conditions, such as uptake of potassium and compatible solutes, the cells also react by altering their gene expression to avoid the negative effects of drying such as DNA damage, oxidative stress, and cell membrane damage.

A study of protein expression in *Enterobacter sakazakii* (*Cronobacter* spp.) upon drying and salt stress (Riedel and Lehner 2007) revealed that adaptation of non-growing cells in a desiccated state results in accumulation of proteins that serve some structural or protective role such as molecular chaperones like elongation factor EF-Tu. The synthesis of these stress proteins including heat shock proteins and chaperones is known to be under the control of the stationary sigma factor encoded by *rpoS* (Dodd and Aldsworth 2002). However, neither accumulation nor synthesis of compatible solutes (including trehalose) was observed in either desiccated or salt-stressed cells. This is unexpected since, in *E. coli*, trehalose synthesis genes were found to be under the control of the rpoS sigma factor (Pichereau et al. 2000; Hengge-Aronis et al. 1991). Interestingly, the genetic response to drying was quite different compared to the response to low water activity, due to addition of salt, the latter including a general downregulation of metabolic pathways such as amino acid biosynthesis and transport proteins.

The induction of genes during desiccation was also studied by scientists at the Nestlé Research Center using cDNA-AFLP (Breeuwer et al. unpublished results). Using this technique, it was determined that more than 90 genes were induced, and from the cDNA fragments obtained in the experiments, 47 had homology with annotated proteins in the BLAST databases (see Table 1).

Most of the induced genes were associated with general stress response mechanisms, such as stringent response, oxidative stress, heat shock proteins, cell wall functions, and the CRP regulon. Interestingly, at least 4 genes involved in galactose metabolism were induced, which together with the observed induction of *otsA* and *yqaB* indicate involvement of trehalose (Fig. 2). However, as indicated earlier, the exact role that trehalose plays in desiccation resistance of *Cronobacter* spp. is not clear.

Table 1 Analysis of cDNA-AFLP fragments induced upon drying and homology with possible genes

Analysis of cDNA-AFLP fragments: categorization					
Induced cDNA fragments	cDNA length (bp)	% identity	Putative gene	Protein	Putative function
Fragment code		DNA/DNA (%):prot/prot (%)	Stringent response		
A01	137	43/48 (89 %):18/20 (85 %)	*dapF*	Diaminopimelate epimerase	Biosynthesis of L-lysine
A11	156	83/97 (85 %):39/42 (92 %)	*ileS*	Isoleucine tRNA synthetase	
C03	65	No homology:20/20 (100 %)	*carB*	Carbamoylphosphate synthetase (large subunit)	Precursor biosynthesis Arg
A02	45	35/37 (97 %):12/12 (100 %)	*argH*	Argininosuccinate lyase	Biosynthesis of arginine
D06	48	38/43 (88 %):15/15 (100 %)	*astA*	Arginine succinyltransferase	Arginine catabolism, production ammonia
B07	62	50/53 (94 %):18/18 (100 %)	*ftsZ*	Cell division protein FtsZ	Cell division
CC08	169	61/68 (89 %):17/32 (53 %)	*Dps*	Starvation-induced DNA protection protein	DNA protection
			CRP regulon		
B09	43	28/31 (90 %):11/11 (100 %)	*Crp*	Cyclic AMP receptor protein (CRP)	Transcription factor
G04	177	139/166 (83 %):49/57 (85 %)	*osmY*	Periplasmic protein	Unclear
E01	154	120/133 (90 %):33/35 (94 %)	*Acs*	Acetyl-coenzyme A synthetase	
D06	48	38/43 (88 %):15/15 (100 %)	*astA*	Arginine succinyltransferase	Arginine catabolism, production ammonia
			Galactose metabolism		
C05	199	129/156 (82 %):34/40 (85 %)	*mglB*	Galactose-binding transport protein	Transport galactose
D12	42	30/33 (90 %):11/12 (91 %)	*galE*	UDP-galactose-4-epimerase	Interconversion galactose–glucose
D07	92	57/65 (87 %):21/21 (100 %)	*galE*	UDP-galactose-4-epimerase	Interconversion galactose–glucose

(continued)

Table 1 (continued)

Analysis of cDNA-AFLP fragments: categorization					
Induced cDNA fragments	cDNA length (bp)	% identity	Putative gene	Protein	Putative function
F07	165	127/158 (80 %):48/53 (90 %)	*galT*	Galactose-1-phosphate uridylyltransferase	Interconversion galactose–glucose
			Cell wall functions		
F10	62	53/56 (94 %):no homology	*nlpD*	Lipoprotein	Hydrolytic function in cell wall formation
D03	74	55/64 (85 %):23/24 (95 %)	*wecA*	Undecaprenyl-phosphate alpha-N-acetylglucosaminyl 1-phosphate transferase	Lipopolysaccharide O-polysaccharide biosynthesis pathways
H04	99	20/20 (100 %):24/31 (77 %)	*yqaB*	Phosphatase (phosphoglucomutase, pgm)	Conversion of glucose-6-phosphate to glucose-1-phosphate
AA05	95	70/77 (90 %):28/28 (100 %)	*cpxR*	CpxR response regulator	Responds to aggregated and misfolded proteins in the bacterial envelope
DD03	81	62/67 (92 %):24/24 (100 %)	*lpxC* (*envA*)	UDP-3-O-acyl-GlcNAc deacetylase	LipidA biosynthesis
			Oxidative stress		
C04	285	188/212 (88 %):62/70 (88 %)	*acnA*	Aconitate hydratase	Protection against oxidative stress
BB03	258	187/218 (85 %):76/85 (89 %)	*acnA*	Aconitate hydratase	Protection against oxidative stress
C06	195	146/182 (80 %):33/41 (80 %)	*katN*	Putative catalase	Protection against oxidative stress
			Heat shock proteins		
D05	193	86/96 (89 %):no homology	*rpoH*	Sigma 32 factor	Involved in heat stress response
G02	39	28/29 (96 %):11/11 (100 %)	*clpB*	ATP-dependent protease	Chaperone function in stress
F11	105	90/96 (93 %):33/33 (100 %)	*dnaK*	Chaperone protein	Chaperone function in stress

G06	126	95/111 (85 %):36/40 (90 %)	*grpE*	Chaperone protein	Chaperone function in stress
F02	130	52/58 (89 %):17/20 (85 %)	*ibpA*	Small heat shock protein	Protection against stress
CC02	54	53/54 (98 %):11/11 (100 %)	*groEL*	Heat shock protein GroEL	Protection against stress
AA03	180	161/172 (93 %):54/56 (96 %)	*groEL* like	GroEL-like protein	Stress protein
			Not categorized		
A07	164	120/133 (90 %):20/21 (95 %)	*infB*	Translation initiation factor 2	Initiation of translation
A08	94	75/84 (89 %):23/23 (100 %)	*Pnp*	Polynucleotide phosphorylase (PNPase)	3′-Exoribonuclease
A12	156	40/46 (86 %):41/49 (83 %)	*nrdI*	Ribonucleotide reductase (RNR)	
C02	78	65/74 (87 %):24/25 (96 %)	*yfiD*	Putative formate acetyltransferase	
H01	133	103/119 (86 %):26/30 (86 %)	*yfiD*	Putative formate acetyltransferase	
C08	156	73/83 (87 %):22/31 (70 %)	*cpxP*	Regulator protein	Regulation of cpxA, in response to misfolding proteins
D01	144	No homology:29/43 (67 %)	*trbJ*	trbJ protein	Conjugative transfer
F04	172	No homology:32/52 (61 %)	*repA*	Plasmid replication protein	Replication of plasmids
D02	135	71/80 (88 %):34/42 (80 %)	*ysgA*	Putative carboxymethylenebutenolidase	Degradation aromatic compounds
E04	76	No homology:17/20 (85 %)	*yihQ*	Putative glycosidase	Unknown
G09	79	44/50 (88 %):16/19 (84 %)	*mutL*	"Molecular matchmaker"	DNA repair
D04	78	42/45 (93 %):17/21 (80 %)	*ygdE*	Putative SAM-dependent methyltransferase	Protection DNA against restriction enzymes
G05	109	90/106 (84 %):33/35 (94 %)	*cafA* (*rnG*)	Ribonuclease G	5′ end processing of 16S rRNA
BB10	167	135/155 (87 %):48/52 (92 %)	*Pps*	Phosphoenolpyruvate synthase	General carbon metabolism
CC07	150	26/28 (92 %):36/43 (83 %)	*otsA*	Trehalose-6-phosphate synthase	Trehalose synthesis
CC09	286	No homology:60/64 (93 %)	*sucA*	2-Oxoglutarate dehydrogenase E1 component	TCA cycle enzyme

One of the induced genes was *osmY*. We determined with an *osmY* knockout mutant that like the *otsA* gene, OsmY was associated with tolerance to salt (unpublished results); however, *osmY* does not have an apparent role in protection against desiccation. OsmY contains two BON domains, which are also present in other osmotic shock proteins. The likely function of the BON domain is attachment to phospholipid membranes, with one hypothesis being that OsmY helps to prevent shrinkage of the cytoplasmic compartment (Yim and Villarejo 1992).

In conclusion, the genetic response of bacteria to drying is very complex and involves multiple regulons. There does not appear to be one single protein that is solely responsible for the survival of pathogens under low-water-activity conditions.

Concluding Remarks

Surviving pathogenic microorganisms in dry products are responsible for numerous foodborne disease outbreaks, but complete understanding of physiological survival mechanisms is still lacking. The response of pathogens to low water activity is very complex and involves immediate physiological actions of the cell as well as a coordinated genetic response. Compatible solutes, such as trehalose, may play an important role in bacterial survival, but as has been shown for *Cronobacter* spp., trehalose-negative strains survived dry stress as well as the wild-type strains, suggesting that other mechanisms may play a role. Another contributing factor thought to be associated with resistance to drying is the production of extracellular polysaccharides (EPS); nevertheless, it has also been shown that the role of EPS in drying tolerance is not clear-cut and that additional research is needed to understand its role in resistance against drying.

The resistance of pathogens to dry conditions is also key for the establishment of such strains in factory environments, from where they can potentially migrate to the product. This is nicely illustrated by the work of Mullane et al. (2007, 2008), who determined the distribution of *Cronobacter* strains in an infant formula powder processing facility. In that study, *Cronobacter* strains were detected in the dry parts of the facility, including the dryer floors, bag filling areas, and filters of the air handling units, and from there they may potentially end up in the product.

Because of the increased awareness of the survival and cross-contamination routes of *Salmonella* and *Cronobacter* during processing, there has been a growing focus by the industry and regulatory authorities to prevent the growth and spread of pathogens in areas where contact with low-a_w finished products can occur. This led to recommendations by the Grocery Manufacturers Association for the control of foodborne pathogens in dry product facilities (Scott et al. 2009a, b; Chen et al. 2009). The main measures to prevent cross-contamination with pathogens such as *Salmonella* and *Cronobacter* include proper application of GMPs and HACCP, combined with strict application of zoning and dry cleaning to avoid growth of bacteria in the processing environment and near the production line.

By applying these preventive measures, tremendous improvements were achieved during the last decade, and, for example, today the low-a_w food industry can confidently release infant formula per the strict Codex requirements of the absence of *Salmonella* in 60×25-g samples per lot and *Cronobacter* spp. in 30×10-g samples per lot (CAC 2008).

References

Arku B, Mullane N, Fox E, Fanning S, Jordan K (2008) *Enterobacter* survives spray drying. Int J Dairy Technol 61:102–108

Barron CB, Forsythe SJ (2007) Dry stress and survival time of *Enterobacter sakazakii* and other Enterobacteriaceae in dehydrated powdered infant formula. J Food Prot 2007:2111–2117

Burnett S, Gehm ER, Weissinger WR, Beuchat LR (2000) Survival of *Salmonella* in peanut butter and peanut butter spread. J Appl Microbiol 89:472–477

Breeuwer P, et al. Unpublished results.

Breeuwer P, Lardeau A, Peterz M, Joosten HM (2003) Desiccation and heat tolerance of *Enterobacter sakazakii*. J Appl Microbiol 95:967–973

CAC (Codex Alimentarius Commission) (2008) Code of hygiene practice for powdered formulae for infants and young children. CAC/RCP 66. World Health Organization-Food and Agriculture Organization of the United Nations, Rome, Italy

Chen Y, Scott VN, Freier TA, Kuehm J, Moorman M, Meyer J, Morille-Hinds T, Post L, Smoot L, Hood S, Shebuski J, Banks J (2009) Control of *Salmonella* in low-moisture foods III: process validation and environmental monitoring. Food Prot Trends. 493–434

Crowe JH, Hoekstra FA, Crowe LM (1992) Anhydrobiosis. Annu Rev Physiol 54:579–599

Dancer GI, Mah JH, Rhee MS, Hwang IG, Kang DH (2009) Resistance of *Enterobacter sakazakii* (*Cronobacter spp.*) to environmental stresses. J Appl Microbiol 107:1606–1614

Diep B (2006) Unpublished results. Nestlé Research Centre, Lausanne, Switzerland

Dodd CER, Aldsworth T (2002) The importance of RpoS in the survival of bacteria through food processing. Int J Food Microbiol 74:189–194

FAO/WHO Food and Agriculture Organization/World Health Organization (2004) *Enterobacter sakazakii* and other microorganisms in powdered infant formula: meeting report. MRA Series 6. www.who.int/foodsafety/micro/meetings/feb2004/en/ (Online)

Garmiri PKE, Coles TJH, Cogan TA (2008) Role of outer membrane lipopolysaccharides in the protection of *Salmonella enterica* serovar Typhimurium from desiccation damage. FEMS Microbiol Lett 281:155–159

Gomez Zavaglia A, Tymczyszyn E, de Antoni G, Anibal Disalvo E (2003) Action of trehalose on the preservation of *Lactobacillus delbrueckii* ssp. *bulgaricus* by heat and osmotic dehydration. J Appl Microbiol 95:1315–1320

Hengge-Aronis R, Klein W, Lange R, Rimmele M, Boos W (1991) Trehalose synthesis genes are controlled by the putative sigma factor encoded by *rpoS* and are involved in stationary phase thermotolerance in *Escherichia coli*. J Bact 173:7918–7924

Hiramatsu R, Matsumoto M, Sakae K, Miyazaki Y (2005) Ability of shiga toxin-producing *Escherichia coli* and *Salmonella* spp. to survive in a desiccation model system and in dry foods. Appl Env Microbiol 71:6657–6663

Kandhai MC, Reij MW, Gorris LGM, Guillaume-Gentil O, van Schothorst M (2004) Occurrence of *Enterobacter sakazakii* in the food production environments and households. Lancet 363:39–40

Knowles EJ, Castenholz RW (2008) Effect of exogenous extracellular polysaccharides on the desiccation and freezing tolerance of rock-inhabiting phototrophic microorganisms: effect of EPS on tolerance of rock-inhabiting phototrophs. FEMS Microbiol Ecol 66:261–270

Komitopoulou E, Peñaloza W (2009) Fate of *Salmonella* in dry confectionary raw materials. J Appl Microbiol 106:1892–1900

Lehner A, Riedel K, Eberl L, Breeuwer P, Diep B, Stephan R (2005) Biofilm formation, extracellular polysaccharide production, and cell-to-cell signaling in various *Enterobacter sakazakii* strains: aspects promoting environmental persistence. J Food Prot 68:2287–2294

Morgan CA, Herman N, White PA, Vesey G (2006) Preservation of microorganisms by drying; a review. J Microbiol Methods 66:183–193

Mullane N, Whyte P, Wall PG, Quinn T, Fanning S (2007) Application of pulsed-field gel electrophoresis to characterize and trace the prevalence of *Enterobacter sakazakii* in an infant formula processing facility. Int J Food Microbiol 116:73–81

Mullane N, Healy B, Meade J, Whyte P, Wall PG, Fanning S (2008) Dissemination of *Cronobacter* spp. (*Enterobacter sakazakii*) in a powdered milk protein manufacturing facility. Appl Environ Microbiol 19:5913–5917

O'Byrne CP, Booth IR (2002) Osmoregulation and its importance to food-borne microorganisms. Int J Food Microbiol 74:203–216

Ophir T, Gutnick DL (1994) A role of exopolysaccharides in the protection of microorganisms from desiccation. Appl Environ Microbiol 60:740–745

Pagotto FJ, Nazarowec-White M, Bidawid S, Farber JM (2003) *Enterobacter sakazakii*: infectivity and enterotoxin production in vitro and in vivo. J Food Prot 66:370–375

Pichereau V, Hartke A, Auffray Y (2000) Starvation and osmotic stress induced multiresistances: influence of extracellular compounds. Int J Food Microbiol 55:19–25

Potts M (1994) Desiccation tolerance of prokaryotes. Microbiol Rev 58:755–805

Potts M (2001) Desiccation tolerance: a simple process? Trends Microbiol 9:553–559

Riedel K, Lehner A (2007) Identification of proteins involved in osmotic stress response in *Enterobacter sakazakii* by proteomics. Proteomics 7:1217–1231

Scott VN, Chen Y, Freier TA, Kuehm J, Moorman M, Meyer J, Morille-Hinds T, Post L, Smoot L, Hood S, Shebuski J, Banks J (2009a) Control of *Salmonella* in low-moisture foods I: minimizing entry of *Salmonella* into a processing facility. Food Prot Trends. 342–353

Scott VN, Chen Y, Freier TA, Kuehm J, Moorman M, Meyer J, Morille-Hinds T, Post L, Smoot L, Hood S, Shebuski J, Banks J (2009b) Control of *Salmonella* in low-moisture foods II: hygiene practices to minimize *Salmonella* contamination and growth. Food Prot Trends. 435–445

Sleator RD, Hill C (2001) Bacterial osmoadaptation: the role of osmolytes in bacterial stress and virulence. FEMS Microbiol Rev 26:49–71

Tamminga SK, Beumer RR, Kampelmacher EH, van Leusden FM (1976) Survival of *Salmonella eastbourne* and *Salmonella typhimurium* in chocolate. J Hygiene 76:41–47

Tamaru Y, Takani Y, Yoshida T, Sakamoto T (2005) Crucial role of extracellular polysaccharides in desiccation and freezing tolerance in the terrestrial cyanobacterium *Nostoc commune*. Appl Environ Microbiol 71:7327–7333

Welsh DT, Herbert RA (1999) Osmotically induced intracellular trehalose, but not glycine betaine accumulation promotes desiccation tolerance in *Escherichia coli*. FEMS Microbiol Lett 174:57–63

Yim HH, Villarejo M (1992) osmY, a new hyperosmotically inducible gene, encodes a periplasmic protein in *Escherichia coli*. J Bacteriol 174:3637–3644

Transcriptomic Responses of *Salmonella* Species to Desiccation and Low-Moisture Environments: Extending Our Knowledge of How Bacteria Cope with Low-Moisture Stress

Sarah Finn, Peter McClure, Alejandro Amézquita, and Séamus Fanning

Abstract *Salmonella* is the bacterial pathogen of greatest concern in low-moisture food products. This genus possesses an innate ability to persist for extended time periods in a dried state within raw material, finished product, and the factory environment. Due to this phenomenon, *Salmonella* has been associated with many foodborne outbreaks associated with low-moisture foods. In this chapter, the importance of *Salmonella* in the context of low-moisture products is discussed. Laboratory methods that can be used to study the behavior of this pathogen in controlled stress-inducing environmental conditions are briefly reviewed. The mechanisms that aid survival of this pathogen within a dried state remain to be fully elucidated. To better understand these mechanisms, a number of recent studies have determined the transcriptomic response of this bacterium when desiccated onto an abiotic surface. Findings from these studies will be reviewed in detail. In addition, the cellular changes occurring when salmonellae are exposed to lowered water activity in a liquid system have been explored. In time, increased knowledge regarding these survival mechanisms may aid in enhancing the safety of food and public health by using this information to develop more effective control strategies.

Keywords *Salmonella* • Desiccation • Low-moisture environments • Transcriptome • Survival mechanisms

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Importance of *Salmonella* in the Context of Low-Moisture Foods

Each year, more than 80 million cases of salmonellosis and an estimated 155,000 deaths occur worldwide that are associated with the ingestion of contaminated food products (Majowicz et al. 2010). Following consumption of contaminated food or water containing *Salmonella*, onset of symptoms occurs after 6–72 h, resulting in abdominal cramping, diarrhea, nausea, and vomiting (Coburn et al. 2007). The infection is self-limiting with symptoms normally resolving within 5–7 days. Intravenous rehydration therapy may be required if significant fluid loss is experienced, notably in the case of infants, the elderly, and immunocompromised individuals. In a minority of cases, salmonellae may pass the intestinal barrier and enter the bloodstream leading to bacteremia. Hence, this bacterium is one of the major concerns for food manufacturers worldwide.

Salmonella are the leading cause of foodborne illness associated with intermediate- and low-moisture food products (Finn et al. 2013a; Beuchat et al. 2013). This bacterium can be found in a variety of raw ingredients and can persist under dry conditions for long periods of time (Finn et al. 2013a; Podolak et al. 2010; Van Doren et al. 2013). A range of *Salmonella enterica* serovars have been linked to foodborne outbreaks associated with reduced-moisture food products. These have been extensively reviewed in recent publications (Beuchat et al. 2013; Finn et al. 2013a, Podolak et al. 2010). The food products in question are diverse in nature and include peanut butter, powdered infant formula (PIF), chocolate, cereal, tea, and spices (Weissman et al. 1977; Gill et al. 1983; Rowe et al. 1987; Rabsch et al. 2005; Julian et al. 2010; Scheil et al. 1998; Werber et al. 2005). Many of the outbreaks resulting from ingestion of contaminated low-moisture foods have been associated with a low infectious dose(s) (10–100 CFU) (Greenwood and Hooper 1983; Rowe et al. 1987). This observation may be attributed to an underestimation of the *Salmonella* cell numbers present, resulting from the suboptimal recovery of cells from food product samples (Kapperud et al. 1990; Werber et al. 2005). However, it is more likely that a high-fat-content diet offers protection to the bacteria during transmission through the GI tract, which has been documented in several studies (Aviles et al. 2013; Possemiers et al. 2010).

Major causes of contamination are improper equipment care, failure to achieve critical limits associated with critical control points, and poor sanitation (Carrasco et al. 2012). The control of *Salmonella* is critically important within manufacturing facilities. The ability to identify the source of any potential contamination is essential and requires the use of effective environmental monitoring programs. Sources and monitoring of *Salmonella* contamination have been described in previous reviews (Finn et al. 2013a; Beuchat et al. 2013; Podolak et al. 2010). In order to trace potential sources, a number of approaches can be taken. Once environmental samples have been processed, direct testing of the *Salmonella* isolates can be done to further characterize them. Phage typing is one of the methods used for subtyping (Barrett et al. 1994; Holmberg et al. 1984). However, the use of more sensitive

chemical analysis such as MALDI-TOF (matrix-assisted laser desorption/ionization-time of flight) mass spectrometry is a highly accurate method (Giebel et al. 2010; Carbonnelle et al. 2011; Sparbier et al. 2012) and is increasingly being used for bacterial identification. For the identification and tracking of persistent bacterial isolates, the methods used often rely on DNA isolation from positive culture samples followed by molecular subtyping. Perhaps the most widely used of these is pulsed-field gel electrophoresis (PFGE) (Morita et al. 2006; Mullane et al. 2007). Alternative methods include ribotyping, multilocus sequence typing (MLST), 16S rDNA sequencing, and optical mapping. As sequencing technologies become increasingly available, it is likely that, in time, whole genome sequencing (WGS) may become a mainstream method by which persistent isolates may be detected throughout the production chain (Wilson et al. 2013). This would offer a number of advantages over traditional typing methods, such as the ability to study phylogenetic changes of a persistent population over time (Wilson et al. 2013). However, it would be necessary to employ trained personnel to analyze and manage the vast amount of data generated when using such technologies (Wilson et al. 2013). Foley et al. have previously reviewed molecular typing methods used for the analysis of *Salmonella* in the food industry (Foley et al. 2007; Wattiau et al. 2011).

Studying Adaptation of Bacteria to Stressful Environments

There are a variety of methods by which the behaviors of bacteria within stressful environments can be studied. Investigation of these adaptive responses can provide vital information regarding condition-specific survival mechanisms induced by external stress. This information can then be used to create targeted treatments such as the application of antibacterial countermeasures and biocidal formulations that ultimately function to prevent growth or inactivate pathogenic microorganisms. A range of mechanisms used in the general study of bacterial adaptation and their specific application in regard to desiccation and low-moisture stress are discussed below and are summarized in Fig. 1.

Genome Sequencing

Next-generation sequencing (NGS) technologies are powerful tools to examine changes occurring within the bacterial genome of interest in response to stressful conditions. For example, identification of mutations, such as single nucleotide polymorphisms (SNPs), occurring within a gene in an antibiotic-resistant isolate may result in structural alterations in the antibiotic target molecule, producing the resistant phenotype (Esaki et al. 2004). While this technique is useful when examining those bacteria carrying beneficial mutations that allow them to outcompete the original wild-type strain, it is limited to conditions where sufficient growth occurs.

Genomics	Transcriptomics	Proteomics
DNA	RNA	Protein
Techniques - SNP analysis - Whole genome sequencing	Techniques - qRT-PCR - Microarray - RNA-Seq	Techniques - 2D-DIGE - Western blotting - Mass spectrum analysis
Advantage In depth analysis of changes with the whole genome	Advantage Targets changes occurring only in surviving cells	Advantage Examination of how stress affects cellular phenotype
Limitation Surviving sub-population isolates in minority. May lose interesting mutations during recovery from the dried state	Limitation Degradation of RNA due to harsh dry conditions may be encountered	Limitation Large population of dead cells present after desiccation may mask changes occurring within surviving cells

Fig. 1 Techniques used to study response of bacteria to low-moisture conditions

When examining how bacteria develop tolerance to survive in the presence of antibiotics or biocidal active agents, what is often measured is how these cells have adapted to *grow* in the presence of these compounds. However, desiccation tolerance and survival within low-a_w environments must focus on how bacteria survive and persist. Currently, due to the high proportion of cellular damage caused by desiccation stress, the analysis of desiccated cells comes with a "trade-off," whereby the surviving subpopulation of cells is in the minority due to extensive cell death occurring upon desiccation (Finn et al. 2013b). Therefore, genome sequencing analysis may not be applicable in this case, because in order to isolate the surviving subpopulation, cells must be recovered and grow in a non-stress-inducing medium. This passaging and removal of stress may give rise to additional mutations, therefore altering the true nature of what occurs within the persisting cells.

Transcriptomic Studies

Transcriptomic analysis focuses on the upregulation and downregulation of RNA transcripts, following gene expression, in a particular test condition, in comparison to an appropriate control. It is hypothesized that increases in expression of specific genes (upregulation) indicate that those genes are required for adaption and survival

within the selected stress condition, whereas a downregulation suggests those genes are no longer necessary. This is a sensitive molecular technique that focuses only on active cells (i.e., those surviving stress-induced conditions). It is possible that the extended RNA half-life of some transcripts may lead to a certain number of transcripts from dead cells being carried over. Although the data generated provide a model of adaptive processes that may be occurring within the bacterial cell, further confirmatory experiments, such as gene deletion studies, are often required to ascertain whether the specific genes are essential for survival or not.

Quantitative reverse transcription-polymerase chain reaction (qRT-PCR) was an early method used in the investigation of transcriptional changes (Gibson et al. 1996; Bustin 2000; Ginzinger 2002). RNA purification is carried out on test and control bacterial cultures. This RNA sample is then reverse transcribed to produce a copy of cDNA which is used for further quantitative analysis. Only a subset of specific genes hypothesized to be involved in the response to the particular environment can be studied using this approach. Oligonucleotide primers are designed to target those genes of interest and that are to be used in real-time PCR reactions. In these reactions, fluorescence is measured (often using SYBR dye) and these reactions correspond directly to the number of transcripts present. Relative fold change in comparison to the control test is often calculated using the $2^{-}\Delta\Delta^{CT}$ method (Livak and Schmittgen 2001).

Fluorescent two-color DNA microarrays are among the most common methods by which transcriptomic changes are examined (Fig. 2). Unlike qRT-PCR analysis, microarray platforms allow for the simultaneous measurement of thousands of genes from the bacterium/organism of interest (Schena et al. 1995; Shalon et al. 1996). In this case, thousands of oligonucleotide probes are directly printed onto a glass slide corresponding to sequences of known genes. Purified RNA, extracted from test and control samples, is converted to cDNA as described above and usually labeled with fluorescent Cy3 or Cy5 dCTP. This is subsequently competitively hybridized to the array slide (Hegde et al. 2000). Alternatively, instead of the traditional RNA-RNA hybridization approach, the use of a fluorescently labeled common reference, such as genomic DNA, can offer a number of advantages (Thompson et al. 2006; Finn et al. 2013b; Yang and Speed 2002). For example, the common reference removes the need for dye-swap experiments, thereby reducing the number of microarrays required and subsequently the cost. This approach also allows for the comparison of data across a variety of different growth conditions (Yang and Speed 2002). Once hybridization occurs, unbound molecules are washed away and the slide is measured using a laser scanner. Further normalization and bioinformatic analysis will identify those genes that are differentially expressed under the conditions tested. Often, qRT-PCR analysis is carried out on a subset of genes to validate the expression profiles observed in the microarray data.

More recently, the development of RNA-seq technology has revolutionized transcriptome analysis (Croucher and Thomson 2010). This technique exploits NGS technology, enabling the study of the entire transcriptome with an approach that is highly reproducible. RNA-seq offers a higher level of sensitivity with the ability to detect genes that exhibit low levels of expression and which would most

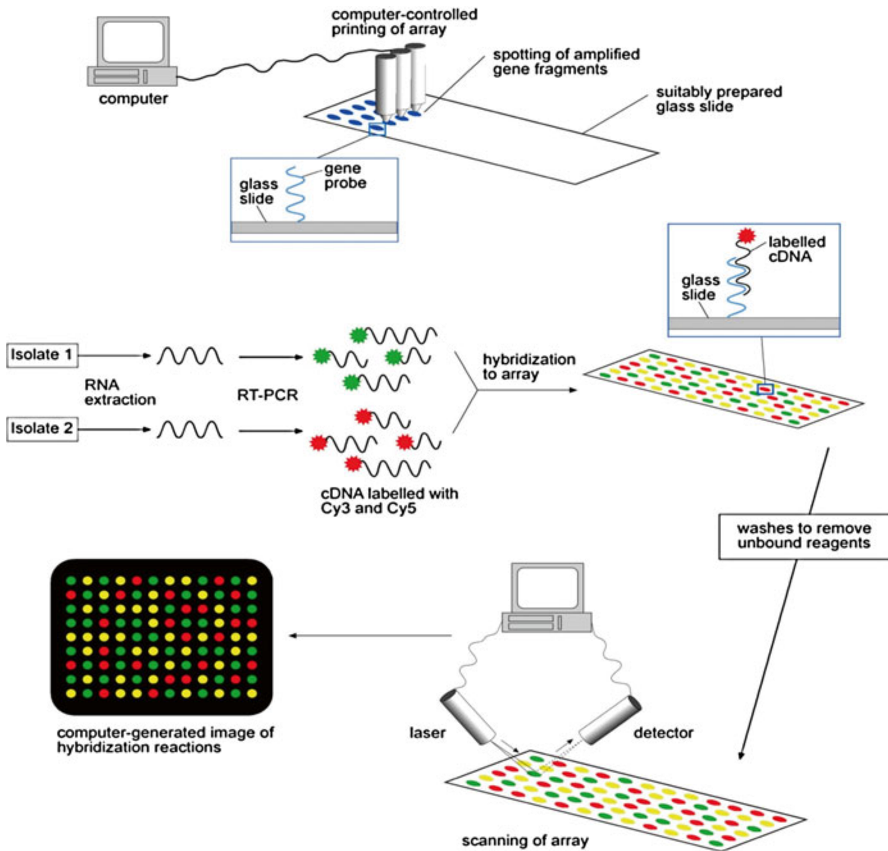

Fig. 2 Outline of workflow used when analyzing the transcriptome by microarray technology. Quinn et al. *Veterinary Microbiology and Microbial Disease*, 2nd edition, Wiley-Blackwell, Oxford, UK, Copyright © 2011 (Quinn et al. 2011)

likely be undetected using other protocols (Croucher and Thomson 2010). It enables the investigation of transcripts at the single nucleotide level (Croucher and Thomson 2010). Furthermore, it offers the possibility of detecting small interfering RNA transcripts that are often not detected when more traditional microarray platforms are used. This methodology has also been applied for the detection of transcriptional start sites (Kröger et al. 2012; Croucher and Thomson 2010). At present, the cost associated with this methodology is a significant drawback, making multiple replicates an expensive endeavor. However, as the technology becomes more readily available, it may gradually replace the microarray approach.

In the context of low-moisture and desiccation studies, transcriptome analysis is the most widely used methodology in the analysis of mechanisms of survival in these conditions (Deng et al. 2012; Li et al. 2012; Balaji et al. 2005; Gruzdev et al. 2012; Finn et al. 2013b). Transcriptome analysis targets the active, surviving subpopulation of cells and reduces the noise generated from dead cells.

Proteomics

Investigation of the total proteome is another useful method to study adaptation of bacteria to stress. While genomic and transcriptomic platforms can allow for the identification of gene alterations occurring within the cell, the proteome will identify how these changes affect the cellular phenotype. 2D differential fluorescence gel electrophoresis (DIGE) is a powerful method used to study the proteomic changes that occur in response to stress (Sonck et al. 2009; Gwendoline et al. 2009; Condell et al. 2012; Lilley and Friedman 2004). The use of proteomic analysis for the investigation of desiccation tolerance has been used to study *Cronobacter* and is discussed in Chap. XX (Breeuwer) (Riedel and Lehner 2007). Although the investigation of the total proteome is an effective method to study the bacterial response to stress, in some cases, where dead cells outnumber the surviving population, this method may not be applicable due to a "masking" effect. This masking effect may be observed, arising from an abundance of proteins originating from the dead cell pool. Therefore, if using proteomic protocols, it may be appropriate to validate those changes in targeted polypeptides by western blotting, as a confirmatory step.

Other Investigative Methods

To obtain the more holistic understanding of how bacteria survive in a harsh environmental condition, it may be prudent to carry out a systems biology-based approach. In addition to genomic, transcriptomic, and proteomic characterization, the use of metabolic profiling by mass spectrometry is another powerful tool to measure the change in metabolites over time. Single cell analysis of desiccated populations using flow cytometry to examine physiological changes may also prove useful in the future. As more sensitive analytical tools become available, isolation of subpopulations capable of surviving long-term desiccation could prove vital in truly pinpointing desiccation survival mechanisms.

To summarize, in order to identify mechanisms employed by *Salmonella* species in response to low-moisture conditions, it is essential to utilize the appropriate scientific tools/techniques. Similar to the desiccation environment, cells exposed to a_w levels occurring in low-moisture products are not capable of growth. Unlike antibiotics and biocidal active agents, the development of tolerance to allow growth in high levels of commonly used humectants, such as NaCl, KCl, and various sugars, has not been observed in *Salmonella* (although survival is possible). Therefore, in order to examine the response in a manner that mirrors the environment found within a low-moisture food matrix, it is not possible to cultivate the bacteria, resulting in only small populations being available for investigation. Hence, the techniques recommended for use would include those outlined above. There is some evidence that increased levels of NaCl increases the resistance of salmonellae to drying. For example, Cole (2005) reported that concentrations of up to 0.2 M NaCl increased the resistance of *S.* Enteritidis E and *S.* Typhimurium SL1344 to drying at 85 % relative humidity (RH). This was attributed to the protective effects of trehalose and

other RpoS-dependent factors, with some evidence of these increasing membrane integrity. The protective effect of trehalose following growth in M9 medium containing up to 0.2 M NaCl was in agreement with findings reported by other groups (Lesne et al. 2000; Welsh and Herbert 1999; Bullifent et al. 2000). However, growth of *Salmonella* at subinhibitory levels of humectant compounds, such as with NaCl, in which the bacteria are still capable of cell division, may provide important information to extend our understanding of how bacteria of interest survive at reduced moisture levels.

Duration of *Salmonella* Species Survival to Desiccation on Abiotic Surfaces

Salmonella can persist in a desiccated state for long periods of time, with survival being affected by several environmental conditions, including temperature and the presence of other compounds, such as nutrients or humectants. It has previously been determined that *Salmonella* can survive in a desiccated state on paper disks for 70 days when stored at ambient temperature (25 °C) (Hiramatsu et al. 2005). However, survival was decreased, to 35 days, when the storage temperature was increased to 35 °C. Conversely, when stored at 4 °C, the numbers of bacteria remained at a constant level for 24 months (Hiramatsu et al. 2005). Addition of sucrose increased persistence of this bacterium on these paper disks up to 79-fold, whereas NaCl had a detrimental effect on bacterial survival (Hiramatsu et al. 2005). Similarly, *Salmonella* can survive for over 100 weeks at 4 °C when desiccated on a plastic surface (Gruzdev and Pinto 2012). Taken together, these results highlight the effect of external environmental conditions on the persistence of an important foodborne pathogen within a desiccated state.

Transcriptomic Studies Examining the Effect of Desiccation on *Salmonella*

Salmonella exposed to desiccation stress on abiotic surfaces have recently been studied (Finn et al. 2013b; Li et al. 2012; Gruzdev et al. 2012). These studies provide the foundation for our early understanding of how these bacteria can persist so successfully in a desiccated state, by mimicking a situation that may be encountered within a low-moisture production facility.

The first of these studies was reported by Gruzdev et al. (2012), whereby a traditional microarray platform was used to examine the transcriptomic response of *S.* Typhimurium 22 h after desiccation on a 90-mm Petri dish. Stationary phase bacterial cells that had been resuspended in double-distilled water were air-dried onto the Petri dish surface and incubated at 25 °C for 22 h and ca. 40 % relative humidity. These results were compared to a control broth culture incubated in double-distilled water at 25 °C for 22 h. After 22 h desiccation, 90 genes were upregulated, whereas

only seven genes were downregulated, with a greater than 1.8-fold change ($P < 0.05$), in comparison to the control culture.

In a study by Li et al. (2012), the response of *Salmonella* Tennessee stationary phase cells suspended in phosphate buffered saline, dehydrated onto filter paper disks, was examined. After 2 h of desiccation at a relative humidity of 11 %, RNA was purified from these cells and analyzed using a microarray similar to that described above. In this case, gene expression alterations were calculated on the basis of functional group changes occurring above a fivefold change ($P < 0.05$) compared to an untreated liquid control culture. A total of 63 upregulated and eight downregulated genes were observed in the *S.* Tennessee isolate. The authors also carried out an identical experiment using *S.* Typhimurium LT2, which is more susceptible to desiccation stress, and this isolate had demonstrated 63 upregulated and nine downregulated genes compared to the control.

Finn et al. (2013b) investigated the transcriptomic response of *S.* Typhimurium ST4/74 to desiccation on a stainless steel surface. Early stationary phase (ESP) cells suspended in Luria-Bertani (LB) medium were dried onto stainless steel (grade 304) for 4 h at 24 °C in a sterile laminar flow cabinet at ca. 45 % relative humidity, after which the RNA was extracted and examined using a microarray protocol. Differentially expressed genes were identified as those having a greater than fivefold change ($P < 0.05$) in expression in comparison to the inoculum culture. After 4 h of desiccation, 266 upregulated and 187 downregulated genes were identified.

The authors also compared the genes upregulated in each of these three reports and discovered that, although some genes were shared for all three studies, the majority of genes showing increased expression were unique to each study. It is likely that these differences are a result of the serovar to serovar variation, time points of RNA purification, and the abiotic surface used for desiccation. It was also postulated by these authors that although *Salmonella* can survive in a desiccated state for extended time periods, the response during the early stages of desiccation, when a limited amount of water is available for biological reactions, may be important for long-term survival. A further conclusion by these authors was that the examination of the transcriptome after long periods of desiccation may cause certain signals to be overlooked due to the effect of RNA degradation.

The transcriptomic responses observed over the three aforementioned investigations are now discussed in more depth. It is important to note that while the results obtained reflect desiccation occurring in an aerated state at ambient temperature, an alternate set of signals are likely to be observed in response to other modes of dehydration such as spray drying (Finn et al. 2013a).

Osmoprotection

Osmoprotectant Transport

The importation of osmoprotectant molecules, such as trehalose, proline, glycine betaine, and other so-called compatible solutes into the bacterial cell, is an important mechanism by which cells respond to a reduction in water availability from the

surrounding environment (Csonka and Hanson 1991). These low-molecular-weight compatible solutes can accumulate to high concentrations within the cell without affecting cellular processes, and they function to limit the subsequent loss of water (Csonka 1989; Csonka and Hanson 1991).

During desiccation stress, two ABC transporters (ATP-binding cassette transporters), *proU* and *osmU*, involved in the uptake of osmoprotectants were upregulated during the early stages of desiccation (Finn et al. 2013b; Li et al. 2012). Deletion of either of these transporters resulted in a marked decrease in survival compared to the wild-type isolate (Finn et al. 2013b). Secondly, the ProP proline permease was determined to be essential for long-term desiccation survival on a stainless steel surface, with no viable cells being detected after 4 weeks of desiccation in mutant isolates lacking *proP* (Finn et al. 2013b). Interestingly, no osmoprotectant transporter genes were identified by Gruzdev et al. (2012) after 22 h of desiccation, perhaps indicating that the cells do not possess the energy required to drive these systems during a later stage in the desiccation response.

Potassium Import

Gruzdev et al. (2012) identified a significant upregulation of the *kdp* genes that encode a major K^+ transporter. This high-affinity K^+ P-type ATPase is often induced during the initial stages of osmotic upshift, where uptake of this ion quickly balances the osmotic pressure in the cell and is later replaced by neutral compounds, such as the osmoprotectants mentioned above (Csonka 1989). Therefore, these results challenge the concept that an increase in K^+ import precedes the induction of transporters such as *proP* and *proU*. This observation is consistent with results obtained by Balaji et al. (2005), who determined that both *proP* and *proU* are induced in the presence of NaCl in advance of an increase in transcription of the *kdpFABC* genes. However, deletion of the *kdpA* gene did not affect dehydration tolerance; therefore whether this gene has a functional role during desiccation stress remains to be determined (Gruzdev et al. 2012).

Trehalose Biosynthesis

Similar to the case of the osmoprotectant transporters, trehalose biosynthesis has been reported to be upregulated during the early stages of desiccation (Li et al. 2012; Finn et al. 2013b). This disaccharide also functions as a compatible solute and plays an important role during the osmotic stress response (Kempf and Bremer 1998; Strom and Kaasen 2006; Balaji et al. 2005). Li et al. (2012) measured the level of trehalose production in a desiccation-tolerant and desiccation-sensitive *Salmonella* isolate and observed a significant increase in trehalose biosynthesis in the tolerant strain, indicating an important role for this compound for survival in a dehydrated state. Due to the anabolic diversion of glucose into trehalose production, it has been postulated that an increase in fatty acid catabolism functions as an

alternative energy source for the bacterial cells (Finn et al. 2013b). Both Finn et al. (2013b) and Li et al. (2012) observed an upregulation of these catabolic genes during the initial stages of desiccation stress.

Amino Acid Biosynthesis

Significant upregulation was observed in histidine biosynthesis in two of the studies investigating the transcriptomic response to desiccation (Gruzdev et al. 2012; Finn et al. 2013b). It is possible that this amino acid possesses a hitherto unrecognized protective role during exposure to low-moisture conditions, possibly by increasing the abundance of histidine-containing proteins that may confer a stabilization effect (Finn et al. 2013b). However, this observation requires further investigation in order to deduce the functional role for this amino acid during desiccation stress. Other amino acids associated with upregulation during desiccation stress include arginine, leucine, and cysteine (Finn et al. 2013b; Gruzdev et al. 2012). However, as with histidine, further investigation is warranted to determine whether these compounds contribute to desiccation resistance.

Central Metabolism

As mentioned earlier, an increase in fatty acid catabolism has been linked to the transition of *Salmonella* into a desiccated state (Finn et al. 2013b; Li et al. 2012). These reactions generate more ATP molecules per carbon atom in comparison to glucose and are therefore an efficient method of energy production (Finn et al. 2013a).

Another change observed, in regard to central metabolism, is an increase of the glyoxylate shunt pathway, which is encoded by the isocitrate lyase (*aceA*) and malate synthase (*aceB*) genes. This pathway was upregulated after 22 h of dehydration in *Salmonella* dried onto plastic and after 2 h when dried onto paper disks (Gruzdev et al. 2012; Li et al. 2012). Gruzdev et al. (2012) postulated that following the induction of this pathway, the metabolites passing through the TCA cycle would be reduced, therefore decreasing the concentration of available reducing power in the form of NADH being produced from glucose. This reduction in NADH may then cause a reduction in the concentration of reactive oxygen species derived from respiration, thereby ultimately limiting oxidative stress (Gruzdev et al. 2012). This shunt is often induced when glucose is in limited supply (Fischer and Sauer 2003). Hence, the induction observed under the desiccation investigation conditions reported by Li et al. (2012) and Gruzdev et al. (2012) may be attributed to the cells being dehydrated in a nutrient-limited medium. This may also explain why no induction was observed by Finn et al. (2013b) when a nutrient-rich medium was used during the desiccation process. However, deletion of *aceA* did lead to a marked reduction in dehydration tolerance over 22 h on plastic compared to the wild-type strain (Gruzdev et al. 2012).

Ribosome Structure

Gruzdev et al. (2012) reported that a number of the genes that were significantly upregulated after desiccation included those that functioned in the translational processes, including genes involved in the ribosomal structure. In contrast, in nutrient-starved environments, this signaling was not observed (Kaczanowska and Rydén-Aulin 2007; Gruzdev et al. 2012). The former authors speculated that this induction is a direct result of the presence of nutrients released from cells that do not survive the dehydration process, which then drive translation in the surviving cells (Gruzdev et al. 2012). Genes of this functional group have not been observed to be upregulated during the earlier stages of desiccation, perhaps indicating a later induction (Finn et al. 2013b; Li et al. 2012).

Transcriptional Regulator σ^E

In the context of desiccation, the sigma factor encoded by the *rpoE* gene has a function in the long-term desiccation survival of *Salmonella* (Gruzdev et al. 2012; Finn et al. 2013b). This sigma factor is involved in the response to environmental factors that cause envelope stress, such as osmotic shock, heat, and starvation (McMeechan et al. 2007; Alba and Gross 2004; Rhodius et al. 2005). The sigma factor σ^E was identified as being significantly upregulated after 4 h of desiccation on stainless steel and also after 22 h of desiccation on a plastic surface (Finn et al. 2013b; Gruzdev et al. 2012). This may indicate that σ^E is required, regardless of the abiotic surface upon which the desiccation occurs. Furthermore, knockout mutations were created in both of these investigations, which subsequently confirmed a role for this regulator on the survival potential of the *Salmonella*, as these mutant isolates had statistically lower desiccation persistence (Gruzdev et al. 2012; Finn et al. 2013b).

Survival Mechanisms Employed by *Salmonella* in Media Containing Moisture-Reducing Compounds

The mechanisms used by bacteria in response to osmotic shock in a broth culture have been extensively studied, particularly for NaCl (Finn et al. 2013a; Podolak et al. 2010; Csonka and Hanson 1991). Many of these studies were conducted using subinhibitory concentrations of those compounds of interest and wherein bacteria were still capable of growth. These mechanisms have recently been reviewed (Finn et al. 2013a) and are discussed in brief below.

As outlined above, when exposed to reductions in the external water activity, bacteria must quickly and efficiently respond in order to preserve turgor, often via the early influx of K^+, followed by compatible solute uptake (Csonka 1989). A number

of different mechanisms that control the transition from K^+ uptake to osmoprotectant accumulation have been proposed and have been reviewed by Finn et al. (2013a). The main osmoprotectants in question are proline, glycine betaine, and ectoine (Csonka and Hanson 1991), and the main transporters involved include ProP, ProU, and OsmU, which have all been linked to survival and adaptation to low-water-activity stress in broth culture (Cairney et al. 1985; Stirling et al. 1989; Frossard et al. 2012). As in the case of desiccation, trehalose is also important for the adaptation to osmotic shock by NaCl (Balaji et al. 2005; Strom and Kaasen 2006; Csonka and Hanson 1991; Kempf and Bremer 1998). The production of this disaccharide is controlled by the alternative sigma factor RpoS, which induces the *otsAB* genes (Kempf and Bremer 1998). Induction of these genes appears to be one of the immediate responses to NaCl shock, with increased expression observed after 6 min of exposure (Balaji et al. 2005).

Regulation of two outer membrane porins, OmpF and OmpC via the EnvZ/OmpR, a two-component regulatory system, is another strategy by which bacteria respond to osmotic changes (Finn et al. 2013a; Feng et al. 2003; Wang et al. 2012; Hall and Silhavy 1981). An increase in external osmolarity leads to higher levels of the phosphorylated OmpR response regulator, eventually causing a downregulation of OmpF with OmpC becoming the most predominant porin (Feng et al. 2003; Balaji et al. 2005). This again allows for the passive diffusion of compatible solutes (Kempf and Bremer 1998).

Most of studies investigating the survival mechanisms following exposure to NaCl have been conducted at a_w levels much higher than those used in a low-moisture food setting. However, Deng et al. (2012) reported the first studies using RNA-seq technology to investigate the transcriptomic response of *Salmonella* using peanut oil (a_w 0.3), a low-moisture food matrix. Under these conditions it was reported that *Salmonella* enter a dormant state in which <5 % of the genome was transcribed. When the same salmonellae were grown in a non-stressful environment (LB), 78 % of the total genome was actively transcribed. Aside from the low-a_w stress, this dormancy may have resulted from nutrient starvation, also leading to an increased level of rRNA degradation (Deng et al. 2012; Lewis et al. 1996; Deutscher 2006). Other genes with increased expression in peanut oil were a selection of genes involved in DNA protection (Deng et al. 2012). Similar to desiccation, the RpoE sigma factor was upregulated in those bacterial cells exposed to peanut oil, as was the heat shock sigma factor RpoH (Deng et al. 2012). This study was the first to investigate the response of *Salmonella* in a low-moisture, food-relevant matrix. Similar research conducted on other reduced-moisture products will undoubtedly extend our early knowledge of how bacteria respond in these stressful circumstances.

NaCl is the most extensively studied ionic compound in the context of cellular osmotic response. However, within the food industry, other humectants may be added to products in order to reduce the moisture content and enhance flavor. The means by which bacteria respond to these compounds have not been thoroughly characterized. A recent investigation described the transcriptomic response of *S.* Typhimurium following 6 h of exposure to NaCl, KCl (ionic-based humectants),

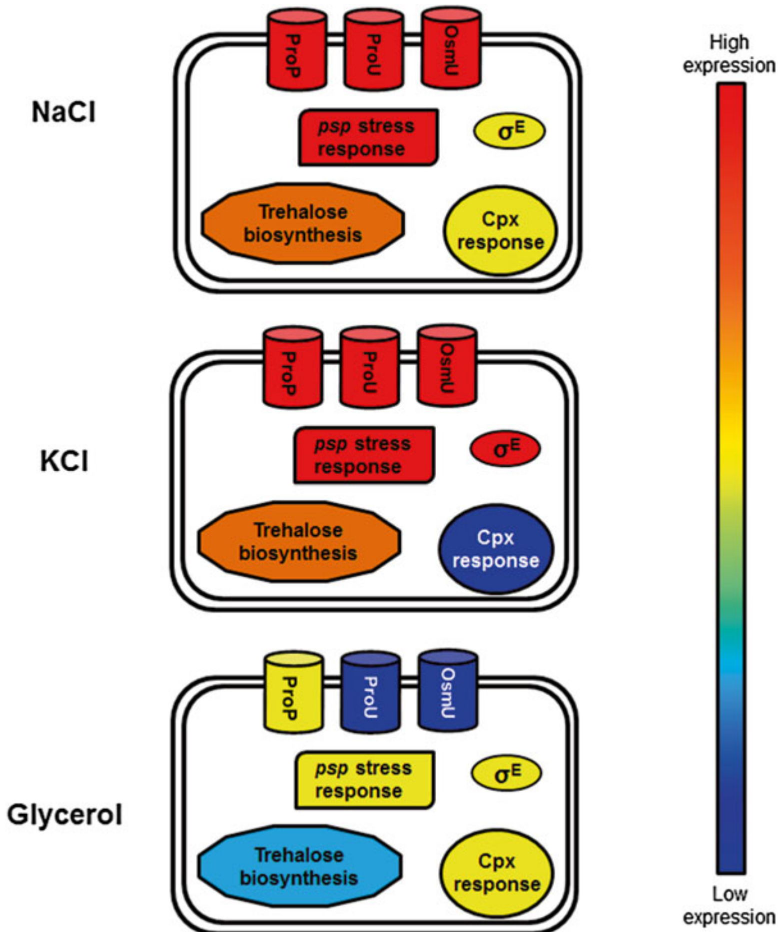

Fig. 3 Example of cellular changes occurring within cells exposed to three different humectants over a 6-h period ($a_w = 0.95$)

and glycerol (nonionic), using a common a_w of 0.95 (Finn et al. unpublished data). This investigation highlighted transcriptional signals used by *Salmonella* in response to alterations in the environment. Results revealed that the bacterial response was dependent on the humectant used. Overall, KCl induced the most extensive changes when compared to an inoculum control after 6 h of exposure. Both NaCl and KCl significantly upregulated osmoprotectant transport systems and trehalose biosynthesis. In contrast, the opposite was observed for cells exposed to glycerol (Fig. 3). The transcriptional σ^E regulator was also induced by KCl, however, but this was not observed in the presence of NaCl and glycerol. Both ionic compounds tested also induced the upregulation of the *psp*- and Cpx-mediated stress response circuits; however, this was not observed in the presence of the nonionic glycerol.

Conclusion

Salmonella is one of the most important pathogens in the context of low-moisture food products. Further characterization of mechanisms used by salmonellae to adapt to dehydrated conditions is essential to gain a complete understanding of how *Salmonella* persists in food manufacturing facilities and food matrices. Combining several methodological platforms for such investigations, including transcriptomics and metabolomics, may help to provide a more holistic view of the bacterial responses occurring under these stressed conditions. Ultimately, it is hoped that the knowledge gained from these studies will aid in the design of new control protocols aimed at reducing *Salmonella* persistence in the food production environment, perhaps by the development of targeted biocidal compounds. The responses of *Salmonella* within a desiccated state are beginning to be elucidated and further characterization will yield even more data, possibly contributing to other hitherto uncharacterized survival mechanisms. Currently, data describing how *Salmonella* responds within a low-a_w food matrix is lacking and will likely become a prevalent research topic in the future.

References

Alba BM, Gross CA (2004) Regulation of the *Escherichia coli* σ^E dependent envelope stress response. Mol Microbiol 52:613–619

Aviles B, Klotz C, Smith T, Williams R, Ponder M (2013) Survival of *Salmonella enterica* serotype Tennessee during simulated gastric passage is improved by low water activity and high fat content. J Food Prot 76:333–337

Balaji B, O'connor K, Lucas JR, Anderson JM, Csonka LN (2005) Timing of induction of osmotically controlled genes in *Salmonella enterica* serovar Typhimurium, determined with quantitative real-time reverse transcription-PCR. Appl Environ Microbiol 71:8273–8283

Barrett T, Lior H, Green J, Khakhria R, Wells J, Bell B, Greene K, Lewis J, Griffin P (1994) Laboratory investigation of a multistate food-borne outbreak of *Escherichia coli* O157: H7 by using pulsed-field gel electrophoresis and phage typing. J Clin Microbiol 32:3013–3017

Beuchat LR, Komitopoulou E, Beckers H, Betts RP, Bourdichon F, Fanning S, Joosten HM, Ter Kuile BH (2013) Low water activity foods: increased concern as vehicles of foodborne pathogens. J Food Prot 76:150–172

Bullifent HL, Dhaliwal K, Howells AM, Goan K, Griffin K, Lindsay CD, Tunnacliffe A, Titball RW (2000) Stabilisation of *Salmonella* vaccine vectors by the induction of trehalose biosynthesis. Vaccine 19:1239–1245

Bustin SA (2000) Absolute quantification of mRNA using real-time reverse transcription polymerase chain reaction assays. J Mol Endocrinol 25:169–193

Cairney J, Booth I, Higgins C (1985) Osmoregulation of gene expression in *Salmonella* Typhimurium: *proU* encodes an osmotically induced betaine transport system. J Bacteriol 164:1224–1232

Carbonnelle E, Mesquita C, Bille E, Day N, Dauphin B, Beretti J-L, Ferroni A, Gutmann L, Nassif X (2011) MALDI-TOF mass spectrometry tools for bacterial identification in clinical microbiology laboratory. Clin Biochem 44:104–109

Carrasco E, Morales-Rueda A, García-Gimeno RM (2012) Cross-contamination and recontamination by *Salmonella* in foods: A review. Food Res Int 45:545–556

Coburn B, Grassl GA, Finlay B (2007) *Salmonella*, the host and disease: a brief review. Immunol Cell Biol 85:112–118

Cole HJ (2005) The survival and mechanisms of inactivation of Salmonella organisms during drying in relation to food safety. Ph.D. Thesis, School of Food Biosciences, University of Reading, UK

Condell O, Sheridan A, Power K, Bonilla-Santiago R, Sergeant K, Renaut J, Burgess C, Fanning S, Nally J (2012) Comparative proteomic analysis of *Salmonella* tolerance to the biocide active agent triclosan. J Proteomics 75:4505–4519

Croucher NJ, Thomson NR (2010) Studying bacterial transcriptomes using RNA-seq. Curr Opin Microbiol 13:619–624

Csonka LN (1989) Physiological and genetic responses of bacteria to osmotic stress. Microbiol Rev 53:121–147

Csonka LN, Hanson AD (1991) Prokaryotic osmoregulation: genetics and physiology. Annu Rev Microbiol 45:569–606

Deng X, Li Z, Zhang W (2012) Transcriptome sequencing of *Salmonella enterica* serovar Enteritidis under desiccation and starvation stress in peanut oil. Food Microbiol 30:311–315

Deutscher MP (2006) Degradation of RNA in bacteria: comparison of mRNA and stable RNA. Nucleic Acids Res 34:659–666

Esaki H, Noda K, Otsuki N, Kojima A, Asai T, Tamura Y, Takahashi T (2004) Rapid detection of quinolone-resistant *Salmonella* by real time SNP genotyping. J Microbiol Methods 58:131–134

Feng X, Oropeza R, Walthers D, Kenney LJ (2003) OmpR phosphorylation and its role in signaling and pathogenesis. ASM News 69:390–395

Finn S, Condell O, Mcclure P, Amézquita A, Fanning S (2013a) Mechanisms of survival, responses and sources of *Salmonella* in low-moisture environments. Frontiers Microbiol 4:331

Finn S, Händler K, Condell O, Colgan A, Cooney S, Mcclure P, Amézquita A, Hinton JCD, Fanning S (2013b) ProP is required for the survival of desiccated *Salmonella enterica* serovar Typhimurium cells on a stainless steel surface. Appl Environ Microbiol 79:4376–4384

Fischer E, Sauer U (2003) Metabolic flux profiling of *Escherichia coli* mutants in central carbon metabolism using GC-MS. Eur J Biochem 270:880–891

Foley SL, Zhao S, Walker RD (2007) Comparison of molecular typing methods for the differentiation of *Salmonella* foodborne pathogens. Foodborne Pathog Dis 4:253–276

Frossard SM, Khan AA, Warrick EC, Gately JM, Hanson AD, Oldham ML, Sanders DA, Csonka LN (2012) Identification of a third osmoprotectant transport system, the OsmU system, in *Salmonella enterica*. J Bacteriol 194:3861–3871

Gibson U, Heid CA, Williams PM (1996) A novel method for real time quantitative RT-PCR. Genome Res 6:995–1001

Giebel R, Worden C, Rust S, Kleinheinz G, Robbins M, Sandrin T (2010) Microbial fingerprinting using matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS): applications and challenges. Adv Appl Microbiol 71:149–184

Gill O, Sockett P, Bartlett C, Vaile M, Rowe B, Gilbert R, Dulake C, Murrell H, Salmaso S (1983) Outbreak of *Salmonella* Napoli infection caused by contaminated chocolate bars. Lancet 1:574

Ginzinger DG (2002) Gene quantification using real-time quantitative PCR: an emerging technology hits the mainstream. Exp Hematol 30:503–512

Greenwood MH, Hooper W (1983) Chocolate bars contaminated with *Salmonella* Napoli: an infectivity study. Br Med J (Clin Res Ed) 286:1394

Gruzdev N, Mcclelland M, Porwollik S, Ofaim S, Pinto R, Saldinger-SELA S (2012) Global transcriptional analysis of dehydrated *Salmonella enterica* serovar Typhimurium. Appl Environ Microbiol 78:7866–7875

Gruzdev N, Pinto R (2012) Persistence of *Salmonella enterica* during dehydration and subsequent cold storage. Food Microbiol 32:415–422

Gwendoline K, Kathleen S, Geert S, David DC, Jos V, Sigrid DK (2009) 2D proteome analysis initiates new Insights on the *Salmonella* Typhimurium LuxS protein. BMC Microbiol 9:198

Hall MN, Silhavy TJ (1981) Genetic analysis of the major outer membrane proteins of *Escherichia coli*. Annu Rev Genet 15:91–142

Hegde P, Qi R, Abernathy K, Gay C, Dharap S, Gaspard R, Hughes J, Snesrud E, Lee N, Quackenbush J (2000) A concise guide to cDNA microarray analysis. Biotechniques 29:548–563

Hiramatsu R, Matsumoto M, Sakae K, Miyazaki Y (2005) Ability of Shiga toxin-producing *Escherichia coli* and *Salmonella* spp. to survive in a desiccation model system and in dry foods. Appl Environ Microbiol 71:6657–6663

Holmberg SD, Wachsmuth I, Hickman-Brenner F, Cohen M (1984) Comparison of plasmid profile analysis, phage typing, and antimicrobial susceptibility testing in characterizing *Salmonella* Typhimurium isolates from outbreaks. J Clin Microbiol 19:100–104

Julian E, Macdonald K, Marsden-Haug N, Saathoff-Huber L, Bonavolante R, Otero S, Nosari J, Austin C, Stein D, Garvey A (2010) *Salmonella* Montevideo infections associated with salami products made with contaminated imported black and red pepper, United States, July 2009–April 2010. MMWR 59:1647–1650

Kaczanowska M, Rydén-Aulin M (2007) Ribosome biogenesis and the translation process in *Escherichia coli*. MMBR 71:477–494

Kapperud G, Gustavsen S, Hellesnes I, Hansen AH, Lassen J, Hirn J, Jahkola M, Montenegro MA, Helmuth R (1990) Outbreak of *Salmonella* Typhimurium infection traced to contaminated chocolate and caused by a strain lacking the 60-megadalton virulence plasmid. J Clin Microbiol 28:2597–2601

Kempf B, Bremer E (1998) Uptake and synthesis of compatible solutes as microbial stress responses to high-osmolality environments. Arch Microbiol 170:319–330

Kröger C, Dillon SC, Cameron ADS, Papenfort K, Sivasankaran SK, Hokamp K, Chao Y, Sittka A, Hébrard M, Händler K, Colgan A, Leekitcharoenphon P, Langridge GC, Lohan AJ, Loftus B, Lucchini S, Ussery DW, Dorman CJ, Thomson NR, Vogel J, Hinton JCD (2012) The transcriptional landscape and small RNAs of *Salmonella enterica* serovar Typhimurium. Proc Natl Acad Sci U S A 109:E1277–E1286

Lesne J, Berthet S, Binard S, Rouxel A, Humbert F (2000) Changes in culturability and virulence of *Salmonella* Typhimurium during long-term starvation under desiccating conditions. Int J Food Microbiol 60:195–203

Lewis DA, Paramathasan R, White DG, Neil LS, Tanner AC, Hill SD, Bruce JC, Stuart JM, Ridley AM, Threlfall EJ (1996) Marshmallows cause an outbreak of infection with *Salmonella* Enteritidis phage type 4. Commun Dis Rep CDR Rev 6:R183–R186

Li H, Bhaskara A, Megalis C, Tortorello ML (2012) Transcriptomic analysis of *Salmonella* desiccation resistance. Foodborne Pathog Dis 9:1143–1151

Lilley KS, Friedman DB (2004) All about DIGE: quantification technology for differential-display 2D-gel proteomics. Expert Rev Proteomics 1:401–409

Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-}\Delta\Delta^{CT}$ method. Methods 25:402–408

Majowicz SE, Musto J, Scallan E, Angulo FJ, Kirk M, O'brien SJ, Jones TF, Fazil A, Hoekstra RM (2010) The global burden of nontyphoidal *Salmonella* gastroenteritis. Clin Infect Dis 50:882–889

Mcmeechan A, Roberts M, Cogan TA, Jørgensen F, Stevenson A, Lewis C, Rowley G, Humphrey TJ (2007) Role of the alternative sigma factors σ^E and σ^S in survival of *Salmonella enterica* serovar Typhimurium during starvation, refrigeration and osmotic shock. Microbiology 153:263–269

Morita T, Kitazawa H, Iida T, Kamata S (2006) Prevention of *Salmonella* cross contamination in an oilmeal manufacturing plant. J Appl Microbiol 101:464–473

Mullane N, Whyte P, Wall P, Quinn T, Fanning S (2007) Application of pulsed-field gel electrophoresis to characterise and trace the prevalence of *Enterobacter sakazakii* in an infant formula processing facility. Int J Food Microbiol 116:73–81

Podolak R, Enache E, Stone W, Black DG, Elliott PH (2010) Sources and risk factors for contamination, survival, persistence, and heat resistance of *Salmonella* in low-moisture foods. J Food Prot 73:1919–1936

Possemiers S, Marzorati M, Verstraete W, Van De Wiele T (2010) Bacteria and chocolate: a successful combination for probiotic delivery. Int J Food Microbiol 141:97–103

Quinn PJ, Markey BK, Leonard F, Fitzpatrick E, Fanning S, Hartigan P (2011) Veterinary microbiology and microbial disease. Wiley, New York, NY

Rabsch W, Prager R, Koch J, Stark K, Roggentin P, Bockemuhl J, Beckmann G, Stark R, Siegl W, Ammon A (2005) Molecular epidemiology of *Salmonella enterica* serovar Agona: characterization of a diffuse outbreak caused by aniseed-fennel-caraway infusion. Epidemiol Infect 133:837–844

Rhodius VA, Suh WC, Nonaka G, West J, Gross CA (2005) Conserved and variable functions of the σ^E stress response in related genomes. PLoS Biol 4:e2

Riedel K, Lehner A (2007) Identification of proteins involved in osmotic stress response in *Enterobacter sakazakii* by proteomics. Proteomics 7:1217–1231

Rowe B, Hutchinson DN, Gilbert RJ, Hales BH, Begg NT, Dawkins HC, Jacob M, Rae FA, Jepson M (1987) *Salmonella* Ealing infections associated with consumption of infant dried milk. Lancet 330:900–903

Scheil W, Cameron S, Dalton C, Murray C, Wilson D (1998) A South Australian *Salmonella* Mbandaka outbreak investigation using a database to select controls. Aust N Z J Public Health 22:536–539

Schena M, Shalon D, Davis RW, Brown PO (1995) Quantitative monitoring of gene expression patterns with a complementary DNA microarray. Science 270:467–470

Shalon D, Smith SJ, Brown PO (1996) A DNA microarray system for analyzing complex DNA samples using two-color fluorescent probe hybridization. Genome Res 6:639–645

Sonck KA, Kint G, Schoofs G, Vander Wauven C, Vanderleyden J, De Keersmaecker SC (2009) The proteome of *Salmonella* Typhimurium grown under in vivo-mimicking conditions. Proteomics 9:565–579

Sparbier K, Weller U, Boogen C, Kostrzewa M (2012) Rapid detection of *Salmonella* sp by means of a combination of selective enrichment broth and MALDI-TOF MS. Eur J Clin Microbiol Infect Dis 31:767–773

Stirling D, Hulton C, Waddell L, Park S, Stewart G, Booth I, Higgins C (1989) Molecular characterization of the *proU* loci of *Salmonella* Typhimurium and *Escherichia coli* encoding osmoregulated glycine betaine transport systems. Mol Microbiol 3:1025–1038

Strom AR, Kaasen I (2006) Trehalose metabolism in *Escherichia coli*: stress protection and stress regulation of gene expression. Mol Microbiol 8:205–210

Thompson A, Rowley G, Alston M, Danino V, Hinton JCD (2006) *Salmonella* transcriptomics: relating regulons, stimulons and regulatory networks to the process of infection. Curr Opin Microbiol 9:109–116

Van Doren JM, Neil KP, Parish M, Gieraltowski L, Gould LH, Gombas KL (2013) Foodborne illness outbreaks from microbial contaminants in spices, 1973–2010. Food Microbiol 36:456–464

Wang LC, Morgan LK, Godakumbura P, Kenney LJ, Anand GS (2012) The inner membrane histidine kinase EnvZ senses osmolality via helix-coil transitions in the cytoplasm. EMBO J 31:2648–2659

Wattiau P, Boland C, Bertrand S (2011) Methodologies for *Salmonella enterica* subsp. *enterica* subtyping: gold standards and alternatives. Appl Environ Microbiol 77:7877–7885

Weissman JB, Doug Deen R, Williams M, Swanston N (1977) An island-wide epidemic of salmonellosis in Trinidad traced to contaminated powdered milk. West Indian Med J 26:135–143

Welsh DT, Herbert RA (1999) Osmotically induced intracellular trehalose, but not glycine betaine accumulation promotes desiccation tolerance in *Escherichia coli*. FEMS Microbiol Lett 174:57–63

Werber D, Dreesman J, Feil F, Van Treeck U, Fell G, Ethelberg S, Hauri A, Roggentin P, Prager R, Fisher I (2005) International outbreak of *Salmonella* Oranienburg due to German chocolate. BMC Infect Dis 5:7

Wilson MR, Allard MW, Brown EW (2013) The forensic analysis of foodborne bacterial pathogens in the age of whole-genome sequencing. Cladistics 29:449–461

Yang YH, Speed T (2002) Design issues for cDNA microarray experiments. Nat Rev Genet 3:579–588

Processing Plant Investigations: Practical Approaches to Determining Sources of Persistent Bacterial Strains in the Industrial Food Processing Environment

Jeffrey L. Kornacki

Abstract Persistent pathogenic bacterial strains may result from many sources, such as repetitively contaminated ingredients brought into the plant environment, unrepaired roof leaks, and workers routinely entering the plant from agricultural or other contaminated areas, as well as from a microbial population that has established itself in the plant environment, perhaps originating from one or more of these venues. Pathogenic bacteria may also adapt to dry conditions and survive for years under these conditions, including within the production environment and in dry raw ingredients, a phenomena documented to occur with strains of *Salmonella*. These can be very difficult situations to effectively investigate and resolve, made even worse by certain false paradigms employed by investigators, Molecular subtyping approaches to identify the occurrence of persistent strains are usually required.

Keywords Investigation • Persistent strain • Adaptation • False assumptions • Molecular subtyping • Salmonella • Indicator

Persistent Strain Defined

Persistent strains have been alternatively described as "endemic strains," "systemic strains," "house bugs," "recurrent strains," and "persistent strains" in this author's experience. Sometimes repetitive findings result in a perspective that the plant environment has become a "harborage" for a particular strain. The principal characteristic which these terms describe is one of repeated isolation of the same strain over time, in a given facility. Kastenberg and Gram (2009) defined the phenomenon of strain persistence as "…the ability to repeatedly isolate a specific molecular subtype or

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strain in the same factory during an extended period of time; typically months or years." These findings become troublesome to regulators and industry alike, especially when such findings occur subsequent to strenuous attempts at corrective action to eliminate the microbe from the processing facility.

Two common assumptions related to these recurring subtypes are that they (1) have become adapted to the food processing plant environment and (2) occurred as a result of negligent control efforts by the food producer. These are possibilities, but neither assumption is necessarily true in every instance, as will be shown.

Adaptation

Some pathogenic bacterial strains can adapt to a processing plant environment, become dislodged by a variety of means, and move through the plant environment. However, it is also possible that a persistent strain may continually or sporadically enter the plant environment from an outside source or sources. There is evidence that strains of *Salmonella* are endemic to bird populations (Lawson et al. 2011). Hence, birds common to the locale of the plant may carry a particular strain and may nest or defecate in (or near) plant air intakes or on roof tops. Kornacki (2012a, b) described poorly maintained roof tops as potential sources of *Salmonella. Salmonella* endemic to such bird populations could thus be the originating source to the plant environment (e.g., through the air in cases where birds may roost in HVAC intakes or more commonly through torn wet roof membranes as alluded to above, thus seeding the plant sporadically). Such strains may also enter the plant environment routinely through contaminated ingredients or employee's shoes or clothing, particularly from those working in agricultural environments.

Negligent Control Efforts or a Difficult Problem

It is not uncommon for food production facilities with strict employee hygiene policies and practices to have persistent bacterial strains, despite rigorous programs to "seek and destroy" such microbes. It is important to recognize that these microbes are 1 millionth the length of a human, thus taking up roughly $1/10^{18}$ the volume of an adult human being. Such tiny microbes, invisible to the naked eye, are able to exist in microscopic areas in the facility. Such facilities may be millions of cubic feet in size. Furthermore, poorly designed equipment and facility structures can create significant challenges to even the best control efforts. Hence, the presence of a particular strain found in a food processing environment two or more times is not necessarily reflective of a facility's lack of commitment to eliminate such harborage points.

Why Dry Foods Present a Microbiological Risk

Although microbiological-induced illnesses have occurred from contaminated low-water-activity foods for many decades (Table 1), it required many years to recognize the significance of this public health risk.

Low-Water-Activity Considerations

The amount of chemically available moisture in a sample is referred to as water activity (a_w). The water activity of a material has a profound effect on the ability of a microbe to grow or survive. a_w values vary between 0.000 and 1.000 and are defined as the ratio of vapor pressure in the target ingredient divided by the vapor pressure of pure water (which is assumed to be 1.00 under standard conditions). The amount of chemically available moisture required for growth varies between microorganisms, all conditions being equal (Fig. 1). a_w values below a minimum level required for microbial growth do not ensure the death of a microbial population

Table 1 Selected *Salmonella*-associated outbreaks (recalls[a]) with ready-to-eat, low-moisture products

Product	Year
Milk powder[b]	1973
Chocolate[b]	1982–1983
	1985–1986, 1987
	2001, 2006
Powdered infant formula[b]	1993, 2008
Paprika-seasoned potato chips	1993
Infant cereals	1995
Peanut butter[b,c]	1996, 2006–2007, 2008–2009, 2011
Breakfast cereal[b,c]	1998, 2008
Raw almonds[b]	2000–2001; 2003–2004
Peanuts[b]	2001
Tahini and halva[b]	2002
Puffed rice and corn children's snack with a vegetative seasoning[b]	2007
Spices[c]	(1980–2000; 2009, 2009–2010)
	2010
Hydrolyzed vegetable protein (HVP)[c]	Recalled at 2010

[a]Recall only designated by parenthesis
[b](Scott et al. 2009)
[c](Kornacki 2012a)

Approximate Minimum a_w for Growth of Certain Groups of Microorganisms Important in Foods

Most spoilage bacteria	0.90-0.91	Most yeasts	0.87-0.94
Bacillus cereus	0.95	Osmophilic	0.60-0.78
Clostridium botulinum	0.90-0.98	Most molds	0.70-0.80
Type A	0.95	Xerophilic	0.60-0.70
Type B	0.94	*Aspergillus*	0.68-0.88
Type E	0.97	*A. flavus*	0.80-0.90
Enterobacter	0.95-0.98	*Fusarium*	0.80-0.92
Escherichia coli	0.94-0.97	*Saccharomyces rouxii*	0.62-0.81
Salmonella	0.93-0.96		
Staphylococcus aureus	0.84-0.92		
Vibrio parahaemolyticus	0.93-0.98		
Halophilic bacteria	0.75		

Adapted from Banwart, 1979

Fig. 1 Approximate minimum a_w for growth of selected microorganisms. Banwart GJ (1979) Conditions that influence microbial growth, Chap. 4. In: Basic food microbiology, AVI Publishing Co., Westport, CT

and, in fact, may induce desiccation-resistance genes, assisting the bacteria in the long-term plant environment survival process. Dry preservation techniques (e.g., freeze-drying), commonly used by laboratories to preserve microbial populations, are illustrative of that fact. Thus, the mere reduction of water activity cannot be relied upon to eliminate microbial populations, contrary to some popular beliefs.

It is well established that as the water activity of a product is reduced, the heat resistance of microbes in that matrix increases (Stumbo 1965; Geopfert et al. 1970).

Microbes That Adapt to Dry Foods and Dry-Food Processing Environments

Some microbial populations are known to have adaptive mechanisms enabling dormant survival in a dry state (see chapter in this book entitled “Adaptation of Pathogenic Microorganisms to Dry Conditions”). *Salmonella* and *Cronobacter* spp. (formerly *Enterobacter sakazakii*; Breeuwer et al. 2003) are among those bacterial genera of particular relevance to dry-food producers. An example of *Salmonella* survival in a dry environment was reported by Robertson (1972) who challenged floor dust (composed mainly of blanket dust but also hair and cigarette ash) from a boy’s boarding school environment. The artificially contaminated dust was sampled

over a 4-year period during which *Salmonella* was recovered. This author is aware of many situations where the same serotype of *Salmonella* has been resident in dry-processing facilities for more than a decade and in some instances two decades.

The Problem of Effective Cleaning

The equipment and facilities for dry-food processing have not historically been designed for traditional wet cleaning (Chen et al. 2009a, b). Generally, microbial growth does not occur in foods with water activity (a_w) values below 0.64. Consequently, microbial spoilage has not been a problem to this segment of the food industry if moisture is properly controlled (Chen et al. 2009a, b). However, in more recent times, concerns related to allergen control have resulted in increased pressure to "wet-clean" processing lines in these particular facilities (Kornacki 2012a). In addition, there is a regulatory perspective that food products produced on a common or shared line between validated cleaning and sanitation events must be considered part of the same lot. Many dry-processing facilities operate continuously for weeks between wet-cleaning events. However, the risk that such companies face is appreciable. For example, consider a company that produces a product over a 30-day period and tests composite samples collected across each 8-h shift. In this hypothetical example, the production facility considers each 8-h shift a "lot." The contamination of a product detected on day 30 would implicate all 30 days of production from a US regulatory position. Further, many food manufacturers have inadequate capacity for on-site storage of up to 30 days of production commodities. Storage in a third-party warehouse could be regarded in regulatory circles as not being under the control of the manufacturer. The statistics of finished product testing does not provide a true indication as to when the product was contaminated, despite the fact that products produced on day 30 were determined to be contaminated. Consequently, the entire lot from the first day to the last 30 days is then implicated. This type of regulatory pressure has also created incentives for increased validated wet cleaning (Kornacki 2012a). However, wet cleaning of these types of processing lines and facilities can result in an increased number of microbial growth niches, thus increasing the risk of finished product contamination as documented by Kornacki (2012a). A more complete discussion of wet and dry cleaning is provided in this book by authors Burnett and Hagberg.

In-Plant Investigations

The topic of in-plant investigations is also covered in greater detail in the book *Principles of Microbiological Troubleshooting in the Industrial Food Processing Environment* (Kornacki 2010). A combination of environmental and in-line and finished product investigatory sampling is often performed.

The Problem of Effective Product Sampling

This topic is covered in more detail in the chapter entitled "Methodological and Sampling Challenges to Testing Spices and Low a_w Foods for the Presence of Foodborne Pathogens" in this book. The low incidence of contamination often associated with foods mitigates against detection, finding low-incidence contamination, even if such lots were homogeneously contaminated (not usually the case) and tested with a perfect method (Kornacki 2011). For example, to find a "positive" sample in a production lot homogeneously contaminated at the 1 % level would require approximately 300 randomly selected samples in order to affect a 95 % chance of detecting one or more samples with a perfect method (Fig. 3). In the case of *Salmonella*, these samples can be composited into 20×375 g samples and is equivalent to 5× the FDA Category I testing level (Figs. 2 and 3). Such an approach can be employed in an investigational context on in-line samples obtained before and after contact with equipment that cannot be adequately broken down for investigation (Kornacki 2010).

The Problem of Perceptions

Direct measurable product contamination typically requires the presence of microbial niche(s) with high populations of CFUs, when large volumes of product are produced. However, a vast number of niches with lower numbers could also have the same effect. It is easier to recover high numbers of a pathogen in an environmental growth niche than low numbers in the finished product. For example, a niche with 10 million cells that sloughs into 10,000 lbs of product will result in an average level of contamination of ca. 2 CFU/g; however, detection of this level by enrichment techniques would require homogeneous distribution of the organism. Hence, it is critical to know where to sample in the environment. The creation and dispersal of such niches into the environment and ultimately the product can result from unsanitary (1) operating practices (including misapplied sanitation), (2) maintenance repair practices, and (3) design of equipment and facility. Pictorial examples of such conditions are found in *Principles of Microbiological Troubleshooting in the Industrial Food Processing Environmen*t (Kornacki 2010).

Among most manufacturers and microbiological investigators, the following paradigm generally holds true: If one believes that a microorganism cannot be present in a particular location, then it is unlikely that an investigation of that site will occur. This is especially true if the site is difficult to sample and extensive efforts are required to gain access to the sampling environment. A review of these assumptions can be found in publications by Kornacki (2009a, b, 2010).

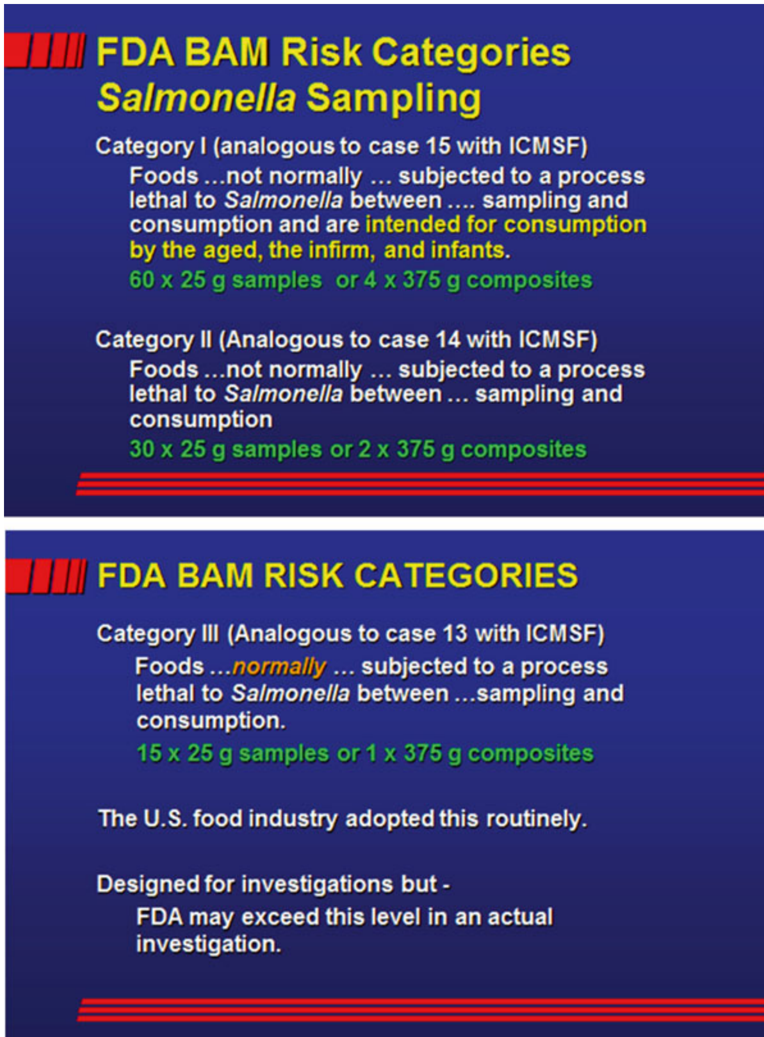

Fig. 2 FDA bacteriological analytical manual risk categories I and II

"Not That Organism" (*Listeria*, *Staphylococcus*, Enterohemorrhagic *E. coli*, *C. perfringens*, *B. cereus*, and Emerging Strains)

Absolutist paradigms regarding microbial contamination should be avoided. Earlier we discussed the false paradigm that microorganisms cannot contaminate dry foods because they will not grow. However, there is a long history of pathogen contamination of dry foods. That is because microorganisms may survive, despite their failure to grow (Scott et al. 2009; Chen et al. 2009a, b; Beuchat et al. 2013).

One such belief is that listeriae do not contaminate dry foods or dry-food processing environments. Nevertheless, the author's personal experience has shown

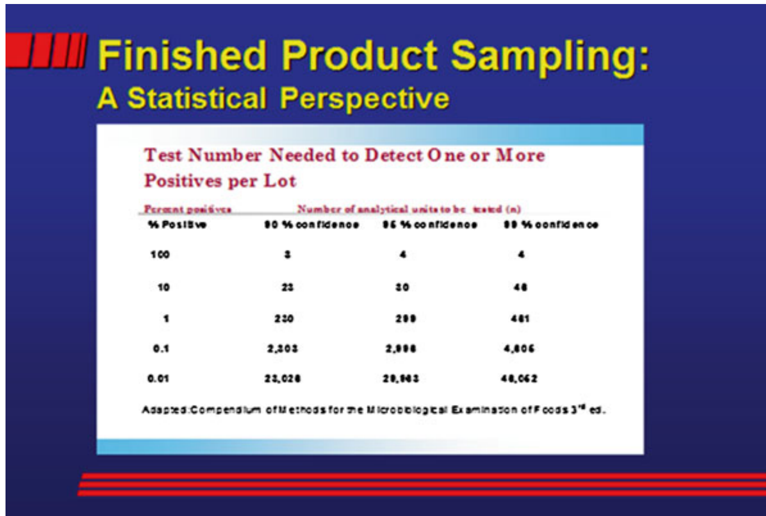

Fig. 3 Probabilities associated with the number of samples needed to detect a contaminant under ideal conditions

otherwise. Hints of this appear in the literature. For example, *Listeria* contamination of spray-drier facilities (Kornacki and Gurtler 2007) and a *Listeria monocytogenes* recall of bay leaves (Vij et al. 2006) have been documented.

Another common perception is that only *Staphylococcus aureus* or only coagulase-positive *Staphylococcus* spp. can produce staphylococcal enterotoxin (SET) in foods when, in fact, other *Staphylococcus* species, including *S. intermedius* and some coagulase-negative strains of *S. hyicus*, *xylosus*, *epidermidis*, *chromogenes*, *warneri*, *sciuri*, *saprophyticus*, *lentus*, *haemolyticus*, *hominis*, and *schleiferi*, have been shown to be SET producers (Udo et al. 1999; Valle et al. 1990; Oliveira et al. 2010; Veras et al. 2008). In addition, a number of commercial kits detect only SETs A, B, C, D, and E. Some may assume that these are the only SETs and that failure to detect these means that a sample does not contain SETs. However, SETs G, H, and I can produce emetic activity, while enterotoxin-like substances J through U have also been found (Seo and Bohach 2007). Hence, the presence of a negative result by a commercial SET assay does not prove the absence of a SET.

Yet another false paradigm is that *E. coli* O157:H7 does not form niches in the processing environment (Behling et al. 2010). Such a paradigm is convenient for sectors of the processing industry who can point blame back to the suppliers of raw ingredients. Another false paradigm encountered by this author is that *E. coli* O157:H7 is an indicator for all STEC that cause human illness. This is, despite its unique characteristics, compared to most other *E. coli* (e.g., its unusual tolerance for low pH, apparent failure to grow at 45.5 °C in EC broth (Behling et al. 2010), and atypical appearance on *E. coli* Petrifilm (Kornacki et al. 2013).

Another false assumption is that effective thermal processing applied to destroy *Clostridium botulinum* spores will also inactivate all other pathogens. Data, however, reveal that rare strains of *C. perfringens* and *Bacillus cereus* exist, which, under some conditions, are capable of surviving heat treatments capable of killing *C. botulinum* spores (Adams 1973; Bradshaw et al. 1975, 1977). Still another false assumption is that all foodborne pathogens have been discovered. However, history would indicate otherwise (Kornacki 2013).

"Not That Equipment or Site"

Manufacturers very often assume that microbes cannot move past a gasket (e.g., on a valve stem or rotary valve) or fail to recognize that pathogens can migrate from microbial niches between the inner and outer torn skin of a host to the food processing environment or pass through a sandwiched area of equipment into the processing environment or product stream. Experience has shown, however, that microbes are quite capable of entering such difficult-to-clean areas (Kornacki 2009a, b, 2010). There is no replacement for rigorous equipment break down by a skilled individual (and reassembly after appropriate cleaning, sanitation drying, or other appropriate remediation).

"Not That Cleaning Regime" (e.g., CIP, COP)

Other misguided assumptions have to do with the falsely assumed efficacy of unvalidated clean-in-place, manual cleaning, and clean-out-of-place approaches. The mere application of a cleaning and sanitization regimen does not ensure that microbial populations have been reduced to acceptable levels. Validation and verification of all such procedures should be done.

"Not That Process" (e.g., Heat Treatments, Gas Treatments)

Sometimes manufacturers mistakenly assume that thermal treatments used to destroy microbes in high-moisture foods can be used with equal efficacy in low-moisture foods. For example, more than 7.5 $\log_{10}$ CFU of most strains of *Salmonella* are destroyed at 158 °F for 15 s in high-a_w foods with pH values between 5.6 and 7.4 (Sörqvist 2003), but only one $\log_{10}$ reduction may occur in milk chocolate in 6.0–17.5 h at 158 °F, which has a much lower a_w (Mitscherlich and Marth 1984). Another assumption is that sterilant gas treatments used to decontaminate spices are equally effective across all spices, regardless of bulk density and gas penetration. In these authors experience, spices that have been gas-sterilized have sometimes been shown to be positive for *Salmonella* spp. Such misconceptions as these can lead to a failure to appropriately question CCPs.

Environmental Sampling Approaches

Many approaches, including but not limited to pre-sterilized sponge, swab, and contact plate sampling, are employed. Critical to this is appropriate training in aseptic techniques. The advantages and disadvantages of these environmental sampling approaches are described by Kornacki (2010).

Testing Approaches

The detection of microbes of interest should be done with a suitable method. These methods should be validated in the specific matrices to be tested and be accredited by an appropriate authoritative body (e.g., AOAC, FDA, USDA, AFNOR, etc). However, it should be acknowledged that some method development may be required in the context of newly recognized or emerging pathogens (Kornacki 2013).

The Importance of Molecular Subtyping

Detecting a microbe of the same genus and species in a raw ingredients or the environment, as in a finished product, does not necessarily prove that the implicated ingredient or environment was the source of contamination of the product. This is because the contaminant species may be common in the natural environment or the raw ingredient or may be one of the positive control microbes used by a testing laboratory. The genetic diversity, even at that species or serotype level, can be too broad to establish a causal link.

The Need for Molecular Subtyping

The aforementioned principles highlight the need for molecular subtyping, even in the case of *Salmonella* contamination, for which subspeciation by serotyping may mistakenly be considered an adequate degree of discrimination. The genus *Salmonella* is classified into two species, namely, *bongori* and *enterica*. The species *S. enterica* is further subdivided into six subspecies, namely, *enterica* (e.g., *Salmonella enterica* subsp. *enterica*), *salamae*, *arizonae*, *diarizonae*, *houtenae*, and *indica*. However, there are more than 2,400 known serovars of *Salmonella* (Brenner et al. 2000) illustrating the subspecies diversity of *Salmonella*.

Nevertheless, with common *Salmonella* isolates, additional discrimination through further subtyping is typically required to determine a plausible route of contamination. For example, *Salmonella enterica* serotype Typhimurium and serotype Enteritidis are two of the most common serotypes isolated in human infection, according to the CDC National *Salmonella* Surveillance Annual Summaries (http://www.cdc.gov/ncidod/dbmd/phlisdata/salmonella.htm) (2012). A high degree of

genetic diversity, as reflected by pulsed-field gel electrophoresis (PFGE) patterns and random fragment length polymorphisms (RFLP) patterns, exists within serotypes Typhimurium and Enteritidis (Bender et al. 2001; Gatto et al. 2006). For example, Bender et al. (2001) reported 174 unique PFGE patterns of *Salmonella* Typhimurium. Hence, finding *Salmonella* Typhimurium in raw materials that are oven treated as well as in finished product prepared from that ingredient does not directly implicate the ingredient apart from further molecular subtyping. There may have been another strain of *Salmonella* Typhimurium present in the post-process environment that cross-contaminated the product. Hence, this serotype-based approach to contamination investigation may be insufficient, apart from determining a plausible route of infection and molecular subtyping.

The Need for Determining a Plausible Route of Infection

A detailed description of the epidemiologic approaches used by the US Centers for Disease Control and Prevention (CDC) to determine what particular food and food manufacturer are responsible for an outbreak is beyond the scope of this chapter. The CDC routinely uses molecular subtyping for source tracking in the context of foodborne illness. Sometimes this approach is referred to as “genetic fingerprinting” (Swaminathan 2001). However, this description can be misconstrued. A fingerprint is unique to an *individual* human when examined in the context of forensic criminal investigations. In microbial source tracking, the term molecular “fingerprint” refers to a DNA pattern associated with a *clonal population*, not an individual. Populations of such organisms may be local or widespread. They may also be rare or common to a particular environment. Hence, finding a bacterium of a specific molecular subtype in one place may be insufficient to infer a causal route to another product produced in another location. To establish a causal connection, an understanding of a plausible route needs to be established, as illustrated in the following two examples.

The same molecular ribotype designated DUP 1053 of *Listeria monocytogenes* was determined to be the most common ribotype isolated from smoked fish in the Netherlands (Thimothe et al. 2004) but was also recovered from ready-to-eat deli turkey in the United States (Olsen et al. 2005; Fugett et al. 2006). However, it is unlikely that there was any connection between the deli meat produced in the United States and the smoked salmon produced in the Netherlands.

Another example is the recovery of *Salmonella s*erotype Agona from cereal in 1998 and again in 2008 (CDC 1998, 2008). Prior to 1969–1970, *Salmonella* Agona was not a public health concern in the United States, the United Kingdom, the Netherlands, or Israel (Clark et al. 1973). This changed after these countries imported *Salmonella* Agona-contaminated fish meal. By 1972, *Salmonella* Agona was the 8th and 2nd most common *Salmonella* serotype isolated in the United States and the United Kingdom, accounting for 500 and 700 human cases, respectively. However, no one has argued that the Peruvian fish meal caused the contamination of ready-to-eat cereal.

Thimothe et al. (2004) wrote, "…Molecular subtyping methods can be a useful tool to monitor and understand L. monocytogenes contamination sources, including where L. monocytogenes persists in the plant environment and how the organisms are spread or move from one area of the plant to another." This statement can be applied to other microbes. The situation in a food processing plant is somewhat similar to that involved in identifying the product and manufacturing facility as sources of the outbreak pathogen. Merely finding a *Salmonella* serovar in the raw materials section of a facility does not necessarily link the microbe to the same serovar obtained from the finished product. Pathogens are typically expected to be present on the raw product side of many food processing facilities. However, they are not expected to be present in the post-critical control point for microbiology (CCPm) side of processing facilities. A couple of possibilities exist for the aforementioned scenario. These are (1) the molecular subtypes are different between the isolates from the ingredient and the finished product and another source should be investigated (e.g., the environment of the post-CCPm side of the plant), or (2) the subtypes are the same.

In the situation wherein the subtypes are the same, the CCP_m (a point at which populations of pathogens are destroyed) should be reevaluated to validate its efficacy. If the CCP_m is not in control or is unreliable, then it may be reasonable to conclude the contaminated ingredient was *a* source of contamination. However, other sources may still exist, as the ingredient may have contaminated other locations in the production environment which may represent another contamination source(s) for the product. It is impossible to know if all the environmental sources of product contamination have been located when the product has not been biocidally treated in its final hermetically sealed container (Kornacki 2010).

Molecular Subtyping Approaches

Many reviews have been written addressing this topic; hence, this section will be brief.

Approaches are available, with each having advantages and disadvantages. A more detailed discussion of these approaches has been described elsewhere (Moorman et al. 2010; Farber et al. 2001). These approaches include, among others, serotyping, phage typing, bacteriocin typing, plasmid profiles, multi-locus enzyme electrophoresis (MEE), restriction endonuclease analysis (REA), pulsed-field gel electrophoresis (PFGE), random amplified palindromic DNA (RAPD), AFLP, multi-locus variable repeat tandem analysis (MLVA), multi-locus sequence typing (MLST), and microarray-based target sequencing (MBMS). The US FDA recently announced a 5-year project with the CDC, the University of California–Davis, and Agilent Technologies to sequence the entire genome of 100,000 foodborne pathogens with the goal to make the results available in a public database (FDA 2012). This database is hoped to help speed the investigation of foodborne outbreaks by linking outbreak-associated pathogens with information in the database. Critical to evaluating a typing method include the following: (1) universality (e.g.,

how many strains can the system type?), (2) reproducibility, (3) discriminatory power, (4) ease of interpretation, (5) speed, (6) expense, and (7) technical simplicity (Farber et al. 2001).

Approaches Used by Government Agencies

The "gold standard" for molecular subtyping for foodborne pathogens (e.g., *Salmonella*, *Shigella*, pathogenic *E. coli*, *Campylobacter*, *Yersinia*, *Vibrio*, and *Listeria monocytogenes*) used by the federal government and internationally is PFGE (Felix et al. 2012; Fields et al. 2011; Peters 2009), despite the relative abundance of other approaches, many of which have been evaluated, utilized by CDC, and shown to have superior discriminatory power over PFGE. For example, MLVA was used along with PFGE in an outbreak investigation associated with peanut butter (http://www.cdc.gov/salmonella/typhimurium/strains_table.html). However, the PFGE approach has remained the "gold standard" due to its epidemiologic concordance and universality (Fields et al. 2011). Despite the advantages of this approach, it is laborious and time-consuming and requires highly standardized methods and analysis algorithms to ensure compatibility between laboratories. Once isolates are obtained and purified, which could take several days, the assay requires 24 h, and only 10–20 isolates can typically be tested and compared at this time with present technology. Some criteria have been developed for the comparison of the degree of similarity between patterns (Tenover et al. 1995). However, these have been challenged, and an understanding of the significance of slight differences in patterns among strains is often not completely acquired (Fields et al. 2011). Given these considerations and the technical skill involved in performing the assay, other more automated systems have been favored by the industry.

Approaches Used by the Food Industry

Food companies who routinely engage in molecular subtyping perform the assay in an off-site, often commercial, laboratory. They typically test for foodborne pathogens from only their general production environment (zone 3) exclusive of food contact (zone 1) and adjacent (i.e., zone 2) areas.

Companies who engage in molecular subtyping of isolates must address several considerations, such as: (1) If a pathogen is to be subtyped, what systems afford rapid, reliable results? And (2) if a pathogen is not to be subtyped, are there other indicator organisms that will provide a conservative approach over testing a pathogen?

Food processors typically require extremely rapid yet reliable testing methods. In the author's experience, many companies have benefited from commercially available automated Rep-PCR systems and ribotyping systems. These are rapid and have been used to determine if laboratory cross-contamination has occurred, as well as actual food cross-contamination events, despite their limitations.

Approaches to Non-crisis Investigations

Sometimes companies need to assess their risk from pathogen contamination in the plant environment; nevertheless, they may have policies in place, which prevent pathogen sampling on product-associated (e.g., zone 1) surfaces and sometimes zone 2 (adjacent) surfaces. In these instances, microbial indicators of hygiene have sometimes been used (Kornacki 2012b). One approach to *Listeria*-related source tracking has been a Rep-PCR technology developed by Global Laboratories (Gainesville, FL) in which *Listeria*-like colonies or *Listeria* spp. recovered from modified Oxford medium are assigned a molecular subtype based upon the use of a proprietary set of restriction endonucleases. This approach allows for more focused hypotheses generation as to the mechanism of movement of a particular subtype through the facility and may result in more refined corrective actions (Wester 2004). A similar approach was shown to be feasible with the HTEB (aka EB (HT) assay of Kornacki Microbiology Solutions (Kornacki 2012a, b). The HTEB assay was shown to recover microbes of similar physiology to *Salmonella* from environmental niches. Microbes recovered from a dry-food processing facility using this method included *Citrobacter freundii*, *Escherichia hermanii*, *Enterobacter amnigenus*-2, *Morganella morganii*, *Proteus mirabilis*, and *Salmonella enterica* subsp. Arizona. Rep-PCR was shown to discriminate between many of these isolates, even those of the same species (Kornacki 2010, 2012a, b). The preferred embodiment of this assay is available through license from Kornacki Microbiology Solutions, Inc. (www.kornackifoodsafety.com).

Summary

Repetitive isolation of the same bacterial strain in a food manufacturing environment is often assumed to be the consequence of negligence by the food processor. However, this may not always be the case. The phenomenon of strain persistence is often more complex than a failure or unwillingness to apply cleaners and sanitizers or a lapse in employee hand washing or failure of an employee to wear a hairnet, though all such practices are important. Persistent strains may result from poor facility or equipment design through no apparent fault of the food manufacturer and/or maintenance practices that create microbial growth niches and/or certain operational practices—including misapplied sanitation, often performed with the best of intentions. Furthermore, the present trend toward increasing wet-cleaning techniques in dry-product processing plants that have not been designed to accommodate water increases the risk of microbiological niche development and product contamination. All the above may render effective cleaning and sanitization impossible, allowing for the persistence of surviving but adapted bacterial subtypes or their biofilms. Strains that have adapted to the dry food processing environment may lie dormant and undetected for years until disturbed. Once these strains are dislocated to areas of high moisture, growth occurs increasing the relative risk of product contamination from the environment. A food manufacturing facility may

also receive ingredients contaminated sporadically with a persistent strain in the ingredient supplier's processing environment. The result is the steady introduction of this strain of pathogen into the ingredient purchaser's production environment.

Those investigating persistent bacterial strains in food plant environments should be aware of false paradigms that prevent their detection as well as appropriate routine pathogen and hygienic indicator testing, including molecular subtyping approaches that can be used to establish plausible contamination routes in the facility. An understanding of the microbial ecology of the food processing environment through the use of molecular subtyping can be used to refine corrective actions, resulting in reduced risk of finished product contamination.

References

Adams DM (1973) Inactivation of *Clostridium perfringens* type A spores at ultrahigh temperatures. Appl Microbiol 26:282–287

Banwart GJ (1979) Basic food microbiology. AVI Publishing Co, Westport, CT

Behling RG, Eifert J, Erickson MC, Gurtler JB, Kornacki JL, Lane E, Radcliff R, Ryser ET, Stawick B, Yan Z (2010) Selected pathogens of concern to industrial food processors: Infectious, toxigenic, toxico-infectious, selected emerging pathogenic bacteria, chapter 2. In: Kornacki JL (ed) Principles of microbiological troubleshooting in the industrial food processing environment. Springer, New York, pp 5–62

Beuchat LR, Komitopoulou E, Beckers H, Betts RP, Bourdichon F, Fanning S, Joosten HM, Ter Kuile BH (2013) Low-water activity foods: increased concern as vehicles of foodborne pathogens. J Food Prot 76:150–172

Bender JB, Hedberg CW, Boxrrd DJ, Besser JM, Wikklurd JH, Smith KE, Ostermlm MT (2001) Use of molecular subtyping in survillance for salmrnella entrica serotype Typhimurium. N Engl J Med 344:189–195

Bradshaw JG, Peeler JT, Twedt RM (1975) Heat resistance of ileal loop reactive *Bacillus cereus* strains isolated from commercially canned food. Appl Microbiol 30:943–945

Bradshaw JG, Peeler JT, Twedt RM (1977) Thermal inactivation of ileal loop-reactive *Clostridium perfringens* type A strains in phosphate buffer and beef gravy. Appl Environ Microbiol 34:280–284

Brenner FW, Villar RG, Angulo FJ, Tauxe R, Swaminathan B (2000) *Salmonella* nomenclature. J Clin Microbiol 38:2465–2467

Breeuwer P, Lardeau A, Peterz M, Joosten HM (2003) Desiccation and heat tolerance of *Enterobacter sakazakii*. J Appl Microbiol 95:967–973

CDC (1998) Multistate outbreak of *Salmonella* serotype Agona infections linked to toasted oats cereal – United States, April–May, 1998. MMWR 47(22):462–464. http://www.cdc.gov/mmwr/preview/mmwrhtml/00053368.htm. Accessed 7 Feb 2013

CDC (2008) Investigation of outbreak of infections caused by *Salmonella* Agona. http://www.cdc.gov/salmonella/agona/. Accessed 7 Feb 2013

Chen J, Scott VN, Freier TA, Kuehm J, Moorman M, Meyer J, Morille-Hinds T, Post L, Smoot L, Hood S, Shebuski J, Banks J (2009a) Control of *Salmonella* in low-moisture foods II: process validation and environmental monitoring. Food Protection Trends 29(7):435–445

Chen Y, Scott VN, Freier TA, Kuehm J, Moorman M, Meyer J, Morille-Hinds T, Post L, Smoot L, Hood S, Shebuski J, Banks J (2009b) Control of *Salmonella* in low-moisture foods III: process validation and environmental monitoring. Food Protection Trends 29(8):493–508

Clark GM, Kaufmann AF, Gangarosa EJ (1973) Epidemiology of an international outbreak of *Salmonella* agona. Lancet 2:490–493

Farber JM, Gendel SM, Tyler KD, Boerlin P, Landry WL, Fritschel SJ, Barrett TJ (2001) Molecular typing and differentiation, chapter. In: Downes FP, Ito K (eds) Compendium of methods for the microbiological examination of foods, 4th edn. American Public Health Association Washington, DC, p 127–156, Chapter 11

FDA (2012) FDA News Release: FDA, UC Davis, Agilent Technologies and CDC to create publically available food pathogen genome database. http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm311661.htm, July 12. Accessed 11 Feb 2013

Felix B, Dao TT, Lombard B, Brisabois A, Roussel S (2012) The use of pulsed-field gel electrophoresis in *Listeria monocytogenes* sub-typing - Harmonization at the European Union level, chapter 14. In: Magdeldin S (ed) Gel electrophoresis – principles and basics. ISBN: 978-953-51-0458-2, InTech, http://www.intechopen.com/books/gel-electrophoresis-principles-andbasics/harmonization-of-listeria-monocytogenes-pfge-sub-typing-at-european-union-level

Fields PI, Fitzgerald C, McQuiston JR (2011) Fast and high throughput molecular typing methods, chapter 5. In: Hoorfar J (ed) Rapid detection, characterization, and enumeration of foodborne pathogens. ASM Press, Washington, DC, pp 81–93

Gatto AJ, Peters TM, Green J, Fisher IST, Gill ON, O'Brien SJ, Maguire C, Berghold C, Lederer I, Gener-Smidt P, Torpdahl M, Siitonen A, Lukinmaa S, Tscäpe H, Prager R, Luzzi I, Dionisi AM, van der Zwaluw WK, Threlfall EJ (2006) Distribution of molecular subtypes with *Salmonella enterica* serotype Enteritidis phage type 4 and *S. Typhimurium* definitive phage type 104 in nine European countries, 2000–2004: results of an international multi-centre study. Epidemiol Infect 134:729–736

Fugett E, Fortes E, Nnoka C, Weidmann M (2006) International Life Sciences Institute North America *Listeria monocytogenes* strain collection: development of standard *Listeria monocytogenes* strain sets for research and validation studies. J Food Prot 69:2929–2938

Geopfert JM, Iskander IK, Amundson CH (1970) Relation of the heat resistance of salmonellae to the water activity of the environment. Appl Microbiol 19:429–433

Kastenberg VG, Gram L (2009) Model systems allowing of sensitivity to disinfectants and comparison of disnifection susceptibilty of persitent and presumed nonpresident Listeria monocytogenes. J Appl Microbiol 106:1667–1681

Kmarik SL (1940) Manufacture of dried beef. US Patent 2:224–397

Kornacki JL (2009a) The missing element in microbiological food safety inspection approaches, part 1. February–March, Food Safety Magazine, Glendale, CA. http://www.foodsafetymagazine.com/magazine-archive1/februarymarch-2009/the-missing-element-in-microbiological-food-safety-inspection-approaches-part-1/. Accessed 9 Nov 2013

Kornacki JL (2009b) The missing element in microbiological food safety inspection approaches, part 2. April–May, Food Safety Magazine, Glendale, CA. http://www.foodsafetymagazine.com/magazine-archive1/aprilmay-2009/the-missing-element-in-microbiological-food-safety-inspection-approaches-part-2/. Accessed 9 Nov 2013

Kornacki JL (2010) Principles of microbiological troubleshooting in the industrial food processing environment. Springer, New York

Kornacki JL (2011) Practical sampling plans, indicator microorganisms, and interpretation of test results from trouble-shooting, chapter 25. In: Hoorfar J (ed) Rapid detection, characterization and enumeration of foodborne pathogens. ASM Press, Washington, DC, pp 373–379

Kornacki JL (2012a) Hygiene control in the dry food products industry: the roles of cleaning methods and hygienic indicators, chapter 28. In: Hoorfar J (ed) Case studies in food safety and authenticity: lessons from real-life situations. Woodhead Publishing, Cambridge, UK, pp 254–266

Kornacki JL (2012b) Looking for love in all the wrong places: experiences with indicators and risk assessment. Presentation at FOODHACCP 7th annual conference for food safety and quality, Elk Grove, IL, 14 Nov 2012

Kornacki JL (2013) Research during microbial food safety emergencies and contaminant investigations, chapter 7. In: Taormina PJ (ed) Microbiological research and development for the food industry. CRC Press, Taylor & Francis Group, New York, pp 185–202

Kornacki JL, Gurtler JB (2007) Incidence and control of *Listeria* in food processing facilities, chapter 17. In: Ryser ET, Marth EH (eds) *Listeria*, listeriosis and food safety, 3rd edn. CRC Press, Taylor & Francis Group, Boca Raton, FL, pp 681–766

Kornacki JL, Gurtler JB, Stawick B (2013) Enterobacteriaceae, coliforms and *Escherichia coli* as quality and safety indicators, chapter 8. In: Downes FP, Ito K (eds) Compendium of methods for the microbiological examination of foods, 5th edn. American Public Health Association, Washington, DC

Lawson B, Hughes LA, Peters T, de Pinna E, John SK, Macgregor SK, Cunningham AA (2011) Pulsed-field gel electrophoresis supports the presence of host-adapted *Salmonella* enterica subsp. enterica Serovar Typhimurium strains in the British Garden Bird population. Appl Environ Microbiol 77:8139–8144

Mitscherlich E, Marth EH (1984) Microbial survival in the environment. Springer, New York, p 584, Table 26

Moorman M, Pruett P, Weidman M (2010) Value and methods for molecular subtyping, chapter 10. In: Kornacki JL (ed) Principles of microbiological troubleshooting in the industrial food processing environment. Springer, New York, pp 157–174

Olsen SJ, Patrick M, Hunter SB, Reddy V, Kornstein L, MacKenzie WR, Lane K, Bidol S, Stoltman GA, Frye DM, Lee I, Hurd S, Jones TF, LaPorte TN, Dewitt W, Graves L, Wiedmann M, Schoonmaker-Bopp DJ, Huang AJ, Vincent C, Bugenhagen A, Corby J, Carloni ER, Holcomb ME, Woron RF, Zansky SM, Dowdle G, Smith F, Ahrabi-Fard S, Ong AR, Tucker N, Hynes NA, Mead P (2005) Multistate outbreak of *Listeria monocytogenes* infection linked to delicatessen turkey meat. Clin Infect Dis 40:962–967

Oliveira AM, Miya NT, Sant'Ana AS, Pereira JL (2010) Behavior and enterotoxin production by coagulase-negative *Staphylococcus* in cooked ham, reconstituted skimmed milk, and confectionery cream. J Food Sci 75:475–481

Peters TM (2009) Pulsed-field gel electrophoresis for molecular epidemiology of food pathogens. Methods Mol Biol 551:59–70

Robertson MT (1972) Survival of *S.* Typhimurium on floor dust. Puht ltlth Lcmd 87:39–45

Scott VN, Chen Y, Freier TA, Kuehm J, Moorman M, Meyer J, Morille-Hinds T, Post L, Smoot L, Hood S, Shebuski J, Banks J (2009) Control of Salmonella in low moisture foods I: minimizing entry of *Salmonella* into a processing facility. Food Protection Trends 9:342–353

Seo KS, Bohach GA (2007) Staphylococcus aureus, chapter 22. In: Doyle MP, Beuchat LR (eds) Food microbiology fundamentals and frontiers, 3rd edn. ASM Press, Washington, DC, pp 493–518

Sörqvist S (2003) Heat resistance in liquids of *Enterococcus spp., Listeria spp., Escherichia coli, Yersinia enterocolitica, Salmonella spp.* and *Campylobacter spp*. Acta Vet Scand 44:1–19

Stumbo CR (1965) Thermobacteriology in food processing. Academic, New York

Tenover FC, Arbeit RD, Goering RV, Mickelsen PA, Murray BE, Persing DH, Swaminathan B (1995) Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: Criteria for bacterial strain typing. J Clin Microbiol 33:2233–2239

Thimothe J, Nightingale KK, Gall K, Scott VN, Wiedmann M (2004) Tracking of *Listeria monocytogenes* in smoked fish processing plants. J Food Prot 67:328–341

Udo EE, Al-Bustan MA, Jacob LE, Chugh TD (1999) Enterotoxin production by coagulase-negative staphylococci in restaurant workers from Kuwait City may be a potential cause of food poisoning. J Med Microbiol 48:819–823

Valle J, Gomez-Lucia E, Piriz S, Goyache J, Orden JA, Vadillo S (1990) Enterotoxin production by staphylococci isolated from healthy goats. Appl Environ Microbiol 56:1323–1326

Veras JF, do Carmo LS, Tong LC, Shupp JW, Cummings C, dos Santos DA, Cerqueica MMOP, Cantini A, Nicoli JR, Jett M (2008) A study of enterotoxigenicity of coagulase-negative staphylococcal isolates from food poisoning outbreaks in Minas Gerais, Brazil. Int J Infect Dis 12:410–415

Vij V, Ailes E, Wolyniak C, Angulo FJ, Klontz KC (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

Wester P (2004) Case study: trending using molecular methods. Presentation in IAFP pre-meeting workshop entitled, out of the filing cabinet into use: real world experience with trending data. Baltimore, MD

Dry Cleaning, Wet Cleaning, and Alternatives to Processing Plant Hygiene and Sanitation

Scott L. Burnett and Robert Hagberg

Abstract Sanitation is a key component of any processing facility's food safety program. There are, however, certain challenges when approaching sanitation in a processing plant that produces low-water-activity food. Methods that are considered conventional in other plant environments may not be suitable here, where the use of water is scrutinized to avoid unnecessary introduction of moisture which can lead to potential microbial growth niches within equipment and the facility infrastructure. This chapter describes the objectives of sanitation in a processing plant that produces low-water-activity (a_w) food and provides guidance where dry or nonaqueous methods are appropriately used, if not recommended, over wet methods. Dry cleaning and sanitizing involve the use of established effective procedures without substantial use of water. In addition to sanitation, controlling environmental hygiene of the processing facility also includes measures such as hygienic zoning and related physical and procedural barriers to the spread of potential pathogens. Considerations for such procedures in an effort to maintain a dry environment are provided.

Keywords Sanitation • Dry cleaning • Wet cleaning • Dry sanitizing • Wet sanitizing • Environmental hygiene control

Introduction

Sanitation is a key component of any food processing facility's food safety program, and processors should implement a documented sanitation program that addresses cleaning and sanitizing schedules and methods, validation and verification monitoring

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of standard sanitation operating procedures, record management to document the application of sanitation methods, and corrective action plans for contamination occurrences. There are, however, certain challenges when approaching sanitation in a low-water-activity (a_w) food plant. Methods that are considered conventional in other food plant environments may not be suitable where use of water is minimized to avoid unnecessary introduction of moisture and which can lead to potential microbial growth niches within equipment and the facility infrastructure. This chapter describes the objectives of sanitation in a low-a_w food processing plant and provides guidance where dry or nonaqueous methods are appropriately used, if not recommended, over wet methods. Dry cleaning and sanitizing involve the use of effective microbial control procedures, without significant use of water. Controlling environmental hygiene in the processing facility also includes, in addition to sanitation, measures such as hygienic zoning and related physical and procedural barriers to the spread of potential pathogens. Considerations for applying such procedures in an effort to maintain a dry environment are provided.

Definitions

Sanitation: Hygienic measures that ensure the safety of food by reducing the risk of food contamination. Sanitation includes the reduction, removal, or elimination of microbes, pests, waste, and unwanted soils from the manufacturing environment.

Cleaning: Use of an effective procedure to remove unwanted soils from equipment or manufacturing facilities. This is done to prevent excessive product buildup and/or to prevent carry-over of allergens, nonorganic ingredients, or flavors to subsequent runs and enhance operational efficiency. Cleaning may also reduce populations resulting from product residues.

Sanitizing: Use of an effective procedure to actively reduce microbial populations on equipment or manufacturing facilities. Active reduction involves the use of chemical sanitizers, heat, and/or other treatments in a manner proven to sufficiently reduce levels of EPA test organisms or other microorganisms of concern by 5 log cycles (i.e., 99.999 %). In general, sanitizing is effective only on clean nonporous surfaces and may have temperature and/or exposure time requirements.

Dry cleaning: Use of an effective cleaning procedure without notable use of water (including aqueous solutions, aqueous suspensions, or steam). Dry cleaning methods may include wiping, scraping, brushing, sweeping, blowing, vacuuming, purging, or flushing. Flushing agents may be nonaqueous liquids or solids. Dry cleaning methods will not add moisture to food processing equipment or facility.

Dry sanitizing: Use of an effective sanitizing procedure without notable use of water (including aqueous solutions, aqueous suspensions, or steam). Dry sanitizing methods may include the wiping or spraying of alcohol-based solutions, application of heat, gaseous antimicrobial agents, or irradiation. Dry sanitizing methods will not add moisture.

Wet cleaning: An effective procedure using water (including aqueous solutions, aqueous suspensions, or steam) for cleaning. Methods include clean-in-place (CIP), clean-out-of-place (COP), water hoses, and scrubbing, utilizing buckets and brushes or wipes. Wet cleaning is followed by a sanitizing step.

Controlled wet cleaning: Use of an effective cleaning procedure with a limited amount of water. Controlled wet cleaning methods include the removal of equipment and wet cleaning in a dedicated clean room and isolated wet cleaning-in-place such that water use is minimized, contained, and channeled directly to drains. It can also be accomplished by scrub cleaning with water-based detergents, which minimize water use on equipment or in the environment. Controlled wet cleaning is followed by a sanitizing step and is always followed by immediate drying.

Wet sanitizing: Use of an effective procedure using water (including aqueous solutions, aqueous suspensions, or steam) for sanitizing. Methods include all wet cleaning application methods, e.g., wall-mounted premixed aqueous sanitizers and hand-pump sanitizers for applications of sanitizing chemicals.

Sanitation Objectives

Understanding the objectives of a sanitation event is essential for the selection of appropriate methodologies, determining requirements for validation and verification/monitoring, and establishing the frequency of cleaning. Sanitation objectives for food safety and quality include:

Lot Definition

Sanitation of processing equipment can help support the definition of a production lot in manufacturing, thereby placing a "sanitation break" prior to the beginning of manufacture of the next production lot. Emphasis of dry sanitation is placed on equipment downstream of the final validated microbicidal processing step, usually included as a critical control point under the in-house HACCP plan, and subsequent equipment along the product stream to packaging. Dry sanitation methods are preferred, where the product stream is typically low a_w. It is important that these methods are validated for efficacy in removing soil and reducing microbial populations and that verification that SSOPs were properly carried out is performed. In some cases, attributes of the finished product and/or equipment design present barriers against effective dry sanitation methods. In these cases, controlled wet sanitation methods are employed. Scientific justification for lot definition may include components, in addition to sanitation, such as ingredient management and handling, finished product testing, and validation of processing steps that result in microbial lethality.

Allergen Removal

Validation and verification of allergen removal by dry sanitation methods can be very difficult and resource-intensive. The goal is for the validated SSOP to remove residues of in-process or finished product to the extent that surfaces are free of detectable allergens, as determined by the SSOP verification process, which may include visual inspection or analysis of allergen presence by use of a commercially available enzyme-linked immunoassay-based kit. The latter is much more sensitive than the former and, thus, preferred. Validated methods for allergen removal are necessary to mitigate cross-contamination of an allergen ingredient-containing product to a product otherwise not containing the allergenic ingredient or processing aid. For example, one may wish to produce a product containing milk powder on a line previously containing soy protein. Milk powder is an allergen but the line should be free of the prior allergen (soy).

Product Carry-Over/Claims/Other Hazards

Product-to-product changeover of a processing line engages sanitation methods effective in reducing the commingling of product to a level acceptable by criteria determined by regulatory and consumer acceptance considerations. An example of product carry-over driven by regulatory compliance is vitamin fortification where the sanitation objective is to reduce the presence of residues in the product that was being processed to an acceptable level such that levels in the subsequent in-process or finished product are at predetermined acceptable safety and/or quality levels.

Functionality of Equipment: Operational Efficiency

Cleaning to remove product residue from equipment, which would otherwise contribute to a lack of process control, is another sanitation objective. For example, dryer slots and pores can become obstructed with in-process product residue, preventing the passage of air needed to dry the product stream bed. Such equipment may require routine and frequent cleaning to ensure adequate operating conditions. This type of sanitation can focus in on specific pieces of equipment, while adjacent equipment may not be cleaned or sanitized; therefore, procedures must be in place to protect adjacent equipment and materials from debris and cleaning chemicals used in the process of a functional clean.

Pest Control

Control of pests within the facility begins with a sound environmental cleaning program, usually structured within a facility's master sanitation schedule, which is described below.

Dry Sanitation Methods

Because microorganisms, including *Salmonella,* cannot grow without adequate moisture, dry sanitation is preferable whenever possible. Certain low a_w foods may begin as or include components which are high a_w. Others may undergo wet processing steps during manufacturing. Assessing the a_w of the product stream from receiving to packaging, much as is done for an HACCP process flow map, is a useful first step in determining where dry sanitation methods or controlled wet sanitation approaches are preferred or more suitable. Foods, including the in-process food stream, that are considered to be low a_w are those at a_w of <0.85 (Beuchat et al. 2013). Sanitation methods employed depend on the sanitation objective, equipment and facility design, and if the nature of the product residue can accommodate a dry method. The sequences of steps applied in dry sanitation are presented in Table 1.

Table 1 Sequence of steps in dry sanitation[a]

Step number	Step	Description
1	Sanitation preparation	• Purge all systems and empty all product reservoirs • Remove all ingredients, packaging, and garbage • Gather and stage all safety equipment, cleaning tools, cleaning chemicals, etc.
2	Secure and disassembly of equipment	• Secure equipment by lockout/tagout (LOTO), confined space entry, and other applicable OSHA regulations • Remove guards, release belt tension from conveyors • Remove applicable parts for clean-out-of-place (COP) • Disassemble all other components
3	Dry clean	• Brush down and vacuum from top to bottom • Use compressed air sparingly and not on floors • Sweep or vacuum soils to remove
4	Detail clean	• Hand-scrape surfaces to remove residues • Detail brush down of equipment • Vacuum remaining product fragments • Wipe down equipment as necessary • Clean framework and equipment legs • Wipe excess grease and fittings
5	Post-inspection and reclean	• Reassemble and run equipment for at least one cycle to dislodge remaining soil • Self-inspect equipment using a flashlight, ensuring all food contact surfaces are free of debris • Reclean as necessary
6	Preoperational inspection reassembly	• Complete preoperational inspection using a flashlight and document an additional verification and documentation • Address noted deficiencies and document • LOTO—reassemble equipment • Remove LOTO
7	Sanitization and final release	• Document preoperational inspection and all corrective actions • Sanitize using a quick-drying EPA-registered sanitizer or another validated approach. Allow time to dry • Release facility for production

[a]Adapted from (Chen et al. 2009)

Dry Cleaning Methods

Several approaches to cleaning residue from product contact surfaces are used in low-a_w food production facilities. The chosen sequence of methods should maximize the efficiency of cleaning procedure, using the resources available, and be matched against the nature of the residue and its interaction with the underlying surface and equipment design. Equipment designed for ease of disassembly for surface inspection and accessibility is critical to accommodate effective removal of all product residues. For example, fine particulate matter (e.g., flour, spices, etc.) in a mixer made of stainless steel may be effectively removed by brushing using dedicated tools for the task. Slotted dryer belts, however, with adherent and dried product residue may require a more aggressive approach. When applicable, purging of the product stream with like product or with a benign ingredient (e.g., salt) can be effective in gross cleaning and may be used as the first step in the process, depending on the sanitation objective.

Cleaning with brushes, scrapers, and other tools is a common practice done and can be effective in adequately removing soil from surfaces. Use of dedicated tools for this purpose is critical. Tools used to clean product contact surfaces after the final microbial lethality step, if there is one, should be kept separate from tools that are used on product contact surfaces prior to the final lethality step. Tools used to clean environmental surfaces (not direct product surfaces) should be dedicated for that purpose as well and ideally confined to pre- and post-lethality areas also. The facility should have in place a program for identifying color-coded tools and for training employees on their use. Tools should be cleaned after each use or, if applicable, in high-risk uses or areas, be discarded and replaced.

Vacuuming is another commonly used approach for dry cleaning. Either a central vacuum system (ideally located outside the processing area) or portable units may be used. Routine and appropriate maintenance programs should be performed on central vacuum systems to prevent accumulation of dust that may entrap moisture and microorganisms and insects. Although the Grocery Manufacturers Association recommends the use of central vacuums with caution due to concerns regarding product accumulation within ducting (Chen et al. 2009), such systems are not uncommon, and with the appropriate level of maintenance, such risks can be avoided. As with manual tools, vacuum attachments should be dedicated to use based on risk assessment to avoid cross-contamination. Adequate filtration of outgoing air is important as well. Portable vacuums equipped with HEPA filters are recommended. Methods and locations for cleaning the portable or central vacuum systems must also be established.

Controlled use of compressed air has been used for removing debris. This is typically discouraged (Chen et al. 2009), because microorganisms associated with product soil can become airborne and settle on surfaces in the surrounding environment as a result of the use of compressed air. Its use should therefore be limited to circumstances in which access to product residue is limited or constrained by equipment design. If compressed air is used, governing the maximum air pressure available for use

in cleaning (e.g., 40 p.s.i) as well as prohibiting its use to clean floors is recommended. Additionally, biological and chemical contaminants from air supply lines can be introduced onto facility surfaces if air-drying and filtration are inadequate or not properly maintained. Microbiological monitoring for indicator organisms (e.g., Enterobacteriaceae) is recommended (Chen et al. 2009).

Abrasive blasting is an approach to remove more adherent soils in which particulates are accelerated in a pressurized airstream directed at a surface. Media employed in abrasive blasting of dry cleaning food processing equipment include sodium bicarbonate, calcium carbonate, and dry ice. The approach is generally effective in removing residue from surfaces, but several drawbacks limit its application. The generation of dust, soil transport, and noise limit blasting with sodium bicarbonate or calcium carbonate. Dry ice blasting may cause formation of condensation on adjacent surfaces. The rate of cleaning depends on the nature of the soil (i.e., generally less effective on elastic or soft soils), target equipment design, and the blasting equipment that is employed.

Because achieving product contact surfaces free of visible residue can be difficult using the dry cleaning methods described above, final cleaning using nonaqueous/alcohol-based chemicals is often necessary. This step involves saturating disposable towelettes and/or microfiber cloths with the cleaning solution and manually wiping down product contact surfaces as much as possible such that all visible product residue is removed. Nonaqueous/alcohol-based solutions offer the benefit of being quick-drying and only introduce very limited amounts of moisture onto the surface being cleaned. Final cleaning with nonaqueous-based chemicals can be slow, and consideration for the appropriate personal protection equipment (PPE) must be given to ensure employee safety when handling and cleaning with these products.

Dry Sanitizing

Alcohol-based sanitizers are used for equipment sanitizing where heating is not a practical approach. A commonly used sanitizer for dry equipment for food contact surfaces is comprised of approximately 150 ppm quaternary ammonium compounds (QAC) plus isopropyl alcohol (IPA), which provide for its quick-drying characteristic once applied. Sanitizer products must be EPA-registered (when used within the United States) and followed per the manufacturer's label to ensure effectiveness and employee safety.

The antimicrobial effectiveness of IPA QAC sanitizer has been studied. Du et al. (2009) evaluated the efficacy of Aqueous QAC and IPA QAC in reducing *Salmonella* in dust associated with almond hulling and shelling facilities. No change in *Salmonella* populations was reported upon exposure to Aqueous QAC (200 ppm) for 10–15 min, whereas populations were reduced to levels below the limit of detection (1.3 log CFU/g) following treatment with IPA QAC (>3.9 log reduction). This study revealed the marked biocidal activity of IPA QAC in the presence of a substantial organic challenge.

Table 2 Reduction of *Salmonella* on stainless steel exposed to dry heat[a]

Temperature (°C)	Exposure time (h)	Initial cell density ($\log_{10}$ CFU/carrier)	Reduction ($\log_{10}$ CFU/carrier)
80	4	6.0	1.85
90	4	6.7	4.28
100	2	5.8	>4.40

[a]Adapted from (McKelvey and Bodnaruk 2011)

IPA QAC sanitizers must be handled with care, using appropriate PPE considering any confined space concerns and their low flash point which can result in flammability. They should not be used adjacent to nearby fire or spark ignition sources. Delivery in a carbon dioxide-propelled stream mitigates this risk and provides for good surface coverage. Employee flame retardant personal protective equipment may also be appropriately worn.

Dry heat can be used to sanitize equipment surfaces and has applicability in ovens, toasters, dryers, and other equipment used to deliver heat to the product stream. McKelvey and Bodnaruk (2011) investigated the efficacy of dry heat in reducing populations of *Salmonella* dried onto stainless steel (Table 2) and concluded that extended time periods at evaluated temperatures (80–90 °C) are required to sanitize equipment in dry environments.

The efficacy of gaseous antimicrobials has been reviewed (Kim et al. 2003; Czarneski et al. 2012), and their application in food processing for routine sanitizing of equipment and/or the environment is somewhat limited. Because gas generation and handling systems and decontamination area containment are resource capital-intensive, treatments with ozone or chlorine dioxide have been largely targeted toward restoring the hygienic level of a food processing environment or specific equipment to undetectable microbial levels concomitant with safe food manufacture, following an unanticipated contamination event. One must also consider the safety of personnel and the compatibility of soft metals to the exposure of the gaseous treatment. Benefits associated with gaseous antimicrobial treatment include antimicrobial efficacy, especially in otherwise inaccessible areas, as well as the fact that gaseous decontamination practices ideally should not introduce moisture to the treated environment.

Wet Sanitation Methods

Aqueous approaches to sanitation have been presented elsewhere (Marriott 1997). In otherwise dry food manufacture, which includes steps in which the product stream is not considered to be at low a_w (i.e., it is >0.85 a_w), wet sanitation may be preferable. The chosen method must be validated and appropriate for the targeted sanitation objective.

Efforts should be made to control exposure of the environment to water, to the extent where it is practical. Water should not be allowed to spray or splash from the

floor or unclean equipment to cleaned and sanitized equipment, or to areas, or onto adjacent processes in operation. Sanitary design of equipment is very important. Water must be allowed to drain thoroughly from all equipment surfaces, and adequate time for thorough drying must be provided to minimize the potential for microbial growth. Sites potentially harboring moisture and/or product residue should be identified and eliminated to the extent possible, and periodic disassembly of equipment for thorough sanitation is beneficial to reduce risks associated with the creation of microbiological harborage niches. When possible, equipment parts should be removed for cleaning in dedicated washrooms and allowed to dry thoroughly prior to reintroduction to the processing environment and reassembly. Schmidt (Schmidt 2013) described the importance of appropriate sanitary design for equipment.

Sanitation SOP Validation and Verification/Monitoring

The method used to validate the effectiveness of SSOPs should be tailored to the sanitation objectives of the event. For example, sanitation applied to reduce the populations of microorganisms on equipment surfaces to support lot definition may be validated by determining its efficacy in reducing aerobic plate counts or other indicator microbes as a result of cleaning and sanitizing. Guidelines for validation of allergen cleaning in nut processing facilities have been published (Grocery Manufacturers 2010). A system for monitoring/verifying and documenting the effectiveness of ongoing SSOPs should be in place and include tools such as visual inspections for cleanliness, indirect measurements of ATP by bioluminescence, allergen detection swabs, and preoperational nonpathogen microbial contact surface environmental monitoring of equipment.

Sanitary Design of Equipment

A successful sanitation program is founded on equipment that is designed and fabricated according to appropriate sanitary design standards. Several organizations publish standards or guidelines and checklists for the sanitary design of equipment and facilities. These include organizations such as the 3-A Sanitary Standards, Inc., the European Hygienic Engineering and Design Group, and the Grocery Manufacturers Association (Chen et al. 2009; Grocery Manufacturers Association).

Processing Plant Hygiene

Measures designed to control the potential spread of pathogenic microorganisms, including *Salmonella*, throughout the processing environment should be applied (Chen et al. 2009). Hygienic zoning of a facility is one approach to containing

materials within raw ingredient handling areas and of protecting the product stream in further-processed areas of the plant. Footwear, equipment wheels, and air currents may serve as vectors for pathogen transmission. Exclusion of personnel and equipment traffic from processing floors is effective for impeding the spread of pathogens (Morita et al. 2006). Where traffic cannot be restricted in areas where product is manufactured, chemical sanitization should be employed. Nonaqueous methods, such as the use of IPA QAC sanitizer or solid QAC products, are preferable to minimize the presence of moisture (Burnett et al. 2013). The microbiological quality of make-up air and air directional flow and balancing should be addressed to avoid contamination of areas requiring a higher level of hygiene (i.e., after the final validated thermal lethality step).

Master Sanitation Schedule

A master sanitation schedule is a useful tool in managing cleaning activities of a facility and should include periodic infrastructure and equipment cleaning over a 52-week cycle, such that all areas specified in the schedule will be cleaned at least annually based on the needs of the facility. Master sanitation schedules should describe what is to be cleaned (e.g., overhead structures, drains, etc.), who will conduct the cleaning, the required frequency of cleaning, and how the cleaning should be done. After cleaning is completed according to the master sanitation schedule, the activity is documented and records are maintained. Audits of the effectiveness and completion of these activities are highly recommended.

Because floor drains can be sources of pathogenic microorganisms and their spread is potentially likely due to footwear and equipment traffic, master sanitation schedules should include a drain preventive maintenance program. Drains should be cleaned with dedicated tools and personal protective equipment on a frequency appropriate to the risk of harborage and presence of moisture. Care must be given to prevent transmission of microorganisms during the drain cleaning process.

Environmental Moisture Leak Management

Quickly responding to leaks found in the processing facility is important to reduce risks associated with the ingress and spread of pathogens. Leaks can originate from external water sources (e.g., roof leaks) and internal sources (e.g., leaky pipes, condensation, etc.). Processing and non-processing areas alike should be frequently monitored (e.g., each shift) for the presence of leaks. If a leak is observed, a risk assessment should follow, considering such factors as the leak source, its proximity to the product stream, extent of product stream coverage or protection, leak amount, degree of foot and/or equipment traffic, and air flow. Holah et al. (2012) described a method for determining risk and identifying sources of contamination and vectors

of transmission to product or the plant environment. The level of corrective and preventative action should be appropriate to the level of risk determined to be present and may include stopping operations, isolation of impacted area, removal of adjacent equipment or staged ingredients, identification and correction of the root cause, cleaning and sanitizing of impacted surfaces, and microbial swabbing for testing to verify adequate sanitization and containment. The risk assessment should also consider the disposition of product lots which may require validated reconditioning or disposal if warranted by the leak-associated risks.

Conclusions

Clearly defining and training employees concerning the objectives of sanitation are important in selecting and executing appropriate cleaning methodologies. Dry sanitation methods are preferred over wet approaches when the nature of the product stream residue, equipment design, and sanitation objectives are considered and can successfully accommodate them. Validation, verification, and monitoring sanitation effectiveness are equally important when employing dry sanitation techniques. Additional programs to ensure environmental hygiene control, including master sanitation schedules and hygienic zoning, are also critical to ensure safe production of low-a_w food.

References

Beuchat LR, Komitopoulou E, Beckers H, Betts RP, Bourdichon F, Fanning S, Joosten HM, Ter Kuile BH (2013) Low-water activity foods: increased concern as vehicles of foodborne pathogens. J Food Prot 76(1):150–172

Chen YH, Scott VN, Freier TA, Kuehm J, Moorman M, Meyer J, Morille-Hinds T, Post L, Smoot L, Hood S, Shebuski J, Banks J (2009) Control of Salmonella in low-moisture foods II: hygiene practices to minimize Salmonella contamination and growth. Food Prot Trends 29:435–445

Du W, Danyluk MD, Harris LJ (2009) Efficacy of aqueous and alcohol-based quaternary ammonium sanitizers for reducing Salmonella in dusts generated in almond hulling and shelling facilities. J Food Sci 75:M7–M13

McKelvey PJ, Bodnaruk PW (2011) Survival of Salmonella species on stainless steel exposed to dry heat. IAFP Annual Meeting, Milwaukee, WI

Kim J, Yousef AE, Khadre MA (2003) Ozone and its current and future application in the food industry. Adv Food Nutr Rsrch 45:167–218

Czarneski M, Hughes M, Oliveras J (2012) Environmental monitoring and decontamination of food processing facilities. Food Protect Trends 9:522–530

Marriott NG (1997) Principles of food sanitation. Chapman and Hall, New York, NY

Schmidt R (2013) Food equipment hygienic design: an important element of a food safety program. Food Safety Mag 18(6):14–17, 52

Grocery Manufacturers Association (2010) Industry handbook for safe processing of nuts. Washington, DC

3-A Sanitary Standards, Inc. 6888 Elm Street, Suite 2D McLean, Virginia, USA 22101. www.3-a.org

European Hygienic Engineering and Design Group. Lyoner, Sraβe 18, 60528 Frankfurt am Main, Germany. www.edehg.org

Grocery Manufacturers Association. The GMA equipment design checklist for low moisture foods. http://www.gmaonline.org/downloads/technical-guidance-and-tools/GMA_Equipment_Design_Checklist_05_14_2010.xls

Morita T, Kitazawa H, Lida T, Kamata S (2006) Prevention of Salmonella cross-contamination in an oilmeal manufacturing plant. J Appl Microbiol 101:464–473

Burnett SL, Egland SJ, McKelvey PJ, Cook FK (2013) Chemical decontamination of footwear soles to limit microbial transfer in a dry environment. Food Prot Trends 33:74–81

Holah J, Margas E, Hagberg R, Warren B, Fraser-Heaps J, Mortimore S (2012) Identifying and controlling microbiological cross-contamination. Food Safety Mag. Feb/Mar:54–61

Part III
Low a_w Food Commodities of Interest

Spices

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Abstract Many spices are grown in developing nations where hygiene and sanitation practices are very different from those in developed countries. Growth, harvest, and production conditions, along with physical and chemical characteristics of a spice, can influence its microbiological quality. *Salmonella* is the pathogen of greatest concern in spices due to its ability to persist in low a_w environments. The application of good agricultural practices and good manufacturing practices throughout the spice supply chain helps to minimize contamination issues. Treatments, including steam, ethylene oxide fumigation, propylene oxide fumigation, and irradiation, are used to reduce microbial loads.

Keywords Spices • Spice safety • Antimicrobial constituents • *Salmonella* • *Bacillus cereus* • *Mycotoxins*

Introduction

Spices are defined by the American Spice Trade Association (ASTA) as "any dried plant product used primarily for seasoning purposes" (American Spice Trade Association 1990). The United States Food and Drug Administration (US FDA) expands the definition to "any aromatic vegetable substance in the whole, broken or ground form, except for those substances which have been traditionally regarded as foods, such as onions, garlic and celery; whose significant function in food is seasoning rather than nutritional; that is true to name; and from which no portion of any volatile oil or other flavoring principle has been removed" (U.S. Food and Drug Administration 2009). This definition was written with the intention of assuring that spices are pure and do not contain the spent material that remains after essential oils are extracted.

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A variety of dried parts of botanically diverse plants fill spice racks around the world. Some spices are fruits, as in the case of black pepper and white pepper, whereas others are rhizomes, such as ginger and turmeric. Cinnamon is the dried bark of a tropical tree, while cloves and saffron are flower parts. Basil, marjoram, and oregano are dried leaves, and caraway, mustard, and cumin are seeds. Some plants produce two spices, e.g., cilantro is the leaf of the *Coriandrum sativum* plant whose dried seeds are coriander (Farrell 1990). Mace and nutmeg also are obtained from one plant. Mace is the lacy aril membrane that surrounds the nutmeg seed. For some uses, spices are added to impart both flavor and color. This is often the case with saffron, paprika, and turmeric.

The spice trade has a fascinating and intriguing history that spans thousands of years. As goods with high commercial value, great wealth was acquired by rulers of towns and kingdoms as spices were transported from China, Indonesia, and India to Egypt, the Eastern Mediterranean and European merchants. It is often noted that the country that controlled the spice trade at any one point in history was usually the wealthiest and most powerful in the world at that time (American Spice Trade Association 1985; Farrell 1990). Mystery enveloped the origin of spices and was perpetuated by merchants until the fifteenth century, to the extent that the majority of people using spices did not know where they originated. Pioneers such as Marco Polo, Christopher Columbus, and Ferdinand Magellan uncovered routes to spice-growing regions, which paved the way for greater accessibility and affordability for all (Farrell 1990). The demand for spices in America grew with the influx of diverse cultures and, with them, their eating preferences.

Fast forward to modern times and it is apparent that the demand for spices continues to increase. Spice consumption in the United States over the past four decades has displayed an upward trend (US Department of Agriculture/Economic Research Service 2013a) that reflects ethnic diversity as well as a desire to experiment with new flavors. Furthermore, the potential health benefits of spices (Bandara et al. 2012; Park et al. 2012) and the need to enhance the flavor of low-salt and low-fat foods help explain why US consumption is increasing. On a volume and value basis, the United States is consistently the world's largest spice importer, with more than 1.2 billion pounds imported in 2011 (U.S. Department of Agriculture/Economic Research Service 2013a). Capsicums, mustard seed, black and white pepper (combined), and ginger accounted for more than half of the total spice imports by weight (U.S. Department of Agriculture/Economic Research Service 2013a). Numerous countries supply spices to the United States with Indonesia, India, China, Vietnam, Brazil, Peru, Mexico, and Spain leading the list in 2012 (U.S. Department of Agriculture/Economic Research Service 2013b).

Raw Material and Processing

Many spices are grown in developing countries in and/or near the tropics, where hygiene and sanitation practices are vastly different from those in most developed nations (American Spice Trade Association 2011; Tainter and Grenis 2001).

Inadequate sanitary conditions are coupled with the fact that spices are agricultural products and, thus, are naturally surrounded by dirt, dust, birds, animals, and insects in the field, resulting in a high risk for foodborne pathogen contamination. Spice farming can be quite different from our concept of traditional field farming (Tainter and Grenis 2001). Many spice farmers grow their crop on a small scale or harvest spices that grow naturally on hills or in jungles (Tainter and Grenis 2001). For example, cardamom grows wild in the rain forests of Sri Lanka (Farrell 1990), whereas rosemary grows wild in the hills of Morocco. Spices are typically harvested by hand and laid on mats or on the ground to dry naturally by the sun and wind, often with little, if any, protection from birds, animals, and pests. It is not surprising, therefore, that these commodities have the potential to be contaminated before and after harvest with a variety of indigenous microorganisms, including *Salmonella*, which is the pathogen of greatest concern in spices.

Following the drying process, growers and harvesters transport their crops to a central collection point where the spices are typically gathered and commingled by a local broker (Tainter and Grenis 2001). The local collector may then sell his material to a larger collector who subsequently sells to a processor who conducts the initial cleaning (American Spice Trade Association 2011). Combining small lots of spices from various locations can increase variations in the microbial populations within the final lot, which is a consideration when applying a microbial sampling plan to raw materials. Additional cleaning and processing can either be conducted close to the source or after arrival in the importing country (Tainter and Grenis 2001).

The application of good agricultural practices (GAPs) (U.S. Food and Drug Administration 1998) and good manufacturing practices (GMPs) (U.S. Food and Drug Administration 2012b) throughout the supply chain helps to minimize contamination issues. In the field, this includes proper employee hygiene, the use of appropriate fertilizers, and clean sources of irrigation water. As the product moves through the supply chain, attention must be paid to how the product is handled, processed, and stored to reduce the potential for spoilage and inadvertent contamination. Dried spices are considered a low-moisture food. The limiting water activity (a_w) for growth of bacteria in foods is approximately 0.85 (Fontana 2007), whereas the limit for molds is lower at approximately 0.60. Since the a_w of dried spices is typically below 0.60, the growth of bacteria is rarely a concern, assuming proper storage conditions are maintained. Guidelines for spice storage typically specify conditions at less than 60 % relative humidity (RH) (American Spice Trade Association 2011; Codex Alimentarius 1995; European Spice Association 2004); however, as with any food, if spices are not dried or stored properly, conditions may arise where a_w levels can be higher. Many spices, such as black pepper, are hygroscopic in nature. When stored in a high humidity environment, the a_w of a hygroscopic spice will reflect the % RH of the storage conditions (Dhas and Koritkanthimath 2003; Keller et al. 2013). In general, the a_w will be equal to the % RH divided by 100 once the product equilibrates with the atmosphere in which it is stored. Black pepper stored in a high humidity environment in excess of 85 % or even 95 % RH will not remain at a low a_w, but will come to equilibrium with the environment and achieve a_w levels of 0.85 or higher. Such an increase in product a_w is not always visible to the naked eye and may allow the growth of mold and, potentially, bacteria (Keller et al. 2013).

Spices that are imported into the United States are required to meet federal regulations for safety and cleanliness under the primary authority of the US FDA (American Spice Trade Association 2011). The American Spice Trade Association has established specifications for extraneous matter in imported and domestic spices that help buyers and users of spices assure cleanliness. The specifications include limits for dead insects, mammalian excreta, other types of excreta, insect infestation, mold, and foreign material (American Spice Trade Association 2007). Limits for insect fragments and rodent hairs are also listed for many spices in the US FDA Food Defect Levels Handbook (U.S. Food and Drug Administration 2011). Both animal and insect debris can be vectors of pathogens, and their presence in spices implies potential for pathogen contamination. However, one study found there was no relationship between the enteric microflora of imported spices and the presence/number of fecal pellets isolated from the spices (Satchell et al. 1989). Although microbiological parameters are not specifically addressed in the various standards, the reasonable certainty standard of safety expressed in US food law for food additives and GRAS (generally recognized as safe) substances can be applied to spices. The understanding is that, with reasonable certainty, pathogens such as *Salmonella* will not be present in spices that have entered into interstate commerce (American Spice Trade Association 2011).

It is important to note that the drying process itself will not assure the reduction to safe levels of pathogens present at harvest (American Spice Trade Association 2011; Dhas and Koritkanthimath 2003). While many spices and seasoning blends are used in cooked applications, they are also frequently used in ready-to-eat foods and cooked foods that are seasoned before consumption. As examples, seasoning blends are often topically applied to crackers, chips, and other snack foods, black pepper and red pepper are sprinkled on foods at the table, dehydrated onion and herbs may be stirred into sour cream and used as a dip, and seasonings may be applied to artisan breads after baking. For this reason, it should be assumed that all retail spices and many spices used in industrial and food service applications may be directly consumed. The US FDA takes this into consideration and places spices into food category I or II for *Salmonella* sampling (U.S. Food and Drug Administration 2003). Category I foods do not normally receive a *Salmonella* inactivation step between the time of sampling and consumption and are intended for sensitive populations. As such, the US FDA indicates that 60 samples should be taken at random from each lot and 25 g removed from each sample to make four 375 g composites that are tested in their entirety. Category II foods are also not typically subjected to a process lethal to *Salmonella* before consumption but are intended for the general population. Thirty samples are taken from each lot and 25 g from each is used to form two 375 g composites that are tested in their entirety.

Although foodborne pathogens will not grow at a low a_w, their presence in a spice and its subsequent use in a high-moisture food that does not undergo a microbiological inactivation step could lead to contamination and growth in the final food product. Spices contaminated with pathogens can then serve as an inoculum for the foods in which they are used. In addition to concerns about pathogens, high bacterial loads in spices used in high-moisture, processed foods may have an impact on the quality of the respective finished product (Witkowska et al. 2011).

To provide assurance of safety, a pathogen reduction treatment that targets the most resistant *Salmonella* found in spices is often applied to either whole or ground spices. The primary types of treatments employed in the spice industry include saturated, dry, or superheated steam, ethylene oxide fumigation, and irradiation (American Spice Trade Association 2011). Propylene oxide fumigation is used to a lesser extent (American Spice Trade Association 2011). The American Spice Trade Association estimates that between 40 and 85 % of the spices in the United States are treated with ethylene oxide each year (American Spice Trade Association 2011). The advantage is that it does not significantly alter the appearance or flavor of the spice. While there are concerns about ethylene oxide residues as a carcinogen and a potent mutagen, an assessment of the lifetime cancer risk from ethylene oxide residues in spices imported into New Zealand were determined to be practically negligible (Fowles et al. 2001). The US Environmental Protection Agency has strict tolerances for ethylene oxide and ethylene chlorohydrin residues in spices at 7 and 940 ppm, respectively (US Environmental Protection Agency 2009). An exception is basil, which cannot be treated with ethylene oxide (U.S. Environmental Protection Agency 2009) because treatment results in the formation of high levels of ethylene chlorohydrin due to the presence of naturally occurring chlorides.

A second type of treatment, steam pasteurization, is gaining popularity because it meets many customers' desires for natural methods of pasteurization. In the United States, steam treatment is often applied to whole spices such as white and black pepper (American Spice Trade Association 2011). The process recipes are carefully designed to effectively reduce microbial pathogens while maintaining key flavor and appearance attributes. Irradiation is also an approved process for spices and has the advantage that the spice can be treated in its final package. It can be particularly effective for spices that are difficult to treat by other means; however, consumer acceptance of irradiation is generally low. The US FDA has established a maximum dose of 30 kGy for the irradiation of spices (U.S. Food and Drug Administration 2008).

Whether a product is steam treated, ethylene oxide treated, or irradiated, it is essential that strict hygiene and sanitation practices are employed during posttreatment processing, blending, handling, packaging, and storage in order to eliminate the opportunity for post-process contamination.

Antimicrobial Constituents

The general microflora/microbiota of spices are related to harvest and production conditions, as well as the physical and chemical characteristics of the spice. Many spices contain antimicrobial compounds, which may provide some, but not complete, protection against foodborne pathogens (Arora and Kaur 1999; Billing and Sherman 1998; Ceylan and Fung 2004; Juneja et al. 2010; Kalemba and Kunicka 2003; Koutsoumanis et al. 1999; Ramos et al. 2012; Rounds et al. 2012; Shan et al. 2007; Weerakkody et al. 2011). Many of the recognized inhibitory components in spices are phenolic constituents of the essential oil fraction (Beuchat and Golden 1989;

Council for Agricultural Science and Technology 1998; Shelef 1983). The mode of action of these antimicrobial compounds is poorly understood (Kalemba and Kunicka 2003). One hypothesis is that microbial membranes are disrupted. Regardless of the mode of action, the antimicrobial compound must be present in situ at concentrations above any minimum inhibitory concentration (MIC) needed to denature the cell membrane or prevent outgrowth of *Salmonella*. A heavily contaminated spice would require higher concentrations of antimicrobial compounds to be effective. Furthermore, the actual concentration of antimicrobial compounds in spices varies dramatically, based on the type of spice, agricultural growth conditions, maturity of the plant at harvest, variety of the spice plant, and storage conditions (Kalemba and Kunicka 2003; McCarron et al. 1995). Regardless of concentrations found in spices, antimicrobials are more likely to be effective prior to the reduction of the moisture level (drying) of the spice. For many compounds, metabolism by the microbe is required for antimicrobial action. Results of a recent study revealed *Salmonella* under desiccation and starvation stress was in a physiologically dormant state (Deng et al. 2012). While in this state, some antimicrobial compounds present in a spice have limited antimicrobial efficacy. This may explain why *Salmonella* has occasionally been detected in spices such as oregano (Van Doren et al. 2013) that contain recognized antimicrobial constituents. There have been several incidents in which *Salmonella*-contaminated oregano prompted market recalls (Vij et al. 2006). Van Doren et al. (2013) found that the presence of antimicrobials in oregano and allspice had no effect on reducing the prevalence of *Salmonella* on imported shipments (Van Doren et al. 2013).

Microbiological Profile of Spices: An Overview

The microbiological quality of spices can vary dramatically as a consequence of their variety, production methods, and the presence/absence of naturally occurring antimicrobial compounds. McKee reviewed spice and herb studies and determined that aerobic mesophilic bacterial plate counts ranged from a low of 2 log CFU/g to greater than 8 log CFU/g, without any relationship to the type of spice (McKee 1995). High counts were observed for all types of spices. In addition, spices and dried herbs were found to contain a variety of foodborne pathogens. Aflatoxins were detected in black pepper, turmeric, coriander, and nutmeg (McKee 1995). The results of surveys conducted more recently revealed an equally broad spectrum of microorganisms and bacterial counts and included pathogens as well (Abou Donia 2008; Banerjee and Sarkar 2003; Hara-Kudo et al. 2006; Moreira et al. 2009; Sagoo et al. 2009; Sospedra et al. 2010; Van Doren et al. 2013; Witkowska et al. 2011).

One of these recent studies was a survey of 180 spice samples, representing 30 types of spices available on the Irish market. Witkowska et al. (2011) found that 20 % of the samples had greater than 6 log total aerobic mesophilic bacteria (TAMB) CFU/g, whereas 40 % had less than 4 log TAMB CFU/g (Witkowska et al. 2011). Paprika, with no detectable TAMB, was the only spice that had been heat-treated.

There were also no detectable TAMB in the mustard seed and white pepper samples. Black pepper samples had a mean TAMB count of 2.44 log CFU/g, which suggested that they may have received a microbial reduction treatment, since counts for this spice are often much higher. Aerobic sporeformers were the predominant bacterial population, with 80 % of the spice varieties containing 1.96 log to 6.09 log TAMB CFU/g and a mean count of 3.95 log CFU/g. Molds (1–3 log CFU/g) were detected in 50 % of the samples, yeasts were only recovered from two samples, and *Enterobacteriaceae* were detected in 23 % of the spices, with counts ranging from 1.86 log to 4.50 log CFU/g. The highest Enterobacteriaceae populations were detected in basil, celery, and fennel. All samples tested negative for *Salmonella* and *Listeria* spp., and *Staphylococcus aureus* and *B. cereus* were only detected in marjoram.

In an assessment of the microbial quality of spices obtained in Brazil, *Salmonella* was found in black pepper and cumin, whereas *B. cereus* was detected in clove, parsley, and dehydrated green onion (Moreira et al. 2009). *Salmonella* was also reported in black and red pepper imported to and examined in Japan (Hara-Kudo et al. 2006). In a study of the microbiological quality of herbs and spices from Mexican markets, the only foodborne pathogen detected in 304 samples of 5 different spices was *B. cereus* (Garcia et al. 2001). Molds were detected in multiple samples (Garcia et al. 2001). A total of 2,833 retail and 132 production batches of dried spices and herbs from production facilities and retail markets in the United Kingdom were also examined (Sagoo et al. 2009). *Salmonella* was detected in 1.5 % of retail and in 1.1 % of production samples. Further examination of *Salmonella*-contaminated batches identified 17 different serovars, including 5 serovars found in black pepper alone. Additionally, *Bacillus cereus*, *Clostridium perfringens*, and *Escherichia coli* were found in some herb and spice samples.

A study by the US FDA of imported spices in the United States from fiscal years 2007–2009 revealed the prevalence of *Salmonella* was 6.6 %, a number which has not changed significantly in the last 30 years (Van Doren et al. 2013). Worth noting is that imported spices were 1.9 times more likely to be contaminated with *Salmonella* when compared to all other FDA-regulated imported foods, including fresh produce (Van Doren et al. 2013). Surprisingly, the prevalence of *Salmonella* in spices that were subjected to a pathogen reduction treatment before import into the United States was 3 % (Van Doren et al. 2013). This suggests either an ineffective pathogen inactivation treatment or post-process contamination.

Pathogens of Concern

Sporeforming Microorganisms: Bacillus cereus, Clostridium perfringens, *and* Clostridium botulinum

Clearly, foodborne pathogens of greatest concern in low-moisture foods are those that persist in a dry environment. The most desiccation-resistant microorganisms include the sporeformers *Bacillus cereus*, *Clostridium perfringens*, and *Clostridium*

botulinum. Spores of these microorganisms typically occur in soil and are capable of surviving for many years in dry environments. Their presence at low levels in spices is, therefore, not unexpected.

In order for the sporeforming foodborne pathogens in spices to cause an illness outbreak, subsequent bacterial growth after a spice is added to a high-moisture food is required. In addition, a thermal treatment may also be necessary to initiate spore germination. Spore germination and growth may require temperature abuse. With *Clostridium*, anaerobic conditions are also required for toxin production to occur or sufficient populations to cause illness. Hence, any foodborne illness resulting from these microorganisms would require a critical breach of food processing parameters or serious under-processing and temperature abuse of a food product. Although sporeformers are present in spices to varying degrees, there have been no reports of foodborne illnesses caused by any of these three microorganisms attributed to dried spices in the United States.

Molds and Mycotoxins

In addition to sporeforming bacteria, desiccation-resistant microorganisms include mold species, many of which are capable of producing mycotoxins. The presence of some mold is not always avoidable in the production of spices. Furthermore, the mere presence of a mold capable of producing mycotoxins does not imply that mycotoxins are present.

Due to the carcinogenic nature of some mycotoxins, many countries, including the United States, have standards specifying limits for mycotoxins in foods, particularly for aflatoxins. Monitoring levels in spices is routine and can prevent the introduction of spices with excessive mycotoxin levels to the market. Although levels of mycotoxins exceeding legal limits have occasionally been found, no outbreaks associated with acute aflatoxin toxicity have been reported for humans in the United States (U.S. Food and Drug Administration 2012a).

The molds most frequently isolated from spices include those from the *Aspergillus* genus. *Aspergillus flavus*, which produces several types of aflatoxin, has been isolated from a variety of spices, and aflatoxins in excess of permissible levels have occasionally been found in spices (Abou Donia 2008; Elshafie et al. 2002; Erdogan 2004; Fazekas et al. 2005; Garcia et al. 2001; Gatti et al. 2003; McKee 1995; Thirumala-Devi et al. 2001). In 2005, the Food Standards Agency published results of a survey for aflatoxins and ochratoxin A in spices (Food Standards Agency (UK) 2005). A total of 61 samples of paprika, chili powder, and cayenne pepper were sampled from warehouse and retail locations. Of those, 4 samples were found to exceed legal limits for aflatoxin B1 and 3 exceeded limits for other aflatoxins.

Salmonella *Species*

Despite the inability to form a spore, this Gram-negative rod-shaped bacterium has proven to be exceptionally tenacious with respect to long-term survival under dry conditions. Salmonellae survival in excess of a year has been described in low-moisture products in several studies (Beuchat and Scouten 2002; Keller et al. 2013; Lehmacher et al. 1995; Tamminga et al. 1976; Uesugi et al. 2006, 2007). The infectious dose of some *Salmonella* species in specific foods has been estimated to be as low as one cell, and it is reported to be the second most common cause of foodborne illness in the United States (U.S. Food and Drug Administration 2012a). With the potential for such a low infectious dose, particularly in high fat foods, *Salmonella* does not have to grow in a spice, or grow in a food that contains a contaminated spice, in order to result in foodborne illness.

Survival of *Salmonella* under dry conditions is not well understood. Among the possible explanations for the extreme desiccation survival displayed by *Salmonella* species may be the involvement of outer membrane lipopolysaccharides (LPS) (Garmiri et al. 2008; Gibson et al. 2006). Strains with LPS mutations were found to be significantly less resistant to desiccation than parental strains. Garmiri et al. (2008) hypothesized that expression of the O polysaccharide was required to enhance resistance to dehydration and that the LPS served as a "buffer" against changes in the water content of the environment. Genes involved in cold shock or heat shock may also play a role in the desiccation resistance of *Salmonella* (Deng et al. 2012; Gruzdev et al. 2012). Cross-protection of different stresses such as desiccation and heat and cold shock has been documented for *Salmonella* (Gruzdev et al. 2011). The fact that genes involved with other stress responses would be associated with a desiccation response is not unexpected. The use of newer molecular sequencing technologies to identify such genes may provide additional information in the future with respect to the mechanisms for survival and persistence in dry environments.

Recalls and Outbreaks

The number of spice-related recalls due to *Salmonella* has increased in recent years, and there have been several high-profile foodborne salmonellosis outbreaks associated with spices. The use of sophisticated microbiological and epidemiologic tools and methods has played a major role in raising the awareness of potential microbiological risks associated with spices.

A FDA review of spice recalls in the United States due to bacterial pathogen contamination revealed 21 incidents involving 12 types of spices between the years 1970 and 2004 (Vij et al. 2006). Twenty of those recalls were attributed to *Salmonella* contamination, with paprika the spice implicated most often (Vij et al. 2006).

The majority of these recalls occurred between the years 2001 and 2004. While many were primarily the result of increased surveillance in one state, no increase in laboratory confirmed salmonellosis was noted in the implicated areas (Vij et al. 2006).

From September 8, 2009 through September 7, 2010, there were 17 spice and easoning-related entries in the FDA Reportable Food Registry, making up 7.4 % of all primary entries (U.S. Food and Drug Administration 2012c). Of these reports, 16 were due to *Salmonella*. In the September 8, 2010 to September 7, 2011 period, the number of spice and seasoning-related entries increased to 25, representing 11.1 % of all primary entries. Twenty-three of these 25 reports were due to *Salmonella* contamination.

Salmonella has been identified as the causative agent of several foodborne outbreaks associated with spices. One of the earliest recorded outbreaks was linked with white pepper contaminated with *S.* Weltevreden (Severs 1974). Years later (November of 1981 to August of 1982) an outbreak of salmonellosis occurred in Norway with 126 cases attributed to black pepper contaminated with *S.* Oranienburg (Gustavsen and Breen 1984). The long duration of the Norwegian outbreak was of particular interest. Spices can have a very long shelf life and remain in consumer's homes for extended periods of time. The long shelf life of dried products coupled with the extensive survival of *Salmonella* in low a_w foods and difficulty in definitively determining a causative agent can extend outbreaks far beyond what would be typical for a "fresh" product.

Another significant outbreak occurred a decade later in Germany involving contaminated paprika used to season potato chips. There were an estimated 1,000 cases of illness, mostly affecting children younger than 14 years of age (Lehmacher et al. 1995). The estimated infectious dose in that outbreak was reported to be 4–45 salmonellae. The potentially high fat content associated with the paprika-powdered chips may have protected the salmonellae from gastric acid in the stomach, resulting in the low infectious dose.

Contaminated anise seed was the vehicle of an outbreak of *S.* Agona infection in Germany, resulting in periodic cases of salmonellosis in infants from October 2002 until July 2003 (Koch et al. 2005). The contaminated anise seed was used in herbal tea preparations and, when tested, was determined to have only an estimated 0.036 salmonellae/g of sample.

More recently, two large outbreaks of salmonellosis in the United States were attributed to the consumption of contaminated white, black, and red pepper (Centers for Disease Control and Prevention 2010; Higa 2011). In 2009, white pepper from Vietnam used as a restaurant table condiment was the source of an outbreak of *Salmonella* Rissen, affecting 87 people in 5 states (Higa 2011). The Rissen serovar was previously uncommon in the United States, but a global *Salmonella* survey revealed that this serovar was fairly common in Southeast Asia and was among the top 10 *Salmonella* isolates in Thailand. The other notable outbreak occurred between July 2009 and April 2010 involving black and red pepper contaminated with *S.* Montevideo (Centers for Disease Control and Prevention 2010). Illnesses were initially associated with salami products, suggesting that salami was the source, but extensive microbiological and epidemiologic investigations identified red and

black pepper that was applied to the salami as the vehicle. The outbreak involved 272 cases in 44 states and the District of Columbia. The median age of those affected was 37, with a range of less than 1–93 years. Twenty-six percent of the patients were hospitalized, and there were no deaths reported (Centers for Disease Control and Prevention 2010).

Emerging Pathogens

Although *Salmonella* remains the primary concern in spices, mainly due to its persistence in dry environments and potential low dose for infection, it is important to note that the microbial world is dynamic. Microorganisms can acquire virulence genes from related species and the environment. *Escherichia coli* O157:H7 is an example of an organism that is assumed to have evolved from less virulent strains (Feng et al. 1998; Kyle et al. 2012; Shaikh and Tarr 2003). Studies reveal that Shiga toxin-producing strains of *E. coli* are either similar in desiccation tolerance to *Salmonella* (Hiramatsu et al. 2005) or are less resistant to desiccation than *Salmonella* (Barron and Forsythe 2007). To date, no recall or outbreak related to spices has been attributed to *E. coli* O157:H7. Nonetheless, *E. coli* O157:H7 has been reported in dry products and environments. In 2011, an outbreak of *E. coli* O157:H7 was reported in hazelnuts (Minnesota Department of Health 2011). To prevent the emergence of additional pathogens and their spread to other food categories, it is critical that appropriate GMPs and food safety standards are applied.

Prevention and Control

Recent recalls and foodborne disease outbreaks associated with spices have reinforced the importance of a comprehensive food safety program to assure that safe foods containing spices reach the consumer. Trade associations and government agencies have developed guidelines and regulations, respectively, to aid in this effort (American Spice Trade Association 2011; Codex Alimentarius 1995; European Spice Association 2004; Grocery Manufacturers Association 2009; U.S. Food and Drug Administration 2012b).

The American Spice Trade Association (2011) advocates a robust supplier approval program and periodic re-qualification to assure adherence to GAPs, GMPs, and product- and process-specific HACCP plans. A clean, sanitary environment for spice production and storage is necessary to minimize the opportunity for contamination. When harvest, production and manufacturing conditions are known to result in possible pathogen contamination or unacceptably high microbial loads, a suitable treatment or kill step must be applied. Several treatment options are available for spices, including ethylene oxide fumigation, propylene oxide fumigation, irradiation, and steam treatment. All of these methods are effective, but validation and verification programs are

required to assure they achieve the appropriate *Salmonella* inactivation (American Spice Trade Association 2011).

Post-processing GMPs are critical to assure that spices are not recontaminated after treatment. This includes adequate barriers between pretreatment and posttreatment areas, positive appropriately filtered air in the posttreatment area relative to the other areas in the plant, the use of dedicated equipment in respective areas, and restricted movement of personnel and equipment on wheels (American Spice Trade Association 2011). Recent data from surveys by the US FDA of spices at import revealed the presence of *Salmonella* in some shipments of treated spices (Van Doren et al. 2013). This suggests either excessively high pathogen loads, improper application of treatment, or post-processing contamination (Van Doren et al. 2013). Environmental monitoring, with the focus on areas near product contact surfaces in the posttreatment area, is advocated to confirm that control steps are appropriate (American Spice Trade Association 2011).

References

Abou Donia MA (2008) Microbiological quality and aflatoxinogenesis of Egyptian spices and medicinal plants. Global Veterinaria 2:175–181

American Spice Trade Association (1985) A history of spices, Englewood Cliffs, NJ

American Spice Trade Association (1990) The foodservice and industrial spice manual, Englewood Cliffs, NJ

American Spice Trade Association (2007) Cleanliness specifications for spices, seeds and herbs, Washington, DC

American Spice Trade Association (2011) Clean, safe spices: guidance from the American Spice Trade Association, Washington, DC. http://www.astaspice.org/i4a/pages/index.cfm?pageid=4200. Accessed 3 Mar 2012

Arora DS, Kaur J (1999) Antimicrobial activity of spices. Int J Antimicrob Agents 12:257–262

Bandara T, Uluwaduge I, Jansz ER (2012) Bioactivity of cinnamon with special emphasis on diabetes mellitus: a review. Int J Food Sci Nutr 63:380–386

Banerjee M, Sarkar PK (2003) Microbiological quality of some retail spices in India. Food Res Int 36:469–474

Barron JC, Forsythe SJ (2007) Dry stress and survival time of *Enterobacter sakazakii* and other Enterobacteriaceae in dehydrated powdered infant formula. J Food Protect 70:2111–2117

Beuchat LR, Golden DA (1989) Antimicrobials occurring naturally in foods. Food Technol 43:134–142

Beuchat LR, Scouten AJ (2002) Combined effects of water activity, temperature and chemical treatments on the survival of *Salmonella* and *Escherichia coli* O157:H7 on alfalfa seeds. J Appl Microbiol 92:382–395

Billing J, Sherman PW (1998) Antimicrobial functions of spices: why some like it hot. Q Rev Biol 73:3–49

Centers for Disease Control and Prevention (2010) *Salmonella* Montevideo infections associated with salami products made with contaminated imported black pepper and red pepper – United States, July 2009–April 2010. Morbidity Mortality Weekly Rep 59:1647–1650

Ceylan E, Fung DYC (2004) Antimicrobial activity of spices. J Rapid Meth Autom Microbiol 12:1–55

Codex Alimentarius (1995) International food standards: code of hygienic practice for spices and dried aromatic plants. CAC/RCP 42-1995. http://www.codexalimentarius.org/standards/list-of-standards/

Council for Agricultural Science and Technology (1998) Naturally occurring antimicrobials in food. Task Force Report No. 132, 1998 CAST, Iowa

Deng X, Li Z, Zhang W (2012) Transcriptome sequencing of *Salmonella enterica* serovar Enteritidis under desiccation and starvation stress in peanut oil. Food Microbiol 30:311–315

Dhas PHA, Koritkanthimath VS (2003) Processing and quality of black pepper – a review. J Spices Aromatic Crops 12:1–13

Elshafie AE, Al-Rashdi TA, Al-Bahry SN, Bakheit CS (2002) Fungi and aflatoxins associated with spices in the Sultanate of Oman. Mycopathologia 155:155–160

Erdogan A (2004) The aflatoxin content of some pepper types sold in Turkey. Chemosphere 56:321–325

European Spice Association (2004) European Spice Association Quality Minima Document. www.esa-spices.org/download/esaqmdrev1-2nov07.pdf. Accessed 18 Oct 2012

Farrell KT (1990) Spices, condiments, and seasonings. Van Nostrand Reinhold, New York, NY, pp 3–14

Fazekas B, Tar A, Kovacs M (2005) Aflatoxin and ochratoxin A content of spices in Hungary. Food Addit Contam 22:856–863

Feng P, Lampel KA, Helge K, Whittam TS (1998) Genotypic and phenotypic changes in the emergence of *Escherichia coli* O157:H7. J Infect Dis 177:1750–1753

Fontana AJ (2007) Appendix D: minimum water activity limits for growth of microorganisms. In: Barbosa-Canovas GV, Fontana AJ, Schmidt SJ, Labuza TP (eds) Water activity in foods: fundamentals and applications. Blackwell Publishing Professional, Ames, IA, p 405

Food Standards Agency (UK) (2005) Survey of spices for aflatoxins and ochratoxin A. http://www.food.gov.uk/science/research/surveillance/fsis2005/fsis7305. Accessed 22 Oct 2012

Fowles J, Mitchell J, McGrath H (2001) Assessment of cancer risk from ethylene oxide residues in spices imported into New Zealand. Food Chem Toxicol 39(11):1055–1062

Garcia S, Iracheta F, Galvan F, Heredia N (2001) Microbiological survey of retail herbs and spices from Mexican markets. J Food Prot 64:99–103

Garmiri P, Coles KE, Humphrey TJ, Cogan TA (2008) Role of outer membrane lipopolysaccharides in the protection of *Salmonella enterica* serovar Typhimurium from desiccation damage. FEMS Microbiol Lett 281:155–159

Gatti MJ, Fraga ME, Magnoli C, Dalcero AM, da Rocha Rosa CA (2003) Mycological survey for potential aflatoxin and ochratoxin producers and their toxicological properties in harvested Brazilian black pepper. Food Addit Contam 20:1120–1126

Gibson DL, White AP, Snyder SD, Martin S, Heiss C, Azadi P, Surette M, Kay WW (2006) *Salmonella* produces an O-antigen capsule regulated by AgfD and important for environmental persistence. J Bacteriol 188:7722–7730

Grocery Manufacturers Association (2009) Control of *Salmonella* in low-moisture foods. http://www.gmaonline.org/downloads/technical-guidance-and-tools/SalmonellaControlGuidance.pdf. Accessed 21 Aug 2012

Gruzdev N, McClelland M, Porwollik S, Ofaim S, Pinto R, Saldinger-Sela S (2012) Global transcriptional analysis of dehydrated *Salmonella enterica* serovar Typhimurium. Appl Environ Microbiol 78:7866–7875

Gruzdev N, Pinto R, Sela S (2011) Effect of desiccation on tolerance of *Salmonella enterica* to multiple stresses. Appl Environ Microbiol 77:1667–1673

Gustavsen S, Breen O (1984) Investigation of an outbreak of *Salmonella oranienburg* infections in Norway, caused by contaminated black pepper. Am J Epidemiol 119:806–812

Hara-Kudo Y, Ohtsuka K, Onoue Y, Otomo Y, Furukawa I, Yamaji A, Segawa Y, Takatori K (2006) *Salmonella* prevalence and total microbial and spore populations in spices imported to Japan. J Food Protect 69:2519–2523

Higa J (2011) Outbreak of Salmonella Rissen associated with Ground White Pepper: The Epi Investigation., Jan 6, 2011 Union International Investigation Meeting. California Department of Public Health http://www.cdph.ca.gov/programs/DFDRS/Documents/QSS_Presentation_SRissen_and_%20white%20pepper_010611.pdf. Accessed 27 Dec 2011

Hiramatsu R, Matsumoto M, Sakae K, Miyazaki Y (2005) Ability of Shiga toxin-producing *Escherichia coli* and *Salmonella* spp. to survive in a desiccation model system and in dry foods. Appl Environ Microbiol 71:6657–6663

Juneja VK, Hwang CA, Friedman M (2010) Thermal inactivation and postthermal treatment growth during storage of multiple *Salmonella* serotypes in ground beef as affected by sodium lactate and oregano oil. J Food Sci 75:M1–M6

Kalemba D, Kunicka A (2003) Antibacterial and antifungal properties of essential oils. Curr Med Chem 10:813–829

Keller SE, Van Doren JM, Grasso EM, Halik LA (2013) Growth and survival of *Salmonella* in ground black pepper (*Piper nigrum*). Food Microbiol 34:182–188

Koch J, Schrauder A, Alpers K, Werber D, Frank C, Prager R, Rabsch W, Broll S, Feil F, Roggentin P, Bockemühl J, Tschäpe H, Ammon A, Stark K (2005) *Salmonella* Agona outbreak from contaminated aniseed, Germany. Emerg Infect Dis 11:1124–1127

Koutsoumanis K, Lambropoulou K, Nychas GJ (1999) A predictive model for the non-thermal inactivation of *Salmonella enteritidis* in a food model system supplemented with a natural antimicrobial. Int J Food Microbiol 49:63–74

Kyle JL, Cummings CA, Parker CT, Quiñones B, Vatta P, Newton E, Huynh S, Swimley M, Degoricija L, Barker M, Fontanoz S, Nguyen K, Patel R, Fang R, Tebbs R, Petrauskene O, Furtado M, Mandrella RE (2012) *Escherichia coli* serotype O55:H7 diversity supports parallel acquisition of bacteriophage at shiga toxin phage insertion sites during evolution of the O157:H7 lineage. J Bacteriol 194:1885–1896

Lehmacher A, Bockemuhl J, Alesksic S (1995) Nationwide outbreak of human salmonellosis in Germany due to contaminated paprika and paprika-powdered potato chips. Epidemiol Infect 115:501–511

McCarron M, Mills A, Whittaker D, Kurian T, Verghese J (1995) Comparison between green and black pepper oils from *Piper nigrum* L. Berries of Indian and Sri Lankan Origin. Flavour Frg J 10:47–50

McKee LH (1995) Microbial contamination of spices and herbs: a review. Lebensmittel Wissenschaft und Technologie 18:1–11

Minnesota Department of Health (2011) MDA laboratory testing confirms *E. coli* O157:H7 in recalled hazelnuts. http://www.health.state.mn.us/news/pressrel/2011/ecoli030911.html. Accessed 30 Oct 2012

Moreira PL, Lourenção TB, Pinto JP, Rall VL (2009) Microbiological quality of spices marketed in the city of Botucatu, Sa˜o Paulo, Brazil. J Food Protect 72:421–424

Park SH, Kyeong MS, Hwang Y, Ryu SY, Han SB, Kim Y (2012) Inhibition of LPS binding to MD-2 co-receptor for suppressing TLR4-mediated expression of inflammatory cytokine by 1-dehydro-10-gingerdione from dietary ginger. Biochem Biophys Res Commun 419:735–740

Ramos C, Teixeira B, Batista I, Matos O, Serrano C, Neng NR, Nogueira JM, Nunes ML, Marques A (2012) Antioxidant and antibacterial activity of essential oil and extracts of bay laurel Laurus nobilis Linnaeus (Lauraceae) from Portugal. Nat Prod Res 26:518–529

Rounds L, Havens CM, Feinstein Y, Friedman M, Ravishankar S (2012) Plant extracts, spices, and essential oils inactivate *Escherichia coli* O157:H7 and reduce formation of potentially carcinogenic heterocyclic amines in cooked beef patties. J Agric Food Chem 60:3792–3799

Sagoo SK, Little CL, Greenwood M, Mithani V, Grant KA, McLauchlin J, de Pinna E, Threlfall EJ (2009) Assessment of the microbiological safety of dried spices and herbs from production and retail premises in the United Kingdom. Food Microbiol 26:39–43

Satchell FB, Bruce VR, Allen G, Anderews WH, Gerber HR (1989) Microbiological survey of selected imported spices and associated fecal pellet specimens. J Assoc Off Anal Chem 72:632–637

Severs D (1974) *Salmonella* food poisoning from contaminated white pepper. Epidemiol Bull 18:80

Shaikh N, Tarr PI (2003) Escherichia coli O157:H7 Shiga toxin-encoding bacteriophages: integrations, excisions, truncations, and evolutionary implications. J Bacteriol 185:3596–1753

Shan B, Cali YZ, Brooks JD, Corke H (2007) Antibacterial properties and major bioactive components of cinnamon stick (Cinnamomum burmannii): activity against foodborne pathogenic bacteria. J Agric Food Chem 55:5484–5490

Shelef LA (1983) Antimicrobial effects of spices. J Food Saf 6:29–44

Sospedra I, Soriano JM, Mañes J (2010) Assessment of the microbiological safety of dried spices and herbs commercialized in Spain. Plant Foods Hum Nutr 65:364–368

Tainter DR, Grenis AT (2001) Spices and seasonings: a food technology handbook. Wiley-VCH, New York, NY, pp 1–23

Tamminga SK, Beumer RR, Kampelmacher EH, Leusden FM (1976) Survival of *Salmonella eastbourne* and *Salmonella typhimurium* in chocolate. J Hyg 76:41–47

Thirumala-Devi K, Mayo MA, Reddy G, Tangni EK, Larondelle Y, Reddy DV (2001) Occurrence of ochratoxin A in black pepper, coriander, ginger and turmeric in India. Food Addit Contam 18:830–835

U.S. Department of Agriculture/Economic Research Service (2013a) Food availability (per capita) data system, Worksheet for spices: supply and disappearance, 16 Sept 2013. http://www.ers.usda.gov/data-products/food-availability-(per-capita)-data-system.aspx. Accessed 3 Dec 2013

U.S. Department of Agriculture/Economic Research Service (2013b) U.S. imports of coffee, tea and spices data 1999–2012. http://www.ers.usda.gov/data-products/us-food-imports.aspx. Accessed 3 Dec 2013

U.S. Environmental Protection Agency (2009) 40 CFR 180.151. Ethylene oxide: tolerances for residues. U.S. Government Printing Office, Washington, DC

U.S. Food and Drug Administration (1998) Guide to minimize microbial food safety hazards for fresh fruits and vegetables, Washington, DC. http://www.fda.gov/downloads/Food/GuidanceComplianceRegulatoryInformation/GuidanceDocuments/ProduceandPlanProducts/UCM169112.pdf. Accessed 27 Aug 2012

U.S. Food and Drug Administration (2003) Bacteriological analytical manual, Chapter 1, Food sampling/Preparation of sample homogenate. http://www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalManualBAM/ucm063335.htm. Accessed 6 Sept 2012

U.S. Food and Drug Administration (2008) 21 CFR 179.26. Foods permitted to be irradiated under FDA regulations. http://www.fda.gov/Food/FoodIngredientsPackaging/IrradiatedFoodPackaging/ucm074734.htm. Accessed 5 Nov 2012

U.S. Food and Drug Administration (2009) Inspections, compliance, enforcement, and criminal investigations: CPG Sec. 525.750 spices – definitions. http://www.fda.gov/ICECI/ComplianceManuals/CompliancePolicyGuidanceManual/ucm074468.htm. Accessed 30 Oct 2012

U.S. Food and Drug Administration (2011) Food defect levels handbook. http://www.fda.gov/food/guidancecomplianceregulatoryinformation/guidancedocuments/sanitation/ucm056174.htm. Accessed 24 Aug 2012

U.S. Food and Drug Administration (2012a) The bad bug book, second edition. Foodborne pathogenic microorganisms and natural toxins handbook. http://www.fda.gov/Food/FoodSafety/FoodborneIllness/FoodborneIllnessFoodbornePathogensNaturalToxins/BadBugBook/default.htm. Accessed 23 Oct 2012

U.S. Food and Drug Administration (2012b) Current good manufacturing practice in manufacturing, packing or holding human food, 21CFR110 food and drugs chapter 1: subchapter B: food and human consumption, http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/cfrsearch.cfm?cfrpart=110. Accessed 18 Oct 2012

U.S. Food and Drug Administration (2012c) Reportable food registry annual report: FDA foods and veterinary medicine program: the reportable food registry: targeting inspection resources and identifying patterns of adulteration: Second annual report: September 8, 2010–September 7, 2011. http://www.fda.gov/Food/FoodSafety/FoodSafetyPrograms/RFR/ucm200958.htm. Accessed 25 May 2012

Uesugi AR, Danyluk MD, Harris LJ (2006) Survival of *Salmonella* Enteritidis phage type 30 on inoculated almonds stored at −20, 4, 23, and 35 °C. J Food Protect 69:1851–1857

Uesugi AR, Danyluk MD, Mandrell RE, Harris LJ (2007) Isolation of *Salmonella* Enteritidis Phage Type 30 from a single almond orchard over a 5-year period. J Food Protect 70: 1784–1789

Van Doren JM, Kleinmeier D, Hammack T, Westerman A (2013) Prevalence, serotype diversity, and antimicrobial resistance of *Salmonella* in imported shipments of spice offered for entry to the United States, FY2007-FY2009. Food Microbiol 34:239–251

Vij V, Ailes E, Wolyniak C, Angulo FJ, Klontz KC (2006) Recalls of spices due to bacterial contamination monitored by the U.S. Food and Drug Administration: The predominance of salmonellae. J Food Protect 69:233–237

Weerakkody NS, Caffin N, Dykes GA, Turner MS (2011) Effect of antimicrobial spice and herb extract combinations on *Listeria monocytogenes*, *Staphylococcus aureus*, and spoilage microflora growth on cooked ready-to-eat vacuum-packaged shrimp. J Food Protect 74:1119–1125

Witkowska AM, Hickey DK, Alonso-Gomez M, Wilkinson MG (2011) The microbiological quality of commercial herb and spice preparations used in the formulation of a chicken supreme ready meal and microbial survival following a simulated industrial heating process. Food Control 22:616–625

Dried Dairy-Based Products

Jeffrey L. Kornacki and Greg Desautels

Abstract Dried dairy products have a very good but not perfect food safety record, compared to many other foods. The greatest microbiological concern with dried dairy powders is *Salmonella* contamination, because of the organism's ability to survive at low water activity in the product and the processing facility's environment. Risk of product contamination is greatest from the dryer through packaging and may be exacerbated by wet cleaning of inadequately dried equipment. Pathogen control for dried dairy products is best achieved by control of the processing environment.

Keywords *Salmonella* • Dry milk • Dry cheese • Whey protein • Dry whey • Spray drier • Environmental sampling • Contaminated equipment • Testing

Types of Dried Dairy Products

A variety of dried dairy products exist, including dried milk powders such as nonfat dry milk (NFDM), dry whole milk, whey protein concentrate (WPC), whey protein isolate (WPI), dry cheese preparations, lactose powder, and sweet whey powder. These products are largely used as ingredients in a variety of foods, many of which are then ready to eat and some that are formulated as ingredients for foods that may or may not be subjected to a microbicidal treatment.

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Ready-to-Cook Dried Dairy Product Ingredients

Some dried dairy powders may be blended with other dry ingredients and used in products which receive a substantial heat treatment (e.g., cake mixes, snack foods, breading for poultry, and other products, candy, sauce mixes, and yogurt). Some of these products may also be used as carriers for other foods, feeds, pet foods, nutraceuticals, and pharmaceuticals.

Ready-to-Eat Products

Ready-to-eat products include those that may be hydrated by the consumer or blended and then consumed directly without a microbicidal step (e.g., protein beverages, reconstituted milk products, some coffee creamers, chocolate, etc.). Some may be blended without a heat treatment into nonsterile infant formula, depending upon the process, in which case *Cronobacter* (described in another chapter of this book) is also a microbiological contamination risk for certain narrow subgroups of the population. Many dairy powders are agglomerated to facilitate ease of hydration, such as instantized dry milk powder.

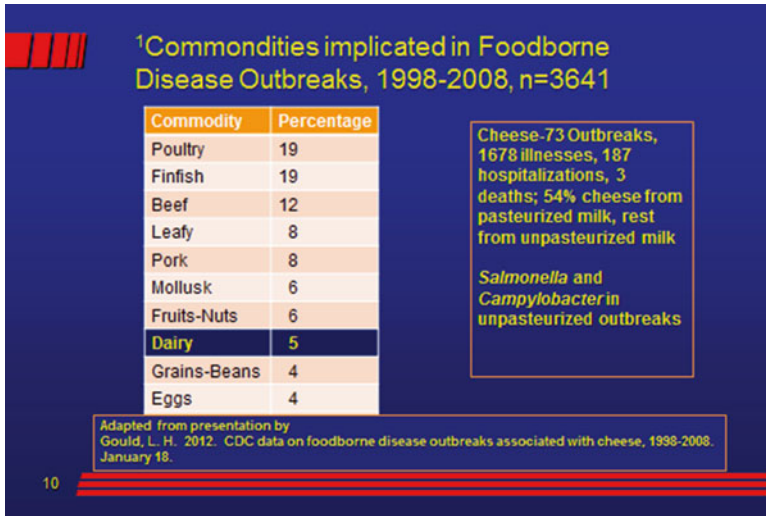
Microbiological Safety

Microbiological Safety Record

Dried dairy products have a relatively safe history, when compared to other foods (Fig. 1), but have been implicated in recalls and foodborne illnesses (Figs. 1 and 2). A voluntary industry survey of the environment and products from approximately 30 dry milk product facilities obtained over a 45-year period (January 1967 to December 2012) revealed that *Salmonella* was isolated from 2.52 % of environmental samples, 0.52 % of sifter tailings, and 0.08, 0.08, and 0.03 % of spray-dried, roller-dried, and instantized milk powders, respectively (ADPI 2013). However, it is notable that *Salmonella* prevalence in facilities was reduced from 2.85 % during the 23-year period from 1967 to 1990 to less than half (1.25 %) in the 22-year period between 1990 and 2012 (ADPI 2013).

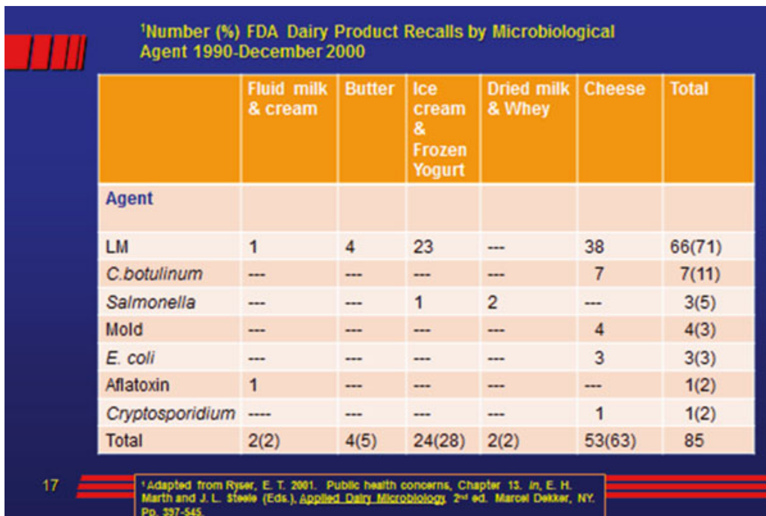
Selected Examples of Foodborne Illness Outbreaks and Recalls from Dry Milk Powder

Salmonella Tennessee, 1993. Salmonella serovar Tennessee was isolated from the stools of two infants in Canada, and one *Salmonella* infection in Illinois was traced to infant formula prepared from spray-dried milk-based powder produced in a Minnesota facility. Further investigation revealed that between November 4, 1992, and June 29,



Commodity	Percentage
Poultry	19
Finfish	19
Beef	12
Leafy	8
Pork	8
Mollusk	6
Fruits-Nuts	6
Dairy	5
Grains-Beans	4
Eggs	4

Fig. 1 Commodities implicated in foodborne disease outbreaks, 1998–2008



	Fluid milk & cream	Butter	Ice cream & Frozen Yogurt	Dried milk & Whey	Cheese	Total
Agent						
LM	1	4	23	---	38	66(71)
C.botulinum	---	---	---	---	7	7(11)
Salmonella	---	---	1	2	---	3(5)
Mold	---	---	---	---	4	4(3)
E. coli	---	---	---	---	3	3(3)
Aflatoxin	1	---	---	---	---	1(2)
Cryptosporidium	----	---	---	---	1	1(2)
Total	2(2)	4(5)	24(28)	2(2)	53(63)	85

Fig. 2 Dairy product recalls 1990–2000, *LM Listeria monocytogenes*

1993, forty-eight cases of *Salmonella* Tennessee infection were reported to CDC, resulting in a recall of a variety of spray-dried products produced at this processing facility. This strain was an unusual lactose-fermenting *Salmonella* strain requiring careful attention to unusual reactions on selective agar media (CDC 1993).

Salmonella, *2009*. USDA recovered *Salmonella* from "dairy shake" powder. A supplier of key ingredients for this product was a Minnesota-based dairy cooperative.

FDA sampling at the cooperative resulted in recovery of *Salmonella* from equipment, in 2009. This finding led to numerous recalls of instant NFDM, whey protein, fruit stabilizers, and gums manufactured over a 2-year period, due to its potential to be contaminated with *Salmonella.* Additional recalls of at least 286 items included cake products, candy, drink mix, NFDM, oatmeal, sauce mix topping, and yogurt products (FDA 2009a, b). No illnesses were reported.

Salmonella Give—2008, France. Salmonella serovar Give was implicated in an outbreak of salmonellosis and a subsequent recall associated with dry infant milk powder manufactured in France, which had been rehydrated before feeding to infants (Jourdan et al. 2008).

Risks

The principal microbiological risk associated with dried dairy products is *Salmonella,* due to its ability to persist in dry environments and foods. In most instances, except lactose powder manufacture, liquid product or slurry is heated to temperatures adequate to destroy populations of vegetative microorganisms, prior to its injection into the drying system. However, once the product enters the dryer, product water activity is instantly reduced to low levels. For example, milk or cheese product at a_w values of 0.98–0.99 will decrease to values often less than a_w 0.3 in instantly. This is because the air temperatures of the dryer are often several hundred degrees Fahrenheit. It is well established that as water activity is reduced, microbial heat resistance increases (Kornacki and Marth 1993; Geopfert et al. 1970). In fact, Kornacki (2012) reported that a 13-min treatment at 240 °F was insufficient to substantially reduce *Salmonella* spp. populations in wheat at ca. a_w 0.2. Hence, *Salmonella* lethality will occur in an operating spray dryer only during those moments when the incoming product is at high water activity. The lack of microbial lethality during spray-drying was evidenced in a study by Doyle et al. (1985), which revealed only a 1–1.5 $\log_{10}$ reduction in the population of *Listeria monocytogenes* during spray-drying, regardless of whether nonfat dry milk was prepared from concentrated (30 %) or unconcentrated (10 %) skim milk. It is well established that *L. monocytogenes* is more heat resistant than most *Salmonella* strains in high water activity matrices. However, *L. monocytogenes* is generally as heat resistant in high-*a*w foods as *S.* Senftenberg 775 W, which is noted for its high heat resistance in high-a_w foods (Sörqvist 2003; Ng et al. 1969). Hence, less reduction of *Salmonell*a populations in spray-dried milk would occur under similar conditions. Should contamination of dried dairy products occur, the spray-drying chamber and subsequent processing and handling areas are those most likely to be sources of contamination, provided milk entering the spray dryer is at a microbicidal temperature. Hence, processors manufacturing dried dairy products typically have robust environmental and product testing program for *Salmonella. Programs should involve the following elements:*

- Weekly sampling of zone 2, 3, and 4 surfaces. Focus should be placed on traffic routes and potential sources of moisture in the environment.
- Routine sampling of sifter tailings.

- Sampling from sanitary vacuums used to clean the environment.
- Finished product composite sampling and testing of every lot through the use of a continuous in-line sample system.

Processors often employ monitoring of food contact and non-food contact surfaces for Enterobacteriaceae as well.

Processing and Equipment

Each of fluid dairy product drying processes involves beginning with a high-moisture product, such as skim milk, whole milk, or cheese slurry and heat treating it, usually through a heat exchanger (plate or shell-in-tube or swept/scraped surface). In some processes, such as that for whey protein concentrate, the product is concentrated in falling film evaporators prior to passing through a final heat exchanger. The product is then injected into a dryer. The dryer may be a drum dryer, spray dryer, or box dryer type. Subsequent processing may involve passing the product through coolers (e.g., fluidized bed coolers). In some instances, bucket elevators, screw conveyors, drag conveyors, and pneumatic conveyance systems are used to move the product throughout the processing environment. Product often passes through sifters and cyclones to recover and recycle product dust. In some instances, a wet scrubber may be used instead of a cyclone to recover fines. Finally, product often passes through a rotary valve and is then packaged. (See example in Fig. 3.)

Cleaning Considerations

The Problem of Effective Cleaning

Procedures for wet cleaning and sanitization of food processing equipment and processing environments are well established. This is also true for such procedures related to high-moisture areas of dairy product drying facilities, including protocols for cleaning and sanitizing dryers. However, downstream dry processing may require dry cleaning approaches that are unique to specific types of equipment and processes. This topic is addressed in greater depth in the chapter by Burnett and Hagberg in this book entitled *Dry Cleaning, Wet Cleaning, and Alternatives to Processing Plant Hygiene and Sanitation*.

Equipment and facilities for dry-food processing have not originally been designed for traditional wet cleaning (Chen et al. 2009). Such equipment may be designed with sandwiched structures that cannot be effectively and efficiently disassembled for wet cleaning, sanitization, and subsequent thorough drying before reassembly. Such unhygienic equipment design will result in the development of microbial growth niches and a higher risk of product contamination when traditional wet cleaning procedures are used. The same is true of other equipment and

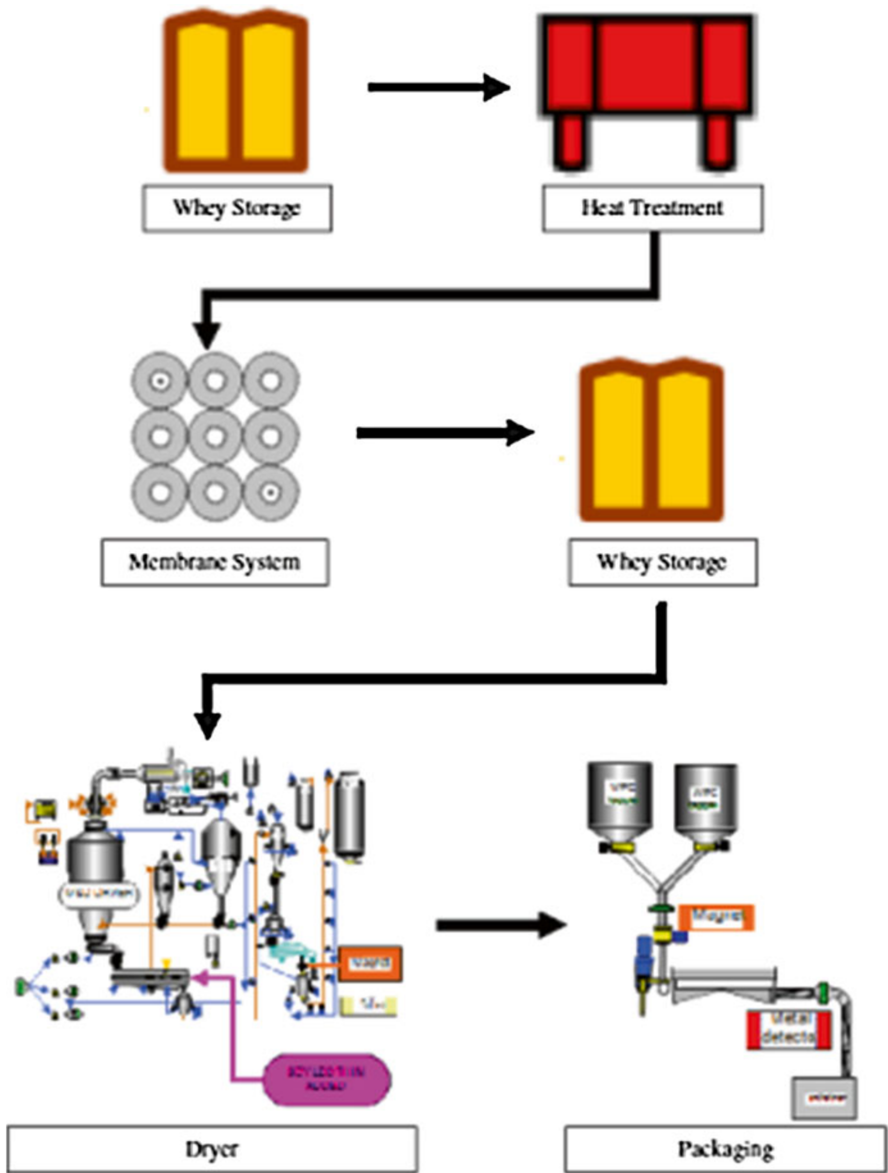


Fig. 3 Flow diagram for production of whey protein concentrates

facility structures that are not hygienically maintained. There is no substitute for an effective preventative maintenance program as well as validated and verified preventive controls that afford hygienic conditions in the plant and equipment before product is produced.

Examples of Areas of Product Contamination Resulting from Drying Equipment

Selected observations of such areas were described previously by Kornacki (2011b). High-risk areas in dry milk processing facilities include:

1. Penetrated insulated double-walled structures in dryers. Wet insulation can result in growth of *Salmonellae* and subsequent contamination. Stress cracks can provide an opportunity for *Salmonellae* to enter the product stream (Rowe et al. 1987, Fig. 4, Kornacki 2011b).
2. Accumulations of powder within cyclone exhausts. If this accumulated material is not effectively removed during cleaning, water vapor from dried product will off-gas and moisten product residues particularly in horizontal cyclone exhausts. After the powder's water activity increases, microbial contaminants can grow. Vibration of the cyclone during operation can cause in powder accumulations at the cyclone/exhaust vent interface to drop back into the product stream, often into the fluid bed cooler (Kornacki 2011b).
3. Weirs in the fluid bed are often sleeved. If these sleeves are not removed or able to be removed, they accumulate moisture during wet cleaning, which becomes niches for microbial growth that can slough off into the product stream during operation (Kornacki 2011b).
4. Rotary valves (Fig. 5) are often Cleaned-In-Place (CIP). Fluid during such cleaning may enter into gasketed flange areas and become a microbial growth niche that can slough off into the product stream (Kornacki 2011b). This highlights the importance of validation and verification of all cleaning and sanitization regimes.

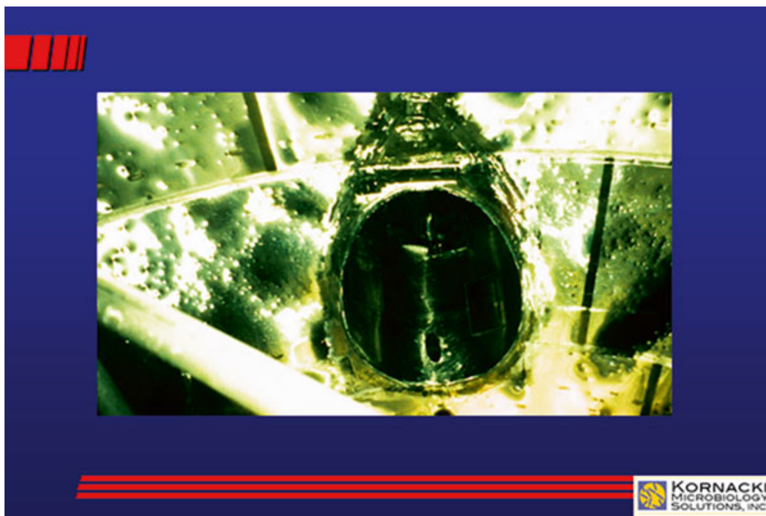


Fig. 4 Pockmarked inner wall of an insulated dryer with likely stress cracks

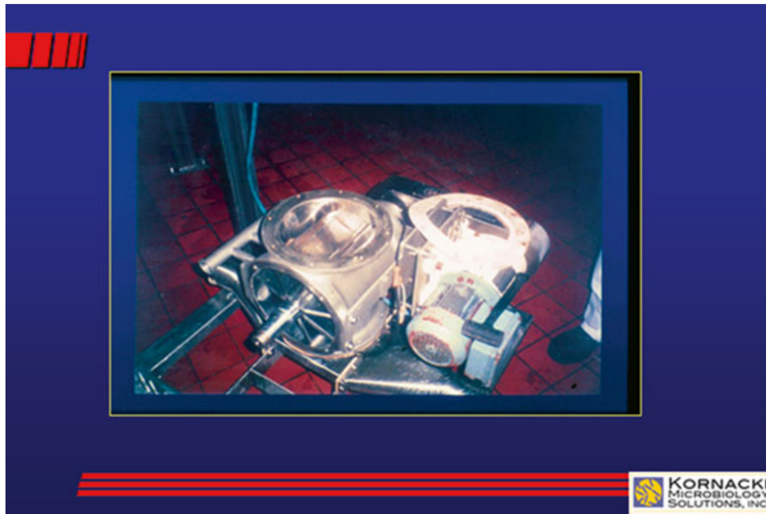


Fig. 5 Rotary value that has been ineffectively CIP cleaned

5. Screw conveyors. A product emerging from dryers is hot. Water vapor emerging from such product may condense on metal screw conveyor flights and conveyor lids and lid-associated gaskets. These moistened areas can grow microbes and result in direct product contamination (Kornacki 2011b). In addition, screw conveyor flights, which are not flushed with conveyer end plates, create a void. Product accumulations in these voids may become moist (e.g., from residual moisture from wet cleaning or other sources of moisture), resulting in microbial growth and product contamination.
6. Stress cracks in bucket elevators, especially near areas that collect moisture (e.g., bucket elevator pads that become wet from cleaning or overspray from cleaning), may grow *Salmonella* that can migrate through stress cracks.
7. Stress cracks in sifter screen rims can collect wash water in which *Salmonella* can grow and contaminate product during sifter operation.
8. Reused metal product totes which have not been thoroughly dried subsequent to cleaning and sanitization can become microbial growth niches resulting in product contamination.
9. Product accumulations on ledges or structures underneath drum dryers may become wet and contact sheets of product emerging from roller drums, subsequently contaminating product.
10. Wet cleaning during operation of roller drum dryers can result in splashing of residues from the floor into dry product sheets, as they are removed from the drum.
11. Hammer mills (Fig. 6) that are manually cleaned and placed on unclean surfaces can become contaminated and contaminate dry product.
12. CIP pop outs. Dried product particles can become wedged, holding the CIP pop-out valve slightly open, and thereby allowing residual rinse water to drip

Fig. 6 Hammer mill placed in unclean area after wet cleaning

into the product stream. Personal experience by the first author of this chapter revealed this as a significant source of coliforms in agglomerated product inside an enclosed product timing belt.

13. CIP spray balls. Failure to remove CIP spray balls during wet cleaning will result in product dust passing into the balls through numerous pores, which are used during wet cleaning to deliver cleaners, sanitizers, and rinse fluid. Failure to remove these spray balls prior to production often results in wet product residues able to contaminate product. Microbial growth and product contamination will occur from such sources.
14. Spray dryer catwalks (Fig. 7). Rusted unclean structures on catwalks can result in product contamination, when these are rolled into the dryer.
15. Wet-cleaned, but not dried, areas of dryer-associated bag houses result in standing water in bag houses which can result in revival of injured cells and development of microbial growth niche in close proximity to product.

Testing Programs

Environmental Monitoring

Both a routine and investigational approach to sampling the production environment for *Salmonella,* coupled with finished product testing, is both common and recommended in the industry. However, there is limited value to finished product testing, as described in chapters by Niemera, Burnett and Hagberg, Cordier, Kornacki, and

Fig. 7 Rusty underside of a catwalk for spray dryer

others in this book. Designing an environmental sampling program should be on a site-by-site basis. *Salmonella* are serologically defined. Hence, a recognized *Salmonella* O and H group should be determined to be certain that this organism has been isolated. If confirmation is desired, presumptive *Salmonella* results (e.g., typical appearance on lysine iron agar, LIA, and/or triple sugar iron agar, TSI) should always be confirmed serologically. Each confirmation of a *Salmonella* isolate that is presumptive positive should be positive by polyvalent O and polyvalent H antisera. There are over 2,400 distinct *Salmonella* serovars (Brenner et al. 2000). However, the food processor's liability may be reduced if a specific O and H group is determined, rather than mere reliance on a single polyvalent O and a single polyvalent H antigen reaction. Some processors may wish to obtain a full serotype (O, H, and single factor antigens determined), and other processors may wish to have the isolates further subtyped by a variety of techniques such as pulsed-field gel electrophoresis typing, repetitive extragenic palindromic (REP) PCR, or phage-typing, for example.

Efforts to remediate areas that test positive for *Salmonella* should be followed by retesting of previously positive areas. Many processors retest such sites at least three times. However, tracking and trending approaches, such as those described by Eifert and Arritt (2002) over a longer period of time, are recommended. In addition, an investigational sampling program, in which samples near the positive sites are taken, an approach often referred to as a "vectoring," should be initiated in response to a previous *Salmonella*-positive sample. Such approaches should recognize that microbes which have contributed to a previous positive sample may be located not only on the same plane but also may be located above or below areas that have tested positive.

Product Testing

Finished product testing in the dried dairy powder industry is typically quite rigorous, usually with each manufacturing production lot tested at the FDA category I, II, or III level, wherein $n = 60$, 30, or 15 samples or composites of samples resulting in 4×375, 2×375, or 1×375 g level, respectively (Andrews, and Hammack 2011). Despite plant lot codes, FDA regards all lots produced within a validated and verified cleanup (including sanitization) as a “lot.” Hence, a 30-day production is likely to be considered adulterated, even if the only positive sample recovered was on day 30 from product produced on a common line, in the absence of special cause that would explain the day 30 positive and exonerate the previous 29 days. An example of a special cause may be a finding that a *Salmonella*-positive roof leak occurred near the production line on the day the sample was taken which has also tested positive for the same *Salmonella* serotype.

In-Plant Investigations

The topic of in-plant investigations is addressed in greater detail in the book *Principles of Microbiological Troubleshooting in the Industrial Food Processing Environment* (Kornacki 2010) and also in Chap. 3 of this book. A combination of environmental and in-line and finished product investigatory sampling is often performed. A sampling-based approach to production line-specific microbiological risk assessment is described by Kornacki (2014).

Summary/Conclusions

Dried milk powder is used in a variety of products, some of which are ready to eat and intended for high-risk populations (e.g., infant formula), whereas other dried milk ingredients may be subjected to a lethal treatment before consumption, as may occur with cake mixes. Dryers used by the industry are unlikely to provide a microbicidal treatment to kill *Salmonella*, as such dryers rapidly reduce the a_w of the product to thermally protective levels. Despite the very good food safety record of dried dairy products, the risk of *Salmonella* contamination of product from the environment of milk and dairy product drying facilities still exists. This is despite a substantial reduction in the prevalence of *Salmonella* contamination in the past 45 years. This reduction is likely due to increased awareness of risk and intensified sampling and control strategies implemented by the industry. Present technologies still cannot guarantee a *Salmonella*-free product apart from diligent efforts by the industry, with regard to environmental control and monitoring.

References

American Dairy Products Institute (ADPI) (2013) ADPI milk manufacturers sampling survey, June 1967 thru January 2012. www.adpi.org Accessed Jan 2014

Andrews WH, Hammack TS (2011) Food sampling and preparation of sample homogenate, Chapter 1. In: FDA bacteriological analytical manual. Online http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm2006949.htm. Accessed 12 Jan 2014

Brenner FW, Villar RG, Angulo FJ, Tauxe R, Swaminathan B (2000) *Salmonella* nomenclature. J Clin Microbiol 38:2465–2467

CDC (1993) *Salmonella* serotype Tennessee in powdered milk products and infant formula – Canada and United States, July 9. Morbidity Mortality Weekly Rep 42(26):516–517

Chen Y, Scott VN, Freier TA, Kuehm J, Moorman M, Meyer J, Morille-Hinds T, Post L, Smoot L, Hood S, Shebuski J, Banks J (2009) Control of *Salmonella* in low-moisture foods III: process validation and environmental monitoring. Food Protection Trends 29(8):493–508

Doyle MP, Meske LM, Marth EH (1985) Survival of *Listeria monocytogenes* during the manufacture and storage of dry milk. J Food Prot 48:740–742

Eifert J, Arritt FM (2002) Evaluating environmental sampling data and sampling plans. Dairy Food Env Sanitat 22(5):333–339

FDA (2009a) Company recalls various products due to potential Salmonella contamination. July 28. http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm169471.htm. Accessed 13 Jan 2014

FDA (2009b) Plainview Milk Cooperative ingredient recall product list. July 29. http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm169471.htm. Accessed 13 Jan 2014

Geopfert JM, Iskander IK, Amundson CH (1970) Relation of the heat resistance of salmonellae to the water activity of the environment. Appl Microbiol 19:429–433

Jourdan N, Le Hello S, Delmas G, Clouzeau J, Manteau C, Désaubliaux B, Chagnon V, Thierry-Bled F, Demare N, Weill FX, de Valk H (2008) Nationwide outbreaks of Salmonella enterica serotype Give infections in infants, September 2008. Eurosurveillance 13(39):1–2, 25 Sept http://www.eurosurveillance.org/images/dynamic/EE/V13N39/art18994.pdf. Accessed 13 Jan 2014

Kornacki JL (2010) Principles of microbiological troubleshooting in the industrial food processing environment. Springer, New York

Kornacki JL (2011a) Practical sampling plans, indicator microorganisms, and interpretation of test results from trouble-shooting, chapter 25. In: Hoorfar J (ed) Rapid detection, characterization and enumeration of foodborne pathogens. ASM Press, Washington, DC, pp 373–379

Kornacki JL (2011b) Symposium S41. Drying equipment (tunnel and spray driers) When and how to clean (…and how not to clean…. and where to clean!) IAFP Annual Meeting, Milwaukee, WI, 3 Aug

Kornacki JL (2012) Hygiene control in the dry food products industry: the roles of cleaning methods and hygienic indicators, chapter 28. In: Hoorfar J (ed) Case studies in food safety and authenticity: lessons from real-life situations. Woodhead Publishing, Cambridge, UK, pp 254–266

Kornacki JL, Marth EH (1993) Thermal inactivation of Salmonella senftenberg and micrococcus freudenreichii in retentates from ultrafiltered milks. Lebensm Wiss U Technol 26:21–27

Ng H, Bayne HG, Garibaldi JA (1969) Heat resistance of *Salmonella*: the uniqueness of *Salmonella* senftenberg 775W. Appl Microbiol 17(1):78–82

Rowe B, Hutchinson DN, Gilbert RJ et al (1987) *Salmonella ealing* infections associated with consumption of infant dried milk. Lancet 2:900–903

Sörqvist S (2003) Heat resistance in liquids of *Enterococcus spp., Listeria spp., Escherichia coli, Yersinia enterocolitica, Salmonella spp.* and *Campylobacter spp*. Acta Vet Scand 44:1–19

Low-Water Activity Meat Products

Peter J. Taormina and John N. Sofos

Abstract Dried edible muscle tissue products are some of the oldest processed food types with their origin lost in time. Today, they exist in various forms and flavors throughout the world. In addition to meat from various animal and bird species, as well as fish, such products may be formulated with salt, sugar, spices, flavorings, and other ingredients, and, in addition to drying, they may undergo fermentation and smoking. Formulations and processes used are selected to improve their eating quality but also to extend the shelf life and assure safety at ambient temperatures. In modern years, they are manufactured according to well-defined and controlled processes for consistency and assured safety and quality in finished products. A major improvement has been the isolation and proper use of microbial starter cultures for consistent fermentation when the products are fermented. This chapter describes the most common dried meat products, their major characteristics, processing procedures, involvement in microbial illness, and approaches for microbial control and assurance of quality and safety.

Keywords Low-water activity meat • Dried meat • Dried poultry • Jerky • Dry sausage

Introduction

Historical Overview of Salting and Drying of Meat

Drying meats is one of oldest, most effective ways of preservation (Gailani et al. 1986). Man learned to hang fish and meats in the air and sun as the easiest way to enable drying (Chang et al. 1996). Since this method was slow, depending on

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climate conditions, and generally not able to lower the moisture content below 15 %, this practice often left fish and meat susceptible to microbiological spoilage or contamination from dust, mold, insects, and rodents (Leistner 1987). Consequently, the introduction of salt, sugar, and smoke led to further advancement of dried meat processing, which improved the safety and quality of the end product. Salting and drying meat is probably the oldest process used to preserve meat from the time of harvest until some future point of consumption, sometimes relatively long into the future. Salt preservation methods of meats and fish are believed to have begun as early as 3,000 BC (Ambrosiadis 2005; Romans et al. 2001; Sofos 1984). Temperate regions of the world, in particular, adopted such practices through trial and error as it was gradually realized that salting and drying preserve and protect meat from spoilage. Warm, humid climates were probably unsuitable for effective drying of meat, so development of drying preservation practices particularly occurred in temperate climates. Unfortunately, the origin of dry-cured meats is lost to antiquity, and so the various specific processing techniques and customs of each country are not available (Toldrá 2004). However, it is likely that each method developed over several generations and was adapted to the unique palettes of those people groups. Indigenous methods of drying meats that resulted in illness or undesirable sensory properties were long ago abandoned, and time-tested methods survived, while processes that permitted survival of microorganisms imparting desirable sensory characteristics were adopted over time.

Today, it is known that low-moisture meats, produced by salting and drying, create selective pressures to kill or reduce Gram-negative bacteria and parasites while permitting survival and even proliferation of certain xerotolerant Gram-positive bacteria, yeasts, and molds. Microorganisms surviving on low-a_w meat, poultry, and fish impart certain flavor characteristics as proteolytic and lipolytic microorganisms become part of the dominant microflora outcompeting spoilage and pathogenic microorganisms (Bennani et al. 1995).

Sodium chloride and moisture content percentages of foods have been important measurements in the modern era for the assessment of microbiological stability, but water activity (a_w) serves as a better general indicator of microbial stability than moisture content (Sperber 1983). The term a_w is defined as the vapor pressure of a food divided by the vapor pressure of pure water at the same temperature, or the equivalent relative humidity/100. In foods, a_w is greatly influenced by the presence of sodium chloride because it binds water very effectively (Chirife and del Pilar Buera 1996). Sodium chloride levels are also used to calculate brine concentrations, which are determined by the equation: $[\% \text{ NaCl}/(\% \text{ NaCl} + \% \text{ H}_2\text{O})] \times 100$ (Cerveny 1980). Brine concentration is also known as water phase salt (WPS), which, along with a_w, is a key measurement for assessing the standards of identity for certain low-moisture meat products as well as the microbial stability of intermediate-moisture meats produced by drying, fermenting, and/or smoking (Ingham et al. 2004) and of salted, smoked fish (Cornu et al. 2006).

Contemporary Methods of Producing Low-a_w Meats

Contemporary methods of producing low-a_w meats involve salting, air-drying, low-temperature slow heating, freeze-drying, and combinations of these methods. Air-drying is still a common practice for commercial and artisanal production of dry sausages in Western Europe (Lücke and Vogeley 2011). Intermediate-moisture meat products are defined as those of a_w 0.60–0.91 (Chang et al. 1996; Feránndez-Salguero et al. 1994; Leistner 1987). Even though NaCl is the main a_w depressor in intermediate-moisture meat products, its molality cannot be used to estimate a_w due to the effects of other solutes in such products (Feránndez-Salguero et al. 1994).

The stability of cured meats depends on a_w, presence of other antimicrobials, packaging, and temperature. High-salt (8–15 %) cured meats with moisture contents of 25–30 % are microbiologically stable. Nevertheless, oxidation in these products slowly leads to color loss and flavor degradation in months, if not years. Intermediate salt-cured meats are processed with lower salt levels (4–8 %); they typically have 35–55 % moisture and require refrigeration to restrict microbial growth unless products are fermented or acidified or dried to low a_w. Lower salt levels (1.5–4 %) and higher moisture levels (60–75 %) lead to decreased stability. However, such cured meats can be kept refrigerated in unopened packages for up to 12–18 weeks when formulated with additional antimicrobials such as organic salts or organic acids (Legan et al. 2004; Seman et al. 2002). These antimicrobials are used to restrict the growth of *Listeria monocytogenes*, which can contaminate cured ready-to-eat meats after heating and prior to or during final packaging.

The speed and effectiveness of drying processes are dependent on air velocity (Lebert et al. 2005) and relative humidity (Arnau et al. 2003). In muscle foods, water is either bound or unbound and the state of water between the two is in flux (Chang et al. 1996). Some of the bound water (ca. 10 %) is believed to be hydrogen bonded with the surface of protein molecules (Chang et al. 1996), while some is simply in interstitial spaces between muscle fibers and connective tissues. Water vapor pressure is a useful measurement of moisture content in meats, and a_w in meat products is a function of the moisture content/solid content, the components and composition of the product, the microstructure of the product, the temperature, and the state of some component solids (like sugar) (Chang et al. 1996). In terms of water removal, there are two terms used: dehydration and drying. Dehydration occurs when moisture is removed from a food via thermal treatment. Mechanical methods of moisture removal from solids, such as pressing, are distinguished from this definition (Chang et al. 1996). Shrinkage of muscle fibers forces loosely held water into the extracellular space. Pressing separates the loosely held liquid from the solids and removes most of the moisture from the muscle with some loss of solids in the pressed liquid. Dehydration is simultaneously a heat and mass transfer operation, which is impacted by multiple factors, including temperature, humidity, meat composition, added solutes and humectants, physical pressure, and air velocity.

It is imperative that moisture is removed as quickly and uniformly as possible from the muscle tissue to control microorganism behavior in and on meats during dehydration. Improper combinations of air velocity, temperature, and RH for certain products can cause problems such as surface crusting (also called "case hardening" in sausages), which traps moisture inside the meat product, permitting microbial survival or growth. Lebert et al. (2005) studied the growth of *Pseudomonas* spp. on raw semi-membranous pork muscles and determined that less than or equal to a 2-log increase occurred within 96 h at 12 °C if the air velocity was equal to 0.2 m/s and RH was below 82 %, or if the RH was as high as 90 % and air velocity was higher at 0.9 m/s (Lebert et al. 2005). As water diffusivity increased under these conditions, a_w decreased, thereby reducing the growth rate of *Pseudomonas*. Although this study was conducted at high a_w levels (>0.9) relative to low-a_w meats, it reveals the impact of air velocity and RH on surface drying of meat.

Many intermediate-moisture and all dried meat products are considered "shelf stable." Shelf stability is defined as the condition under which a food product is unable to support the growth of foodborne bacterial pathogens and spoilage microorganisms and can, therefore, be stored at ambient temperature indefinitely or until quality degradation renders the product no longer fit for purpose.

Impact of a_w *Depression on the Microbiota of Meat*

At the point when muscle foods begin to lose moisture, a change in microbiota ensues. Addition of ingredients such as sodium chloride, nitrate, nitrite, and sugars to meat causes the a_w to decrease rapidly from 0.98 to 0.99 down to ca. 0.96. The effect of a_w depression on the behavior of microorganisms in meat systems is usually to impart additional stress on cells through osmotic pressure. These stresses also interact with other factors in meat products—primarily with pH. Since many low-a_w meat products are also acidic due to fermentation, the interacting effects of pH and a_w work in consort to induce selective pressures that permit the survival of Gram-positive bacteria, yeasts, and molds while inhibiting Gram-negative bacteria (Blackburn et al. 1997). In the resulting reduced-moisture meat products, including fermented sausage, these conditions favor the growth and survival of lactic acid bacteria, micrococci, and nonpathogenic staphylococci (Feránndez-Salguero et al. 1994; Kotzekidou 1992). Through multiplication of these organisms, mainly *Lactobacillus*, *Pediococcus*, and *Micrococcus*, the pH value of these products decreases, which favors the selection of microbiota that compete well and survive under acidic conditions. Therefore, acid-tolerant bacteria, yeast, and molds will primarily persist in such meat products.

During the production of cured meats, microorganisms are subjected to changes in a_w. During the initial stages of drying processes, conditions are favorable for microbial growth and survival, but as moisture is removed from meat and the a_w decreases, microorganisms become stressed and begin to die off. Reduction of a_w by 0.05–0.13 units causes an increase in cellular stress and adaptive processes in

Escherichia coli and *Salmonella*, which is not as pronounced in bacteria such as *Staphylococcus aureus* and *Listeria monocytogenes* (Mellefont et al. 2003, 2004). A study by Tiganitas et al. (2009) revealed that as the a_w decreases from 0.98 to 0.94 in increments of 0.01, the maximum specific growth rate of *L. monocytogenes* at 10 °C is reduced linearly. It was also determined that preadaptation of cells to pH 5.0 or 6.0 in the presence of a_w 0.995 enabled subsequent growth at pH 7.2, a_w 0.940 at 10 °C, following a lag period of many weeks, whereas cells grown at pH 7.2 without preadaptation were subsequently not capable of growth at a_w 0.940.

Dry salt application to animal-derived products is particularly lethal to bacterial, parasitic, and viral pathogens. Salt-cured hams are preserved from bacterial pathogen growth during the curing and drying process, largely due to NaCl (Reynolds et al. 2001). NaCl alone did not destroy *Trichinella spiralis* in dry-cured hams through a curing process of 40 days, except on surface muscles when the brine content approached 8 % (Zimmermann 1971). However, 5 % NaCl, 10 % moisture, and 8.5 % brine concentration were determined to be the thresholds of nonviability of all parasites. The process of dry-salting natural sausage casings for 30 days at temperatures greater than 4 °C sufficiently reduces infectivity of zoonotic viruses that cause foot-and-mouth disease (Wijnker et al. 2007) and classical swine fever (Wijnker et al. 2008).

Types of Products and Reported Water Activities

There are a variety of low-a_w meat and poultry products, which can often be traced to early historical development in various geographic regions (Table 1). Many of these products share similarities in ingredients used in marinades, pickling solutions, and curing agents, and the temperature, humidity, and times used for drying are also similar. Only a few of the many dried meat products available throughout the world are described briefly in the following paragraphs.

Pork Products

There are multiple pork products originating in various regions of the world, many of which are summarized in Table 1. In most cases, these products are dry-cured, often using the ham portion of pork. These products are often considered delicacies due to the unique flavor and texture profiles and the investment in monthlong processes of production. Dry-cured ham is one of the main products, with variations produced in different geographic regions, for example, Spanish (Iberian, Serrano jamón), Italian (prosciutto, coppa, Parma, San Daniele), French (Bayonne), German (Westphalia), and American (country-style Smithfield hams). Traditional country ham production practiced by agriculturalists in the USA, especially in the mountainous Appalachian region, is a time-honored tradition. Hams are stuffed and

Table 1 Traditional dried or dry-cured meats and their regions of origin, processing methods, and typical water activities

Name of product	Region	Traditional method of production	Water activity	References
Pastirma Basturma	Turkey and Greece	Thin strips (50 × 60 × 5 cm) of meat are dry-cured at ambient temperature; strips are salted, dried, and pressurized, then covered in paste of garlic and other spices and dried for 1 day in a pile, and hung 5–12 days in a ventilated space. It is produced in September to November when flies are not prevalent and air temperature is mild and humidity moderate	0.85–0.90	(Aksu et al. 2008; Ambrosiadis 2005; Chang et al. 1996; Ingham et al. 2006; Leistner 1987)
Dendling	China Malaysia	Paper-thin (0.2 cm) slices of lean pork or beef are cut parallel to muscle fibers and are cured in sugar, salt, soy sauce, monosodium glutamate, and spices. After curing 24–36 h at 4 °C, slices are laid on an oiled bamboo basket or wire rack and dried at 50–60 °C until 50 % of weight is lost. Further drying occurs by cooking over charcoal or deep fat frying. Some variations may utilize nitrate and nitrite	0.69	(Chang et al. 1996; Darmadji et al. 1990; Leistner 1987)
Cooked and dried meat cubes	China Malaysia	Whole-muscle beef, pork, or chicken is boiled 40–45 min and then cut in 5 × 5 × 10-cm cubes. Cubes are added to a cure in a steam kettle and cooked until nearly all the cure has evaporated. Then cubes are dried in an air dehydrator	<0.69 <0.61 for mold control	(Chang et al. 1996; Leistner 1987)
Zousoon	China Malaysia	Pre-rigor pork is boned; cut into pieces parallel to muscle fibers; added to a gas-fired scraping frying pan along with water, sugar, and salt; and then cooked until semidry	0.60–0.65	(Chang et al. 1996)
Dried pork floss	China Malaysia	Pre-rigor pork is boned; cut into pieces parallel to the muscle fibers; added to a gas-fired scraping frying pan along with water, sugar, and salt; and then cooked until dry	≤0.40	(Chang et al. 1996)
Lup cheong (Cantonese) La zang (Mandarin)	China	Raw, nonfermented sausage made from coarsely ground pork (usually ham) and pork fat is mixed with sugar, salt, soy sauce, Chinese wine, potassium nitrate, five spice powder (anise, clove, fennel, and watchau), and monosodium glutamate. Up to 25 % water can be added which affects the appearance of wrinkles after drying. The mixture is stuffed into small hog casing and tied at 15-cm pieces, which are punctured to allow air to escape during drying. Pieces are dried by heating over charcoal at 45–50 °C for 1–2 days and then holding at room temperature for 2–3 days	0.75	(Chang et al. 1996; Leistner 1987)
Nikku	Arctic Canada	Seal meat is cut into pieces, placed flat on wire-bottom trays, and dried under ambient outdoor conditions until roughly 65 % weight is lost	[b]	(Forbes et al. 2003)
Cervelat	Germany[a]	Ground beef and pork are seeded with a starter culture and combined with a mixture of salt, sugar, nitrite, and whole black peppers, and then fermented, smoked, and dried	<0.85	(Chang et al. 1996; Romans et al. 2001)
Pepperoni	Italy[a]	Pork, beef, salt, sugar, nitrite, spice, and usually starter culture are combined, fermented, smoked, and then dried	0.65	(Chang et al. 1996; Romans et al. 2001)

Farmer sausage	Northern Europe	A blend of 65 % beef and 35 % pork is chopped fine, seasoned, stuffed into beef middles, and heavily smoked, followed by drying	0.85	(Chang et al. 1996; Romans et al. 2001)
Mortadella	Italy[a]	A blend of 75 % pork and 25 % beef, sometimes with turkey, chicken, goat, or mutton, is combined with garlic and sometimes with green pistachio nuts and/or olives, fermented, and dried	>0.85	(Chang et al. 1996; Romans et al. 2001)
Sobrasada	Spain	A blend of 50 % lean pork and 40 % pork fat is ground and mixed with 5 % paprika, 2 % salt, and 5 % white pepper and stuffed into natural casings, held at 4 °C for 24 h, and then ripened at ambient winter temperatures (8–15 °C) and 60–85 % RH for ca. 4 months until 15 % water is lost	0.83	(Chang et al. 1996; Rossello et al. 1995)
Biltong	Africa	Long strips of beef are cured with salt, sugar, and spices (nitrate or nitrate may or may not be included). Potassium sorbate (0.1 %) is sometimes added to control mold growth. Traditionally, pieces are sun-dried by hanging on barbed wire or galvanized fences	0.64–0.85	(Burnham et al. 2006; Chang et al. 1996)
Kilishi and bande	Africa	Lean beef or goat meat cut into thin strips are dried in the sun or smoked/cooked over a low fire. Sometimes these are salted	0.59	(Chang et al. 1996; Chukwu and Imodiboh 2009);
Pemmican	North America	Native Americans used bison meat or venison, which was sun-dried or smoked at low temperatures followed by pounding the dried meat into shreds. Then dried fruit was pounded into the dried meat and this was embedded in melted fat before the combination was sewed into rawhide bags for portability	[b]	(Chang et al. 1996)
Charqui	Brazil	Fresh beef *semitendinosus* muscle is cured in dry salt, washed, and sun-dried or heated with smoking. Pieces can be stacked and restacked with inverting uppermost pieces to reposition on the bottom which helps press moisture out of all the pieces	0.70–0.75	(Chang et al. 1996; Lara et al. 2003)
Cecina	Mexico	Fresh lean beef from hindquarters is sliced parallel to muscle fiber grain in long strips of about 5-mm thickness and immersed in brine at 1:2 meat to brine ratio for 4 h. Strips are oven-dried at 50 °C for 1 h or sun-dried for several days. Oil or vinegar is sometimes added, which aids in drying	0.85	(Chang et al. 1996; Garcia et al. 1997)
Salchichon	Spain	Lean beef, lean pork, or both are combined with pork back fat, a seasoning of 1–4 % salt, nitrate, nitrite, and white pepper and 1 % sugar and then held at 25–30 °C and 80–90 % RH for 5 days, followed by maturation for 60 days at 30–37 °C and 70–80 % RH. Variations are made with venison and ostrich	0.80–0.87	(Benito et al. 2007; Capita et al. 2006; Chang et al. 1996; Feránndez-Salguero et al. 1994)

(continued)

Table 1 (continued)

Name of product	Region	Traditional method of production	Water activity	References
Cavourmas	Greece	Bone-in pieces of pork, beef, mutton, and goat meat from neck, shoulder, and breast regions are cut into small pieces and slowly cooked with some water with seasonings, salt, and sometimes leek or onion. The water is evaporated and the meat begins to fry in its own melted fat. Modern-day methods stuff the meat into artificial casings, filling the voids with melted fat. Cavourmas can be shelf stable depending on whether the method leads to aw below 0.95	0.94–0.96	(Ambrosiadis 2005)
Lefkada (salami)	Greece	Ground pork meat, pork back fat, salt, nitrite, nitrate, sugar, seasoning, and local herbs of the Ionian island of Lefkada are comminuted and stuffed into natural casing from pig small intestine. The sausage is fermented and dried to low a_w	[b]	(Ambrosiadis 2005)
Soutzoukakia	Greece	Beef and sheep meat and fat (no pig meat allowed) are combined with wheat flour, garlic, red pepper, cumin, salt, and sodium nitrite, comminuted, and then stuffed into natural casing. The sausage is fermented to pH 5.3–5.6 and then dried to low a_w	0.93	(Ambrosiadis 2005)
Apaki	Greece	Fillet of lean pork meat in thick strips (2–3 cm) is placed into salt for 1 day and then soaked in vinegar for 2–3 days. After removal from vinegar, it is dried with towels and covered with cinnamon for 6 h. It is then covered with ground black pepper, dry savor, and more cinnamon and hung to dry for many weeks, or it may undergo a drying process followed by hot smoking for 1 day	[b]	(Ambrosiadis 2005)
Parma and San Daniel hams	Italy		0.92–0.94	(Giovannini et al. 2007)
Serrano hams	Spain		0.80	(García-Esteban et al. 2004)
Jamón	Spain		[b]	
Iberian hams	Spain		[b]	
Bayonne	France		[b]	
Westphalia	Germany		[b]	
Country hams	North America		0.80–0.85	(Ng et al. 1997; Portocarrero et al. 2002)
Lebanon bologna			0.85	
Jerky			0.70–0.75	

[a]Now widely produced in throughout the world
[b]Water activity not reported, but assumed to be below 0.90

packed with a mixture of salt, brown sugar, and potassium nitrate (typically known as saltpeter). Rectangular-shaped pork belly slabs of meat are also treated with the same combination of three mixed curing agents. Pork bellies are set (skin/rind side down) and curing agents are spread evenly across the meat/fat surface. Finally, another pork belly of the same size is laid (meat side down) on top of the curing agents on top of the first pork belly. Both hams and sandwiched pork bellies are placed in a cold environment, which should not drop below the freezing point, and a flat board is set on top of the pork bellies along with a heavy weight, such as two concrete blocks. During the curing period, the hams and bacons lose a significant amount of water weight, which drains out the sides of the curing meat. After 2–5 months of curing, the hams and bacon slabs are soaked in warm water to remove residual curing agents. Hams are then placed inside of cotton or nylon netting for hanging. The bacon slabs are also hung from hooks. These are then smoked and are considered ready to eat, after slicing, although many people soak the ham pieces again prior to cooking and eating.

Dry-cured Iberian (Spanish) hams are noted for their distinctive flavor. There are a wide variety of staphylococci and micrococci that can be isolated from these hams during the 18 to 24 monthlong maturation process (Rodríguez et al. 1994). *Penicillium chrysogenum* and *Staphylococcus xylosus* are also known to impart flavor characteristics in Iberian hams during ripening (Rodríguez et al. 1998). Italian Parma hams are also considered a delicacy for their distinctive flavor characteristics. These hams grow predominant species of yeast populations during the entire maturation process that include *Debaryomyces hansenii*, *Candida zeylanoides*, *Debaryomyces marasmus*, and, to a lesser extent, *Candida famata* and *Hyphopichia burtonii* (Simoncini et al. 2007). The species *Candida catenulata*, *Candida guilliermondii*, and *Candida edax* and other genera such as *Cryptococcus* and *Wingea* were occasionally detected. Similar yeast dynamics were observed in the Norwegian specialty "Fenalår" dry-cured leg of lamb (Asefa et al. 2009a, b), and *C. zeylanoides* levels were notably higher during pre-salting and *D. hansenii* increased during post-salting and drying.

The potential for Na^+ replacement in the salting process for dry-cured hams was studied by Blesa et al. (2008). Hams were salted at 2 % of weight using traditional NaCl and two experimental formulations containing 50 % NaCl and KCl, and 55 % NaCl, 25 % KCl, 15 % $CaCl_2$, and 5 % $MgCl_2$. It was concluded that hams using either reduced sodium salt mixture, especially the calcium and magnesium, needed more time to reach similar a_w values than hams salted with 100 % NaCl. Mesophilic, salt-tolerant microorganisms were generally at the same population at 20 and 50 days of ripening. By 50 days, lactic acid bacteria counts were higher on hams salted with 100 % NaCl than on other hams. Furthermore, *S. aureus* counts were higher on hams salted with the mixtures compared to the NaCl-treated hams but remained below 10^5 CFU/g at 80 days.

Several other pork products are produced by cooking and rendering. Salted pork is a product used mostly as a flavoring ingredient for cooking soup stock, soups, and greens.

Canned, pre-fried bacon has been produced for several years under the following criteria: moisture/salt × protein index of ≥0.4, brine ratio (moisture/salt) of ≤9.0, brine concentration (salt/(moisture + salt)) of ≥10 %, and maximum 40 % cook yield (U.S. Department of Agriculture-Food Safety and Inspection Service 2005). Cooked bacon is currently more commonly packaged in barrier packaging film with a modified atmosphere or vacuum rather than by canning. Cooked bacon produced in this manner must only meet the ≤40 % yield standard, i.e., ≥60 % shrink loss during cooking, but does not necessarily need to meet the specific moisture protein ratio or brine concentration requirements that are for canned pre-fried bacon. The maximum 40 % cooking yield can be achieved by traditional means of heating such as frying or, more commonly, by microwave processing, with either method resulting in large quantities of grease as a by-product.

Cooked bacon can support *Staphylococcus aureus* growth and toxin formation at certain a_w and non-refrigeration temperature combinations (Lee et al. 1981; Silverman et al. 1983). Industrially produced cooked bacon is exposed to the environment after receiving a lethality treatment and is handled as it exits continuous cooking ovens prior to final packaging. Therefore, there is a risk for recontamination by *L. monocytogenes*; however, cooked bacon does not support the growth of *L. monocytogenes* and the pathogen gradually dies off during storage (Taormina and Dorsa 2010). Freezing of cooked bacon results in greater survival of *L. monocytogenes* than does refrigerated or ambient storage. Survival under frozen conditions was unaffected by the cooking yields of bacon (≤40 %) and the resultant physical properties, such as a_w and percent moisture. A $1-\log_{10}$ reduction of the pathogen on all frozen, cooked bacon products was ultimately reached over several months. Rates of death on cooked bacon cubes were relatively low at −20 °C, whereas the pathogen population steadily decreased on the lower a_w cooked bacon strips and bits that were stored under refrigeration or ambient temperatures.

Lard is a rendering product from pork produced by cooking fatty pork trimmings to very high temperatures sufficient for melting fat and separating from protein and collagen. Lard lacks the basic nutrients essential for the growth of human bacterial pathogens in the limited water phase. Proteins, carbohydrates, and vitamins, which are necessary for microbial growth, are virtually absent from animal-derived fats after processing. Another physical property of lard that provides a hurdle against microbial growth is a_w (Sperber 1983). In an industrial laboratory overseen by one of the authors of this chapter, thirty lard samples ranging in moisture content from 0.04 to 0.16 % were analyzed for a_w, and the average a_w of the 30 samples was 0.605, with none exceeding an a_w of 0.796 (data not published). The minimum a_w requirement for growth of *Staphylococcus aureus* is 0.860 at otherwise optimal conditions of nutrient levels, pH, and temperature (Sperber 1983; Troller 1971). All other foodborne bacterial pathogens require a higher a_w for growth (Sperber 1983; Troller 1986). Given the heat processing parameters for this product, the lack of nutrients, and the low a_w, survival and growth of bacterial pathogens is not a hazard that is reasonably likely to occur.

Pork rinds or the skin of pigs, also known as pork scratchings or pork crackling in the UK or pork cracklings in the Southeast USA (http://store.goldenflake.com/

ProductDetails.asp?ProductCode=431), is a fried or roasted product used as snack or as an ingredient to improve sausage consistency. Variations of this product by various names exist throughout the world, and it is frequently a by-product of the lard industry. Frying or roasting shrinks the product in size because most of the fat that is attached to the inside of the skin melts and is removed during processing.

Beef Products

Beef jerky is a popular low-a_w meat product in the USA. It is processed using heat to attain a desired dryness, texture, and shelf stability. Thus, jerky is classified by the US Department of Agriculture (USDA) as a heat-treated and shelf-stable, ready-to-eat meat product. In terms of lethality against pathogens, a 5.0-log reduction of *Salmonella* serovars and *E. coli* O157:H7 must be achieved by the meat jerky-making process according to the USDA (United States Department of Agriculture-Food Safety and Inspection Service 2012), and for poultry jerky, a 7.0-log reduction of *Salmonella* must be achieved as required in 9 CFR 381.150. During the processing of meat and poultry jerky-log reduction of *L. monocytogenes*, preferably a 5.0-log reduction should be achieved (United States Department of Agriculture-Food Safety and Inspection Service 2012). Various home-style dehydrators were used to produce jerky from inoculated whole-muscle beef, and reductions of *E. coli* O157:H7 averaged 4.81, *Salmonella* 5.07, *L. monocytogenes* 5.10, and *S. aureus* 5.55 log (Dierschke et al. 2010).

Pasterma or pastirma is one of the oldest dry-cured, intermediate-moisture meat products of Middle Eastern countries (e.g., Egypt, Turkey, etc.). Production involves rubbing of salt and nitrate (or nitrite) onto meat pieces, which are then dried, compressed, and coated with a garlic-containing paste. The process is completed in about 5–6 weeks when a reduction in weight of about 35–40 % has occurred. The resulting low-a_w fermented (natural or starter culture) product is shelf stable and is served as thin slices. The quality of sliced pasterma is preserved by packaging and storage at <10 °C (Aksu et al. 2005; Katsaras et al. 1996). Phenotypic, biochemical, and ribotyping identification revealed that pasterma isolates are mostly *Lactobacillus plantarum*, with *Lactobacillus sakei*, *Enterococcus faecium*, *Pediococcus acidilactici*, and *Weissella confusa* also present (Dincer and Kivanc 2012).

Bündnerfleisch is an air-dried meat product of Switzerland and is also known as Bindenfleisch or Viande des Grisons. It is believed that real Bündnerfleisch is produced only at altitudes 800–1,800 m above sea level, which is the altitude of the villages in Grisons, Switzerland. Main ingredients are lean beef (fat and sinew are removed) from the upper shoulder of the animal. The meat is treated with white wine, salt, and seasonings and then dried, initially for 3–5 weeks, in sealed containers at a temperature near freezing. The meat is rearranged regularly to ensure even distribution and absorption of the salt and seasonings. This is followed by drying through hanging in the air at 9–14 °C. The meat is periodically pressed to remove moisture and loses about 50 % of its original weight.

Bündnerfleisch is sliced very thinly and consumed with bread or used in soups as strips or cubes. A meat product related to Bündnerfleisch may be "bresi" from the Besançon region of France (Hammes et al. 2003).

Bresaola is a fermented dried product, produced mainly in the Italian province of Valtellina. It is made of meat (usually semitendinosus muscle) from young bulls. Bresaola is similar to Bündnerfleisch but it is not pressed. Various similar products may be made with meat derived from cattle, bison, wild boar, deer, goat, horses, and potentially donkeys (Braghieri et al. 2009; Marino et al. 2009; Paleari et al. 2003). Biltong is a dried meat product of South Africa (Hammes et al. 2003). Traditionally, long strips of beef are cured and hung on barbed wire or galvanized fences (Burnham et al. 2006).

Chipped beef, or dried beef, is thinly sliced or pressed, salted in brine and partially dried, and in some cases smoked and is marketed, mostly compressed together in jars or in flat plastic packets. It has been popular in the USA for a long time, and it is served in casseroles or dips or on toast, biscuits, English muffins, or fried potatoes. It has been described also as similar to bresaola (Komarik 1940).

Kippered beef is a cured, dried product that is similar to beef jerky but not as hard or dry (Jacob et al. 2009). It is produced by a process similar to jerky in that it is marinated whole-muscle strips of beef that are cooked and dried with or without smoking until reaching a moisture/protein ratio of $\leq$2.03:1 and $\geq$0.76:1 (U.S. Department of Agriculture-Food Safety and Inspection Service 2005).

Tallow is animal fat defined by technical criteria such as melting point and is produced by rendering beef or mutton fatty scraps (may also contain pork fat or oil from plant sources). The product is solidified at low temperatures and can be stable during long-term storage even at higher temperatures if the presence of oxygen is avoided. Tallow is used mostly as a component in animal feeds and in the manufacture of soap. Large amounts of tallow are also used in the production of shortening. Tallow is the main ingredient of pemmican, a native American food. A nonfood use of tallow is in the production of biodiesel. Additional applications include or have included candles, lubricants and motor oil, and medicinal products (Morton et al. 1988).

Poultry Products

Jerky is also made with poultry meat and is available as a dried, shelf-stable, ready-to-eat product. Poultry jerky should be processed to destroy at least 7.0-log units of *Salmonella*. Potential biological hazards that may be present in poultry jerky are the same as those in beef jerky. Important pathogens of concern in jerky products include *Salmonella*, *Listeria monocytogenes*, *Staphylococcus aureus*, and *Escherichia coli* O157:H7, which is mostly associated with beef. These pathogens need to be eliminated during the processing of jerky products. The main control points for microbial hazards are application ingredients that enhance destruction by heat, lethality heating, and drying. Drying also reduces the a_w of jerky to values below 0.85, which make the product shelf stable. Jerky production usually involves

(1) meat strip preparation, (2) marination, (3) application of interventions such as acid dips, (4) lethality treatments such as cooking/heating, and (5) drying. Turkey breast meat was inoculated with various pathogens and used to make jerky with different combinations of marinade and cooking/drying time/temperature combinations (Porto-Fett et al. 2009). The only turkey jerky production process that met the USDA Food Safety and Inspection Service (FSIS) standards of identity of moisture/protein ratio ≤0.75:1.0 or shelf stability ($a_w \leq 0.80$) was marinated strips that are cooked and dried for 3.5 h at 73.8 °C, which resulted in ≥7.1-log CFU/strip reduction of *E. coli* O157:H7, *Salmonella* Typhimurium, and *L. monocytogenes*.

Products with Meat from Other Species

Dried, shelf-stable meat products are prepared throughout the world with available meat from various species, such as lamb and mutton, goat, bison, ducks, deer, elk, alligator, bear, kangaroo, crocodile, and other wild game, formulated with variations of spice and flavoring (Chawla and Chander 2004; Kanatt et al. 2002). Preservation of these products is based on multiple hurdle technology (Leistner and Gould 2001). Regardless of the species used, proper hurdles to reduce, control, or eliminate *Salmonella* and other bacterial pathogens must be applied.

Dry and Semidry Sausages

Dry and semidry sausages can be considered low- and intermediate-a_w products, respectively. Such products are produced from many combinations of comminuted meat and poultry, spices, sugar, flavorings, and curing agents. Many of the current processing practices for dry sausage originated in the Mediterranean region (Ricke et al. 2007). Sausages derived their name from the Latin term “salsus,” meaning salted. Several types of dry and semidry sausages from throughout the world are listed in Table 1. Some of the most popular types of sausages include the dry French Saucisson sec; German Dauerwurst; the Italian salamis Lombardia, Genoa, Milano, and Siciliano; and Italian pepperoni. Salchichon (aka salchichón) is a pork sausage similar to the Italian salami. Chorizo is a Spanish-type sausage that can be fermented but is frequently acidified and dried. The fermentation of sausage involves lactic acid bacterial conversion of hexose sugars into acid using homofermentative metabolism, resulting in the production of lactic acid, which lowers the pH. The decrease in pH causes a reduction in the water-binding capacity of the meat, accelerating the drying process during aging (Tyopponen nee Erkkila et al. 2003). Semidry sausages are sometimes acidified with citric acid or other acids to reduce the pH.

The production of dry and semidry sausage involves grinding or comminution and blending of meat and nonmeat ingredients, usually with the addition of a lactic acid bacteria starter culture such as *Pediococcus acidilactici*, *P. pentosaceus*,

Lactobacillus curvatus, and *L. sakei*, which are often accompanied with nitrate-reducing bacteria such as *Staphylococcus xylosus* and *S. carnosus* to aid in color development. If a starter culture is not used, as in some regions, particularly in Europe (Comi et al. 2005; Holley et al. 1988), then the method of backslopping is used, which was also common in the USA until the 1960s and 1970s. Backslopping relies on the natural microflora of the meat from prior production and the processing environment to seed the blend with the appropriate lactic acid-producing bacteria. After stuffing the mixture into casings, the incubation proceeds in smokehouses or ripening rooms at moderate to warm temperatures, depending on the preference of the starter cultures. Typically, in the USA, rapid fermentation at higher temperatures of 32–46 °C (90–115 °F) is employed in industrial production of sausage and pepperoni to quickly lower the pH to 5.3 or below in a certain number of degree-hours so that the next step can begin. Degree-hours are a calculation used to define the time and temperature limits that meat may spend within the growth temperature range of *S. aureus* during fermentation in order to ensure the absence of growth in excess of 3 logs, which may be accompanied by enterotoxin formation. A process more commonly used in Europe than in the USA is low-temperature "ripening" at 26 °C (78 °F) with 88 % relative humidity for 3 days until an end point pH of 4.7–4.8 is attained.

Drying procedures often occur in separate chambers or rooms than those used for fermentation. Fermented sausages that are dried to below a_w 0.90 are considered "dry" (Hammes et al. 2003; Lücke 2000). These products are fermented or acidified prior to undergoing a drying or aging process, which usually occurs at 12–15 °C (Lücke 2000). a_w is considered the most important criterion for the classification of fermented sausages because it determines market value as well as microbial stability (Lücke 2000). Dry sausages contain 35 % moisture or less, corresponding to a weight loss of 25 % or more, and a_w of <0.90 and may be stored at ambient temperature (e.g., 20–25 °C). Semidry sausages are considered to be within the a_w range of 0.90–0.95, and these should be stored below 15 °C (Lücke 2000). Semidry sausages are dried to a moisture content of about 40 %, corresponding to a weight loss of about 18 % and a_w of about 0.93. The compositional characteristics of dry sausage produced in the USA, such as moisture/protein ratios (MPR) and pH limits, as well as acceptable ingredients, are defined by the USDA/FSIS in the Food Standards and Labeling Policy Book (U.S. Department of Agriculture-Food Safety and Inspection Service 2005). Non-refrigerated, semidry, shelf-stable sausage must have an MPR of 3.1:1 or less and a pH of 5.0 or less, unless it is commercially sterilized or unless it is specified otherwise. Alternatively, non-refrigerated, semidry, shelf-stable sausages must be fermented to a pH of 4.5 or lower or a combination of pH 4.6 and a_w no higher than 0.91. Whereas semidry sausages tend to have ca. 50 % moisture and a_w of 0.90–0.94, dry sausages such as hard salami tend to have ca. 30 % moisture and a_w of 0.91 or less. Reduced fat versions of dry salamis may also reach ca. 50 % moisture and a_w of 0.93, and these are distributed and displayed at refrigeration temperatures.

The USDA/FSIS published a proposed rule "Performance Standards for the Production of Processed Meat and Poultry Products" (US Department of Agriculture-Food Safety and Inspection Service 2001) which states that for beef-based RTE fermented products, the process is required to provide 5-$\log_{10}$ reductions of *E. coli*

O157:H7. Also, the agency requires the use of a validated manufacturing process for RTE dry and semidry fermented acidified sausages. USDA/FSIS clarified the definition of a "validated manufacturing process" as a process that:

- Applies one of the heat treatments prescribed in regulation 9 CFR 318.17 or 9 CFR 318.23.
- Applies a 5-D process (5-log reduction in pathogenic populations).
- Includes checking of the product for *E. coli* O157:H7 (30 samples per lot with zero positives).
- Uses raw ingredients prepared under hazard analysis and critical control point (HACCP) principles that verify less than or equal to 1 *E. coli* O157:H7/125 g (95 % confidence level) and applies a validated 2-D process.
- Uses other methods that would ensure equivalent safety.

The Canadian Food Inspection Agency (CFIA) has similar process requirements for fermented beef-containing products, which were developed in response to an outbreak of *E. coli* O157:H7 infection from the consumption of fermented sausage in Canada (Canadian Food Inspection Agency 2006).

After the 1994 US multistate outbreak of illnesses (20 confirmed human cases) caused by *E. coli* O157:H7 in dry-cured salami (Centers for Disease Control and Prevention 1995a), USDA/FSIS met regularly with scientists from the USDA Agricultural Research Service (ARS), representatives of the meat and poultry industry, and members of the National Advisory Committee on Microbiological Criteria for Foods to develop a policy for ensuring the safety of shelf-stable, RTE fermented sausages. The National Cattlemen's Beef Association (NCBA) convened a "Blue Ribbon Task Force" to address the issue via research and awarded a research project to the University of Wisconsin's Food Research Institute (FRI) (Nickelson et al. 1996). This research group, along with inputs from industry scientists, developed several processing options that would ensure a 5-log relative reduction of *E. coli* O157:H7 in fermented sausages. The USDA/FSIS approved a processing option developed by the Blue Ribbon Task Force on *E. coli* O157:H7 of the NCBA. The Blue Ribbon Task Force addressed Option 2—a validated 5-log inactivation treatment—and focused on the processing parameters of heat and acid sensitivity of the pathogen.

Dry and semidry meat products are traditionally preserved by the addition of NaCl and reliance on inoculated or native populations of lactic acid bacteria to ferment the available carbohydrate, thereby lowering the pH. Due to efforts to reduce the sodium intake in diets, sodium reduction research has been conducted in sausage products. The survival of lactic acid bacteria and native *Micrococcaceae* during fermentation of reduced-sodium Spanish dry sausages (chorizo de Pamplona) was not significantly different than full-sodium control products (Gimeno et al. 1999). The normal sausage formulation contained 2.6 % NaCl, whereas the experimental formulation was comprised of 1.0 % NaCl, 0.55 % KCl, and 0.74 % $CaCl_2$. These findings were in agreement with others who also determined that partial Na^+ replacement in fermented sausages could result in similar *Enterobacteriaceae* and lactobacilli levels during ripening, compared to the normal formulation (Ibanez et al. 1995).

Separately, it was reported that partial substitution of NaCl by KCl, potassium lactate (K-lactate), and glycine had little effect on the microbiological stability of fermented sausages (Gelabert et al. 2003). However, flavor and/or textural defects were detected by sensory analysis with substitution levels of 40 % by KCl, 30 % with K-lactate, and 20 % with glycine. The partial substitution (above 40 %) of NaCl with different mixtures of KCl/glycine and K-lactate/glycine resulted in important flavor and textural defects, which did not permit an increase in the level of substitution compared to those obtained with the individual components.

Studies using microbiological broth revealed that low to medium NaCl concentrations (2.5–4.5 % w/v) provided a protective effect against inhibition of *L. monocytogenes* by 50–100 IU/ml nisin (Boziaris and Nychas 2006). Additional broth studies were undertaken to determine the effects of Na^+ replacement and nisin presence on *L. monocytogenes* in fermented sausages (Boziaris et al. 2007). At pH 4.5, no growth was observed while in the presence of 50 IU/ml nisin and/or 1 mol/l salts of both NaCl and KCl, and *L. monocytogenes* Scott A was inactivated by >5 log. Equal molar concentrate ions of NaCl or KCl at similar a_w exerted similar effects against *L. monocytogenes* in terms of lag-phase duration and growth or death rate. Levels of *L. monocytogenes* inoculated on dry salami (presumably with $a_w < 0.92$) declined by more than 3 logs by 40 days, regardless of air or vacuum packaging, refrigerated or ambient storage, or inoculation levels (Gounadaki et al. 2007).

The importance of the maturation stage of fermented sausage was highlighted when levels of *E. coli*, *L. monocytogenes*, and *Yersinia enterocolitica* were not reduced to acceptable levels with during the maturation processes (Lindqvist and Lindblad 2009). At 8 °C, the time required for a 1-log reduction in sausage ranged from 21 days for *E. coli*, >16 days for *L. monocytogenes*, to 18 days for *Y. enterocolitica*, but maturation at 20–22 °C induced reductions of these bacteria within 7–11 days, 4–7 days, and 1–4 days, respectively. Ultimately, there were ca. 4-log reductions of these bacteria at 20–22 °C by about 3 weeks of maturation and storage as compared to only marginal reductions at 8 °C. The a_w at 20–22 °C decreased from 0.98 to 0.80 and a moisture content of 24 % within 19.7 days, while during the same amount of time at 8 °C, the a_w decreased only to 0.93 with water content of 41 %, before finally reaching a_w 0.81 and 32 % moisture content at 27.7 days. Reductions of *Salmonella* and *L. monocytogenes* during fermentation and drying of Italian-style salami were not significantly different when salami was dried to an MPR of 1.4:1 compared to when dried to an MPR of 1.9:1 (Nightingale et al. 2006). When enumerated directly on selective media, *Salmonella* populations were reduced from 1.6 to 2.4 log CFU/g and from 3.6 to 4.5 log CFU/g for fermentation alone and fermentation followed by drying, respectively. *L. monocytogenes* populations were only reduced by <1.0 log CFU/g following all fermentation and combined fermentation-drying treatments.

Mathematical models have been constructed to predict the inactivation of foodborne bacterial pathogens, including *E. coli* O157:H7, *Salmonella*, and *L. monocytogenes*, in fermented and dried sausages, including soudjouk-style sausages (Hwang et al. 2009), chorizos (Hajmeer et al. 2006a, b), and other fermented low-a_w sausages (Naim et al. 2003). These models suggest that pH and a_w are the most commonly used factors associated with reductions of pathogens during fermentation, drying, and storage.

Dried Meat and Poultry Powders

Examples of such products include collagen extracts and bouillon, spray-dried chicken fat, and powdered, cooked turkey. Collagen is used as an ingredient in food products for thickening and stabilization. Production of collagen from beef hides involves first the acidification of hides, followed by grinding into a paste. The paste is transferred to heated rollers simultaneously exposing it to lethal heating to destroy vegetative bacterial cells such as *Salmonella* and *E. coli* O157:H7 and drying the collagen into flakes or powder form. The finished product is a hygroscopic powdered material that is sifted and portioned into bags. Like many dry food ingredients, the accumulation of dust from the finished product into the environment and the survivability of *Salmonella* under dry conditions create the potential for post-processing recontamination. Proper facility design and upkeep, worker and equipment traffic patterns, process flow, and dry sanitation procedures can greatly reduce the risk of *Salmonella* in such environments. However, the presence of *Salmonella* and the risk to contaminate the product are increased by substandard plant design, contamination of adjacent environments, equipment and employees, and the use of wet sanitation. In its hydrolyzed form, collagen is known as gelatin, and they both are components of meat extracts, concentrates, soup stocks, and flavorings.

Meat extracts are derived from high-heat (130–140 °C) liquid extraction of beef muscle, liver, or bones followed by vacuum concentration to a pasty solid of about 20 % moisture (Lund et al. 2000; Ockerman and Pellegrino 1988). Meat extracts may include 12–20 % added salt and have a_w <0.80. Meat extracts are commonly used as flavoring ingredients in bouillon cubes, broth, and gravies (Aberle et al. 2001). The composition of meat extracts is usually 18–20 % moisture, 56–60 % protein, 9–25 % ash, 1–2 % water-soluble materials, and 1–2 % fat. The low-moisture content allows meat extracts to be microbiologically stable. Thermophilic or thermoduric bacteria may survive the process but are not expected to grow during storage; however, they may serve as inocula to other foods of higher moisture when extracts are used in formulations. When diluted in water, meat extracts become excellent media for microbial growth. No growth or toxin production should occur when the a_w is maintained at <0.80.

Illnesses and Microbial Contamination of Low-a_w Meats

Summaries of Illness Events and Outbreaks

Even though dried meat products are considered shelf stable, they may be vehicles of foodborne pathogens for human illness, especially when growth is not necessary for infection. Several outbreaks and illnesses caused by biological hazards such as *E. coli* O157:H7, *Salmonella*, *L. monocytogenes*, *S. aureus*, and *Clostridium botulinum* have been attributed to the consumption of low-a_w fermented meats, such as Genoa salami, dry-cured salami, and salami sticks (Hartog et al. 1987;

Moore 2004). In 1966, in California, a type F botulism outbreak was associated with the consumption of homemade venison jerky (Centers for Disease Control and Prevention 1966). In addition, a small outbreak of *E. coli* O157:H7 hemorrhagic colitis was attributed to the consumption of a pork meat-based, dry salami product (Conedera et al. 2007).

In New Mexico, the consumption of whole-muscle meat jerky led to a series of outbreaks of gastroenteritis between 1966 and 1995 (Centers for Disease Control and Prevention 1995b; Eidson et al. 2000). Six of these outbreaks were associated with contamination with *Salmonella* and two were attributed to *S. aureus*. As a result of these outbreak investigations, the New Mexico Environment Department developed regulations for jerky processing in 1989, which included cooking so that meat jerky reaches an internal temperature of 63 °C within 3 h and poultry jerky reaches 74 °C within 3 h. The regulations stated that finished a_w should be ≤0.85. In 2003, the issue reemerged in New Mexico with at least 22 cases of salmonellosis being attributed to commercially produced beef jerky (ProMED-mail 2003). Although the jerky was dried to $a_w < 0.3$, it was suspected that the very slow drying process conditions under low humidity (1 % RH—82 °C oven dry bulb, 30 °C wet bulb) allowed *Salmonella* to dehydrate during heating and become more resistant to the dry heat.

Homemade venison jerky was the vehicle of an outbreak of at least 11 *E. coli* O157:H7 illnesses in Oregon in 1995 (Keene et al. 1997). Investigators were able to detect the pathogen on deer jerky, deer meat, and the deer carcass, as well as nine percent of sampled deer fecal pellets obtained from the area where the deer was harvested. The venison jerky was dried in a home-style dehydrator set at between 51.7 and 57.2 °C for 12–14 h. Laboratory studies revealed survival of *E. coli* O157:H7 on inoculated jerky strips dried at up to 62.8 °C. Precooking of deer meat to 74 °C prior to jerky processing was recommended as a result of these findings.

Microbial Contamination

Enterohemorrhagic *Escherichia coli*

Enterohemorrhagic *E. coli* are associated with cattle, which carry these microbes asymptomatically, and it is therefore associated with raw beef materials that are used to produce low-a_w meats or are used as ingredients in comminuted low-a_w meats. The pathogen is present at lesser frequencies in pork, deer, sheep, and poultry than in beef. *E. coli* O157:H7 is distinguished by its acid tolerance, as are some of the non-O157 Shiga toxin-producing *E. coli* (STEC). STECs were equally or less resistant than *E. coli* O157:H7 to the processing steps involved in pepperoni production (Glass et al. 2012). Nonacid-resistant STEC strains did not survive a French-style sausage fermentation and drying (13–14 °C up to 60 days) process, but acid-resistant STECs, although reduced to low levels, were still detectable after 60 days (Montet et al. 2009). STEC with and without acid resistance gradually but continuously declined by ca. 3 log in fermented sausage during a 30-day drying phase as a_w decreased from 0.95 to 0.89.

Unheated cells and heat-stressed cells of *E. coli* O157:H7 inoculated on salami slices adjusted to a_w 0.90 decreased more rapidly during storage at refrigeration and ambient temperature than cells on samples adjusted to a_w 0.95 (Clavero and Beuchat 1996). This effect was more apparent for heat-stressed cells than unheated cells. Increasing NaCl concentrations from 0.5 to 8.5 % increased the $D_{64.5\,°C}$ values for *E. coli* O157:H7 at pH 4.5 (Blackburn et al. 1997). The effect of the type of starter culture and pH may have an impact on the reduction of *E. coli* O157:H7 during fermentation and during drying of dry-cured salami (Lahti et al. 2001).

The impact of dry yellow mustard supplementation of fermented sausage on the survival of *E. coli* O157:H7 was studied by Graumann and Holley (2008). Deheated mustard, mustard in which myrosinase is inactivated, is commonly used in fermented sausage as filler without imparting strong characteristic mustard flavor. Control and mustard-supplemented sausage had similar a_w during the 4-day fermentation step and the subsequent 44-day drying step; generally, a_w declined from about 0.95 at day 0–0.89 by day 48. Non-deheated mustard powder at 2, 4, and 6 % in dry sausage resulted in reductions of *E. coli* O157:H7 of 3.4, 4.4, and 6.9 log CFU/g, respectively, within 30 days of drying. In the US meat industry, 1.6 % non-deheated mustard is considered unacceptably high due to flavor. A related study measured the combined effects of a_w level, added allyl isothiocyanate (AIT), and pH on reduction of *E. coli* O157:H7 during fermentation and drying of sausage (Chacon et al. 2006). Fermentation to pH 4.93 and drying to a_w 0.87 in 40 days provided a 2.43-log reduction, whereas the addition of 750 and 1,000 ppm AIT provided a 6.5-log reduction in sausages after 21 and 16 days of processing, respectively. *E. coli* O157:H7 cell numbers were reduced by 4.75 log CFU/g after 28 days of processing in treatments with 500 ppm AIT, and the pathogen was not recovered from this treated sausage beyond 40 days. The sensory characteristics of only 500-ppm AIT samples were deemed acceptable by panelists.

E. coli O157:H7 was surface-inoculated and inoculated internally into Westphalian ham during needle tenderization. While the pathogen applied on the surface died during the 112-day ripening period, inclusive of 79 days of drying, the pathogen inoculated internally survived and was recovered at 3.1 log CFU/g after 112 days of ripening (Graumann and Holley 2007).

Salmonella

Cooking beef may cause drying of the surface, thereby enhancing survival of *Salmonella* on the surface, although lethality is achieved in the center of the meat (Blankenship 1978; Blankenship et al. 1980; Goodfellow and Brown 1978; Shigehisa et al. 1985). It was determined that steam applied as a part of a cook process can substantially enhance *Salmonella* inactivation on the surface of beef by increasing the humidity (Blankenship et al. 1980; Goodfellow and Brown 1978). These findings have raised concern over the estimation of *Salmonella* lethality on the surface of any exposed meat product using compliance guidelines from USDA/FSIS Appendix A or from the American Meat Institute (AMI) Process Lethality

Determination Spreadsheet. Estimates of lethality for both guidelines were derived from *D*-values of *Salmonella* under moist conditions. *D*-values at various temperatures for *Salmonella* in low-a_w environments have been summarized (Doyle and Mazzotta 2000), and, in general, *Salmonella* cells tend to be more resistant at a low a_w than at a higher a_w.

The resilience of salmonellae at low and intermediate a_w has been reviewed (Podolak et al. 2010). Survival of the pathogen varies depending on the food matrix and medium composition. Over 14 weeks at 21 °C, *Salmonella* survived better on low-a_w food products (0.40 and 0.52) compared to intermediate-moisture food products with an a_w of 0.75 (Juven et al. 1984). Survival of acid-adapted and unadapted *Salmonella* inoculated on whole-muscle beef jerky after drying was studied. An approximately 5-log reduction in 14–60 days of storage was achieved if jerky products were marinated with a marinade (pH 3.0) modified with acid and ethanol, subjected to a two-step process involving dipping into household vinegar (5 % acetic acid) followed by marination with a traditional marinade, or subjected to a three-step process involving sequential dipping into 1 % Tween-20 solution and 5 % acetic acid followed by marination with a traditional marinade (Calicioglu et al. 2003a, b, c). Treatments were more lethal to acid-adapted inocula than non-adapted. The a_w of jerky produced using the different process variables ranged from a_w 0.595 to 0.688.

Listeria monocytogenes

While *L. monocytogenes* is capable of surviving and establishing niches in processing environments, generally, the pathogen does not grow or survive well on low-a_w meats. The pathogen is present in dry sausage processing environments (Thevenot et al. 2005) and with some frequency in dry sausages (De Cesare et al. 2007), but growth is not often possible due to low pH and a_w. Most studies done on low-a_w meats inoculated with *L. monocytogenes* reveal a more rapid rate of decline as storage temperature increases from 4 to 25 °C. Sometimes studies are necessary to validate lack of growth or even death. In order to perform such studies, it is important that challenge study inocula are prepared in such a way as to avoid excessive osmotic shock at the point of inoculation, such as by a nutritionally depleted, dustlike vector (De Roin et al. 2003). Acid adaptation might also be necessary prior to inoculation into acidified or fermented, low-a_w meat.

Storage after processing of dried and fermented meat products is known to result in reduction of bacterial pathogen populations (Byelashov et al. 2009; Simpson et al. 2008). Death rates (log CFU/cm^2/day) of *L. monocytogenes* inoculated on slices of commercial salami increased as storage temperature increased, with the exception of the acid-adapted cells, which decreased more rapidly at 4 °C than at 12 or 25 °C. Overall, the results revealed that risk of listeriosis associated with commercially produced salami, and probably other dry-fermented sausages, may be significantly reduced by simply holding the final product prior to distribution or use (Simpson et al. 2008). In a related study, Byelashov et al. (2009) evaluated the fate of the same pathogen on sliced inoculated pepperoni during vacuum-packaged storage

and potential differences in survival among three types of inocula, including nonacid-adapted, acid-adapted, and pepperoni extract-habituated cultures. Commercial pepperoni slices were inoculated at 3–4 log CFU/cm^2, before vacuum-packaging and storage for up to 180 days at 4, 12, or 25 °C. The pathogen died slower at 4 than at 12 or 25 °C, but at 12 and 25 °C, the death rates were similar. Death rates of nonacid-adapted inoculum were generally greater than acid-adapted and extract-habituated inocula. Death rates of extract-habituated inocula were generally slightly greater than acid-adapted inocula. At 60 days, pathogen levels were below the detection limit, regardless of inoculum type and storage temperature. It was concluded that storage of pepperoni before distribution or retail sale, especially at ambient temperature, might substantially reduce or eliminate *L. monocytogenes* from the product.

In dry-fermented sausages, the influence of acid- and bacteriocin-producing strains of lactic acid bacteria starter culture, such as *Lactobacillus plantarum* (Tolvanen et al. 2008) and *Lactobacillus sakei* (Ravyts et al. 2008), often makes it difficult to distinguish the effects of drying, alone, on the reductions of *L. monocytogenes*. During fermentation and drying, the effects of bacteriocins and decreasing pH are the principal reasons for the reduction of *L. monocytogenes* (Tyopponen nee Erkkila et al. 2003), with low a_w having an effect during the drying phase.

Ingham et al. (2004) studied the behavior and survival of *L. monocytogenes* on several low-a_w meat products ranging from pork rinds and cracklings at a_w 0.27 to higher a_w products such as smoked, cured beef at a_w 0.96–0.98. Products such as pork rind (a_w 0.27–0.29), cracklings (a_w 0.28), and beef jerky (a_w 0.75) were considered least likely to support the growth of *L. monocytogenes*, with the pathogen decreasing by 1.2–2.1 log CFU/piece in the first week of storage at 21 °C.

Staphylococcus aureus

Due to its relative salt tolerance, compared to other foodborne bacterial pathogens, *S. aureus* is a particular concern in low-a_w meat products. *S. aureus* in low-a_w meats must be controlled by intrinsic factors such as pH or competing microflora or extrinsic factors such as reduced oxygen atmosphere packaging or refrigeration.

Counts of *S. aureus* declined throughout during the salting phase (ca. 0.5-log decrease) and during the sun-drying phase (ca. 2-log decrease) of production of charqui as a_w decreased from 0.821 to 0.752 (Lara et al. 2003). In mutton cubes with an a_w of 0.85, *S. aureus* counts decreased within a few months of storage at ambient temperatures (Chawla and Chander 2004; Kanatt et al. 2002). Reductions of about 3–5 log of inoculated *S. aureus* were observed on jerky (a_w 0.68–0.82), beef snack sticks (a_w 0.85–0.88), pepperoni (a_w 0.76–0.88), and dry salami (a_w 0.76–0.92) stored under vacuum at 21 °C for 28 days (Ingham et al. 2005). A lack of increase of *S. aureus* counts in Serrano dry-cured ham slices packaged under vacuum or modified atmosphere during 8 weeks of storage at 4 °C was attributed to the a_w being below 0.80 (García-Esteban et al. 2004). The production of enterotoxin in dry-cured ham by *S. aureus* growth appears to be a_w and temperature dependent

(Untermann and Müller 1992). Enterotoxin was produced in ham at a_w 0.95, but not when ham was matured at a_w 0.92 and a temperature of 20 °C within 7 days. When held at 35 °C for 7 days, enterotoxin was produced at an a_w of 0.89.

Clostridium botulinum and *C. perfringens*

Clostridial species are ubiquitous and their spores will survive the salting, drying, marination, or cooking used to produce low-a_w meats. However, germination and outgrowth of spores is restricted by the low a_w, and, therefore, such products pose little risk of becoming vehicles of *C. botulinum*- or *C. perfringens*-related illness.

Molds and Mycotoxins

Low a_w (<0.9) and low pH values (<6.0) are particularly favorable for mold proliferation (Hanssen 1995). Molds compete better with bacteria on meat when storage temperatures are lowered to 0 °C or below or when the meat is dried to an a_w that inhibits bacteria and allows fungi to grow (Hernandez and Huerta 1993; Pitt and Hocking 2009). The dominant mold genera of cured meats are *Penicillium* and *Aspergillus*. Mold growth on dry-cured meat products can be beneficial for several reasons: antioxidative effects that contribute to color and flavor stability, preventing surface sliminess, and development of characteristic flavor due to decomposition of lipids and proteins (Rodríguez et al. 1998; Tabuc et al. 2004). The time and temperature of ripening periods for dry sausages and dry-cured meats are optimal for mold development, with *Penicillium*, *Aspergillus*, and *Cladosporium* being three of the main genera identified in such products. The molds present in such products are responsible for substantial contributions to flavor, due to proteolytic and lipolytic enzymes (Galvalisi et al. 2012; Rodríguez et al. 1998). *Penicillium nalgiovense* is commonly isolated from low-a_w salamis and other dry-fermented sausages (Andersen 1995; Asefa et al. 2009a, b; Galvalisi et al. 2012; Iacumin et al. 2009; Sonjak et al. 2010) where it is sometimes used as a starter culture. The species is also routinely part of the mycoflora of whole-muscle, dry-cured pork products and dried lamb legs (Asefa et al. 2009a, b). *Penicillium chrysogenum* has a major influence on the proteolysis of dry-cured Iberian ham during ripening (Rodríguez et al. 1998). Selected strains of *P. chrysogenum* and *D. hansenii* on dry-cured ham produced higher levels of long-chain aliphatic and branched hydrocarbons, furanones, long-chain carboxylic acids and esters, and lower levels of short-chain hydrocarbons than produced by wild fungal populations (Martín et al. 2006).

Aspergillus ochraceus, *Penicillium nordicum*, and *Penicillium verrucosum* are some of the molds of concern, because they are capable of producing ochratoxin A (OTA) (Iacumin et al. 2009; Sonjak et al. 2011). *Penicillium nordicum* is the most important OTA-producing species isolated from dry-cured meat. It has been shown to heavily contaminate dry-cured-meat-processing environments in Slovenia

(Sonjak et al. 2010). In a survey of fermented, nonheat-treated meats from the Czech Republic, *Penicillium* spp., *Acremonium* spp., *Cladosporium* spp., and *Aspergillus* spp. were most frequently isolated (Mižáková et al. 2002). Spices, principally milled black pepper, garlic powder, nutmeg, and crushed caraway, were heavily contaminated and considered a major source for mold contamination of meat. Sea salt used in the production of dry-cured meat was proposed as a contamination source of OTA-producing *P. nordium* (Sosa et al. 2002). *Eurotium* spp., *Aspergillus* spp., and *Penicillium* spp. were the main strains isolated during the preripening and ripening period of Istrian hams (Comi et al. 2004), none of which is a mycotoxin producer. *P. nordicum* was the most frequently isolated mold species from dry-cured pork neck in casing, smoked dry-cured ham, and a dry salami product produced in a Slovenian meat-processing plant (Sonjak et al. 2010). Mold such as *Eurotium*, *Aspergillus*, and *Penicillium* and yeast such as *Debaryomyces hansenii* and *Yarrowia lipolytica* can be frequently detected in dried beef jerky and African biltong (i.e., dried meat strips) (Pitt and Hocking 2009).

In terms of potential for production of mycotoxin in meat and meat products, the presence of oxygen, temperature between 4 and 40 °C, pH value between 2.5 and 8 (optimally between 5 and 8), minimum a_w of 0.80, and maximum salt concentration of 14 % has been proposed (Iacumin et al. 2009; Mižáková et al. 2002). Mycotoxigenic molds can produce mycotoxins on the sausage casings but not inside the meat due to lack of oxygen below the surface (Moss 2000). Several yeast isolates from dry-cured ham can be antagonistic against *P. nordicum* growth and OTA production. Particularly, *Hyphopichia burtonii* and *Candida zeylanoides* exhibited the greatest inhibitory activity against *P. nordicum* and salt enhanced this effect (Virgili et al. 2012). Tabuc et al. (2004) isolated several molds from dry-cured hams and sausages purchased in Southwestern France, several of which were known mycotoxin producers. However, citrinin, ochratoxin A, and cyclopiazonic acid were not assayed in the commercial meat products. In culture media, only *P. cyclopium* and *P. viridicatum* produced mycotoxins, with low levels of ochratoxin A and cyclopiazonic acid, respectively. Bailly et al. (2005) have observed mycotoxin production in dry-cured ham when inoculated with 100 μl of a 10^6 mold spore suspension. *Penicillium citrinum* produced citrinin on dry-cured ham at 86.9 mg/kg after 16 days at 20 °C. *P. viridicatum* produced 50 mg/kg of cyclopiazonic acid on dry-cured ham after 16 days; however, no mycotoxin was produced on samples held at 4 °C. When artificially introduced, cyclopiazonic acid remained stable in dry-cured ham during refrigerated and ambient storage, with roughly 80 % being detectable after 8 days. The *A. ochraceus* strain used did not produce detectable OTA on dry-cured ham, but did produce it on a culture medium. When OTA, citrinin, and patulin were introduced into dry-cured ham, the compounds were not as stable as cyclopiazonic acid. Inoculated *P. polonicum* grew but did not produce the potent neurotoxin, verrucosidin, on dry-cured ham at a_w 0.95 (Núñez et al. 2000).

Ochratoxin A (OTA) is a particularly potent mycotoxin with nephrotoxic, nephrocarcinogenic, teratogenic, neurotoxic, and immunotoxic effects on animals (Cabañes et al. 2010; EFSA 2006). OTA-producing fungi are members of the genera

Aspergillus and *Penicillium* and there are an estimated 20 species known to produce OTA (Cabañes et al. 2010). OTA contamination of meat and poultry products can be due to carryover from animals exposed to naturally contaminated feed or direct contamination with molds (Gareis 1996). Much research has been published on the potential for OTA (and other mycotoxin) production in dry-cured meats at various points during production. Some researchers have even published a HACCP plan for mycotoxigenic fungal hazards in dry-cured meats (Asefa et al. 2011.). The moist, humid conditions of the early stages of preparation of some dry-cured products and the temperature during the drying over long periods of time with high initial humidity render the processing environment and the product good growth conditions for mold (Asefa et al. 2010). Some researchers report actual OTA detected in commercially produced, dry-cured meat products or in meat test substrates. Aflatoxigenic strains of *Aspergillus* spp. were detected in 17.8 % of 420 samples of various smoke-dried meat products in Croatia (Cvetnic and Pepeljnjak 1995). All 75 isolates produced aflatoxin in moist shredded wheat, but aflatoxin formation did not occur in a meat matrix. Several molds were isolated from the Egyptian basterma, including *Aspergillus* and *Penicillium*, and total aflatoxins were detected at 2.8–47 μg/kg (Refai et al. 2003). However, aflatoxins were also found in pepper, garlic, and fenugreek at nearly 100 times higher levels than in basterma, suggesting that mycotoxin development was not necessarily attributable to mold growth during basterma production or storage but was already present in dry ingredients. OTA production is drastically reduced at below 18 °C or by NaCl levels of 10 % or more (Battilani et al. 2010). In a model system, a_w had a profound effect on the growth of *P. nordicum* and OTA production (Battilani et al. 2010). However, it was determined that the toxigenic potential of *P. nordicum* was very low in aged, dry-cured meat products due to surface layers being generally below an a_w of 0.90. Dry-cured ham slices with low carbohydrate levels inoculated with 20 terverticillate *Penicillium* subgenus *Penicillium* isolates and incubated at 25 °C for 20 days supported the synthesis of small amounts of secondary metabolites such as cyclopiazonic acid and rugulovasin A (*P. commune*), arisugacin (*P. echinulatum*), puberulin and anacine (*P. polonicum*), and cyclopeptin and cyclopenol (*P. solitum*) (Núñez et al. 2007). Secondary metabolite levels were considerably less in dry-cured ham compared to those in agar media and other carbohydrate-rich substrates.

Data collected from 160 samples of artisanal and industrial-fermented sausages revealed that levels of OTA detected by ELISA were not significantly different than that using a HPLC method (Iacumin et al. 2009). Forty-five percent of the samples were positive for OTA on the casing, with levels between 3 and 18 μg/kg reported. The authors reported that brushing or washing to remove visible mold was effective at reducing OTA levels and that OTA was not detected inside the sausages. Sun-dried beef liver, kidney, and heart (presumably of low a_w) obtained from markets from the northern and eastern parts of Nigeria were contaminated with various molds, including the mycotoxin producers *Aspergillus* and *Penicillium* (Oyero and Oyefolu 2010). However, aflatoxin B and G levels in raw and sun-dried meat were not very different, suggesting that animals were exposed via feed rather than molds growing during the sun-drying process.

Parasitic Pathogens

Because many dried meat products, particularly dry-cured products, are produced without the use of lethal heating (i.e., cooking), survival and transmission of parasitic pathogens to humans have been a concern. The combination of high salt and mild heating has been utilized as a means to kill parasites that may be present such as *T. spiralis* in pork and *Trichinella nativa* in seals (Forbes et al. 2003). US regulations contain certain combinations prescribed to kill *T. spiralis* in the production of dry-cured pork products in 21 CFR 318.10. Finisher pigs, sows, sheep, goats, and other meats such as kangaroo impose a medium to high relative risk of carrying *Toxoplasma gondii*, whereas beef has a very low prevalence (Mie et al. 2008). Heat treatments that are effective against encysted *T. spiralis* are also effective against *T. gondii* tissue cysts (Dubey et al. 1990). Tissue cysts of *T. gondii* remained viable in pork heated at 52 °C for 9.5 min, but were inactivated when heated to 58 °C and held for 9.5 min or heated to 61 °C for 3.6 min. Freezing raw meat prior to processing reduces the risk of transmission of *T. gondii* (Mie et al. 2008). The processing conditions for uncooked comminuted salami containing beef and sow meat were determined to have a medium risk, despite water loss of 40 % during the process, and a reduced-salt version of that product would likely increase the risk. A 6-month prosciutto curing process at 10 °C was considered low risk if salting exposed raw meat to at least 6 % NaCl for at least 3 days or 3.3 % salt for at least 28 days.

Yeasts

Yeasts, such as *D. hansenii*, *Candida saitoana*, *C. zeylanoides*, and *C. famata*, occur primarily on the surfaces of ripening hams and can cause rancidity as lipolytic activity ensues (Pitt and Hocking 2009). Other yeasts, such as *Hansenula* spp. (now *Pichia*), *Geotrichum candidum* (a yeast-like mold), *Rhodotorula glutinis*, *Cryptococcus albidus*, and *Trichosporon* spp., can also be found on hams. Samples of biltong, dry sausage, salami, and cabanossi with a_w of 0.72, 0.80, 0.87, and 0.88, respectively, were determined to contain *D. hansenii*, *Cryptococcus laurentii*, *C. hungaricus*, *Torulaspora delbrueckii*, *Rhodotorula mucilaginosa*, *Sporobolomyces roseus*, *D. vanriji*, *Trichosporon beigelii*, *Yarrowia lipolytica*, *Saccharomyces cerevisiae*, and *Candida zeylanoides* (Wolter et al. 2000).

Spoilage

Given that bacteria are inhibited in low-a_w meat products, yeasts and molds are typically associated with spoilage. Yeasts and molds can cause spoilage of dry-cured ham products and sausages, especially during maturation (Pitt and Hocking 2009). The most common molds on raw hams during ripening are *Aspergillus* and *Penicillium* (Pitt and Hocking 2009). *P. nordium* can grow and produce ochratoxin A on prosciutto ham if there are air gaps in the center of the ham. Also *P. commune*

growth during ham ripening can cause the "phenol defect," characterized by a phenol-like odor. At an a_w less than 0.85, *Eurotium* and *Aspergillus* are more dominant on meats and are the most common spoilage fungi on dried beef jerky and kangaroo biltong (Pitt and Hocking 2009).

Interventions Against Pathogens in Low-a_w Jerky

Jerky, one of the most common dried, ready-to-eat meat products, has been historically considered a safe product. As indicated, however, in recent years, jerky consumption has been associated with several foodborne disease outbreaks (Centers for Disease Control and Prevention 1995b; Eidson et al. 2000; Gailani et al. 1986; Keene et al. 1997), and questions have been raised about its microbial safety. The USDA/FSIS reported cumulative prevalence rates of 0.31 and 0.52 % for *Salmonella* and *Listeria monocytogenes,* respectively, in jerky produced in federally inspected plants for the period of 1990–1999 (Levine et al. 2001). It appears that some traditional, especially home-drying, practices may not be sufficient to kill *E. coli* O157:H7 in jerky.

Drying in home-type dehydrators for 4 h at 52.9 °C, followed by 4 h at 48.2 °C, reduced initial counts (4–5 log CFU/g) of *Salmonella*, *Staphylococcus aureus*, *Clostridium perfringens*, and *Bacillus subtilis* on jerky by 2–4 log CFU/g (Holley 1985b). Survivors were present during high-relative humidity storage at 20 °C for up to 28 days or for a longer period at lower relative humidity and 2.5 °C. Another study (Holley 1985a) revealed that drying beef inoculated with *S. aureus* at 68.3 °C for 4 h, followed by 4 h at 60 °C, resulted in survival of 15 % of the initial bacterial population, which was reduced to 5 % after a week of storage at 25 °C. Based on these studies, it was suggested that homemade jerky could be assured as stable if initial drying is rapid and at 53 °C or higher, preferably 55–60 °C.

While in previous centuries, jerky-type products were produced by smoking and sun-drying of meat slices, in recent years, there are numerous procedures available to manufacture jerky commercially or in the home. Jerky production may vary by type of meat used (e.g., beef, poultry, or game meat), method of meat preparation (e.g., thick or thin slices or ground beef), marinating formulation and procedure (e.g., ingredients, amount, temperature, and time) and drying method (e.g., smokehouse, oven, food dehydrator, and temperature), and manufacturer preferences.

Food safety concerns for jerky in the 1990s led to various studies conducted to evaluate pathogen inactivation during jerky processing. Since drying at temperatures up to 62.8 °C was not effective to sufficiently reduce *E. coli* O157:H7 in marinated (pH 4.2) whole-muscle venison jerky, it was recommended to precook meat to 74 °C before drying (Keene et al. 1997). The New Mexico Department of the Environment guidelines, developed following such outbreaks, for the production of jerky, require cooking and drying of jerky within 3 h to an internal temperature of 63 °C for beef, lamb, and fish and 74 °C for poultry and a final a_w of 0.85 (Eidson et al. 2000).

Harrison and Harrison (1996) reported that drying at 60 °C for 10 h achieved a 5-log unit reduction of inoculated *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* in marinated whole-muscle beef jerky. Pathogen reduction was also evaluated in ground beef jerky, which may be of greater risk due to possible spreading of pathogen cells throughout the product (Faith et al. 1998; Harrison et al. 1997, 1998). The use of traditional marinade, heating at 71.1 °C before drying, or heating at 57.4 °C for 10 min after drying beef strips at 60 °C for 10 h, resulted in reductions of 5.8, 3.9, and 4.6 log units for *E. coli* O157:H7, *L. monocytogenes*, and *Salmonella*, respectively (Harrison et al. 2001). Reduction of *E. coli* O157:H7 by 5 log units in 5 % fat ground beef jerky was achieved by drying in home-type dehydrators at 52, 57, 63, and 68 °C for 10, 10, 8, and 4 h, respectively (Faith et al. 1998). An increase in fat content of jerky from 5 to 20 % had no effect on the destruction rate of the pathogen at 63 or 68 °C. Required drying times for a 5-log unit reduction in higher-fat (20 %) product increased by 6 and 10 h at 57 and 52 °C, respectively, compared to low-fat (5 %) ground beef jerky (Faith et al. 1998). For a 5-log reduction of *E. coli* O157:H7 in home-style dehydrators, there is a need for approximately 12 h of drying at 57.2 °C, approximately 8 h at 62.8 °C, or approximately 4 h at 68.3 °C, whereas sensitivity of the pathogen increased in meat with 5 % compared to 20 % fat (Buege and Luchansky 1999). Reductions of 5 log units were reached in 10 and 20 h in low- and high-fat products, respectively, at 52 °C. Corresponding times at 57 °C were 10 and 16 h, whereas the same inactivation at 63 and 68 °C required 8 and 4 h, respectively, for products with both fat levels (Faith et al. 1998).

The USDA/FSIS, based on the above and other similar studies, recommended cooking of meat to 71.1 °C before drying in order to assure inactivation of 5 log units of pathogen cells in the finished product (United States Department of Agriculture-Food Safety and Inspection Service 1998). It is important to recognize that preheating of meat, drying of jerky at high temperatures for longer periods of time, and post-drying heat treatments may change product quality characteristics and consumer acceptance compared to traditional jerky.

Reductions of *Salmonella* and *L. monocytogenes* were greater (>4 log units) in unheated jerky treated with a cure mix and dried at 60 °C for 8 h than in uncured samples (2.5–4.0 log units) dried under the same drying conditions (Harrison et al. 1997). Inactivation of *E. coli* O157:H7 also increased in cured ground beef jerky (Harrison et al. 1998). Heating of meat to 71.1 °C before drying did not enhance pathogen reduction in uncured jerky, but it increased the reduction of *Salmonella* in cured product (Harrison et al. 1997). Pathogen reduction in whole jerky strips was not different between regular and low-salt samples. However, pathogens died at a lower rate in reduced-salt, ground beef jerky. Thus, there may be greater risk associated with reduced-salt, ground beef jerky. Sugar addition also increased the lethality of the drying process. Overall, it appears that the curing agents sodium nitrite, salt, and sugar can increase pathogen destruction during jerky processing, but their effects may be variable and rather limited for assurance of safety (Nummer et al. 2004).

Albright et al. (Albright et al. 2003) determined that marination alone did not reduce *E. coli* O157:H7 in whole-meat jerky, but seasoning of meat with spices at 4 °C for 24 h followed by immersion in pickling brine of 78 °C for 90 s increased

destruction of *E. coli* O157:H7 in beef jerky. Marinades acidified with added ascorbic acid (7.7 % by weight) or citric acid (3.7 % by weight), generally, enhanced inactivation of *E. coli* O157:H7 during drying of beef slices marinated for 1 h at 48 °C and dried for 10 h at 62.5 °C. Thus, marinade acidified with household acidulants may enhance bacterial pathogen destruction during home jerky processing. Inactivation of *E. coli* O157:H7, *Salmonella*, and *L. monocytogenes* was increased by various heating and marination treatments before drying (Harrison et al. 2001).

In addition to marination and curing agents (Harrison et al. 1997, 1998) discussed above, the use of other chemical interventions (e.g., organic acids such as acetate and lactate, ethanol, and food-grade surfactants such as polysorbates) before drying has also been evaluated, in order to use lower temperatures during drying for potential enhancement of pathogen inactivation in jerky. In addition, such interventions may provide residual antimicrobial effects during product storage. Since consumers may object to the cooked flavor of jerky if the meat is heated prior or after drying, studies have evaluated the effect of pre-drying treatments in the form of multiple hurdles for the enhancement of pathogen destruction during jerky processing. Studies by Calicioglu et al. (2002a, b, 2003a, b, c) revealed that inclusion of acetate, lactate, and Tween 20 as marinade ingredients increased the inactivation of pre- or post-drying inoculated *E. coli* O157:H7, *Salmonella*, and *L. monocytogenes* during drying and storage of beef jerky.

Yoon et al. (2005) evaluated the influence of an inoculum level of 4 and 7 log CFU/cm^2 *E. coli* O157:H7 on the pathogen's inactivation during drying and storage of beef jerky. Overall, the results revealed that inclusion of an acetic acid treatment before marination and drying of beef jerky not only enhanced *E. coli* O157:H7 destruction but also could be valuable in inactivating a higher level of contamination when present.

Yoon et al. (2006) modeled the effect of drying temperatures (52, 57, and 63 °C) and pre-drying treatments on the inactivation of *L. monocytogenes* on beef jerky. No significant inactivation differences were observed related to drying temperatures in the range tested. Pre-drying acidification resulted in higher pathogen inactivation during drying than other treatments, regardless of drying temperature. Yoon et al. (2009) also modeled the effect of drying temperature in combination with pre-drying marination treatments on the inactivation of *Salmonella* on beef jerky. The models developed in these studies may be useful in selecting appropriate drying temperatures and times in combination with pre-drying treatments in beef jerky processing for adequate inactivation of pathogens.

As indicated, a concern exists that pathogens such as *E. coli* O157:H7 may exhibit increased survival or resistance and cross-protection to other stresses when exposed to sublethal stresses such as acid (Samelis and Sofos 2003). Addressing this issue, studies by Calicioglu et al. (2002a, b, 2003a, b, c) evaluated the effectiveness of various pre-drying modified marinades on acid-adapted or non-adapted pathogen cells during preparation, drying, and storage of whole-muscle beef jerky. In general, it was concluded that previous acid adaptation did not increase survival of pre- or post-drying inoculated *E. coli* O157:H7, *Salmonella*, and *L. monocytogenes* on beef jerky. The use of acidic marinades pre-drying and drying to a low a_w

enhanced antimicrobial activity. Acid adaptation did not increase survival but may have enhanced pathogen death during jerky storage.

The USDA/FSIS, in addition to preheating (71.1 °C) meat slices in marinade before drying for jerky processing, has also issued compliance guidelines that recommend dipping the meat slices in 5 % acetic acid for 10 min before marination and drying. Following the *Salmonella* Kiambu jerky-associated outbreak in New Mexico in 2003, USDA/FSIS issued in March 2004 a compliance guideline for jerky processing, establishing that time–temperature processes used for pathogen destruction during jerky processing should consider oven humidity, especially in high-altitude and low-relative humidity areas (United States Department of Agriculture-Food Safety and Inspection Service 2004).

Validation of pathogen interventions is a necessary step before or during implementation in a food process. For low-a_w meat products, examples of biological hazards and processing steps in need of validation include lethality and *S. aureus* control during fermentations, lethality of marinades as part of jerky processing, validation of drying to cause reductions in a_w coupled with reductions in pathogens, validation of mild heating as a finishing step to destroy parasites, and validation of refrigerated or ambient storage of finished products for growth control of pathogens. To validate safe storage of low-a_w meat products, it is sometimes appropriate to inoculate with vegetative cells of bacterial pathogens. It would be important to precondition cells to low-a_w environments so as to avoid shocking or stressing cells upon introduction to meat. Some methods have been developed to prepare dry inocula of pathogens such as *L. monocytogenes* (De Roin et al. 2003). Hinkens et al. (1996) evaluated processes for a 5-log unit reduction in counts of *E. coli* O157:H7 required by USDA/FSIS following the 1994 outbreak associated with consumption of dry-cured salami. The results revealed that a traditional nonthermal process for pepperoni was sufficient to kill only approximately 2 log CFU/g of the pathogen, whereas heating to internal temperatures of 63 °C instantaneous or to 53 °C for 60 min inactivated 5–6 log units of the pathogen.

Summary and Conclusion

A variety of dried edible muscle tissue products are produced worldwide from various animal and bird species, as well as fish. Formulations can include the addition of salt, sugar, spices, flavorings, and other ingredients. In addition to drying as the key process step, low-a_w muscle foods may undergo fermentation and smoking. Low- to intermediate-a_w meats, induced by salting and drying, create selective pressures to kill or reduce Gram-negative bacteria and parasites while permitting survival and even proliferation of certain xerotolerant Gram-positive bacteria, yeasts, and molds. Most surviving bacteria are innocuous and some may even impart positive organoleptic effects on the product. Molds, such as *Penicillium* spp., commonly grow on surfaces of low-a_w meats, particularly where oxygen levels are supportive. However, the mycotoxigenic potential of such molds on dry meat products is low.

It is generally advantageous to remove moisture from meat as quickly as possible during the drying process in order to restrict the growth and survival of bacterial pathogens. This may mean that traditional processes require modification to ensure safety. Some outbreaks of illness have occurred in low- to intermediate-a_w products such as jerky and hard salami. Therefore, levels of contamination of the raw material must be estimated through hazard analysis, and each process should be designed and validated to achieve appropriate log reduction of bacterial pathogens or destruction of parasitic pathogens. Pathogen inactivation during drying and processing, as well as growth control or death during storage of finished product, should be validated with inoculation studies and verified through appropriate testing of representative finished production lots.

References

Aberle ED, Forrest JC, Gerrard ED, Mills EW (2001) Principles of meat science, 4th edn. Kendall/Hunt Publishing Co., Dubuque, IA

Aksu Mİ, Kaya M, Ockerman HW (2005) Effect of modified atmosphere-packaging and temperature on the shelf life of sliced pastirma produced from frozen/thawed meat. J Muscle Foods 16:192–206

Aksu MI, Kayam M, Oz F (2008) Effects of *Lactobacillus sakei* and *Staphylococcus xylosus* on the inhibition of *Escherichia coli* 0157:H7 in pastirma, a dry-cured meat product. J Food Safety 28:47–58

Albright SN, Kendall PA, Avens JS, Sofos JN (2003) Pretreatment effect on inactivation of *Escherichia coli* O157:H7 inoculated beef jerky. Lebensm Wissensch Technol 36:381–389

Ambrosiadis I (2005) New production technologies for traditional Greek meat products. Biotechnol Anim Husbandry 21:321–330

Andersen SJ (1995) Compositional changes in surface mycoflora during ripening of naturally fermented sausages. J Food Prot 58:426–429

Arnau J, Gou P, Comaposada J (2003) Effect of the relative humidity of drying air during the resting period on the composition and appearance of dry-cured ham surface. Meat Sci 65:1275–1280

Asefa DT, Gjerde RO, Sidhu MS, Langsrud S, Kure CF, Nesbakken T, Skaar I (2009a) Moulds contaminants on Norwegian dry-cured meat products. Int J Food Microbiol 128:435–439

Asefa DT, Kure CF, Gjerde RO, Langsrud S, Omer MK, Nesbakken T, Skaar I (2011) A HACCP plan for mycotoxigenic hazards associated with dry-cured meat production processes. Food Control 6:831–837

Asefa DT, Kure CF, Gjerde RO, Omer MK, Langsrud S, Nesbakken T, Skaar I (2010) Fungal growth pattern, sources and factors of mould contamination in a dry-cured meat production facility. Int J Food Microbiol 140:131–135

Asefa DT, Møretrø T, Gjerde RO, Langsrud S, Kure CF, Sidhu MS, Nesbakken T, Skaar I (2009b) Yeast diversity and dynamics in the production processes of Norwegian dry-cured meat products. Int J Food Microbiol 133:135–140

Bailly JD, Tabuc C, Quérin A, Guerre P (2005) Production and stability of patulin, ochratoxin A, citrinin, and cyclopiazonic acid on dry cured ham. J Food Prot 68:1516–1520

Battilani P, Formenti S, Toscani T, Virgili R (2010) Influence of abiotic parameters on ochratoxin A production by a *Penicillium nordicum* strain in dry-cured meat model systems. Food Control 21:1739–1744

Benito MJ, Martin A, Aranda E, Perez-Nevado F, Ruiz-Moyano S, Cordoba MG (2007) Characterization and selection of autochthonous lactic acid bacteria isolated from traditional Iberian dry-fermented salchichon and chorizo sausages. J Food Sci 72:M193–201

Bennani L, Zenati Y, Faid M, Ettayebi M (1995) Physico-chemical and microbiological characteristics of a dried salted meat product (Kaddid) in Morocco. Z Lebensm Unters Forsch 201:528–532

Blackburn CW, Curtis LM, Humpheson L, Billon C, McClure PJ (1997) Development of thermal inactivation models for *Salmonella enteritidis* and *Escherichia coli* O157:H7 with temperature, pH and NaCl as controlling factors. Int J Food Microbiol 38:31–44

Blankenship LC (1978) Survival of a *Salmonella typhimurium* experimental contaminant during cooking of beef roasts. Appl Environ Microbiol 35:1160–1165

Blankenship LC, Davis CE, Magner GJ (1980) Cooking methods for elimination of *Salmonella* Typhimurium experimental surface contaminant from rare dry-roasted beef roasts. J Food Sci 45:270–273

Blesa E, Aliño M, Barat JM, Grau R, Toldrá F, Pagán MJ (2008) Microbiology and physico-chemical changes of dry-cured ham during the post-salting stage as affected by partial replacement of NaCl by other salts. Meat Sci 78:135–142

Boziaris IS, Nychas GJ (2006) Effect of nisin on growth boundaries of *Listeria monocytogenes* Scott A, at various temperatures, pH and water activities. Food Microbiol 23:779–784

Boziaris IS, Skandamis PN, Anastasiadi M, Nychas GJE (2007) Effect of NaCl and KCl on fate and growth/no growth interfaces of *Listeria monocytogenes* Scott A at different pH and nisin concentrations. J Appl Microbiol 102:796–805

Braghieri A, Girolami A, Carlucci A, Piazzolla N, Riviezzi AM, Napolitano F (2009) Sensory properties affecting acceptability of "Bresaola" from Podolian young bulls. J Sensory Stud 24:677–697

Buege D, Luchansky J (1999) Ensuring the safety of home-prepared jerky. Meat Poult 45:56–59

Burnham GM, Hanson DJ, Koshick CM, Ingham SC (2006) Death of *Salmonella* serovars, *Escherichia coli* O157:H7, *Staphylococcus aureus* and *Listeria monocytogenes* during the drying of meat: a case study using Biltong and Droewors. J Food Safety 28:198–209

Byelashov OA, Carlson BA, Geornaras I, Kendall PA, Scanga JA, Sofos JN (2009) Fate of post-processing inoculated *Listeria monocytogenes* on vacuum-packaged pepperoni stored at 4, 12 or 25 °C. Food Microbiol 26:77–81

Cabañes FJ, Bragulat MR, Castellá G (2010) Ochratoxin a producing species in the genus *Penicillium*. Toxins (Basel) 2:1111–1120

Calicioglu M, Sofos JN, Samelis J, Kendall PA, Smith GC (2002a) Inactivation of acid-adapted and nonadapted *Escherichia coli* O157:H7 during drying and storage of beef jerky treated with different marinades. J Food Prot 65:1394–1405

Calicioglu M, Sofos JN, Samelis J, Kendall PA, Smith GC (2002b) Destruction of acid- and non-adapted *Listeria monocytogenes* during drying and storage of beef jerky. Food Microbiol 19:546–559

Calicioglu M, Sofos JN, Kendall PA (2003a) Fate of acid-adapted and non-adapted *Escherichia coli* O157:H7 inoculated post-drying on beef jerky treated with marinades before drying. Food Microbiol 20:169–177

Calicioglu M, Sofos JN, Kendall PA (2003b) Influence of marinades on survival during storage of acid-adapted and nonadapted *Listeria monocytogenes* inoculated post-processing on beef jerky. Int J Food Microbiol 86:283–292

Calicioglu M, Sofos JN, Kendall PA, Smith GC (2003c) Effects of acid adaptation and modified marinades on survival of postdrying *Salmonella* contamination on beef jerky during storage. J Food Prot 66:396–402

Canadian Food Inspection Agency (2006) Animal products – food of animal origin. Inspection procedures, dispositions, and monitoring and controls, meat hygiene manual of procedures. CFIA, Dartmouth, Canada

Capita R, Llorente M, Mez S, Prieto M, Alonso-Calleja C (2006) Microbiological profiles, pH, and titratable acidity of chorizo and salchichon (two Spanish dry fermented sausages) manufactured with ostrich, deer, or pork meat. J Food Prot 69:1183–1189

Centers for Disease Control and Prevention (1966) Botulinum type F. Morbid Mortal Weekly Rep 15:359

Centers for Disease Control and Prevention (1995a) Escherichia coli O157:H7 outbreak linked to commercially distributed dry-cured salami – Washington and California, 1994. Morbid Mortal Weekly Rep 44:157–160
Centers for Disease Control and Prevention (1995b) Outbreak of salmonellosis associated with beef jerky – New Mexico. Morbid Mortal Weekly Rep 44:785–788
Cerveny J (1980) Effects of changes in the production and marketing of cured meats on the risk of botulism. Food Technol 34:240–243
Chacon PA, Muthukumarasamy P, Holley RA (2006) Elimination of *Escherichia coli* O157:H7 from fermented dry sausages at an organoleptically acceptable level of microencapsulated allyl isothiocyanate. Appl Environ Microbiol 72:3096–3102
Chang SF, Huang TC, Pearson AM (1996) Control of the dehydration process in production of intermediate-moisture meat products: a review. Adv Food Nutr Res 39:71–161
Chawla SP, Chander R (2004) Microbiological safety of shelf-stable meat products prepared by employing hurdle technology. Food Control 15:559–563
Chirife J, del Pilar Buera M (1996) Water activity, water glass dynamics, and the control of microbiological growth in foods. Crit Rev Food Sci Nutr 36:465–513
Chukwu O, Imodiboh LI (2009) Influence of storage conditions on shelf-life of dried beef product (Kilishi). World J Agric Sci 5:34–39
Clavero MRS, Beuchat LR (1996) Survival of *Escherichia coli* O157:H7 in broth and processed salami as influenced by pH, water activity, and temperature and suitability of media for its recovery. Appl Environ Microbiol 62:2735–2740
Comi G, Urso R, Iacumin L, Rantsiou K, Cattaneo P, Cantoni C, Cocolin L (2005) Characterisation of naturally fermented sausages produced in the North East of Italy. Meat Sci 69:381–392
Comi G, Orlic S, Redzepovic S, Urso R, Iacumin L (2004) Moulds isolated from Istrian dried ham at the pre-ripening and ripening level. Int J Food Microbiol 96:29–34
Conedera G, Mattiazzi E, Russo F, Chiesa E, Scorzato I, Grandesso S, Bessegato A, Fioravanti A, Caprioli A (2007) A family outbreak of *Escherichia coli* O157 haemorrhagic colitis caused by pork meat salami. Epidemiol Infect 135:311–314
Cornu M, Beaufort A, Rudelle S, Laloux L, Bergis H, Miconnet N, Serot T, Delignette-Muller ML (2006) Effect of temperature, water-phase salt and phenolic contents on *Listeria monocytogenes* growth rates on cold-smoked salmon and evaluation of secondary models. Int J Food Microbiol 106:159–168
Cvetnic Z, Pepeljnjak S (1995) Aflatoxin-producing potential of Aspergillus flavus and Aspergillus parasiticus isolated from samples of smoked-dried meat. Nahrung 39:302–307
Darmadji P, Izumimoto M, Miyamoto T, Katoaka KEI (1990) Lactic fermentation effects on preservative qualities of Dendeng Giling. J Food Sci 55:1523–1527
De Cesare A, Mioni R, Manfreda G (2007) Prevalence of *Listeria monocytogenes* in fresh and fermented Italian sausages and ribotyping of contaminating strains. Int J Food Microbiol 120:124–130
De Roin MA, Foong SCC, Dixon PM, Dickson JS (2003) Survival and recovery of *Listeria monocytogenes* on ready-to-eat meats inoculated with a desiccated and nutritionally depleted dustlike vector. J Food Prot 66:962–969
Dierschke S, Ingham SC, Ingham BH (2010) Destruction of *Escherichia coli* O157:H7, *Salmonella*, *Listeria monocytogenes*, and *Staphylococcus aureus* achieved during manufacture of whole-muscle beef Jerky in home-style dehydrators. J Food Prot 73:2034–2042
Dincer E, Kivanc M (2012) Characterization of lactic acid bacteria from Turkish Pastirma. Ann Microbiol 62:1155–1163
Doyle ME, Mazzotta AS (2000) Review of studies on the thermal resistance of salmonellae. J Food Prot 63:779–795
Dubey JP, Kotula AW, Andrews CD, Lindsay DS (1990) Effect of high temperature on infectivity of *Toxoplasma gondii* tissue cysts in pork. J Parasitol 76:201–204
EFSA (2006) Opinion of the scientific panel on contaminants in the food chain on a request form the commission related to ochratoxin A in food. EFSA J 365:1–56
Eidson M, Sewell CM, Graves G, Olson R (2000) Beef jerky gastroenteritis outbreaks. J Environ Health 62:9–13

Faith NG, Le Coutour NS, Alverenga MB, Calicioglu M, Buege DR, Luchansky JB (1998) Viability of *Escherichia coli* O157:H7 in ground and formed beef jerky prepared at levels of 5 and 20 % fat and dried at 52, 57, 63, or 68 °C in a home, style dehydrator. Int J Food Microbiol 41:213–221

Feránndez-Salguero J, Gómez R, Carmona MA (1994) Water activity of Spanish intermediate-moisture meat products. Meat Sci 38:341–346

Forbes LB, Measures L, Gajadhar A, Kapel C (2003) Infectivity of *Trichinella nativa* in traditional northern (country) foods prepared with meat from experimentally infected seals. J Food Prot 66:1857–1863

Gailani MB, Fung DYC, Kraft AA (1986) Critical review of water activities and microbiology of drying of meats. Crit Rev Food Sci Nutr 25:159–183

Galvalisi U, Lupo S, Piccini J, Bettucci L (2012) *Penicillium* species present in Uruguayan salami. Rev Argent Microbiol 44:36–42

García-Esteban M, Ansorena D, Astiasarán I (2004) Comparison of modified atmosphere packaging and vacuum packaging for long period storage of dry-cured ham: effects on colour, texture and microbiological quality. Meat Sci 67:57–63

Garcia I, Diez V, Zumalacarregui JM (1997) Changes in proteins during the ripening of Spanish dried beef "Cecina". Meat Sci 46:379–385

Gareis M (1996) Fate of ochratoxin A on processing of meat products. Food Add Contam 13(Suppl):35–37

Gelabert J, Gou P, Guerrero L, Arnau J (2003) Effect of sodium chloride replacement on some characteristics of fermented sausages. Meat Sci 65:833–839

Gimeno O, Astiasaran I, Bello J (1999) Influence of partial replacement of NaCl with KCl and $CaCl_2$ on texture and color of dry fermented sausages. J Agric Food Chem 47:873–877

Giovannini A, Migliorati G, Prencipe V, Calderone D, Zuccolo C, Cozzolino P (2007) Risk assessment for listeriosis in consumers of Parma and San Daniele hams. Food Control 18:789–799

Glass KA, Kaspar CW, Sindelar JJ, Milkowski AL, Lotz BM, Kang J, Faith NG, Enache E, Kataoka AI, Henry C (2012) Validation of pepperoni process for control of Shiga toxin-producing *Escherichia coli*. J Food Prot 75:838–846

Goodfellow SJ, Brown WL (1978) Fate of *Salmonella* inoculated into beef for cooking. J Food Prot 41:598–605

Gounadaki AS, Skandamis PN, Drosinos EH, Nychas G-JE (2007) Effect of packaging and storage temperature on the survival of *Listeria monocytogenes* inoculated postprocessing on sliced salami. J Food Prot 70:2313–2320

Graumann GH, Holley RA (2007) Survival of *Escherichia coli* O157:H7 in needle-tenderized dry cured Westphalian ham. Int J Food Microbiol 118:173–179

Graumann GH, Holley RA (2008) Inhibition of *Escherichia coli* O157:H7 in ripening dry fermented sausage by ground yellow mustard. J Food Prot 71:486–493

Hajmeer M, Basheer I, Cliver DO (2006a) Survival curves of *Listeria monocytogenes* in chorizos modeled with artificial neural networks. Food Microbiol 23:561–570

Hajmeer M, Basheer I, Hew C, Cliver DO (2006b) Modeling the survival of *Salmonella* spp. in chorizos. Int J Food Microbiol 107:59–67

Hammes WP, Dirk Haller, Gänzle MG (2003) Fermented meat. In: Farnworth ER (ed) Handbook of fermented functional foods. CRC Press, Boca Raton, FL, pp 251–275

Hanssen HP (1995) Mould control in the meat processing industry. Fleischwirtschaft 75:52

Harrison JA, Harrison MA (1996) Fate of *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella typhimurium* during preparation and storage of beef jerky. J Food Prot 59:1336–1338

Harrison JA, Harrison MA, Rose RA (1997) Fate of *Listeria monocytogenes* and *Salmonella* species in ground beef jerky. J Food Prot 60:1139–1141

Harrison JA, Harrison MA, Rose RA (1998) Survival of *Escherichia coli* O157:H7 in ground beef jerky assessed on two plating media. J Food Prot 61:11–13

Harrison JA, Harrison MA, Rose RA, Shewfelt RL (2001) Home-style beef jerky: Effect of four preparation methods on consumer acceptability and pathogen inactivation. J Food Prot 64:1194–1198

Hartog BJ, de Boer E, Lenssinck JB, de Wilde GJ (1987) The microbiological status of dry sausage in East Netherlands. Tijdschr Diergeneeskd 112:322–333

Hernandez E, Huerta T (1993) Evolution of the microbiological parameters of cured ham. Microbiologia 9 Spec No:10–19

Hinkens JC, Faith NG, Lorang TD, Bailey P, Beuge D, Kaspar CW, Luchansky JB (1996) Validation of pepperoni processes for control of *Escherichia coli* O157:H7. J Food Prot 59:1260–1266

Holley RA (1985a) Beef jerky: fate of *Staphylococcus aureus* in marinated and corned beef during jerky manufacture and 2.5 °C storage. J Food Prot 48:107–111

Holley RA (1985b) Beef jerky: viability of food-poisoning microorganisms on jerky during its manufacture and storage. J Food Prot 48:100–106

Holley RA, Lammerding AM, Tittiger F (1988) Microbiological safety of traditional and starter-mediated processes for the manufacture of Italian dry sausage. Int J Food Microbiol 7:49–62

Hwang CA, Porto-Fett AC, Juneja VK, Ingham SC, Ingham BH, Luchansky JB (2009) Modeling the survival of Escherichia coli O157:H7, Listeria monocytogenes, and Salmonella Typhimurium during fermentation, drying, and storage of soudjouk-style fermented sausage. Int J Food Microbiol 129:244–252

Iacumin L, Chiesa L, Boscolo D, Manzano M, Cantoni C, Orlic S, Comi G (2009) Moulds and ochratoxin A on surfaces of artisanal and industrial dry sausages. Food Microbiol 26:65–70

Ibanez C, Quintanilla L, Irigoyen A, Garciajalon I, Cid C, Astiasaran I, Bello J (1995) Partial replacement of sodium-chloride with potassium-chloride in dry fermented sausages – influence on carbohydrate fermentation and the nitrosation process. Meat Sci 40:45–53

Ingham SC, Buege DR, Dropp BK, Losinski JA (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

Ingham SC, Engel RA, Fanslau MA, Schoeller EL, Searls G, Buege DR, Zhu J (2005) Fate of Staphylococcus aureus on vacuum-packaged ready-to-eat meat products stored at 21 °C. J Food Prot 68:1911–1915

Ingham SC, Searls G, Buege DR (2006) Inhibition of *Salmonella* serovars, *Escherichia coli* O157:H7 and *Listeria monocytogenes* during dry-curing and drying of meat: a case study with basturma. J Food Safety 26:160–172

Jacob R, Porto-Fett AC, Call JE, Luchansky JB (2009) Fate of surface-inoculated *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella typhimurium* on kippered beef during extended storage at refrigeration and abusive temperatures. J Food Prot 72:403–407

Juven BJ, Cox NA, Bailey JS, Thompson JE, Charles OW, Shutze JV (1984) Survival of *Salmonella* in dry food and feed. J Food Protect 47:445–448

Kanatt SR, Chawla SP, Chander R, Bongirwar DR (2002) Shelf-stable and safe intermediate-moisture meat products using hurdle technology. J Food Prot 65:1628–1631

Katsaras K, Lautenschlager R, Boschkova K (1996) Physical-chemical processes in the production of pasterma. Fleischwirtschaft 76:136

Keene WE, Sazie E, Kok J, Rice DH, Hancock DD, Balan VK, Zhao T, Doyle MP (1997) An outbreak of *Escherichia coli* 0157:H7 infections traced to jerky made from deer meat. JAMA 277:1229–1231

Komarik SL (1940) Manufacture of dried beef. In: United States Patent & Trade Office (ed)

Kotzekidou P (1992) Identification of staphylococci and micrococci isolated from an intermediate moisture meat product. J Food Sci 57:249–251

Lahti E, Johansson T, Honkanen-Buzalski T, Hill P, Nurmi E (2001) Survival and detection of *Escherichia coli* O157:H7 and *Listeria monocytogenes* during the manufacture of dry sausage using two different starter cultures. Food Microbiol 18:75–85

Lara JAF, Senigalia SWB, Oliveira TCRM, Dutra IS, Pinto MF, Shimokomaki M (2003) Evaluation of survival of *Staphylococcus aureus* and *Clostridium botulinum* in charqui meats. Meat Sci 65:609–613

Lebert I, Baucour P, Lebert A, Daudin J-D (2005) Assessment of bacterial growth on the surface of meat under common processing conditions by combining biological and physical models. J Food Eng 68:89–98

Lee RY, Silverman GJ, Munsey DT (1981) Growth and enterotoxin a production by *Staphylococcus aureus* in precooked bacon in the intermediate moisture range. J Food Sci 46:1687–1692

Legan JD, Seman DL, Milkowski AL, Hirschey JA, Vandeven MH (2004) Modeling the growth boundary of *Listeria monocytogenes* in ready-to-eat cooked meat products as a function of the product salt, moisture, potassium lactate, and sodium diacetate concentrations. J Food Prot 67:2195–2204

Leistner L (1987) Shelf stable products and intermediate moisture foods based on meat. In: Rockland LB, Beuchat LR (eds) Water activity: theory and application in foods. Marcel Dekker, New York, NY, pp 295–327

Leistner L, Gould GW (2001) Hurdle Technologies: combination treatments for food stability, safety, and quality. Kluwer Academic/Plenum Publishers, New York, NY

Levine P, Rose B, Green S, Ransom G, Hill W (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

Lindqvist R, Lindblad M (2009) Inactivation of *Escherichia coli, Listeria monocytogenes* and *Yersinia enterocolitica* in fermented sausages during maturation/storage. Int J Food Microbiol 129:59–67

Lücke F-K (2000) Fermented meats. In: Lund BM, Baird-Parker TC, Gould GW (eds) The microbiological safety and quality of food. Aspen, Gaithersburg, MD

Lücke FK, Vogeley I (2011) Traditional "air-dried" fermented sausages from Central Germany. Food Microbiol 29:242–246

Lund BM, Baird-Parker TC, Gould GW (2000) The microbiological safety and quality of food. Aspen, Gaithersburg, MD

Marino R, della Malva A, Gliatta G, Muscio A, Sevi A (2009) Quality of donkey bresaola. Ital J Anim Sci 8:715–717

Martín A, Córdoba JJ, Aranda E, Córdoba MG, Asensio MA (2006) Contribution of a selected fungal population to the volatile compounds on dry-cured ham. Int J Food Microbiol 110:8–18

Mellefont LA, McMeekin TA, Ross T (2003) The effect of abrupt osmotic shifts on the lag phase duration of foodborne bacteria. Int J Food Microbiol 83:281–293

Mellefont LA, McMeekin TA, Ross T (2004) The effect of abrupt osmotic shifts on the lag phase duration of physiologically distinct populations of *Salmonella typhimurium*. Int J Food Microbiol 92:111–120

Mie T, Pointon AM, Hamilton DR, Kiermeier A (2008) A qualitative assessment of *Toxoplasma gondii* risk in ready-to-eat smallgoods processing. J Food Prot 71:1442–1452

Mižáková A, Pipová M, Turek P (2002) The occurrence of moulds in fermented raw meat products. Czech J Food Sci 20:89–94

Montet MP, Christieans S, Thevenot D, Coppet V, Ganet S, Muller MLD, Dunière L, Miszczycha S, Vernozy-Rozand C (2009) Fate of acid-resistant and non-acid resistant Shiga toxin-producing *Escherichia coli* strains in experimentally contaminated French fermented raw meat sausages. Int J Food Microbiol 129:264–270

Moore JE (2004) Gastrointestinal outbreaks associated with fermented meats. Meat Sci 67:565–568

Morton ID, Gray JI, Tybor PT (1988) Edible tallow, lard and partially defatted tissues. In: Pearson AM, Dutson TR (eds) Edible meat by-products. Advances in meat research. Elsevier Applied Science, London, UK, pp 275–302

Moss MO (2000) Toxigenic fungi and mycotoxins. In: Lund BM, Baird-Parker TC, Gould GW (eds) The microbiological safety and quality of food. Aspen, Gaithersburg, MD, pp 1490–1571

Naim F, Messier S, Saucier L, Piette G (2003) A model study of Escherichia coli O157:H7 survival in fermented dry sausages–influence of inoculum preparation, inoculation procedure, and selected process parameters. J Food Prot 66:2267–2275

Ng WF, Langlois BE, Moody WG (1997) Fate of selected pathogens in vacuum-packaged dry-cured (country-style) ham slices stored at 2 and 25 °C. J Food Prot 60:1541–1547

Nickelson R, II, Kaspar CW, Johnson EA, Luchansky JB (1996) Update on dry fermented sausage *Escherichia coli* 0157:H7 validation research. An executive summary update by the Blue

Ribbon Task Force of the National Cattlemen's Beef Association with the Food Research Institute, University of Wisconsin-Madison. Research report no. 11–316. National Cattlemen's Beef Association, Chicago, IL

Nightingale KK, Thippareddi H, Phebus RK, Marsden JL, Nutsch AL (2006) Validation of a traditional Italian-style salami manufacturing process for control of *Salmonella* and *Listeria monocytogenes*. J Food Prot 69:794–800

Nummer BA, Harrison JA, Harrison MA, Kendall PA, Sofos JN, Andress EL (2004) Effects of preparation methods on the microbiological safety of home-dried meat jerky. J Food Prot 67:2337–2341

Núñez F, Díaz MC, Rodríguez M, Aranda E, Martín A, Asenio MA (2000) Effects of substrate, water activity, and temperature on growth and verrucosidin production by *Penicillium polonicum* isolated from dry-cured ham. J Food Prot 63:231–236

Núñez F, Westphal CD, Bermúdez E, Asenio MA (2007) Production of secondary metabolites by some terverticillate Penicillia on carbohydrate-rich and meat substrates. J Food Prot 70:2829–2836

Ockerman HW, Pellegrino JM (1988) Meat extractives. In: Pearson AM, Dutson TR (eds) Edible meat by-products. Advances in meat research. Elsevier Applied Science, London, UK, pp 303–339

Oyero OG, Oyefolu AB (2010) Natural occurrence of aflatoxin residues in fresh and sun-dried meat in Nigeria. Pan Afr Med J 7:14

Paleari MA, Moretti VM, Beretta G, Mentasti T, Bersani C (2003) Cured products from different animal species. Meat Sci 63:485–489

Pitt JI, Hocking AD (2009) Fungi and food spoilage, 3rd edn. Springer, New York, NY

Podolak R, Enache E, Stone W, Black DG, Elliott PH (2010) Sources and risk factors for contamination, survival, persistence, and heat resistance of *Salmonella* in low-moisture foods. J Food Prot 73:1919–1936

Porto-Fett AC, Call JE, Hwang CA, Juneja V, Ingham S, Ingham B, Luchansky JB (2009) Validation of commercial processes for inactivation of *Escherichia coli* O157:H7, *Salmonella* Typhimurium, and *Listeria monocytogenes* on the surface of whole-muscle turkey jerky. Poultry Sci 88:1275–1281

Portocarrero SM, Newman M, Mikel B (2002) Reduction of *Listeria monocytogenes*, *Salmonella* spp., and *Escherichia coli* O157:H7 during processing of country-cured hams. J Food Sci 67:1892–1898

ProMED-mail (2003) *Salmonella* Kiambu, beef jerky-USA (New Mexico), Archive no. 20031001.2471. International Society for Infectious Diseases

Ravyts F, Barbuti S, Frustoli MA, Parolari G, Saccani G, De Vuyst L, Leroy F (2008) Competitiveness and antibacterial potential of bacteriocin-producing starter cultures in different types of fermented sausages. J Food Prot 71:1817–1827

Refai MK, Niazi ZM, Aziz NH, Khafaga NE (2003) Incidence of aflatoxin B1 in Egyptian cured meat basterma and control by gamma-irradiation. Nahrung 47:377–382

Reynolds AE, Harrison MA, Rose-Morrow R, Lyon CE (2001) Validation of dry cured ham process for control of pathogens. J Food Sci 66:1373–1379

Ricke SC, Diaz IZ, Keeton JT (2007) Fermented meat, poultry, and fish products. In: Doyle MP, Beuchat LR (eds) Food microbiology: fundamentals and frontiers, 3rd edn. ASM, Washington, DC, pp 795–815

Rodríguez M, Núñez F, Córdoba JJ, Bermúdez ME, Asensio MA (1998) Evaluation of proteolytic activity of micro-organisms isolated from dry cured ham. J Appl Microbiol 85:905–912

Rodríguez M, Núñez F, Córdoba JJ, Sanabria C, Bermúdez E, Asensio MA (1994) Characterization of *Staphylococcus* spp. and *Micrococcus* spp. isolated from Iberian ham throughout the ripening process. Int J Food Microbiol 24:329–335

Romans JR, Costello WJ, Carlson CW, Greaser ML, Jones KW (2001) The meat we eat, 14th edn. Interstate, Danville, IL

Rossello C, Barbas JI, Berna A, Lopez N (1995) Microbial and chemical changes in "Sobrasada" during ripening. Meat Sci 40:379–385

Samelis J, Sofos JN (2003) Strategies to control stress-adapted pathogens and provide safe foods. In: Yousef AE, Juneja VK (eds) Microbial adaptation to stress and safety of new-generation foods. CRC Press, Boca Raton, FL, pp 303–351

Seman DL, Borger AC, Meyer JD, Hall PA, Milkowski AL (2002) Modeling the growth of *Listeria monocytogenes* in cured ready-to-eat processed meat products by manipulation of sodium chloride, sodium diacetate, potassium lactate, and product moisture content. J Food Prot 65:651–658

Shigehisa T, Nakagami T, Taji S, Sakaguchi G (1985) Destruction of salmonellae, *Escherichia coli*, and *Staphylococcus aureus* inoculated into and onto beef during dry-oven roasting. Jpn J Vet Sci 47:251–257

Silverman GJ, Munsey DT, Lee C, Ebert E (1983) Interrelationship between water activity, temperature and 5.5 percent oxygen on growth and enterotoxin A secretion by *Staphylococcus aureus* in precooked bacon. J Food Sci 48:1783–1795

Simoncini N, Rotelli D, Virgili R, Quintavalla S (2007) Dynamics and characterization of yeasts during ripening of typical Italian dry-cured ham. Food Microbiol 24:577–584

Simpson CA, Geornaras I, Yoon Y, Scanga JA, Kendall PA, Sofos JN (2008) Effect of inoculum preparation procedure and storage time and temperature on the fate of *Listeria monocytogenes* on inoculated salami. J Food Prot 71:494–501

Sofos JN (1984) Antimicrobial effects of sodium and other ions in foods: a review. J Food Safety 6:45–78

Sonjak S, Ličen M, Frisvad JC, Gunde-Cimerman N (2010) The mycobiota of three dry-cured meat products from Slovenia. Food Microbiol 28:373–376

Sonjak S, Ličen M, Frisvad JC, Gunde-Cimerman N (2011) Salting of dry-cured meat – a potential cause of contamination with the ochratoxin A-producing species *Penicillium nordicum*. Food Microbiol 28:1111–1116

Sosa MJ, Córdoba JJ, Díaz C, Rodríguez M, Bermúdez E, Asenio MA, Núñez F (2002) Production of cyclopiazonic acid by *Penicillium commune* isolated from dry-cured ham on a meat extract-based substrate. J Food Prot 65:988–992

Sperber WH (1983) Influence of water activity on foodborne bacteria – a review. J Food Prot 46:142–150

Tabuc C, Bailly JD, Bailly S, Querin A, Guerre P (2004) Toxigenic potential of fungal mycoflora isolated from dry cured meat products: preliminary study. Rev Méd Vét 156:287–291

Taormina PJ, Dorsa WJ (2010) Survival and death of *Listeria monocytogenes* on cooked bacon at three storage temperatures. Food Microbiol 27:667–671

Thevenot D, Delignette-Muller ML, Christieans S, Vernozy-Rozand C (2005) Prevalence of *Listeria monocytogenes* in 13 dried sausage processing plants and their products. Int J Food Microbiol 102:85–94

Tiganitas A, Zeaki N, Gounadaki AS, Drosinos EH, Skandamis PN (2009) Study of the effect of lethal and sublethal pH and aw stresses on the inactivation or growth of *Listeria monocytogenes* and *Salmonella* Typhimurium. Int J Food Microbiol 134:104–112

Toldrá F (2004) Curing | Dry. In: Jensen WK (ed) Encyclopedia of meat sciences. Elsevier, Oxford, UK, pp 360–366

Tolvanen R, Hellstrom S, Elsser D, Morgenstern H, Bjorkroth J, Korkeala H (2008) Survival of *Listeria monocytogenes* strains in a dry sausage model. J Food Prot 71:1550–1555

Troller JA (1971) Effect of water activity on enterotoxin B production and growth of *Staphylococcus aureus*. Appl Microbiol 21:435–439

Troller JA (1986) Water relations of foodborne bacterial pathogens – an updated review. J Food Prot 49:656–670

Tyopponen nee Erkkila S, Markkula A, Petaja E, Suihko ML, Mattila-Sandholm T (2003) Survival of *Listeria monocytogenes* in North European type dry sausages fermented by bioprotective meat starter cultures. Food Control 14:181–185

U.S. Department of Agriculture-Food Safety and Inspection Service (2001) Performance standards for the production of processed meat and poultry products; proposed rule. Fed Reg 66: 12590–12636

U.S. Department of Agriculture-Food Safety and Inspection Service (2005) Food standards and labeling policy book. In: Office of Policy Program and Employee Development (ed) United States Department of Agriculture, Food Safety and Inspection Service, Washington, DC

United States Department of Agriculture-Food Safety and Inspection Service (1998) Consumer education and information. USDA-FSIS, Washington, DC

United States Department of Agriculture-Food Safety and Inspection Service (2004) Compliance guideline for meat and poultry jerky, Washington, DC

United States Department of Agriculture-Food Safety and Inspection Service (2012) FSIS compliance guideline for meat and poultry jerky produced by small and very small establishments. In: U.S. Department of Agriculture-Food Safety and Inspection Service (USDA-FSIS), Washington, DC

Untermann F, Müller C (1992) Influence of a_w value and storage temperature on the multiplication and enterotoxin formation of staphylococci in dry-cured raw hams. Int J Food Microbiol 16:109–115

Virgili R, Simoncini N, Toscani T, Camardo Leggieri M, Formenti S, Battilani P (2012) Biocontrol of *Penicillium nordicum* growth and ochratoxin A production by native yeasts of dry cured ham. Toxins (Basel) 4:68–82

Wijnker JJ, Depner KR, Berends BR (2008) Inactivation of classical swine fever virus in porcine casing preserved in salt. Int J Food Microbiol 128:411–413

Wijnker JJ, Haas B, Berends BR (2007) Removal of foot-and-mouth disease virus infectivity in salted natural casings by minor adaptation of standardized industrial procedures. Int J Food Microbiol 115:214–219

Wolter H, Laing E, Viljoen BC (2000) Isolation and identification of yeasts associated with intermediate moisture meats. Food Technol Biotechnol 38:69–75

Yoon Y, Geornaras I, Kendall PA, Sofos JN (2009) Modeling the effect of marination and temperature on Salmonella inactivation during drying of beef jerky. J Food Sci 74:M165–M171

Yoon Y, Calicioglu M, Kendall PA, Smith GC, Sofos JN (2005) Influence of inoculum level and acidic marination on inactivation of *Escherichia coli* O157:H7 during drying and storage of beef jerky. Food Microbiol 22:423–431

Yoon Y, Skandamis PN, Kendall PA, Smith GC, Sofos JN (2006) A predictive model for the effect of temperature and predrying treatments in reducing *Listeria monocytogenes* populations during drying of beef jerky. J Food Prot 69:62–70

Zimmermann WJ (1971) Salt cure and drying-time and temperature effects on viability of *Trichinella spiralis* in dry-cured hams. J Food Sci 36:58–62

Dried Ready-to-Eat Cereal Products

Scott K. Hood

Abstract Ready-to-eat (RTE) cereal and snack bars are consumed by people of all ages due to their nutritional density and the wide variety of choices in flavor and texture. Grains such as wheat, oats, corn and rice are the foundational ingredients for these products. The unit operations involved in the production of RTE cereals include a cooking step followed by flaking, puffing, or shredding. Additional ingredients may be added to create the final product. *Salmonella* presents the greatest microbiological risk for these products, and outbreaks of salmonellosis have been documented. The water activity of RTE cereal and snack bars is well below the level needed to support the growth of pathogens. *Salmonella* mitigation strategies include ingredient control programs, validation of the lethality of processing steps, and a robust environmental monitoring program.

Keywords Cereal • Grains • Snack bars • *Salmonella*

Introduction

Ready-to-eat (RTE) cereal products are among the most common breakfast foods in the United States, Canada, the United Kingdom, and Australia. The annual consumption in each of those four countries is more than 4 kg (8.8 lbs) per person, and it is estimated that these products generate 10 billion dollars in retail sales in the United States, annually (General Mills Annual Report 2012). These foods provide a convenient source of nutrition for millions of people every day. In addition to their convenience and nutritional density, RTE cereals can be found in a wide range of flavors that appeal to a wide range of consumers, from very young children to older adults.

Snack bars have become a very popular food due to their convenience and variety of marketplace options. A discussion of the microbiological safety of these products has been included in this chapter because the main components of snack bars are often

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produced by a process similar or identical to RTE cereal unit operations. Snack bars generally fall into one of two main categories—baked bars or cold-formed bars.

The processing steps involved in the production of RTE cereals include cooking and drying grains to yield products with very low water activities. The products, per se, do not support the growth of any microorganisms, including pathogens. When production conditions include good hygienic practices, there are no major microbiological hazards (ICMSF 2011); however, there have been outbreaks of *Salmonella* Agona traced back to the consumption of RTE cereal products (CDC 1998, 2008).

In this chapter, the microbiological safety of RTE cereal products from field to consumption will be discussed. Snack bars made from components produced by the same process as cereal products will also be addressed.

Ingredients

The foundational material for RTE cereal products is typically a grain such as wheat, oats, corn, rice, or barley. In addition to grains, the other classes of ingredients include the following broad categories: water, sweeteners, macro ingredients for flavoring and providing texture, and micro ingredients for flavor, color, nutritional fortification, and shelf life preservation. Sweetener and macro ingredients may include sugar, corn syrup, malt, and molasses. Many RTE cereals may also have particulate ingredients that are blended with the processed cereal pieces. The particulates may be processed foods such as marshmallow pieces, chocolate, nuts, and cereal clusters. Table 1 provides a summary of the categories of ingredients and the examples of the specific ingredients in each category.

Snack bars are typically produced with similar ingredients as those used in RTE cereals. It is common to use components such as puffed or flaked grains as the major component of snack bars, and nuts are often another significant ingredient. Other ingredients may include binders and coatings. Binders typically include sugar or syrups and function to provide both sweetness and to hold the particulate together. Coatings are typically comprised of chocolate or another confectionary blend.

The basic principles of HACCP require a hazard analysis of all ingredients used in the production of any food product (Mortimore and Wallace 2001). Minimally processed agricultural products, such as whole and milled grains, are known to harbor *Salmonella* (Berghofer et al. 2003, Sperber 2007). Microbiological risks

Table 1 Categories and examples of ingredients used in the production of RTE cereals and snack bars

Foundational grains	Sweeteners	Liquids	Micro ingredients	Particulates
Oats	Sugar	Water	Vitamins	Marshmallows
Wheat	Corn syrup	Vegetable oil	Minerals	Nuts
Rice	Molasses		Preservatives	Dried fruit
Corn			Starch	Chocolate
Barley				Cereal clusters

associated with these ingredients will likely be controlled during the cereal manufacturing process. When considering the microbiological risks of RTE cereal, many of the ingredients will be subjected to a thermal process that reduces the risk of pathogen contamination. Control of these ingredients will be discussed later in the chapter.

Ingredients added after any cooking or heating steps must have control programs in place prior to their inclusion in the RTE cereal or snack bar. Nuts, chocolate, and confections have all been identified as ingredients that may be associated with *Salmonella* contamination (ICMSF 2005).

RTE Cereal Processing

Cooking

A simplified view of the unit operations for the production of RTE cereals is depicted in Fig. 1. After mixing of primary ingredients, a cooking process is needed to achieve the desired physical properties. Cooking imparts desirable flavor and color characteristics and is needed to create the appropriate texture. Cooking can occur as a batch or as a continuous unit operation. In both cases, ingredients are subjected to temperatures high enough to gelatinize starch in the grains. The gelatination temperature range for the commonly used grains is between 51 and 71 °C. Typical processing temperatures are much higher and provide a high level of lethality for pathogens. Typical cooking temperatures and times range from 1 h at 100 °C to a few seconds at 175 °F (Fast 2000). The material created by cooking is often extruded through a die to create pellets of low a_w that will be used in downstream unit operations.

Drying

The material resulting from the cooking process must go through further processing to make a finished product. At this point, the product already has a low moisture content, but needs to be dried further, prior to the next unit operation. The time and temperatures in this drying step can vary greatly. It is unlikely that this step provides any substantive lethality for the pathogens of concern.

Flaking, Puffing, and Shredding

The next unit operation in place is to create the unique shape and texture for the final products. There are three basic types of processing, which include one of the following unit operations: flaking, puffing, or shredding.

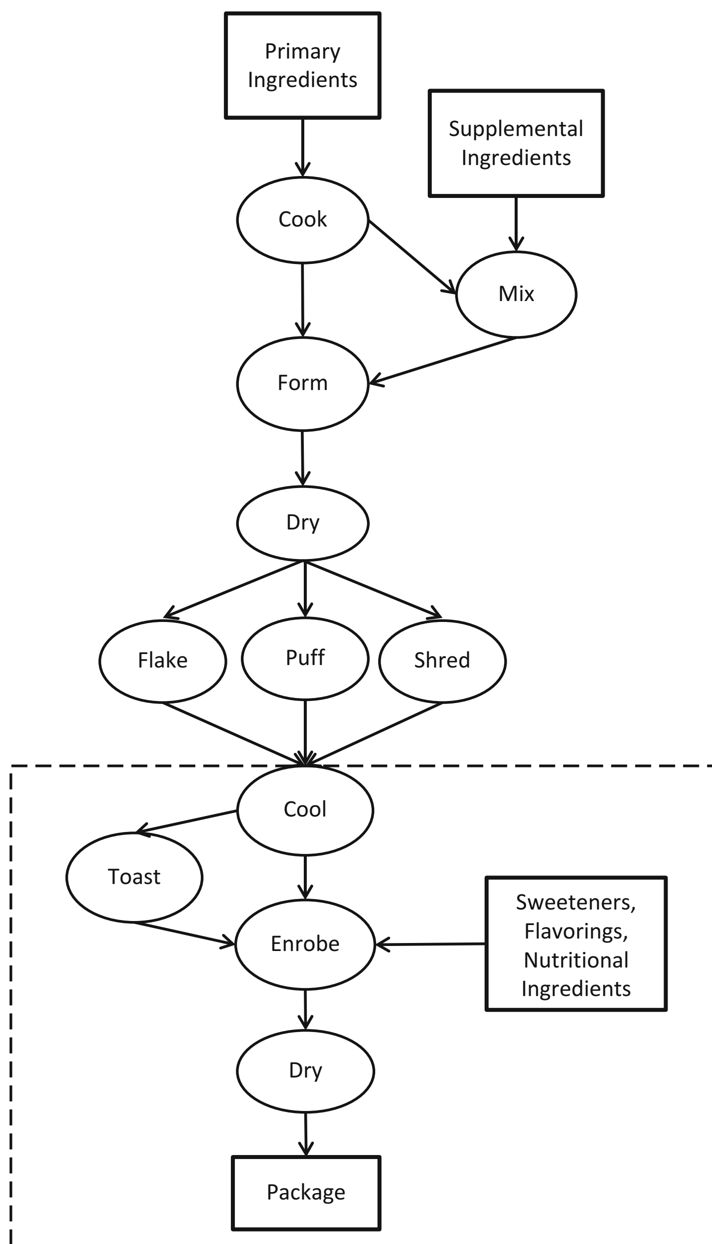

Fig. 1 Simplified flow diagram of RTE cereal process unit operations (*dashed lines* indicate the unit operations after the last potential lethality step)

Flaking subjects pellets to a roller that flattens the pellets for further processing. The flaking step does not provide sufficient heat to kill pathogens.

The puffing treatment converts residual moisture to steam and expands the product to produce a unique texture. Puffing can be accomplished by high-pressure extrusion ("gun puffing") or by ovens. The creation of steam means the product will be at temperatures greater than 100 °C. Gun puffing is done at high pressures of greater than ten bars, and oven puffing temperatures are typically greater than 250 °C (Fast 2000). The heat of this process may provide lethality for vegetative pathogens that might be present. Validation studies would be needed to determine the actual log reduction that may occur during this step. Lethality validation may be conducted through studies in the production facility using nonpathogenic surrogates or through calculations using lethality data appropriate for the product and processing conditions. Studies should follow recognized validation protocols (NACMCF 2006).

Shredding

Shredding is similar to flaking in that the cooked base is rolled to form sheets. The sheets are then cut or shredded to create the desired texture. This process is done at near-ambient temperature and would not kill pathogens of significance. However, subsequent oven treatments may provide a degree of pathogen lethality.

Toasting

The final unit operation, toasting, is applied to create the unique color and texture of the cereal piece. During this process, each individual cereal piece is exposed to high temperature typically above 175 °C for an appropriate period of time to achieve desired sensory characteristics. As with other unit operations, this step may provide lethality, but validation studies would be needed to determine the actual pathogen log reduction that occurs.

Enrobing

Coatings are used to add sweetness, flavorings, and nutritional supplements. Sugar coating may be added as a slurry or in granulated form using a drum enrober. Slurry application temperatures can vary, and in some cases, temperatures may be high enough to provide some level degree of lethality for pathogens. In addition, heat-sensitive vitamins may be sprayed or enrobed on the product. Care must be taken when preparing the slurry or vitamin solutions to avoid contamination. These intermediate processing steps require a hazard analysis to understand the microbiological risks that may be associated with the enrobing applications. If an enrobing solution supports the growth of any pathogens, controls must be in place to prevent pathogen growth.

Packaging

Packaging is the final unit operation in the overall RTE cereal production process. It is also the last point in the process where pathogenic microorganisms may be introduced given that the package is hermetically sealed.

Snack Bar Processing

Simplified processes for making snack bars are shown in Fig. 2. In oven-baked bars, the primary ingredients and a binder are mixed together. This mixture is formed into sheets and baked. While granola may be more like RTE cereal in the way it is sold, the process for making granola is more similar to the process of making baked bars than making cereal. The baking is done to impart flavor, color, and texture to the bars. The time and temperature of this unit operation are very specific to the finished product characteristic. To fully understand the food safety risks of the process and product, validation of lethality for any cooking or heating steps is essential.

In the production of cold-formed bars, the primary ingredients and a binder are also mixed together, with the mix being formed into the specific shape desired. There is no additional heating step.

For both oven-baked and cold-formed bars, there may be a unit operation in which enrobing or coating is applied to the bar and potentially a drying step. The final unit operation is packaging.

Microbiological Hazards

Salmonella

Salmonella presents the most significant microbiological risk for RTE cereal products and snack bars. Outbreaks of disease associated with RTE cereals were considered uncommon until a large, multistate salmonellosis outbreak was identified in the United States and traced to a toasted oat cereal in 1998 (CDC 1998). The likely source of contamination was *Salmonella* in the production facility environment (Breuer 1999). Snack products have also been implicated in Salmonella outbreaks (Sotir et al. 2009).

RTE Cereal products have water activity levels well below those required for the growth of any microorganisms, including pathogens. Although pathogens may not be able to grow in cereal, *Salmonella* can survive for extended periods of time in these products (Podolak et al. 2010). In addition, *Salmonella* can survive in the environment of the production facility for long periods of time (Beuchat et al. 2011; Margas et al. 2011).

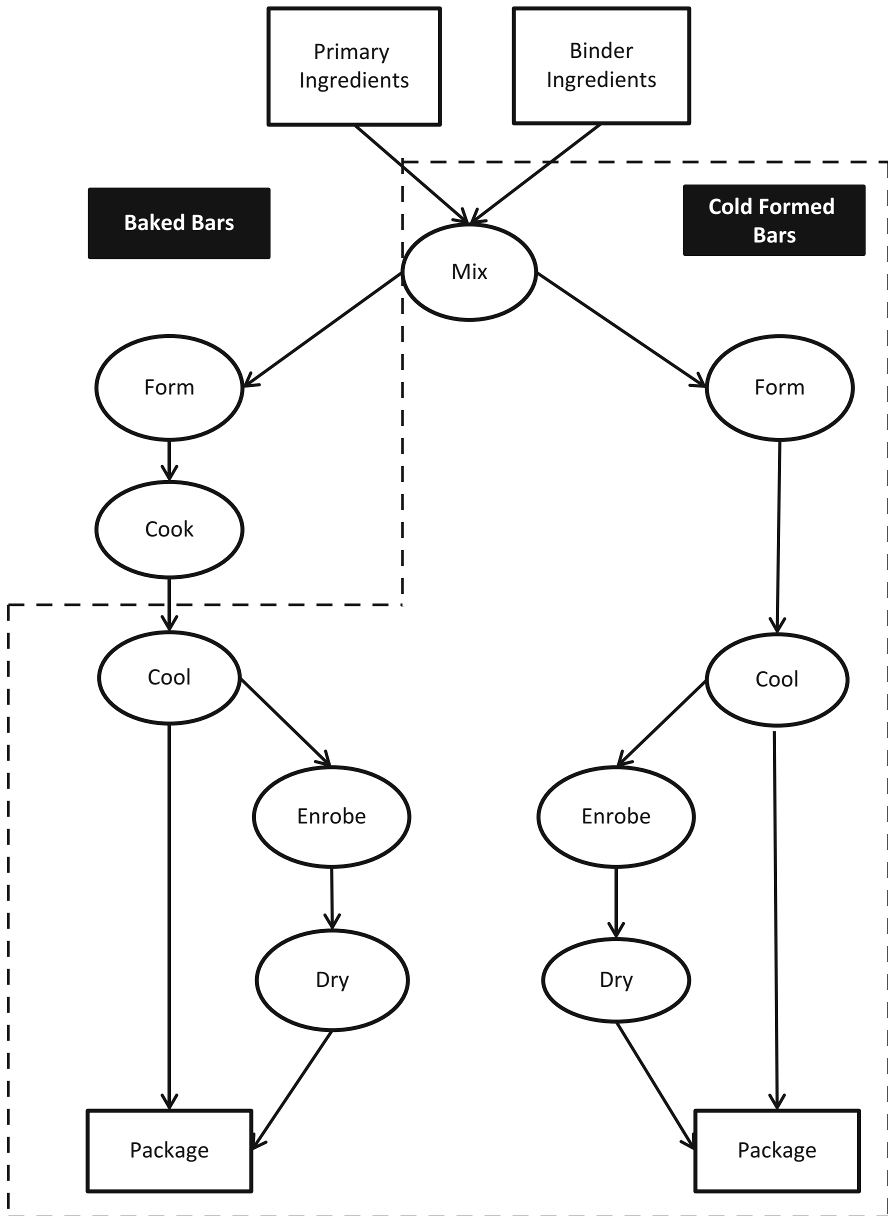

Fig. 2 Simplified flow diagram of baked snack bar and cold-formed snack bar unit operations (*dashed lines* indicate the unit operations after the last potential lethality step)

Pathogens Other Than Salmonella

There is no documented evidence that any pathogens other than *Salmonella* have caused foodborne illnesses as a result of the consumption of RTE cereals. However, there is a risk of other pathogens being present in the finished product.

Listeria monocytogenes is a pathogen that, some have reported, may require growth to large populations in order to cause illness (McLauchlin et al. 2004). Although *Listeria monocytogenes* will not grow in RTE cereal due to its low water activity, listeriae may be present in the wet areas of a cereal plant. Recalls of low-moisture food products due to *L. monocytogenes* contamination have occurred in the United States (FDA 2012). The presence of this pathogen may indicate sanitation failures and could trigger regulator action.

Mycotoxins

Mycotoxins are often not considered when evaluating the microbiological risks of RTE cereal products. In a typical HACCP hazard analysis, they may be considered as chemical hazards (Mortimore and Wallace 2001). Mycotoxins are produced by fungi and can occur in grains that would be used for the production of RTE cereals. The most common mycotoxins associated with cereal grains are aflatoxins, fumonisins, ochratoxin A, nivalenol, deoxynivalenol (DON), and other trichothecenes (ICMSF 2005). A code of practice has been developed to prevent and reduce mycotoxins (Codex Alimentarius 2003). Implementation of those practices is important in mitigating mycotoxin risks associated with RTE cereals and snack bars.

Control of Salmonella

Many factors must be considered when conducting an overall risk assessment of RTE cereal and snack bar products, because there are many unit operations that involve both the destruction of pathogens and the potential growth or reintroduction of pathogens. Hence, the concept of a food safety objective may be appropriate (Stringer 2005).

The basic principles for the control of *Salmonella* in low-moisture foods apply to the production of RTE cereal and snack bars. Like many other low-moisture foods, contamination of RTE cereal products and snack bars is likely to be the result of incoming ingredients and/or the production facility environment. In addition, if contamination occurs during production and there is the opportunity for pathogen growth, the risk increases for that processing step. Seven elements of control (Table 2) have been proposed to reduce the risk of *Salmonella* contamination in low-moisture foods (Scott et al. 2009). An in-depth discussion of each of the seven control elements is presented by Scott et al. (2009) and Chen et al. (2009a, b).

Table 2 Seven elements for the control of *Salmonella* in low-moisture foods

Element	Control measure
1	Prevent ingress or spread of *Salmonella* in the processing facility
2	Enhance the stringency of hygiene practices and controls in the primary *Salmonella* control area
3	Apply hygienic design principles to building and equipment design
4	Prevent or minimize growth of *Salmonella* within the facility
5	Establish a raw material/ingredient control program
6	Validate control measures to inactivate *Salmonella*
7	Establish procedures for verification of *Salmonella* controls and corrective actions

There are some unique considerations, when applying the seven control elements to the production of RTE cereal and snack bars. Many of the ingredients with a known risk for *Salmonella* contamination will be added at the cooking step and will be controlled at that point. Most cooking or heating operations during the production of RTE cereal or baked snack bars are included to create flavors, texture, and color and are not specifically designed for the destruction of pathogenic microorganisms. However, in many cases, they can serve that purpose through high temperatures and long dwell times.

Another consideration is that early in the processing of RTE cereals, the combination of grains, water, and other ingredients is likely to create a mixture that has a water activity greater than 0.95. The validation of the safety of these unit operations may involve referencing scientific data for products with a similar composition. Collection of data for well-documented validations is critical for every individual product and unit operation. Models for lethality during extrusion unit operations have been developed to help determine the lethality of a processing step (Bianchini et al. 2012).

Later in the overall process, the intermediate material has a water activity lower than 0.85. The validation of these later unit operations may be more complex due to the limited data available on similar food compositions. *Salmonella* has significantly greater heat resistance at low water activities than at high a_w (Chick 2011; Doyle and Mazzotta 2000). Following recognized protocols for validation is critical in documenting the level of pathogen reduction that may occur during an individual unit operation (NACMCF 2006).

Ingredients added after a lethal unit operation could introduce pathogenic microorganisms into the products; thus, the microbiological safety of these ingredients needs to be controlled at the supplier level. Control programs may include facility audits and supplier verification testing (Scott et al. 2009). In the production of snack bars, coatings that include cocoa, chocolate, or other confection ingredients are common. *Salmonella* is considered a risk for cocoa and chocolate-containing ingredients (ICMSF 2005).

Controls applied to the facility environment are key prerequisite programs required to prevent contamination. *Salmonella* may enter the facility in a variety of ways, requiring such control, including from minimally processed grains which are

the foundational ingredients for the products. In addition, *Salmonella* may enter the facility through infrastructure breaches, employees, and pests. Implementing a monitoring program based on hygienic zoning within the plant is a well-established approach to preventing contamination (Holah 2005; Chen et al. 2009b). The dashed lines as shown in Figs. 1 and 2 indicate the unit operations after the last potential lethality step and are therefore the zones of greatest concern for potential environmental contamination of the final product.

Verification is one of the principles of HACCP and is another important element to control *Salmonella* in RTE cereal and snack bars. Environmental monitoring plays a role in verification of cleaning and sanitizing practices in the production facility. Testing may be another component of the verification element. Sampling plans have been proposed to cover both product and environmental sampling; routine finished product testing is not recommended unless process deviations or other test results indicate a possible food safety issue (ICMSF 2011). If *Salmonella* testing is included, method validation is needed to assure the test method is appropriate for the food matrix. Positive pathogen findings are an indication that control programs are not functioning properly and any positive finding should be fully investigated to determine the root cause and corrective action taken to mitigate risk.

References

Berghofer LK, Hocking AD, Miskelly D, Jansson E (2003) Microbiology of wheat and flour milling in Australia. Int J Food Micro 85(1):137–149

Beuchat L, Komitopoulou E, Betts R et al (2011) Persistence and survival of pathogens in dry foods and dry food processing, environments. http://dare.uva.nl/document/489334

Bianchini A, Stratton J, Weier S, Hartter T et al (2012) Validation of extrusion as a killing step for *Enterococcus faecium* in a balanced carbohydrate-protein meal by using a response surface design. J Food Prot 75(9):1646–1653

Breuer T (1999) CDC Investigations: the May 1998 outbreak of *Salmonella agona* linked to cereal. Cereal Foods World 44:185–186

CDC (US Centers for Disease Control and Prevention) (1998) Multistate outbreak of Salmonella serotype Agona infections linked to toasted oat cereal – United States. Morbid Mortal Wkly Rep 47:462–464

CDC (2008) Investigation of outbreak of infections caused by Salmonella Agona. http://www.cdc.gov/salmonella/agona/. Accessed 1 Dec 2013

Chen Y, Scott V, Freier T, Kuehm J, Moorman M, Meyer J, Morille-Hinds T, Post L, Smoot L, Hood S, Shebuski J, Banks J (2009a) Control of *Salmonella* in low-moisture foods II: hygiene practices to minimize *Salmonella* contamination and growth. Food Protection Trends 29(7):435–445

Chen Y, Scott V, Freier T, Kuehm J, Moorman M, Meyer J, Morille-Hinds T, Post L, Smoot L, Hood S, Shebuski J, Banks J (2009b) Control of *Salmonella* in low-moisture foods III: process validation and environmental monitoring. Food Protection Trends 29(8):493–500

Chick M (2011) Thermal inactivation kinetics of *Salmonella* serovars on dry cereal, Dissertation, University of Minnesota

Codex Alimentarius (2003) Code of practice for prevention and reduction of mycotoxin contamination in cereals, including annexes on ochratoxin A, zearalenone, fumonisins and trichothecenes. (CAC/RCP 51–2003) Joint FAO/WHO Food Standard Program, FAO, Rome

Doyle M, Mazzotta A (2000) Review of studies on the thermal resistance of salmonellae. J Food Prot 63:779–795

Fast R (2000) Manufacturing technology of ready-to-eat cereals. In: Fast R, Caldwell E (eds) Breakfast cereals and how they are made, 2nd edn. American Association of Cereal Chemists, St. Paul, Minnesota, pp 15–54

FDA (2012) Recall notice. http://www.fda.gov/Safety/Recalls/ucm321955.htm. Accessed 12 Dec 2013

General Mills Annual Report (2012) Growing cereal sales around the world. pp 8–9. http://www.generalmills.com/Home/Media/Download_library/Reports_Publications. Accessed 1 Nov 2012

Holah J (2005) Improving zoning with food processing plants. In: Lelieveld HLM, Mostert MA, Hohal J (eds) Handbook of hygiene and control in the food industry. Woodhead Publishing in Food Science and Technology, Philadelphia, PA

ICMSF (2005) Microorganisms in foods 6: microbial ecology of food commodities, 2nd edn. Kluwer Academic/Plenum Publishers, New York

ICMSF (2011) Microorganisms in foods 8: cereal and cereal products. Springer, New York, pp 209–225

Margas E, Alstrom-Moore A, Dodd C, Holah J (2011) Salmonella survival in low aw environment. http://www.icef11.org/content/papers/mfs/MFS1156.pdf. Accessed 1 Nov 2012

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

Mortimore S, Wallace C (2001) HACCP. Blackwell, Oxford

NACMCF (2006) Requisite scientific parameters for establishing the equivalence of alternative methods of pasteurization. J Food Prot 69:1190–1216

Podolak R, Enache E, Stone W, Black D, Elliott P (2010) Sources and risk factors for contamination, survival, persistence, and heat resistance of *Salmonella* in low moisture foods. J Food Prot 73(10):1919–1936

Scott V, Chen Y, Freier T, Kuehm J, Moorman M, Meyer J, Morille-Hinds T, Post L, Smoot L, Hood S, Shebuski J, Banks J (2009) Control of *Salmonella* in low-moisture foods I: minimizing entry of *Salmonella* into a processing facility. Food Protection Trends 29(6):342–353

Sotir MJ, Ewald G, Kimura AC, Higa JI, Seth A, Troppy S, Myer S, Hoekstra RM, Austin J, Archer J, Spayne M, Daly ER, Griffin PM (2009) Outbreak of *Salmonella* Wandsworth and Typhimurium infections in infants and toddlers traced to a commercial vegetable-coated snack food. Ped Infect Dis J 28:1041–1046

Sperber W (2007) Role of microbiological guidelines in the production and commercial use of milled cereal grains: a practical approach for the 21st century. J Food Prot 70(4):1041–1053

Stringer M (2005) Food safety objectives – role in microbiological food safety management. Food Control 16:775–794

Powdered Infant Formula

Stephen Forsythe

Abstract "Powdered infant formula" (PIF) is a term used to cover both breast milk fortifiers and breast milk substitutes. Both are given to neonates who are immune-deficient. Although their low water activity stops bacterial growth during storage, bacteria are able to grow rapidly following reconstitution. Such organisms may be present through intrinsic contamination or through extrinsic contamination from the water, preparation equipment or carer. This chapter focuses on *Cronobacter* spp. and *Salmonella* serovars due the substantiated cases of neonatal infections through the ingestion of contaminated PIF, as emphasized by the three FAO-WHO risk assessments on the microbiological safety of PIF.

The taxonomic re-evaluations of the *Cronobacter* genus are presented, emphasizing the improved understanding using Next Generation Sequencing, along with multilocus sequence typing to reveal clonal lineages. One particular lineage, *C. sakazakii* clonal complex 4, is particularly associated with neonatal meningitis cases. It is also one of the dominant sequence types that has been isolated from PIF manufacturing plants and PIF. The organism can survive spray-drying and persist for long periods in the dried state. *Salmonella* is another organism of concern for neonatal infections, and although better understood than *Cronobacter* there have been rare outbreaks due to atypical, lactose-fermenting strains.

Keywords *Cronobacter* • *Salmonella* • Powdered infant formula • Desiccation persistence • Thermotolerance

Introduction

Food safety is important to everyone, especially the highly vulnerable members of our society. Subsequently, there are a number of methods used for preserving food and reducing the risk of foodborne illness. Reducing the available water in food is

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one such long-established method used to control bacterial growth. Most Enterobacteriaceae grow optimally at water activity (a_w) values above 0.95 and have a minimum value *ca.* 0.94. Reducing the a_w to 0.6 or less will inhibit microbial growth, although organisms such as *Cronobacter* and *Salmonella* will survive for years under such conditions. Consequently, foodborne outbreaks due to consumption of low-moisture ($a_w < 0.85$) and powdered food products have been reported. This chapter concerns the bacterial aspects of powdered infant formula (PIF) which is ingested by the most vulnerable within our population. This is a low water activity non-sterile product, and intrinsically the lack of available water prevents the growth of bacteria. Nevertheless, any organisms present (sporeformers and non-sporeformers) are able to persist and then grow after reconstitution. Particular attention will be given to *Cronobacter* spp. and *Salmonella* serovars due to the substantiated cases of neonatal infections through the ingestion of contaminated PIF.

The term "powdered infant formula" covers a range of products and can refer to breast milk fortifiers as well as the breast milk substitutes. The former are nutritional supplements added to breast milk which, if the baby was born prematurely, may not yet be sufficiently nutritious for the baby. In general, the term "infant" refers to children less than 1 year in age. However, this adds a complication as the *Salmonella* and *Cronobacter* spp. microbiological criteria for powdered infant formula are for products with an intended target age up to 6 months, after which a more varied diet is introduced at the "weaning" phase. In some countries, there are commercially available PIF products for infants over 6 months, which are called either "follow-up formulas" or "follow-on formulas," for which the current microbiological criteria for "powdered infant formula" do not apply.

The heightened awareness of *Cronobacter* spp. infections of neonates has brought considerable attention to the general microbiological aspects of PIF, since some cases, including fatalities, were attributable to the feeding regime. This product has been the focus of attention for reducing infection risk to neonates, because a large proportion of neonates are fed reconstituted PIF and because the number of exposure routes of these infants to this organism is limited. This resulted in significant international re-evaluations of safety procedures in its production, led to revisions of manufacturer's microbiological criteria, and new guidance for its hygienic reconstitution and feeding.

Three risk assessments were undertaken by the Food and Agriculture Organization-World Health Organization (FAO-WHO 2004, 2006, 2008), and other assessments were undertaken by other regulatory authorities and advisory groups, including the European Food Safety Authority and the Codex Alimentarius Commission. The reviews also considered the risks associated with other organisms which cause neonatal infections and can be isolated from PIF, such as *Salmonella* and other Enterobacteriaceae. Hence, a range of organisms other than *Salmonella* and *Cronobacter* spp. are also considered in this chapter.

The first FAO-WHO (2004) expert committee evaluating the risk assessment of *Cronobacter* spp. in PIF recommended that research should be promoted to gain a better understanding of the taxonomy, ecology, virulence and other characteristics of *Cronobacter* spp. This chapter addresses a number of these topics and will draw

upon recent findings on the genomic analysis of the organism. It will also briefly refer to the wider range of sources for the bacterium and also the low incidence of PIF-associated neonatal infections as compared to adult infections (FAO-WHO 2008). This chapter does not attempt to give an extensive historical overview of *Cronobacter* spp. but, instead, presents the current perspective on the organism and related issues with PIF and similar products. For more informative reviews of *Cronobacter* spp., the reader should consult Kucerova et al. (2011), Joseph et al. (2012a) and Joseph et al. (2012c).

Acquisition of Intestinal Flora by Neonates

Since a newborn baby is uncolonised, it is reasonable to propose that they acquire their microbiota from the mother, caregivers, diet and environment. The contribution of each has been difficult to determine, due to limitations of conventional microbiology, based on cultivation on agar plates and phenotypic tests for identification. Advances in DNA sequencing have enabled a far greater understanding of the intestinal flora. The international studies of the "Human Microbiome Project" (US; http://www.hmpdacc.org/) and "MetaHIT" (EU; http://www.metahit.eu/) have been instrumental in our improved understanding of the intestinal flora. This has led to a large number of publications, substantial DNA sequence databases and software development for analysis and visualisation of the data. It is now well established that the infant intestinal flora is influenced by the feeding regime and takes more than 3 years to become "adult-like." The initial flora is influenced by the mode of delivery with caesarean-section infants having an intestinal flora more similar to the maternal skin flora than vaginal and faecal and containing less bifidobacteria Martín et al. 2004. Thereafter, breastfed infants tend to develop an intestinal flora predominated by *Bifidobacterium* spp., such as *B. breve*, whereas exclusively formula-fed infants develop a less diverse intestinal flora, with a higher level of Proteobacteria, *Bacteroides,* as well as *B. longum* (Ottman et al. 2012). *Bacteroides* and bifidobacteria are well adapted to the degradation of human milk oligosaccharides (diverse glycans). It should be noted that one research group claims babies may not be born sterile but have an initial flora from the meconium which is either composed of enteric bacteria or lactic acid bacteria (Gosalbes et al. 2013), the former being associated with a history of atopic eczema in the mother, whereas the lactic acid bacteria flora was associated with respiratory problems in the infant. As given above, the neonatal intestinal flora is in part influenced by their feeding regime. Breast milk is not sterile and can contain bacteria from the nipples and skin cells and can be intrinsically contaminated if the mother has mastitis. The *C. malonaticus* type strain LMG 23826^T is from a breast abscess. Human mastic breast milk can contain various opportunistic bacterial pathogens which, in many countries, is fed to neonates (Fernández et al. 2013).

Liquid, ready-to-feed infant formula is sterile, but PIFs are not, and they can contain a range of bacterial genera. The levels of bacteria must meet microbiological criteria,

set by the Codex Alimentarius Commission (CAC). The current criteria were decided in 2008 and replaced the former ones from 1979. These criteria were revised, following raised awareness of microbiological issues associated with PIF and infant health, due to cases of neonatal infections by *Cronobacter* spp. In the most recent criteria, the general aerobic plate count is a 3-class plan (n=5 c=2 m=500/g M=5,000/g), and there are only two named bacteria for two-class (presence/absence) sampling plans, including *Salmonella* serovars (2-class plan n=60 c=0 m=0/25 g) and *Cronobacter* spp. (2-class plan n=30 c=0 m=0/10 g). The standard analytical method of presence/absence testing, with a set volume of material for *Cronobacter* spp. in PIF, has no requirement to additionally determine the level of *Cronobacter* spp. in the sample. Table 1 collates a number of studies from different countries, which have reported *Cronobacter* spp. in PIFs. The values should be seen as indicative, as different detection and identification methods have been used, which vary in their accuracy and are not an exhaustive collation of surveys.

In 2004 and 2006, the FAO-WHO compared the range of bacteria that may be isolated from PIF with those which have been epidemiologically linked with infant infections. This resulted in three categories of organisms. The two organisms of most concern were *Salmonella* serovars and *Cronobacter* spp. Other organisms considered were:

Other Enterobacteriaceae: *Pantoea* spp., *Citrobacter koseri*, *C. freundii*, *E. cloacae* and *E. hormaechei*.

Non-Enterobacteriaceae: *Acinetobacter* spp., *Bacillus* spp., *Listeria* spp., *Lactobacillus* spp. and probiotics.

Table 1 Prevalence of *Cronobacter* spp. in powdered infant formula[a]

Country of analysis[b]	Prevalence	Prevalence (%)	Level	Reference
Bangladesh	1/32	3	nd	Hoque et al. (2010)
Brazil	12/42	29	0.54 MPN/100 g	Santos et al. (2013)
Indonesia	10/74	14	nd	Estuningsih et al. (2006)
Jordan	1/69	1	nd	Jaradat et al. (2009)
Japan	4/61	7	0.36 MPN/100 g	Oonaka et al. (2010)
Non-Japanese	5/88	6	0.36–0.91 MPN/100 g	Oonaka et al. (2010)
Korea	4[c]/75	5	3.6–23 cfu/100 g	Kim et al. (2011)
Korea	7/102	7	nd	Park et al. (2010)
Nigeria	20/70[d]	29	nd	Aigbekaen and Oshoma (2010)
UK	2/82	2	nd	Iversen and Forsythe (2004)

[a]Many of the referenced articles do not describe the intended target age range of the sampled PIFs, and therefore, unless otherwise indicated, it is uncertain whether the products are for infants aged 0–6 months or older

[b]Country of analysis does not indicate country of manufacture

[c]Four isolates were from three types of formula: two from PIF (n=23) for intended target age <6 months, one from follow-up formula for intended target age 6–24 months and one from formula for infants with acute diarrhoea

[d]Products with intended target age from birth to 1 year in age

There are a number of similarities between *Cronobacter* spp. and *Salmonella* serovars, with respect to their associated presence in low-moisture products, environments and infections. These products do not support the growth of either organism, but both have been implicated in outbreaks of foodborne infections through similar products. Most of our knowledge is with respect to *Salmonella* serovars. Reports of low-moisture products contaminated with *Salmonella* are given in Table 3. Outbreak investigations have implicated cross-contamination as a major contributing factor (Reij et al. 2004). Both *Cronobacter* and *Salmonella* are able to survive on surfaces for extended periods of time and, therefore, can be transferred to food via contact. Due to this body of evidence, *Salmonella* and *Cronobacter* spp. will be considered in more detail than other Enterobacteriaceae.

Cronobacter spp.

Our current knowledge regarding the epidemiology of *Cronobacter* spp. is limited. Although some infections have been associated with intrinsically and extrinsically contaminated PIF, other environmental sources are possible and several non-infant formula-associated cases have been reported (Bowen and Braden 2008). It should be noted that neonatal infections by *Cronobacter* spp. are rare, and not all have been associated with reconstituted formula ingestion. In fact (a) breast milk is also not sterile, (b) *C. malonaticus* LMG 23826^T (species type strain) was isolated from a breast abscess, (c) *Cronobacter* spp. have been isolated from breast milk, (d) breast milk from mastic mothers is used to feed neonates, (e) the organism has been isolated from enteral feeding tubes from neonates on non-formula diets and (f) the majority of *Cronobacter* infections, albeit less severe, are in the adult population (FAO-WHO 2008). A stratified age profiling of *Cronobacter* carriage by hospital outpatients over the 7-year period 2005–2011 has been published by Holý et al. (2014) and revealed the majority of isolates (63.7 %, $n=91$) were from children aged 1–14 years.

The Cronobacter *Genus*

The *Cronobacter* genus is composed of Gram-negative, facultative anaerobic rods which are members of the Enterobacteriaceae family and closely related to the genera *Enterobacter* and *Citrobacter*. The genus *Cronobacter* was defined and created originally by the reclassification of the species *Enterobacter sakazakii*. It belongs to the taxonomic class Gammaproteobacteria and to the family Enterobacteriaceae. It includes facultatively anaerobic, Gram-negative, oxidase-negative, catalase-positive, non-sporeforming rods, which are generally motile, able to reduce nitrate to nitrite, produce acetoin and are negative for the methyl red test. Following the initial proposal of the *Cronobacter* genus, there have been three further

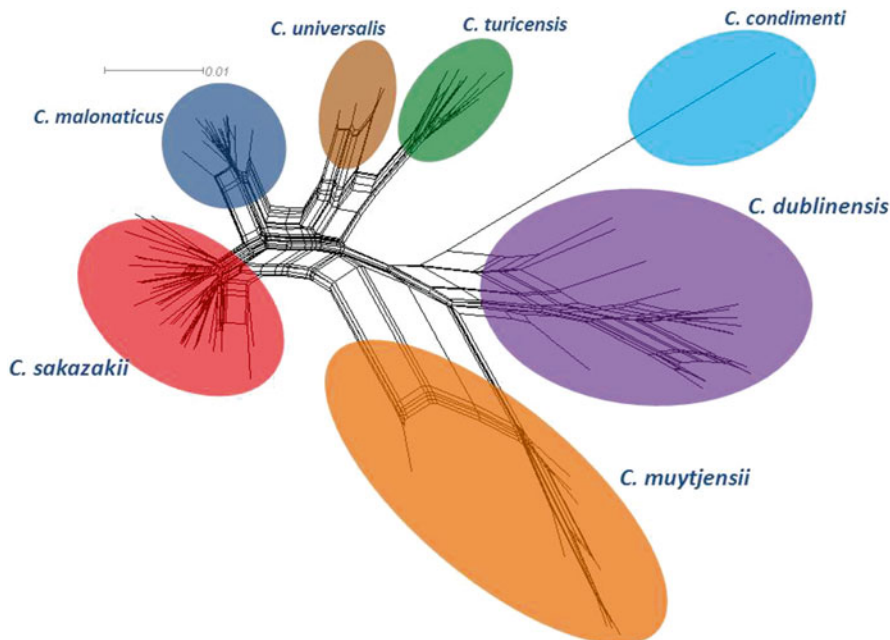

Fig. 1 Neighbor net of the concatenated 7-locus sequence alignment generated for the *Cronobacter* MLST data set, indicating diversity and recombination events. The figure has been drawn to scale using Splitstree4. The numbers at the tips of the branches indicate STs. The formation of parallelograms indicates possible recombination events. Copyright © American Society for Microbiology, Joseph et al. (2012a) *J. Clin. Microbiol*. 50, 3031-3039. DOI: 10.1128/JCM.00905-12

taxonomic revisions, and the genus is currently composed of 7 species: *C. sakazakii*, *C. malonaticus*, *C. turicensis*, *C. universalis*, *C. muytjensii*, *C. dublinensis* and *C. condimenti,* (Iversen et al. 2008; Joseph et al. 2012b; Brady et al. 2013). Phylogenetic analysis, based on whole-genome sequencing and multilocus sequence typing (MLST), shows that *C. sakazakii*, *C. malonaticus*, *C. turicensis* and *C. universalis* appear to be more closely related to each other and, it is hypothesised, separated from the remaining *Cronobacter* species about 41 million years ago (Joseph et al. 2012a; Fig. 1). Accurate bacterial taxonomy is essential for regulatory control, because the detection methods must be based on a thorough understanding of the diversity of the target organism.

Detection and Identification of Cronobacter *spp.*

In recent years, there have been numerous studies on the detection of *Cronobacter* spp. in PIF. A detailed coverage of this topic is not warranted for this chapter, but a number of pertinent issues need to be considered. Initial *Cronobacter* detection methods were reminiscent of the stages for *Salmonella* isolation from milk powders. In brief, the

steps were pre-enrichment in water or buffered peptone water (BPW), followed by enrichment (EE broth), then cultivation on the Enterobacteriaceae-selective agar Violet red bile glucose agar (VRBGA), picking off five colonies from the selective agar and transferring to TSA for phenotypic identification of any yellow-pigmented colonies. It is now recognised that there are a number of limitations with this method. There is no initial selection for *Cronobacter* spp.; instead, any Enterobacteriaceae present could be enriched in EE broth, which would grow on VRBGA, leading to possible overgrowth of *Cronobacter* spp., if present. Furthermore, *ca.* 20 % of *Cronobacter* strains are non-pigmented and would be overlooked on TSA plates. Finally, phenotype databases do not adequately cover the genus, resulting in conflicting results between commercial kits. Even the biotyping scheme, which was used to speciate *Cronobacter* isolates, has now been shown to be inadequate with no more than 50 % of biotypes being unique to any given *Cronobacter* species (Joseph et al. 2013a).

Cronobacter spp. are notably resistant to osmotic stresses, which may be linked to their ecology, and this trait has been used in the design of improved enrichment broths, such as modified lauryl sulphate tryptose broth, containing 0.5 M NaCl, and *Cronobacter* screening broth with 10 % sucrose. Following enrichment, the next step in the isolation of *Cronobacter* spp. is the use of chromogenic agar (primarily based on the α-glucosidase reaction) to differentiate *Cronobacter* spp. from other Enterobacteriaceae, which will have also grown in the enrichment broth and may be present on the isolation plate. Such agars were a major advance in the development of robust and reliable detection procedures. These days, improved detection and identification methods for *Cronobacter* spp. use chromogenic agars, along with DNA-based identification and fingerprinting techniques (Joseph and Forsythe 2012; Chen et al. 2012).

The Codex Alimentarius Commission (CAC) (2008) microbiological criteria are applied to PIF, with the intended age <6 months; however, PIF may be marketed in some countries as a product with intended age up to 12 months, but in other countries a separate product is marketed called "follow-up formula" for consumption by infants older than 6 months. A number of methods for the recovery of desiccation-stressed *Cronobacter* cells from PIF have been developed and used to survey PIF samples (Table 1). Because the organism has only been reported at low numbers (<1/100 CFU/g), a large volume of material needs to be tested. Subsequently, the CAC requirement is to test thirty 10-g PIF quantities, using a presence/absence approach after a resuscitation step is applied rather than direct enumeration of bacteria in the sample.

In addition to testing PIF for *Cronobacter* spp., samples are taken from the production environment and from ingredients (especially starches and other plant-derived material). In addition, production facilities and processes should be already designed and operated to control enteric pathogens, especially *Salmonella*. Commercial companies producing phenotyping kits need to ensure their databases are updated following the taxonomic revisions; for example, the former *E. sakazakii* Preceptrol™ strain ATCC 51329 has been reclassified as *C. muytjensii* and should not be confused with *C. sakazakii*. Currently these online databases have retained the pre-2007 name "*Enterobacter sakazakii*" and do not accurately identify all 7

Cronobacter species. The specificity of some earlier DNA-based PCR probes for *E. sakazakii* need to be re-evaluated for the defined 10 *Cronobacter* species, and new species-specific *Cronobacter* probes have been developed. This is especially pertinent since *E. helveticus, E. pulveris and E. turicensis* were used as negative control strains in the development of many of these methods were briefly, but no longer, classified as *Cronobacter* (Brady et al. 2013).

Pulsed-field gel electrophoresis (PFGE) is commonly used to determine if bacterial strains from related sites are distinguishable. The technique is widely used and is applicable for transnational investigations, as per PulseNet (http://www.cdc.gov/pulsenet/), because the gel results can be electronically analysed. For many years PFGE has been considered the gold standard for genetic typing in epidemiology and is used in investigations of sources of outbreaks. However, it does have limitations as not all bacterial strains give banding patterns and cannot be used to study the relatedness of strains. XbaI is the first-choice restriction enzyme with Enterobacteriaceae; however, investigations should also use a second restriction enzyme (e.g. SpeI) for confirmation and greater resolution. For example, with *C. sakazakii* strains in serotypes O:2 and O:7 are indistinguishable using XbaI restriction digestion despite differing in all eight genes of the O-PS biosynthetic pathway (Joseph et al. 2012c).

Multilocus Sequence Typing of **Cronobacter** *spp.*

16S rRNA gene sequence analysis has been frequently used to determine phylogenetic relationships between organisms in the Enterobacteriaceae and the Ribosomal Database Project (http://rdp.cme.msu.edu/), which contains >2.5 million sequences. However, 16S rDNA sequencing is of limited use for very closely related organisms because there are only minor differences in the rDNA sequence. There have been reported difficulties in distinguishing between *C. sakazakii* and *C. malonaticus* (Iversen et al. 2008). This was probably due to the use of biotype profiles to designate the species, as some of the biotype index strains have subsequently been shown to be assigned the wrong species and many of the biotypes are not specific to any particular *Cronobacter* species (Baldwin et al. 2009; Joseph et al. 2013a). Secondly, there are seven copies of the rDNA gene in *Cronobacter* spp. and intrageneric differences can lead to uncertain and inconsistent base sequence determinations, which subsequently have been deposited in the publicly available databases.

16S rDNA sequence analysis has been applied to early strains of *E. sakazakii* and various misidentifications have been revealed:

1. The strain from a fatal case of neonatal *E. sakazakii* sepsis infection in a neonatal intensive care unit (NICU) was re-identified as *E. cloacae* (Caubilla-Barron et al. 2007).
2. A strain from a NICU outbreak initially identified as *E. sakazakii* was re-identified as *E. hormaechei* (Townsend et al. 2008).

3. A nosocomially spread quinolone-resistant strain of *E. sakazakii* was re-identified as *E. hormaechei* (Poirel et al. 2007).
4. The strain ZORB A 741 used to describe the species oligo-polysaccharide structure of *E. sakazakii* was re-identified as *E. ludwiggi* (Szafranek et al. 2005).
5. The *C. malonaticus* strain HPB 3267 used to describe oligo-polysaccharide structure was re-identified as *E. asburiae* (MacLean et al. 2009; Ivy et al. 2013).

Analysis based on the sequences of housekeeping genes (multilocus sequence analysis, MLSA) has proven to be a useful tool to understand the evolution and diversity of bacterial pathogens, for example, *E. coli* ST131, MRSA-15 ST22 and *Klebsiella pneumoniae* ST258. Baldwin et al. (2009) applied an MLSA based on seven housekeeping genes (*atpD, fusA, glnS, gltB, gyrB, infB* and *ppsA*) to *C. sakazakii* and *C. malonaticus* and demonstrated a robust phylogenetic framework to separate the two species (Fig. 1). The latter study also showed that some previous confusion between the two species may have been due to incorrect speciation of some biotype index strains (Baldwin et al. 2009), as well as many of the biotypes not being unique to any specific species (Joseph et al. 2013a).

PFGE and PCR probes are useful for small-scale studies of strains from a limited region or source. However, they are of limited use when studying bacterial populations, as they do not reflect relatedness, and, instead, DNA sequence-based databases are more applicable. Fortunately, a seven-allele multilocus sequence typing (MLST) scheme has been established and is hosted by the University of Oxford through Professor Keith Jolley. The protocols and database are publically accessible online at www.pubMLST.org/cronobacter. The database contains the profiles and details of over 1000 strains. Additionally, the same approach has been used for multilocus sequence analysis (MLSA) for phylogenetic construction of the *Cronobacter* genus. The concatenated sequence of the seven loci gives a total sequence length of 3,036 bp. The phylogenetic analysis of *Cronobacter* spp. using MLSA is supported by whole-genome phylogenetic analysis (Joseph et al. 2012a, c) (Fig. 1). MLSA supported the recent naming of five novel species: *C. universalis* and *C. condimenti* (Joseph et al. 2012b). There are two expansions of the *Cronobacter* MLST scheme. Tax-MLST includes *rpoB* and *ompA* sequences for the taxonomic assignment of isolates using single locus and *Cronobacter* BIGSdb (Jolley and Maiden 2010, Maiden et al. 2013) whereby large numbers of loci across the genome can be used for genotyping since the database now includes >100 *Cronobacter* genomes and covers the seven species of the genus.

Following a multiple-strain *Cronobacter* outbreak at a French NICU, it became apparent that possibly not all *Cronobacter* strains were equally virulent (Caubilla-Barron et al. 2007). Further interrogation of the MLST database for patterns of association between sequence type and source has revealed that the majority of meningitis cases in the past 30 years across six countries were due to one clonal lineage (CC4), composed of 8 out of the currently recognised 217 sequence types (Joseph and Forsythe 2011, 2012; Joseph et al. 2012a) (Fig. 2). This retrospective observation was confirmed by the highly publicised US cases in 2011, as the meningitis-associated strains were also CC4 (Hariri et al. 2013). It is notable that

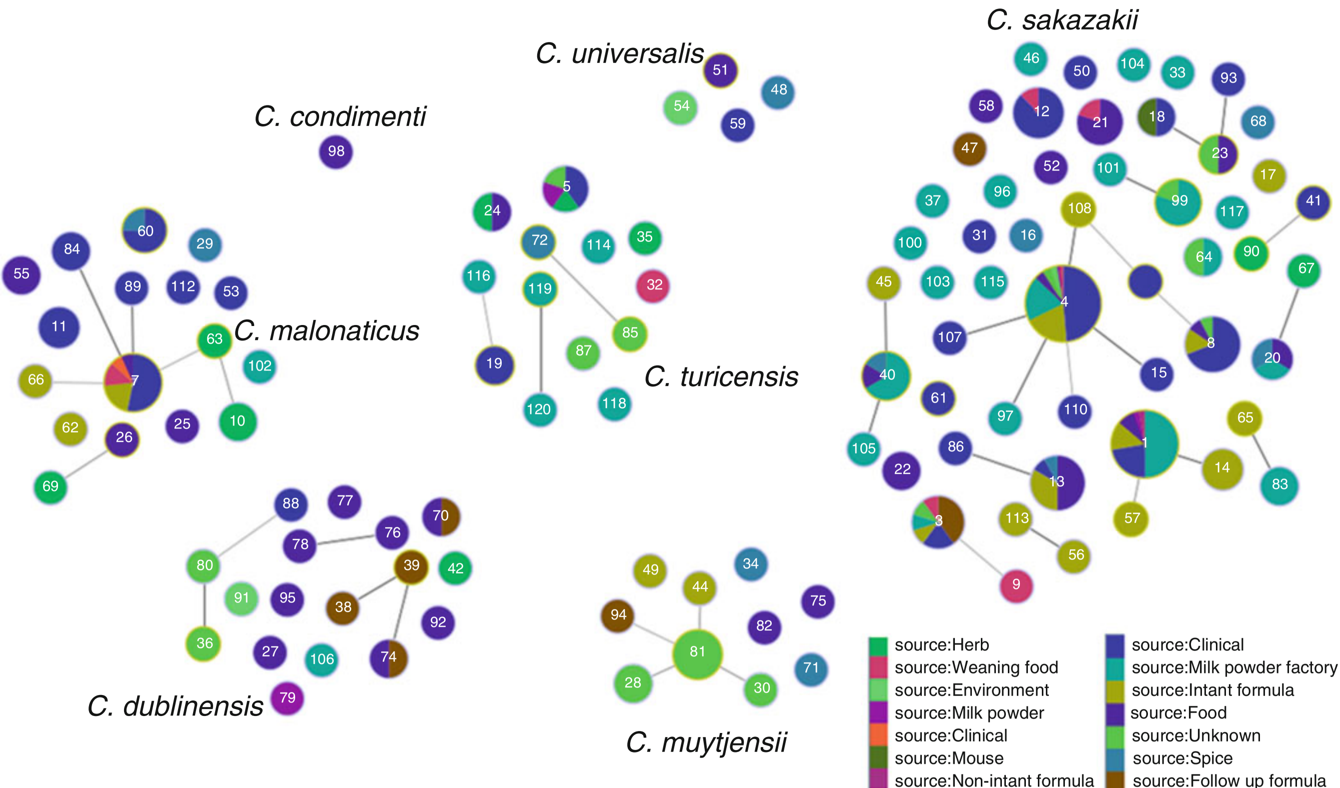

Fig. 2 Population snapshot of the *Cronobacter* MLST database generated using the goeBURST algorithm, indicating the clonal complexes and the diversity of the sources of the strains. The threshold for the output was set to triple locus variation. The dominant STs are represented by the circles with larger diameters. Image from Joseph and Forsythe (2012). Copyright retained

this clonal complex has been reported to be frequently isolated from milk powder factories and PIF processing plants in Ireland, Switzerland, Germany and Australia (Power et al. 2013; Müller et al. 2013; Sonbol et al. 2013). Power et al. (2013) reported a particularly persistent thermotolerant *C. sakazakii* ST4 strain (SP291), being isolated from a PIF manufacturing plant. Sonbol et al. (2013) reported that 24 % of strain isolates from the environment of six milk powder manufacturing plants in Australia and Germany were *C. sakazakii* ST4. Indistinguishable *C. sakazakii* ST4 strains were isolated from both the manufacturing plant and finished PIF product (Müller et al. 2013).

This remarkable discovery indicates that severity of illness appears to be linked to the clonal lineage of the infecting *Cronobacter* strain and gives a clear direction for further *Cronobacter* research. Applying MLST to non-clinical strains has shown that *C. sakazakii* CC4 was isolated from milk powder in 1951, hence giving us a 60-year timescale of such isolates. To date, there does not appear to be such a clear link between sequence type and other *Cronobacter*-associated infections such as necrotising enterocolitis.

Pathogenicity of **Cronobacter** *spp. and Infant Infections*

Cronobacter strains vary in their virulence, as determined by epidemiological studies and in-house mammalian tissue culture, but their virulence mechanisms are poorly understood. The bacteria can attach to intestinal cells and survive in macrophages (Townsend et al. 2007), but the specific receptors involved remain to be determined. To date, only strains from *C. sakazakii*, *C. malonaticus* and *C. turicensis* have been associated with neonatal infections.

Cronobacter spp. have been shown to invade human intestinal cells, replicate in macrophages and invade the blood–brain barrier (Townsend et al. 2007). Comparative analysis of sequenced genomes for strains across the seven species has been used to describe a range of virulence traits in *Cronobacter* spp., including iron acquisition mechanisms, fimbriae and macrophage survival (Kucerova et al. 2010, 2011; Joseph et al. 2012a, c). The sequenced genomes of *Cronobacter* species have revealed an array of adhesins, outer-membrane proteins, efflux systems, iron-uptake mechanisms, haemolysins and type VI secretion systems virtually. Yet, there is considerable diversity within the genus. Sialic acid utilisation is unique to *C. sakazakii* (Joseph et al. 2012c, 2013b) and this may be an important virulence trait since sialic acid is found in breast milk, infant formula, gut mucosa and brain cells, corresponding to the clinical presentations of infection. This may, in part, explain why the *C. sakazakii* species predominates clinical neonatal isolates.

Infections, not necessarily foodborne, with *Cronobacter* spp. occur across all age groups, and most infections, albeit less severe, are in the adult population (FAO-WHO 2008). However, neonates, particularly those of low birthweight, are the major identified group at risk, because the organism can cause meningitis, necrotising enterocolitis (NEC) and sepsis in patients and has a high infant mortality

rate (Bar-Oz et al. 2001; Lai 2001; Caubilla-Barron et al. 2007; Bowen and Braden 2008). Infections with these presentations result in exceptionally high mortality rates, ranging from 40 to 80 % (Lai 2001). In recent years, some outbreaks of bacterial infection in NICUs have been traced to powdered formula contaminated with *Cronobacter* spp. (Table 3). In the USA, the reported *Cronobacter* spp. infection incidence rate is *ca.* 1 per 100,000 infants. This incidence rate increases to 9.4 per 100,000 in infants of very low birthweight, i.e. <1.5 kg (Stoll et al. 2003).

The first neonatal deaths attributed to *Cronobacter* spp. were in 1958. Since then, according to the FAO-WHO (2008), around the world there have only been 120 documented cases of neonatal and infant *Cronobacter* spp. infections and 27 deaths. This, undoubtedly, is an underestimate, due to unreported and misidentified cases. Fatal infant infections have followed cases of necrotising enterocolitis (NEC), septicaemia and meningitis. Infections in older age groups are principally bacteraemias as well as urosepsis and wound infections. NEC is non-invasive (and is multifactorial), whereas in septicaemia and meningitis, the organism has attached and invaded the body, presumably through the intestinal epithelial layer. Due to the understandable sensitivity towards neonatal infections, this aspect of the bacterium has been emphasised more than infections in other age groups.

NEC is a common gastrointestinal illness in neonates and can be caused by a variety of bacterial pathogens. The incidence of NEC is 2–5 % of premature infants and 13 % in those weighing <1.5 kg at birth. It is 10 times more common in infants fed formula milk compared with those fed breast milk. Necrotising enterocolitis due to *Cronobacter* spp. has a high mortality rate, 10–55 % of cases. In *Cronobacter* meningitis, there is gross destruction of the brain, leading, sadly, to either death (40–80 % of cases) or severe neurological damage. The pathogenesis of the meningitis is different to that due to *Neisseria meningitidis* and neonatal meningitic *E. coli* and is similar to that of the closely related bacterium *Citrobacter koseri.* Infants can be colonised by more than one strain of *Cronobacter* (Caubilla-Barron et al. 2007), and in a number of outbreaks, there have been asymptomatic neonates, which could act as a reservoir for the organism. Therefore, during epidemiological studies, a number of isolates should be profiled from a number of sources. In some cases, the organism has been excreted from infants with persistent diarrhoea for a number of months (Block et al. 2002; Hariri et al. 2013).

Sources of Cronobacter *spp.*

Table 2 summarises the range of foods and food ingredients from which *Cronobacter* spp. have been isolated. As already covered, early surveillance studies used methods which probably underestimated the organism's prevalence and concentration in food, and therefore, the incidence frequency should only be regarded as indicative.

The organism is widespread in the natural environment. One probable ecosystem for *Cronobacter* spp. is plant material, as it has been isolated from a wide range of plant products and plant-derived food ingredients: cereals, wheat, corn, soy, rice,

Table 2 Survey of dry food products and food ingredients from which *Cronobacter* spp. have been isolated

Food product or ingredient	Number of positive samples	Total number of samples	%	Reference
Follow-up formula	10	74	14	Estuningsih et al. (2006)
Follow-up formula	3	91	3	Chap et al. (2009)
Cereal-based follow-up formula	6	100	6	Kim et al. (2011)
Dry infant food	5	49	10	Iversen and Forsythe (2004)
Dry infant food	24	199	12	Chap et al. (2009)
Dry infant cereals	2	6	33	Restaino et al. (2006)
Milk powder	3	72	4	Iversen and Forsythe (2004)
Milk powder	2	20	10	Hochel et al. (2012)
Milk powder and derived products	1	55	2	Turcovský et al. (2011)
Starches	40	1,389	3	FAO-WHO (2004)
Corn, soy, wheat and rice	14	78	18	Restaino et al. (2006)
Rice flour	6	16	38	Hochel et al. (2012)
Saengsik	41	86	48	Park et al. (2010)
Dry food ingredients	15	66	23	Iversen and Forsythe (2004)
Herbs and spices	40	122	33	Iversen and Forsythe (2004)
Herbs and spices	26	67	39	Jaradat et al. (2009)
Spices	13	21	62	Turcovský et al. (2011)
Spices	14	71	20	Hochel et al. (2012)
Spices and dried herbs	7	26	27	Baumgartner et al. (2009)
Dried powdered vegetables	1	50	2	Kim et al. (2008)
Nuts	2	2	100	Turcovský et al. (2011)
Instant soups	2	13	15	Turcovský et al. (2011)
Instant soups	6	10	60	Hochel et al. (2012)
Lentils	1	11	9	Hochel et al. (2012)
Oat flakes	1	10	10	Hochel et al. (2012)
Wheat sprout	1	9	11	Hochel et al. (2012)
Tea	3	5	60	Turcovský et al. (2011)
Confectionary	3	42	7	Baumgartner et al. (2009)
Chocolate products	11	37	30	Turcovský et al. (2011)
Seeds	14	34	41	Hochel et al. (2012)
Desiccated coconut	1	10	10	Hochel et al. (2012)
Coconut biscuits	1	1	100	Hochel et al. (2012)
Dried fish (inc. shrimp)	13	50	26	Kim et al. (2008)
Sunsik	17	36	47	Kim et al. (2008)
Tofu	4	11	36	Hochel et al. (2012)
Desiccated foods	18	115	16	Chon et al. (2012)

starch, herbs and spices, vegetables and salads (Iversen and Forsythe 2003; Table 2). Rats and flies may be additional sources of contamination (Pava-Ripoll et al. 2012). The organism has been isolated from a range of other foods, including meats, cheese and milk powder. It is notable that, when *E. sakazakii* was defined, it included a strain which had been isolated from dried milk in 1950. Therefore, *Cronobacter* spp. have been present in dried milk products for many decades. The organism is also recovered from water and seafood (Table 2). Asymptomatic human carriage has also been reported (Block et al. 2002). The bacterium has been isolated from the hospital environment and clinical samples: cerebrospinal fluid, blood, bone marrow, sputum, urine, faeces, inflamed appendix, neonatal enteral feeding tubes and conjunctivae (Hurrell et al. 2009). Nevertheless, it is its presence in PIF which has raised the most concern (Forsythe 2005; Himelright et al. 2002; Van Acker et al. 2001). Unlike commercially ready-to-feed liquid formula, PIF are not sterile and must conform to national and international microbiological criteria (CAC 2008) as will be covered later.

A number of outbreaks of *Cronobacter* spp. have been reported in neonatal intensive care units (NICUs), and such infections have been directly linked to reconstituted PIF, which may have been contaminated intrinsically or during preparation and administration. A common feature, in some of these outbreaks, is the opportunity for temperature abuse of the prepared feed, which would permit bacterial growth. It is pertinent to note that the bacterium is isolated from the tracheae and has been recovered from the feeding tubes of neonates fed breast milk and ready-to-feed formula, not reconstituted infant formula. Therefore, wider sources of the organism during a NICU outbreak need to be investigated, not just the reconstituted PIF and original powder. Infants can be colonised by more than one strain of *Cronobacter*, and therefore, multiple isolates need to be characterised in epidemiological investigations (Caubilla-Barron et al. 2007).

Cronobacter *spp. in PIF, Follow-Up Formula and Milk Powder Manufacturing Plants*

PIF has different microbiological criteria depending upon whether it is intended for infants less than 6 months or more than 6 months. *Cronobacter* spp. being specified as absent in 30 × 10 g quantities in PIF intended to be consumed by infants <6 months and not for formula to be consumed by older infants. In some countries, the latter type of PIF is marketed as “follow-up” or “follow-on” formula. Due to the commonality in composition, these two products may be processed on the same production line and, if this was the case, then strict environmental control and hold and test sampling would be applied by the PIF manufacturer to reduce the chance of microbiological contamination. As shown in Table 2, an international study reported that *Cronobacter* spp. were isolated from 3/91 (3 %) of “follow-up formulas” compared

to 24/199 (12 %) of infant foods and drinks, which is more frequent than that isolated from PIF for the younger age group (Chap et al. 2009).

The production of PIF involves a number of procedures, simplified as mixing of ingredients, homogenisation, pasteurisation treatment, spray drying and packaging. The exact manufacturing process varies between products and manufacturers and may involve wet mixing or dry mixing. Intrinsic contamination could occur prior to packaging the finished product (Müller et al. 2013). Reducing the survival, growth and colonisation opportunities for *Cronobacter* spp. in production facilities requires environment control, including zoning to physically separate high- and low-hygiene areas, maintaining a low-moisture environment (reducing water ingress), effective cleaning routines and control of dust and waste powder (Cordier 2008). PIF ingredients that may contain *Cronobacter* spp. include starch, rice flour, oat flour and maltodextrin (FAO-WHO 2006; Fu et al. 2011; Cetinkaya et al. 2012). Although there are heating steps, which would be expected to kill *Cronobacter* spp., the organism is more heat tolerant in the desiccated state. *Cronobacter* spp. can survive spray drying, and *C. sakazakii* ST4 has been isolated from spray-dried samples (Sonbol et al. 2013).

The spatial distribution and prevalence of *Cronobacter* spp. in milk powder and infant formula processing environments has been reported several times. Isolates have been profiled using PFGE, BOX-PCR and MLST. Mullane et al. (2007) profiled strains which had been isolated over a 1-year period from PIF and the processing environment. The bacterium was isolated from 2.5 % of intermediate and final products and 31 % at specific locations in the processing environment. PFGE was used to genotype the isolates, and 19 pulsetypes, which formed six clusters, were identified. No particular pulsetypes were associated with a particular location. Proudy et al. (2008) genotyped, using BOX-PCR, 200 *Cronobacter* isolates which had been recovered from a PIF factory. The majority (70 %) were deemed clonally related, indicating the persistence of a particular strain in the processing environment. Some contamination of the finished product was traced to the dry blending of ingredients. A number of studies have shown that the supply air is a potential vehicle for *Cronobacter* contamination and confirmed that the organism is dispersed widely in milk powder factories (Mullane et al. 2008; Hein et al. 2009; Craven et al. 2010; Reich et al. 2010; Jacobs et al. 2011). These early studies on strain persistence should be considered along with more recent observations of *C. sakazakii* ST4 presence in manufacturing plants and finished products.

Craven et al. (2010) identified 49 pulsetypes for 126 isolates of *Cronobacter* spp. from 100 locations of five milk powder factories in Australia; there were additional strains which could not be pulsetyped. Similarly, Jacobs et al. (2011) analysed environmental and final product samples from a milk powder manufacturing plant over a 4-year period (2005–2009) in Germany. Eighty-one *Cronobacter* strains were isolated from the spray-drying area and the roller-drying area. These were divided into 13 pulsetypes. Although the original studies by Craven et al. (2010) and Jacobs et al. (2011) did not speciate the *Cronobacter* strains, these have now been reanalysed using MLST (Sonbol et al. 2013). It revealed that *C. sakazakii* was the predominant *Cronobacter* species isolated from the 6 factories and that nearly a third of the strains (21/72) were *C. sakazakii* clonal complex ST4, the lineage associated

with neonatal meningitis. These strains were isolated from various locations, including factory roof, tanker bay, roller dryer and drying tower. The study by Sonbol et al. (2013) also included strains from Muytjens et al. (1988), and some of these were also *C. sakazakii* ST4. Hence, this has been a major *C. sakazakii* clonal lineage over the past 60 years in several parts of the world.

Physiological Attributes of Cronobacter *spp. Tolerance to Stress*

Desiccation Tolerance and Osmotolerance

Compared with *Salmonella*, there have only been a limited number of studies on *Cronobacter* spp. persistence in low-moisture food products. This is surprising, given the organism is primarily associated with neonatal infections through powdered infant formula. Arku et al. (2008) reported the organism can survive in milk during spray drying. In this study, the inlet and outlet dryer temperatures were 160 and 90 °C, respectively, and the organism was recovered after the dried milk was stored for 12 weeks at ambient temperature, demonstrating the persistence of the bacterium under an additional stress condition.

Cronobacter spp. can survive for prolonged periods in infant formula and cereals (Caubilla-Barron et al. 2007; Gurtler and Beuchat 2005, 2007). Their persistence depends upon the storage conditions with longer persistence in infant formulas with low water activities, a_w 0.25–0.30, and at low storage temperature (4 °C). The survival rate is irrespective of the formula types, i.e. milk based or soybean based. Populations of the bacterium survived in infant cereals at 4 °C for up to 12 months at low water activities (a_w 0.30–0.69), but their viability decreased at higher water activity (a_w 0.82–0.83). The survival was also affected by the storage temperature, with higher numbers of *Cronobacter* spp. surviving in cereals (a_w 0.63–0.83) stored at 4 °C than at 21 or 31 °C. Similar to persistence in infant formula, the organism's survival in infant cereal was not affected by composition.

Caubilla-Barron and Forsythe (2007) and Gurtler and Beuchat (2007) have studied the persistence of *Cronobacter* spp. under desiccated conditions. Caubilla-Barron and Forsythe (2007) showed that, in desiccated infant formula, some encapsulated strains of *Cronobacter* were still recoverable after 2.5 years. The viability of the organism decreased during the first month an average of 0.58 log_{10} cycles (0.26–1.15 log_{10} cycles). This value is similar those reported by Gurtler and Beuchat (2007). A larger decrease was observed during the first 6 months, where recovery of the organism declined by 3.34 log cycles. The average recovery then decreased by a further 1.88 log_{10} cycles over the next year. Overall, there was a total decline in the viable counts by 4.52 log_{10} cycles. Unlike the rest of the *Cronobacter* strains in the study, the *C. sakazakii* type strain, NCTC 11467^T, was no longer recoverable after 12 months. Whether this greater desiccation susceptibility is strain specific or typical of the ST8 clonal lineage is uncertain. After 2 years, four of the five recoverable *Cronobacter* strains were encapsulated, and after a further 6 months, the only

recoverable strains were encapsulated. Therefore, capsule production may be an environmental fitness trait, which enables prolonged persistence under low water activity conditions.

Riedel and Lehner (2007), who used a proteomic approach to study *C. sakazakii* strain z236, reported a large number of proteins associated with desiccation resistance and osmotic stress adaptation. However, only these few detailed laboratory studies have been undertaken on *Cronobacter* response to desiccation, despite its obvious relevance to the association of infection through the ingestion of reconstituted PIF. Consequently, our understanding of the organism's ability to persist for prolonged periods in the desiccated state and factors which may enhance this persistence is very limited.

Microbial bioinformatics has enabled some insight into this topic through the recent whole-genome sequencing of all ten species in the *Cronobacter* genus and the role of homologous genes in related organisms, i.e. *Salmonella* and *E. coli*. A specific study of osmotolerance gene homologues has been published by Feeney and Sleator (2011) which goes into more detail than can be covered in this chapter and should be consulted for more information. Their study was based on the earlier *C. sakazakii* BAA-894 genome and comparative genome hybridisation study of Kucerova et al. (2010). Since then, genomes representing all species have been published and await similar detailed studies (Joseph et al. 2012c). A number of key stress response genes have been identified in the *Cronobacter* genomes. A gene encoding a homologue of the universal stress protein *uspA* was detected in all *Cronobacter* species. Similarly, homologues have been found for stringent starvation response, carbon starvation sensing protein *rspA* and carbon starvation protein in all *Cronobacter* species. *Cronobacter* is known for its ability to survive desiccation for up to 2 years (Caubilla-Barron and Forsythe 2007; Osaili and Forsythe 2009).

In response to low water availability, various stress responses are switched on in bacteria to increase the intracellular osmotic pressure. These include increased internal concentration of potassium ions, and its counter ion glutamic acid, and the synthesis of compatible solutes glycine, betaine, proline, carnitine and trehalose. These osmoprotectants are polar, highly soluble compounds which can counteract osmotic pressure and drying by stabilising proteins and membranes. Genes involved in desiccation resistance and osmotic stress adaptation have been located in the genomes of all *Cronobacter* species (Kucerova et al. 2010; Feeney and Sleator 2011; Joseph et al. 2012c).

Three low-affinity K^+ transport systems (TrkG, TrkH, Kup) have been found in the genome of *C. sakazakii* BAA-894. One of these systems, TrkG, is encoded on the large plasmid (pESA3) instead of the chromosome and is absent in the species type strain ATCC 29544^T. The low-affinity transport systems are encoded close to a large-conductance mechanosensitive channel (MscL), which could be co-regulated. This is one of three such channels (MscL, S and K) encoded by *Cronobacter* spp., which enables the bacterial cell to efflux osmolytes in response to a hypo-osmotic shock and consequently to reduce turgor pressure in the cytoplasm. There is also one high-affinity system (Kdp) which has the unusual feature of using the alternative

start codon "GTG." This has been proposed by Feeney and Sleator (2011) as having a role in transcriptional regulation.

Seven putative genes encoding for the osmoregulated permease ProP have been found in the *Cronobacter* genome. This is a transmembrane protein for the uptake of osmoprotectants from the bacterial environment. Whether these contribute to the organism's desiccation survival has not been confirmed. A *proU* operon consisting of *proVWX* is also encoded on the genome. This is an ATP-requiring multicomponent osmoprotectant uptake system. Furthermore, the genome encodes for the osmoprotectant ABC transporter OpuC (Feeney and Sleator 2011). This operon encodes for three components: OpuCA (ATP-binding protein), OpuCC (extracellular substrate-binding protein) and OpuCB/CD (two membrane-associated proteins). As well as encoding for the uptake of osmoprotectants, *Cronobacter* also encodes for the de novo synthesis of proline, betaine and trehalose and is probably under translational control.

Thermotolerance

There are two reasons for considering the thermotolerance of *Cronobacter* spp. as a significant physiological trait in the context of its presence in desiccated foods. Firstly, spray drying involves temperatures normally high enough to kill vegetative bacteria in the dryer injection stream, and secondly, reconstitution of PIF may involve the use of hot water which may reduce the impact of post-dryer environmental contamination. Initially, *Cronobacter* spp. were reportedly the most thermotolerant Enterobacteriaceae (Nazarowec-White and Farber 1997), and although the authors later clarified this statement, there has been a tendency to link the reported "unusual" thermotolerance with survival during PIF production and therefore presence in the product. In support of this, Arku et al. (2008) reported that the organism can survive spray drying, and in their study the inlet and outlet temperatures were 160 and 90 °C, respectively. However, due to crust formation of the powder particles, the organism was effectively exposed to dry and not wet heat, and these are not comparable.

Furthermore, thermal tolerance in *Cronobacter* spp. has been controversial due to conflicting reports. Williams et al. (2005) used mass spectrometry to detail the proteins expressed in 12 *Cronobacter* spp., seven of which were later identified as *C. sakazakii* (6) and *C. muytjensii* (1). Three of the thermotolerant *C. sakazakii* strains (ST4 and ST8) expressed a protein which was a homologue of a hypothetical protein in the thermal-tolerant bacterium *Methylobacillus flagellatus* (KT). This protein was up-regulated in response to desiccation stress in *C. sakazakii* z236 (Riedel and Lehner 2007) and is absent in the genome-sequenced ST1 strain, *C. sakazakii* BAA-894 (Kucerova et al. 2010). The presence of a protein homologue to *Methylobacillus flagellatus* KT protein MFlag020121 in some *Cronobacter* strains correlated with thermal resistance, according to Williams et al. (2005). Further studies have shown that the homolog is present in certain *C. sakazakii* lineages. It is also present in *E. cloacae, C. braakii, C. freundii* and *E. vulneris* and not *E. aerogenes, E. amnigenus, E. cancerogenus, E. gergoviae, C. koseri* or *E. coli,* but

no experimental correlation with thermal resistance has been attempted (FSA 2009). Currently, no function has been directly attributable to the protein and the link with thermal resistance is uncertain. The initiation translation factor (InfB) is proposed by Asakura et al. (2007) to be a marker to "unequivocally detect and identify heat-resistant clones for epidemiological purposes," as they reported it is highly expressed in the heat-tolerant strains. This has not been followed up by any further studies. The *infB* gene is found in all *Cronobacter* spp., and the authors also reported the sequence variation, which is one of the alleles used in the *Cronobacter* spp. MLST scheme. In contrast, the proposed large thermoresistance gene cluster (or most components) identified by Gajdosova et al. (2011) is only present in certain strains of *C. sakazakii* and *C. malonaticus* (Joseph et al. 2012c). Unfortunately, this region is poorly annotated and the association with thermal resistance is currently not supported by experimental data in these strains. Together with biofilm formation, the ability to adapt and persist under stressed conditions enables the bacterium to survive in dry food ingredients.

The FAO-WHO (2004, 2006) and WHO (2007) recommend that PIF should be reconstituted with hot water (>70 °C). This would reduce the risk of *Cronobacter* infections, as it would reduce the general bacterial load. It is, therefore, important to consider the effect of desiccation on thermal tolerance in case the prior history of the organism affects its susceptibility to heat. It should be pointed out that scald-associated injury would result unless the hydrated and heated formula is appropriately cooled prior to feeding it to infants. The difficulties of preparing feed in this manner in neonatal intensive care units are further considered by Holý and Forsythe (2014).

Osaili et al. (2009) found that environmental stresses decreased the thermal resistance of *C. sakazakii* and *C. muytjensii*. For example, extended dry storage of *Cronobacter* spp. in infant milk formula increased its susceptibility to heat during rehydration with hot water. Further studies by Shaker et al. (2008) determined the effect of desiccation on the thermal inactivation of *C. sakazakii* and *C. muytjensii* in reconstituted PIF. Stressed cells in reconstituted PIF were exposed to 52–58 °C for various time periods, which caused significant decrease in *D*-values. Therefore, there was an antagonistic affect of pre-stress on *Cronobacter* heat tolerance.

Salmonella Serovars

Salmonella is one of the most well-known and best-studied bacterial causes of foodborne illness. Although poultry products are the primary vehicle of infection, the organism can cause severe infection through a large number of other food sources, including dried milk powder and chocolate (Collins et al. 1968; Werber et al. 2005). Infants are prone to salmonellosis, with an incidence of 121.6 laboratory-confirmed infections per 100,000 infants (US data), which is *ca.* 8 times greater than the incidence in other age groups. In addition, infants can suffer substantial morbidity from invasive *Salmonella*, and there is a greater risk of complications from the diarrhoeal form of illness.

The *Salmonella* is a far better understood bacterial genus than *Cronobacter*, and there have been a larger number of studies regarding its control in low-moisture products, such as powdered milk, chocolate, peanut butter and cereals (GMA 2009; Podolak et al. 2010). Although *Salmonella* cannot grow in these products, a significant risk can occur through cross-contamination. This section will primarily be limited to the similarities between *Salmonella* with *Cronobacter* and less of a description of the organism itself. There have been a number of *Salmonella* outbreaks which have been traced to contaminated low-moisture foods, including milk powders, peanut butter, paprika-powdered potato chips, herbal teas and chocolate (Marth et al. 1969; Sheth et al. 2011; Sotir et al. 2009). Although most cases of salmonellosis are traced back to eggs, meat consumption and other food stuffs of animal origin, an increasing number of outbreaks are due to *Salmonella* contamination of plant-originated food stuffs (Krtinić et al. 2010). The organism can be found on the surface of plants, as the result of secondary contamination, but they can also occur in soil that is treated with a natural fertiliser, i.e. animal manure, a known *Salmonella* source, as well as contaminated water used for irrigation. Insects can also harbour *Salmonella* and *Cronobacter* (Crumrine et al. 1971; Kopanic et al. 1994; Pava-Ripoll et al. 2012). Therefore, pest control is an important part of food safety.

Salmonella *Serovars in Powdered Infant Formula-Associated Infant Infections*

There have been a number of outbreaks of *Salmonella* infection among infants that were attributable to contaminated PIF (Table 3). These infants had clinical presentations, including diarrhoea, bacteraemia and meningitis. These outbreaks are of particular concern since the consumed reconstituted PIF appeared to be in compliance with current international standards. Additionally, these products are in international trade, being distributed around the world. Therefore, the microbiological safety of PIF with respect to *Salmonella* was reconsidered at the same time as *Cronobacter* by FAO-WHO in 2004 and was also the subject of a published review by Cahill et al. (2008).

Reviews of the data showed that some outbreaks involved very low levels of *Salmonella* that would have been difficult to detect by the test procedures of that time. Also, the outbreaks of *S.* Tennessee and *S.* Virchow were the more rare lactose-fermenting strains (Table 3). Since products may be first screened for Enterobacteriaceae, any lactose-fermenting isolates might be assumed not to be *Salmonella* as it is a very unusual trait in the genus. Therefore, the isolate might not be characterised further, as there is a permissive number of Enterobacteriaceae in PIF. It is of note that many of the PIF-associated outbreaks were traced because the *Salmonella* strains were notable, for example, an uncommon serotype or phenotype (i.e. lactose fermentation). Therefore, these unusual traits may have drawn the attention of investigators. However, such cases may be missed as not all countries

Table 3 Outbreaks of foodborne pathogen infections associated with the consumption of contaminated powdered infant formula, infant cereals and milk powders

Product[a]	Year	Organism	Country	Number of cases[b]	Reference
PIF[c]	1986	*Cronobacter* spp.[d]	Iceland	3	Biering et al. (1989)
PIF	1988	*Cronobacter* spp.	USA	4	Simmons et al. (1989)
PIF	1998	*Cronobacter* spp.	Belgium	12	van Acker et al. (2001)
PIF	2001	*C. sakazakii*	USA	11	Himelright et al. (2002)
PIF	2004	*Cronobacter* spp.	France	9	Coignard et al. (2006)
PIF	1993	*S.* Tennessee	Canada, USA	>3	CDC (1993)
PIF	1996	*S.* Virchow	Spain	>48	Usera et al. (1996)
PIF	1996–1997	*S.* Anatum	UK and France	17	Threlfall et al. (1998)
PIF	2000	*S.* London	Korea	31	Park et al. (2004)
PIF	2004–2005	*S.* Agona	France	141	Brouard et al. (2007)
PIF	2008	*S.* Give	France	8	Jourdan et al. (2008)
PIF	2008	*S.* Kedougou	Spain	42	Rodriguez-Urrego et al. (2008)
Milk powder	1985	*S.* Ealing	UK	76	Rowe et al. (1987)
Milk powder	1973	*S.* Derby	Trinidad	3000	Weissman et al. (1977)
Milk powder	2004	*St. aureus*	China	150	Anon (2004)
Infant cereal	1995	*S.* Senftenberg	UK	5	Rushdy et al. (1998)

[a]Product may have been contaminated intrinsically or extrinsically. For details readers should consult the original article

[b]Number of cases includes asymptomatic subjects who were identified during the study and were therefore not ill

[c]PIF, powdered infant formula

[d]Originally identified as *Enterobacter sakazakii* and therefore *Cronobacter* species has not been determined

have an active surveillance capability, with analytical facilities for serotyping and genotyping (i.e. PFGE). Hence, *Salmonella* outbreaks, which are either geographically or temporally diffuse, will be difficult to detect and control. To the writer's knowledge, there are no data available which describes the distribution of salmonellae in PIF, but it is likely to be sporadic. An example of the difficulty in detecting *Salmonella* spp. is illustrated by the outbreak caused by *S*. Ealing (Rowe et al. 1987). The possible cause was a hole in the spray drier, which allowed the organism to migrate into the drier and contaminate the milk powder. Because this involved only intermittent contamination, it resulted in 33 laboratories initially being unable to find the organism in 4,554 samples from 658 batches of the product. Eventually, the organism was isolated from an opened packet of PIF obtained from an infected infant's home. Since the package had the manufacturer's production code, more focused testing of 267 unopened milk powder packages revealed the organism in four of them.

Desiccation Tolerance of Salmonella *Serovars*

Salmonella serovars are able to persist in the desiccated state. The maximum survival in dried milk is at a_w 0.05–0.20. They can survive for long periods on work surfaces and in foods with low moisture content, especially those with a high fat content. It is therefore important for food manufacturers to minimise numerous risk factors during processing. Plant processing areas can become contaminated due to dust through poor facility design and maintenance, poor equipment design for hygienic cleaning and opportunities for cross-contamination. *Salmonella* can survive for very long periods of time under dry conditions in foods such as chocolate, peanut butter and pepper. Janning et al. (1994) reported that the viability of *Salmonella* strains at a_w 0.2 and 22 °C only decreased by one log cycle over 248–1,351 days. Also *Salmonella* persistence under dry conditions is prolonged in the presence of sucrose (Hiramatsu et al. 2005). Ray et al. (1971) reported the persistence of *Salmonella* in dried milk products as up to 10 months. Caubilla-Barron and Forsythe (2007) compared the persistence of *Salmonella* Enteritidis and various *Cronobacter* spp. in desiccated infant formula. The initial culture size was very high (10^{11} CFU/ml), and after an initial die-off, *S.* Enteritidis persisted in the dry state and was detectable for up to 15 months.

Salmonella cells can adhere to hydrophobic and hydrophilic (i.e. stainless steel) surfaces. Kusumaningrum et al. (2003) showed that *Salmonella* can persist on dry stainless steel surfaces, and as a consequence, there could be transference of the organism to food, following contact. For example, *S.* Enteritidis at 10^5 CFU/cm^2 is recoverable from dry stainless steel for at least 4 days. It is uncertain if *Salmonella* cells produce biofilms in low-moisture environments, though they do under high-moisture conditions, along with cellulose production. Hence, ineffective wet cleaning techniques applied to otherwise dry equipment may lead to biofilm formation. Under stress conditions, *Salmonella* may enter a viable but non-culturable (VBNC) state as a survival strategy (Lesne et al. 2000; Oliver 2010). Whether this affects its pathogenicity capacity is unknown (Passerat et al. 2009).

Salmonella adapts to osmotic stress by the accumulation of betaine via specific transporters. The cells also show cross-resistance to other stresses such as dry heat, UV irradiation, hydrogen peroxide and ethanol (Gruzdev et al. 2011). This indicates the limitations of commonly used chemical and physical treatments used by the food industry to kill foodborne pathogens.

Thermotolerance of Salmonella *Serovars*

The thermal tolerance of *Salmonella* is dependent on many factors, including strain and serovar variation, prior growth conditions and recovery methods. Bacteria are more heat resistant when the water activity is lower than that which is required to support growth. The degree of increased thermoresistance varies according to the

organism and its local environment, i.e. the presence of sugar is protective. *Salmonella* in chocolate can require several minutes at 100 °C to reduce the viable count by one log cycle.

Other Enterobacteriaceae

The FAO-WHO (2004) and (2006) categorised the other Enterobacteriaceae and *Acinetobacter* spp. found in PIF as "Causality plausible, but not yet demonstrated" with respect to neonatal infections. Numerous species of Enterobacteriaceae have been isolated from PIF in various studies (Table 4). Fu et al. (2011) reported that *E. cloacae, E. agglomerans, C. sedlakii* and *K. pneumoniae* were also detected in the PIF samples. Giammanco et al. (2011) recovered *C. freundii* (initially misidentified as *Cronobacter* spp.) and *E. hormaechei* from 32 out of 75 PIF samples (representing 12 brands). Townsend et al. (2008) reported that a three-month outbreak in a NICU due to *E. hormaechei* had been misidentified as *Cronobacter* spp. Antibiotic-resistance profiles of the strains were determined and extended-spectrum β-lactamase (ESBL) activity was detected. In laboratory studies, all strains were shown to invade both gut epithelial and blood–brain barrier endothelial cells and to persist in macrophages. Due to inadequate identification schemes, they proposed that *E. hormaechei* might be an under-reported cause of bacterial infection, especially in neonates. Also, its isolation from various sources, including powdered infant milk formula, makes it a cause for concern and warrants further investigation.

Caubilla-Barron and Forsythe (2007) compared the persistence of various Enterobacteriaceae in desiccated infant formula for up to 30 months. The strains

Table 4 Gram-negative bacteria other than *Cronobacter* and *Salmonella* which have been isolated from PIF, follow-up formulas and weaning foods

Product	Organisms	References
Powdered infant formula	*Klebsiella ozaenae, K. pneumoniae, K. planticola*[a], *E. cloacae, Pantoea* spp., *Serratia ficaria, Rahnella aquatilis*, *E. hormaechei*, *C. freundii, Leclercia adecarboxylata, E. coli, E. vulneris, E. hermannii, Ewingella americana, Cedecea davisae, C. lapagei, Pseudomonas aeruginosa, Pasteurella pneumotropica/haemolytica* and *Stenotrophomonas maltophilia*	Iversen and Forsythe (2004), Oonaka et al. (2010)
Follow-up formula	*Pantoea* spp., *E. hermannii*, *E. cloacae*, *E. amnigenus, K. pneumoniae*, *Citrobacter* spp., *Serratia* spp., *E. coli* and *Acinetobacter baumannii*	Estuningsih et al. (2006), Chap et al. (2009)
Weaning foods	*Aeromonas sobria, K. pneumoniae, Pantoea* spp., *E. cloacae, Stenotrophomonas maltophilia, A. baumannii, P. oryzihabitans, C. amalonaticus, E. agglomerans, E. vulneris, E. coli, K. terrigena, Pantoea* spp., *S. ficaria, S. plymuthica, S. rubidaea, C. freundii, Leclercia adecarboxylata* and *E. hormaechei* subsp. *steigerwaltii*	Iversen and Forsythe (2004), Chap et al. (2009)

included typical Enterobacteriaceae that can be isolated from PIF: *Cronobacter* spp., *E. cloacae*, *S*. Enteritidis, *C. koseri*, *C. freundii*, *E. coli*, *E. vulneris, Pantoea* spp., *K. pneumoniae* and *K. oxytoca*. The initial culture size was very high at 10^{11} CFU/ml. The persistence of *Cronobacter* species has already been described above. They reported that the Enterobacteriaceae could be divided into four groups, with respect to their long-term survival in the desiccated state. Group 1 was composed of *C. freundii*, *C. koseri* and *E. cloacae*. These organisms were no longer recoverable after 6 months. Group 2 organisms were *S.* Enteritidis, *K. pneumoniae* and *E. coli* and could not be recovered after 15 months. The third group consisting of *Pantoea* spp., *K. oxytoca* and *E. vulneris* persisted over 2 years and some capsulated strains of *C. sakazakii* which were still recoverable after 2.5 years. The importance of the capsule in desiccation survival is supported by the persistence of capsulated strains of *K. oxytoca*, *E. vulneris* and *Pantoea* spp. over the 2-year period. Further, none of the ten *C. koseri* or *E. cloacae* strains were capsulated and did not persist over this period of time. Comparison of *Cronobacter* spp. with *C. koseri* is pertinent, as some reports suggest a similarity between the tropism of *Cronobacter* and *C. koseri* for invasion and infection of the central nervous system. It was noted that brain abscesses due to *Cronobacter* and *C. koseri* were morphologically similar and may be due to similar virulence mechanisms (Kleiman et al. 1981). The thermal resistance of these organisms varies considerably, with one strain of *E. hormaechei* isolated from a neonatal feeding tube reported being more thermoresistant than *S.* Senftenberg (FSA 2009).

Acinetobacter spp. have been isolated from PIF and are of potential concern, as strains may be resistant to a number of clinically important antibiotics. The organism is highly resistant to desiccation and can persist in PIF for several years (Juma, pers. comm). Wang et al. (2012) recovered *S. aureus* from sixteen out of 143 PIF samples (11.2 %) and 14 out of 224 infant rice cereal samples (6.3 %). The authors determined antibiograms for the *St. aureus* isolates. Over one third (35.2 %) of strains were resistant to three or more antimicrobials and in particular to erythromycin (75.9 % of strains). The strains were also screened for toxin production and sixty-three percent of strains were positive for one or more toxin, with Panton-Valentine leukocidin 1 (40.7 %), SEG (38.9 %) and SEC (18.5 %) predominating. PFGE revealed the strains were genetically diverse.

Regulatory and Advisory Issues

There have been a number of changes in regulatory and advisory matters related to PIF manufacture and preparation. These are summarised below with respect to the FAO-WHO, WHO and Codex Alimentarius Commission. However, issues related to labelling have been controversial and are regarded as outside the scope of this chapter and readers should consult their local governmental regulatory body for up-to-date information.

FAO/WHO (2004, 2006, 2008)

Although many *Cronobacter* outbreaks had occurred prior to 2001, it was the highly publicised NICU outbreak in Tennessee (USA) in 2001 which led to international response regarding the microbiological safety of PIF (Himelright et al. 2002). Ironically this outbreak was misattributed to PIF, when in fact the formula was not intended for use by infants less than 6 months in age but was used under the instruction of the dieticians in the neonatal intensive care ward to reduce the likelihood of NEC. Using pulse-field gel electrophoresis (PFGE) to genotype isolates, the strain in the cerebrospinal fluid was indistinguishable from that isolated from a previously unopened tin of powdered formula. Six other neonates in the intensive care unit were asymptomatically colonised by the strain and two more had respiratory infections. Hence, instead of a general "nosocomial"-attributed cause of infection, a source could be named and therefore a control measure could be applied. It should be noted, however, that not all neonatal cases of *Cronobacter* spp. infections have been linked to contaminated powdered formula. The organism has also been isolated from hospital air, breast milk, human intestines and throats. So, control of microbiological content of PIF will not necessarily eliminate the risk of neonate infection by this bacterium.

The first and second FAO-WHO meetings (2004 and 2006) reviewed the organisms associated with neonatal infections, those found in powdered infant formula and also those that had been epidemiologically linked. Subsequently, *Salmonella* and *Cronobacter* were designated Category A (clear evidence of causality), and others named Enterobacteriaceae and *Acinetobacter* were in Category B (causality plausible but not yet demonstrated). The prevalence of *Cronobacter* in powdered infant formula has been determined many times and varies between 2 and 14 % (Table 1). There are no published reports of *Cronobacter* in powdered infant formula exceeding 1 cell/g. In fact, the likely level is *ca.* 1 cell/100 g when it occurs. Hence, there is a need to consider opportunities for extrinsic bacterial contamination and multiplication.

To date, the heightened awareness of the organism has focussed on infant infections and has resulted in changes in the microbiological criteria for PIF and reconstitution procedures. In other words, there have been required changes on two sides of the same coin: manufacturing practices and hygienic preparation practices. Such requirements need regulatory enforcement and support but must be based on robust reliable information. Consequently, there have been three FAO-WHO risk assessment meetings on the microbiological safety of PIF (FAO-WHO 2004, 2006, 2008). Those individuals identified as being at high risk for *Cronobacter* infection are neonates (especially low birthweight) for whom their source of nutrition will be limited to breast milk, fortified breast milk or breast milk replacement. Hence, hygienic preparation of feed is essential due to their immature immune system and lack of competing intestinal flora. Key advice from these FAO-WHO risk assessments was that PIF should be reconstituted with water at >70 °C, minimise any storage time by not preparing formula in advance and, if storage for short periods is necessary, then

the temperature should be <5 °C. The high water temperature will drastically reduce the number of vegetative bacteria present, and minimising the storage period will reduce the multiplication of any surviving organisms.

These recommendations have been well addressed by the WHO "Guidelines for the safe preparation, storage and handling of powdered infant formula" (2007), which are available online and can be downloaded using the URL given in the Reference section. It should be noted, however, that the recommendation to reconstitute with hot water has not been adopted in many countries. In addition, the Committee on Nutrition of the European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN 2004) and the French Food Safety Agency (Afssa 2005) disagree with the recommendation due to concerns over scalding the baby and vitamin losses.

Codex Alimentarius Commission (2008): Microbiological Criteria

Consequently, the Codex Alimentarius Commission (CAC) started a review of the microbiological guidelines for powdered infant formula and, in 2004, the FAO-WHO undertook the first of three risk assessment evaluations of *Cronobacter*. At that time, the microbiological criteria for powdered infant formula had a strict two-class plan for *Salmonella* and a three-class plan for coliforms. *Cronobacter* would be within the coliform count. These criteria had been set in 1979, and the Tennessee outbreak prompted their re-evaluation. In fact, *Cronobacter* was not the only organism reviewed, with respect to possible infection through reconstituted infant formula. Since *Salmonella* infections of powdered infant formula had also been reported, the 2006 FAO-WHO meeting reviewed the current relevant knowledge. It was plausible that lactose-fermenting *Salmonella* colonies on commonly used isolation media could be overlooked. Despite the FAO-WHO risk assessment meetings, starting in 2004, the CAC microbiological criteria did not change until 2008. These criteria now apply to powdered infant formula for a target age of up to 6 months. The criteria were not applied for formula commonly known as "follow-on formula," which is used at weaning, as there was insufficient epidemiological evidence.

Other Low-Moisture Products

While this chapter is concerned with low-moisture foods, it is of interest to note the similarity between *Salmonella* and *Cronobacter* spp. extends beyond their presence in food. *Salmonella* can also colonise a variety of mammals, birds, reptiles, amphibians and insects. Because of its ubiquity, it can be present in many raw food materials. *Cronobacter* is probably a commensal in a portion of the human population, but its carriage by other organisms is unknown.

Salmonella-contaminated low-moisture food products have been associated with a number of salmonellosis outbreaks. The foods include chocolate, dried milk, cereal products, peanut butter and dried ingredients, such as black pepper, paprika and desiccated coconut. The presence of sucrose, and high fat, may have a synergistic effect on *Salmonella* persistence in low-moisture foods. *Cronobacter* has been isolated from many of the same types of foods as *Salmonella* and food ingredients (Iversen and Forsythe 2003). An extensive, though not exhaustive, list of dry products that *Cronobacter* spp. have been isolated from is given in Table 2.

Like *Cronobacter*, *Salmonella* can occur on herbs and spices, and if these are added directly to a food product without a kill step, then they could cause infections. An outbreak of *S.* Agona in Germany was attributed to contaminated aniseed. This ingredient had been used in the production of several types of herbal teas linked to the outbreak (Koch et al. 2005). There has been an outbreak due to *Salmonella*-contaminated paprika-powdered potato chips, which resulted in 1,000 cases of salmonellosis (Lehmacher et al. 1995). Children, aged less than 14 years, were the majority of cases. The outbreak was due to the relatively rare *Salmonella* serotypes *S.* Saintpaul, *S.* Rubislaw and *S.* Javiana. These were at levels of 0.04–0.45 cells/g in the snacks. These levels were sufficient to cause illnesses because of the high fat content of the food product, protecting the bacterial cells from stomach acidity. The infective dose was estimated at 4–45 organisms, with an attack rate of 1 in 10,000 exposed persons, and the organisms survived for 8 months on the product. More recently, an outbreak involving at least 69 cases was linked to another rare *Salmonella* serotype, *S.* Wandsworth. The source was traced to a contaminated puffed vegetable-coated ready-to-eat snack food (Sotir et al. 2009), which also contained *Cronobacter* spp. and *S.* Typhimurium. Subsequently, this product was also linked to 18 cases of *S.* Typhimurium infection. Flour would normally be regarded as an ingredient that would be heat treated during food production; however, it can contain *Salmonella* and *Cronobacter* (Joseph et al. 2012b; Cetinkaya et al. 2012) and may be used as a carrier for nutraceuticals and pharmaceuticals and as a bulking agent in foods for infants and the elderly. The FAO-WHO (2004) and (2006) reports show a reduction in starch contamination by *Cronobacter* spp. during this period.

In 1995 there were eight reported cases of *S.* Senftenberg infections in infants (Table 3). These were traced to one brand of baby cereal and were due to cross-contamination of heat-treated and non-heat-treated ingredients during processing (Rushdy et al. 1998). Kandhai et al. (2004) detected *Cronobacter* spp. in a cereal factory. Chap et al. (2009), in their international survey of *Cronobacter* incidence in follow-up formulas and weaning foods, recovered the bacterium from 24/199 (12 %) of infant foods and drinks, which was much higher than in follow-up formulas (3 %).

The presence of *Cronobacter* spp. in herbal teas has been reported by a number of researchers (Tamura et al. 1995; Chap et al. 2009; Stojanović et al. 2011). Given the probable plant-based ecosystem for *Cronobacter* spp. and preferential survival under desiccated conditions, the presence of *Cronobacter* spp. is not surprising. The largest study reported, to date, was by Stojanović et al. (2011), who isolated *C. sakazakii* from 48/150 (32 %) of samples. The organism can also persist in prepared tea at 21 and 37 °C. Since these tea-based products are sometimes given to

infants as medicine to settle intestinal disturbances, their presence in herbal remedies warrants further assessment of risk. Ironically, given the concern of *Cronobacter* spp. as a foodborne pathogen, the patent describing the production of a thickening agent used a strain of *Cronobacter* spp. isolated from Chinese tea (Scheepe-Leberkühne and Wagner 1986).

Another plant-based food product that can contain *Cronobacter* and *Salmonella* is chocolate. Kandhai et al. (2004) detected *Cronobacter* in two chocolate factories. Baumgartner et al. (2009) isolated *Cronobacter* strains with identical PFGE profiles from five samples of two types of confectionaries over an 11-month period, although whether the contamination occurred at the factory or the distributor could not be determined. Turcovský et al. (2011) isolated *C. sakazakii* and *C. malonaticus* from 11/37 chocolate products, and at least one strain was *C. sakazakii* ST4 (Joseph and Forsythe 2012; Joseph et al. 2012a). To the author's knowledge, the level of *Cronobacter* spp. in chocolate has not been published. Interestingly, in some cases of salmonellosis linked to contaminated chocolate, the level of *Salmonella* contamination was very low (1–3 cells/g); however, the fat content may protect the *Salmonella* from the gastric acid in the stomach. The fat content may also increase the organisms' thermal resistance during chocolate production. Cases of salmonellosis have been traced back to chocolate which had been manufactured several months previously. This protracted period for chocolate-associated outbreaks is due to the combination of many factors, including a long product shelf life, large distribution area, common exposure and the long survival period of the organism.

Whether *Cronobacter* spp. cause infections of non-neonates through foods other than PIF has not been established; however, there are parallels with *Salmonella,* with respect to potential presence in a wide range of foods, especially through plant-derived ingredients, as well as stress survival mechanisms, and protection from thermal treatments due to low-water content and fat content in the food matrix. The latter may also contribute to survival through the acidic environment of the stomach.

Conclusions

The FAO-WHO (2004) expert committee recommended that research should be promoted to gain a better understanding of the taxonomy, ecology, virulence and other characteristics of *Cronobacter* spp. in the context of powdered infant formula safety. While this has been addressed with respect to taxonomy, our knowledge of the organism's ecology and virulence are still rudimentary. This may be due to, in part, a perceived presumption of the organism being exclusively linked to powdered infant formula, instead of considering the wider environments that it has been isolated from. It is surprising, given the emphasis that has been placed on *Cronobacter* spp. in powdered infant formula, that there has been a dearth of detailed experimentation into the mechanisms of the organism's persistence under desiccated state. Yet this trait is central to the concern over this member of the Enterobacteriaceae, compared to the others which may also be recovered from PIF, and other dried food

products. In contrast, *Salmonella* is a much better understood organism and is well controlled by the food industry through validated detection methods and HACCP implementation, which includes the control of cross-contamination by airborne bacteria from contaminated powders. These control measures are also important to control the dispersal of *Cronobacter* spp. in food-processing environments.

References

van Acker J, de Smet F, Muyldermans G, Bougatef A, Naessens A, Lauwers S (2001) Outbreak of necrotizing enterocolitis associated with *Enterobacter sakazakii* in powdered milk formula. J Clin Microbiol 39:293–297

Aigbekaen BO, Oshoma CE (2010) Isolation of *Enterobacter sakazakii* from powdered foods locally consumed in Nigeria. Pakistan J Nutr 9:659–663

Afssa (Agence française de sécurité sanitaire des aliments) (2005) Hygiene recommendations for the preparation, handling and storage of feeding bottles. http://www.cclinparisnord.org/Guides/AFSSA_bib.pdf

Arku B, Mullane N, Fox E, Fanning S, Jordan K (2008) *Enterobacter sakazakii* survives spray drying. Intl J Dairy Tech 61:102–108

Asakura H, Morita-Ishihara T, Yamamoto S, Igimi S (2007) Genetic characterization of thermal tolerance in *Enterobacter sakazakii*. Microbiol Immunol 51:671–677

Baldwin A, Loughlin M, Caubilla-Barron J et al (2009) Multilocus sequence typing of *Cronobacter sakazakii* and *Cronobacter malonaticus* reveals stable clonal structures with clinical significance which do not correlate with biotypes. BMC Microbiol 9:223

Bar-Oz B, Preminger A, Peleg O, Block C, Arad I (2001) *Enterobacter sakazakii* infection in the newborn. Acta Paediatr 90:356–358

Baumgartner A, Grand M, Liniger M, Iversen C (2009) Detection and frequency of *Cronobacter* spp. (*Enterobacter sakazakii*) in different categories of ready-to-eat foods other than infant formula. Intl J Food Microbiol 136:189–192

Block C, Peleg O, Minster N, Bar-Oz B, Simhon A, Arad I, Shapiro M (2002) Cluster of neonatal infections in Jerusalem due to unusual biochemical variant of *Enterobacter sakazakii*. Eur J Clin Microbiol Infect Dis 21:613–616

Bowen AB, Braden CR (2008) *Enterobacter sakazakii* disease and epidemiology. In: Farber JM, Forsythe SJ (eds) Emerging issues in food safety. Enterobacter sakazakii. ASM Press, Washington, Chapter 4

Brady C, Cleenwerck I, Venter S, Coutinho T, De Vos P (2013) Taxonomic evaluation of the genus *Enterobacter* based on multilocus sequence analysis (MLSA). Syst Appl Microbiol 36:309–319

Brouard C, Espie E, Weill FX et al (2007) Two consecutive large outbreaks of *Salmonella enterica* serotype Agona infections in infants linked to the consumption of powdered infant formula. Pediatr Infect Dis J 26:148–152

Cahill SM, Wachsmuth IK, Costarrica Mde L, Ben Embarek PK (2008) Powdered infant formula as a source of *Salmonella* infection in infants. Clin Infect Dis 46:268–273

Caubilla-Barron J, Forsythe S (2007) Dry stress and survival time of *Enterobacter sakazakii* and other Enterobacteriaceae. J Food Prot 70:2111–2117

Caubilla-Barron J, Hurrell E, Townsend S, Cheetham P, Loc-Carrillo C, Fayet O, Prere M-F, Forsythe SJ (2007) Genotypic and phenotypic analysis of *Enterobacter sakazakii* strains from an outbreak resulting in fatalities in a neonatal intensive care unit in France. J Clin Microbiol 45:3979–3985

Centers for Disease Control and Prevention (CDC) (1993) *Salmonella* serotype Tennessee in powdered milk products and infant formula – Canada and the United States. MMWR 42:516–517

Cetinkaya E, Joseph S, Ayhan K, Forsythe SJ (2012) Comparison of methods for the microbiological identification and profiling of *Cronobacter* species from ingredients used in the preparation of infant formula. Mol Cell Probes 27:60–64

Chap J, Jackson P, Siqueira R et al (2009) International survey of *Cronobacter sakazakii* and other *Cronobacter* spp. in follow up formulas and infant foods. Intl J Food Microbiol 136:185–188

Chen Y, Lampel K, Hammack K (2012) Cronobacter, chapter 29. In: Bacteriological analytical manual. U.S. Food and Drug Administration. http://www.fda.gov/food/foodscienceresearch/laboratorymethods/ucm289378.htm

Chon J-W, Song K-Y, Kim S-Y, Hyeon J-Y, Seo K-H (2012) Isolation and characterisation of *Cronobacter* from desiccated foods in Korea. J Food Sci 77:M354–M358

Codex Alimentarius Commission (2008) Code of hygienic practice for powdered formulae for infants and young children. CAC/RCP 66-2008

Coignard B, Vaillant V, Vincent J-P, Leflèche A, Mariani-Kurkdjian P, Bernet C, L'Hériteau F, Sénéchal H, Grimont P, Bingen E, Desenclos J-C (2006) Infections sévères à *Enterobacter sakazakii* chez des nouveau-nés ayant consommé une préparation en poudre pour nourrissons. Bulletin Épidémiologique Hebdomadaire 2–3:10–13

Collins RN, Treger MD, Goldsby JB, Boring JR, Coohon DB, Barr RN (1968) Interstate outbreak of *Salmonella newbrunswick* infection traced to powdered milk. JAMA 203:838–844

Cordier J-L (2008) Production of powdered infant formulae and microbiological control measures. In: Farber JM, Forsythe SJ (eds) Emerging issues in food safety. *Enterobacter sakazakii*. ASM Press, Washington, DC, USA

Craven HM, McAuley CM, Duffy LL, Fegan N (2010) Distribution, prevalence and persistence of *Cronobacter* (*Enterobacter sakazakii*) in the nonprocessing and processing environments of five milk powder factories. J Appl Microbiol 109:1044–1052

Crumrine MH, Foltz VD, Harris JO (1971) Transmission of *Salmonella* Montevideo in wheat by stored-product insects. J Appl Microbiol 22:578–580

ESPGHAN Committee on Nutrition (2004) Preparation and handling of powdered infant formula: a commentary by the ESPGHAN Committee on Nutrition. J Pediatr Gastroenterol Nutr 39:320–322

Estuningsih S, Kress C, Hassan AA, Akineden Ö, Schneider E, Usleber E (2006) Enterobacteriaceae in dehydrated powdered infant formula manufactured in Indonesia and Malaysia. J Food Protect 69:3013–3017

Feeney A, Sleator RD (2011) An in silico analysis of osmotolerance in the emerging gastrointestinal pathogen *Cronobacter sakazakii*. Bioeng Bug 2:260–270

Fernández L, Langa S, Martín V, Maldonado A, Jiménez E, Martín R, Rodríguez JM (2013) The human milk microbiota: origin and potential roles in health and disease. Pharm Res 69:1–10

Food and Agriculture Organization-World Health Organization (FAO-WHO) (2004) *Enterobacter sakazakii* and other microorganisms in powdered infant formula, Meeting report, MRA series 6. World Health Organization, Geneva, Switzerland. http://www.who.int/foodsafety/publications/micro/mra6/en/index.html

Food and Agriculture Organization-World Health Organization (FAO-WHO) (2006) *Enterobacter sakazakii* and *Salmonella* in powdered infant formula, Second Risk Assessment Workshop. Meeting Report, MRA Series 10, World Health Organization, Geneva, Switzerland. http://www.who.int/foodsafety/publications/micro/mra10/en/index.html

Food and Agriculture Organization-World Health Organization (FAO-WHO) (2008) *Enterobacter sakazakii* (*Cronobacter* spp.) in powdered follow-up formulae. MRA series. http://www.who.int/foodsafety/publications/micro/MRA_followup.pdf

Food Standards Authority (FSA) (2009) Bacteriocidal preparation of powdered infant formula. http://www.foodbase.org.uk/results.php?f_category_id=andf_report_id=395

Forsythe S (2005) *Enterobacter sakazakii* and other bacteria in powdered infant milk formula. Mother Child Nutr 1:44–50

Fu S, Gao J, Liu Y, Chen H (2011) Isolation of *Cronobacter* spp. isolates from infant formulas and their survival in the production process of infant formula. Czech J Food Sci 29:391–399

Gajdosova J, Benedikovicova K, Kamodyova N, Tothova L, Kaclikova E et al (2011) Analysis of the DNA region mediating increased thermotolerance at 58 °C in *Cronobacter* sp. and other enterobacterial strains. Antonie Van Leeuwenhoek 100:279–289

Giammanco GM, Aleo A, Guida I, Mammina C (2011) Molecular epidemiological survey of *Citrobacter freundii* misidentified as *Cronobacter* spp. (*Enterobacter sakazakii*) and *Enterobacter hormaechei* isolated from powdered infant milk formula. Foodborne Pathog Dis 8:517–525

GMA (2009) Control of *Salmonella* in low-moisture foods. http://www.gmaonline.org/downloads/technical-guidance-and-tools/SalmonellaControlGuidance.pdf

Gosalbes JM, Llop S, Vallès Y, Moya A, Ballester F, Francino MP (2013) Meconium microbiota types dominated by lactic acid or enteric bacteria are differentially associated with maternal eczema and respiratory problems in infants. Clin Exp Allergy 43:198–211

Gruzdev N, Riky Pinto R, Sela S (2011) Effect of desiccation on tolerance of *Salmonella enterica* to multiple stresses. Appl Environ Microbiol 77:1667

Gurtler JB, Beuchat LR (2005) Performance of media for recovering stressed cells of *Enterobacter sakazakii* as determined using spiral plating techniques. Appl Environ Microbiol 71: 7661–7669

Gurtler JB, Beuchat LR (2007) Survival of *Enterobacter sakazakii* in powdered infant formula as affected by composition, water activity, and temperature. J Food Protect 70:1579–1586

Hariri S, Joseph S, Forsythe SJ (2013) Predominance of *Cronobacter sakazakii* ST4 clonal complex strains in *Cronobacter* neonatal meningitis infections in US 2011. Emerg Infect Dis 19:175–177

Hein I, Gadzov B, Schoder D, Foissy H, Malorny B, Wagner M (2009) Temporal and spatial distribution of *Cronobacter* isolates in a milk powder processing plant determined by pulsed-field gel electrophoresis. Foodborne Path Dis 6:225–233

Himelright I, Harris E, Lorch V, Anderson M (2002) *Enterobacter sakazakii* infections associated with the use of powdered infant formula – Tennessee, 2001. MMWR 51:298–300

Hiramatsu R, Masumoto M, Sakae K, Miyazaki Y (2005) Ability of shiga toxin-producing *Escherichia coli* and *Salmonella* spp. to survive in a desiccation model system and in dry foods. Appl Environ Microbiol 71:6657–6663

Hochel I, Růžičková H, Krásný L, Demnerová K (2012) Occurrence of *Cronobacter* spp. in retail foods. J Appl Microbiol 112:1257–1265

Holý O, Forsythe SJ (2014) *Cronobacter* species as emerging causes of healthcare-associated infection. J Hosptl Infect 86:169–177

Holý O, Jana Petrželová J, Vojtěch Hanulík V, Magdaléna Chromá M, Ivanka Matoušková I, Forsythe SJ (2014) Epidemiology of *Cronobacter* spp. isolates from hospital patients from the University Hospital Olomouc (Czech Republic). Epidemiol Mikrobiol Immunol 63:69–72

Hoque A, Ahmed T, Shahidullah M, Hossain A, Mannan A, Noor K, Nahar K, Ilias M, Ahmed D (2010) Isolation and molecular identification of *Cronobacter* spp. from powdered infant formula (PIF) in Bangladesh. Intl J Food Microbiol 142:375–378

Hurrell E, Kucerova E, Loughlin M et al (2009) Neonatal enteral feeding tubes as loci for colonisation by members of the *Enterobacteriaceae*. BMC Infect Dis 9:146

Iversen C, Forsythe S (2003) Risk profile of *Enterobacter sakazakii,* an emergent pathogen associated with infant milk formula. Trends Food Sci Technol 14:443–454

Iversen C, Forsythe SJ (2004) Isolation of *Enterobacter sakazakii* and other Enterobacteriaceae from powdered infant formula milk and related products. Food Microbiol 21:771–776

Iversen C, Mullane N, McCardell B et al (2008) *Cronobacter* gen. nov., a new genus to accommodate the biogroups of *Enterobacter sakazakii*. Intl J Syst Evol Microbiol 58:1442–1447

Ivy RA, Farber JM, Pagotto F, Wiedmann M (2013) International Life Science Institute North America *Cronobacter* (formerly *Enterobacter sakazakii*) isolate set. J Food Prot 76:40–51

Jacobs C, Braun P, Hammer P (2011) Reservoir and routes of transmission of *Enterobacter sakazakii* (*Cronobacter* spp.) in a milk powder-producing plant. J Dairy Sci 94:3801–3810

Janning B, In't Veld PH, Notermans S, Krammer J (1994) Resistance of bacterial strains to dry conditions: use of anhydrous silica gel in a desiccation model system. J Appl Bacteriol 77:319–342

Jaradat ZW, Ababneh QO, Saadoun IM, Samara NA, Rashdan AM (2009) Isolation of *Cronobacter* spp. (formerly *Enterobacter sakazakii*) from infant food, herbs and environmental samples and the subsequent identification and confirmation of the isolates using biochemical, chromogenic assays, PCR and 16S rRNA sequencing. BMC Microbiol 9:225

Jolley KA, Maiden MC (2010) BIGSdb: scalable analysis of bacterial genome variation at the population level. BMC Bioinformatics 11:595

Joseph S, Forsythe S (2011) Association of *Cronobacter sakazakii* ST4 with neonatal infections. Emerg Infect Dis 17:1713–1715

Joseph S, Forsythe SJ (2012) Insights into the emergent bacterial pathogen *Cronobacter* spp., generated by multilocus sequence typing and analysis. Frontiers Food Microbiol 3:397

Joseph S, Sonbol H, Hariri S, Desai P, McClelland M, Forsythe SJ (2012a) Diversity of the *Cronobacter* genus as revealed by multi locus sequence typing. J Clin Microbiol 50:3031–3039

Joseph S, Cetinkaya E, Drahovske H, Levican A, Figueras MJ, Forsythe SJ (2012b) *Cronobacter condimenti* sp. nov., isolated from spiced meat and *Cronobacter universalis* sp. nov., a novel species designation for *Cronobacter* sp. genomospecies 1, recovered from a leg infection, water, and food ingredients. Intl J Syst Evol Microbiol 62:1277–1283

Joseph S, Desai P, Li Y et al (2012c) Comparative analysis of genome sequences covering the seven *Cronobacter* species. PLoS One 7:e49455

Joseph S, Hariri S, Forsythe SJ (2013a) Lack of continuity between *Cronobacter* biotypes and species as determined using multilocus sequence typing. Mol Cell Probes 27:137–139

Joseph S, Hariri S, Masood N, Forsythe S (2013b) Sialic acid utilization by *Cronobacter sakazakii*. Microbial Inform Expt 3:3

Kandhai MC, Reij MW, Gorris LG, Guillaume-Gentil O, van Schothorst M (2004) Occurrence of *Enterobacter sakazakii* in food production environments and households. Lancet 363:39–40

Kleiman MB, Allen SD, Neal P, Reynolds J (1981) Meningoencephalitis and compartmentalization of the cerebral ventricles caused by *Enterobacter sakazakii*. J Clin Microbiol 14:352–354

Kim K, Jang SS, Kim SK, Park J-H, Heu S, Ryu S (2008) Prevalence and genetic diversity of *Enterobacter sakazakii* in ingredients of infant foods. Intl J Food Microbiol 122:196–203

Kim SA, Oh SW, Lee YM, Imm JY, Hwang IG, Kang DH, Rhee MS (2011) Microbial contamination of food products consumed by infants and babies in Korea. Lett Appl Microbiol 53:532–538

Koch JA, Schrauder A, Alpers K, Werber D, Frank C, Prager R, Rabsch W, Broll S, Feil F, Roggentin P, Bockemuhl J, Tschape H, Ammon A, Stack K (2005) *Salmonella* Agona outbreak from contaminated aniseed. Germany Emerg Infect Dis 11:1224–1127

Kopanic RJ, Sheldon BW, Wright CG (1994) Cockroaches as vectors of *Salmonella*: laboratory and field trials. J Food Protect 57:125–132

Krtinić G, Đurić P, Ilić S (2010) Salmonellae in food stuffs of plant origin and their implications on human health. Eur J Clin Microbiol Infect Dis 29:1321–1325

Kucerova E, Clifton SW, Xia XQ et al (2010) Genome sequence of *Cronobacter sakazakii* BAA-894 and comparative genomic hybridization analysis with other *Cronobacter* species. PLoS One 5:e9556

Kucerova E, Joseph S, Forsythe S (2011) *Cronobacter*: diversity and ubiquity. Qual Assur Safety Foods Crops 3:104–122

Kusumaningrum HD, Riboldi G, Hazeleger WC, Beumer RR (2003) Survival of foodborne pathogens on stainless steel surfaces and cross-contamination to foods. Intl J Food Microbiol 85:227–236

Lai KK (2001) *Enterobacter sakazakii* infections among neonates, infants, children, and adults. Case reports and a review of the literature. Medicine (Baltimore) 80:113–122

Lehmacher A, Bockemuhl J, Aleksic S (1995) Nationwide outbreak of human salmonellosis in Germany due to contaminated paprika and paprika-powdered potato chips. Epidemiol Infect 115:501–511

Lesne J, Berthet S, Binard S, Rouxel A, Humbert F (2000) Changes in culturability and virulence of *Salmonella typhimurium* during long-term starvation under desiccating conditions. Intl J Food Microbiol 60:195–203

MacLean LL, Vinogradov E, Pagotto F, Farber JM, Perry MB (2009) Characterization of the O-antigen in the lipopolysaccharide of *Cronobacter (Enterobacter) malonaticus* 3267. Biochem Cell Biol 87:927–932

Maiden MC, van Rensburg MJ, Bray JE, Earle SG, Ford SA, Jolley KA, McCarthy ND (2013) MLST revisited: the gene-by-gene approach to bacterial genomics. Nat Rev Microbiol 11:728–736

Martín R, Langa S, Reviriego C, Jiménez E, Marín ML, Olivares M, Boza J, Jiménez J, Fernández L, Xaus J, Rodríguez JM (2004) The commensal microflora of human milk: new perspectives for food bacteriotherapy and probiotics. Trends Food Sci Technol 15:121–127

Marth EH (1969) Salmonellae and salmonellosis associated with milk and milk products: a review. J Dairy Sci 52:283–315

Mullane NR, Whyte P, Wall PG, Quinn T, Fanning S (2007) Application of pulsed-field gel electrophoresis to characterise and trace the prevalence of *Enterobacter sakazakii* in an infant formula processing facility. Intl J Food Microbiol 116:73–81

Mullane NR, Healy B, Meade J, Whyte P, Wall PG, Fanning S (2008) Dissemination of *Cronobacter* spp. (*Enterobacter sakazakii*) in a powdered milk protein manufacturing facility. Appl Environ Microbiol 74:5913–5917

Müller A, Stephan R, Fricker-Feer C, Lehner A (2013) Genetic diversity of *Cronobacter sakazakii* isolates collected from a Swiss infant formula production facility. J Food Prot 76:883–887

Muytjens HL, Roelofs-Willemse H, Jaspar GH (1988) Quality of powdered substitutes for breast milk with regard to members of the family *Enterobacteriaceae*. J Clin Microbiol 26: 743–746

Nazarowec-White M, Farber JM (1997) Thermal resistance of *Enterobacter sakazakii* in reconstituted dried-infant formula. Lett Appl Microbiol 24:9–13

Oliver JD (2010) Recent findings on the viable but nonculturable state in pathogenic bacteria. FEMS Microbiol Rev 34:415–425

Oonaka K, Furuhata K, Hara M, Fukuyama M (2010) Powder infant formula milk contaminated with *Enterobacter sakazakii*. Jpn J Infect Dis 63:103–107

Osaili T, Forsythe S (2009) Desiccation resistance and persistence of *Cronobacter* species in infant formula. Intl J Food Microbiol 136:214–220

Osaili TM, Shaker RR, Al-Haddaq MS, Al-Nabulsi AA, Holley RA (2009) Heat resistance of *Cronobacter* species (*Enterobacter sakazakii)* in milk and special feeding formula. J Appl Microbiol 107:928–935

Ottman N, Smidt H, de Vos WM, Belzer C (2012) The function of our microbiota: who is out there and what do they do? Frontiers Cell Inf Microbiol 2:1–11

Park JK, Soek WS, Choi BJ et al (2004) *Salmonella enterica* serovar London infections associated with consumption of infant formula. Yonsei Med J 45:43–48

Park JH, Lee YD, Ryu TW, Chang HI (2010) Identification and classification of *Cronobacter spp.* isolated from powdered food in Korea. J Microbiol Biotechnol 20:757–762

Passerat J, Got P, Dukan S, Monfort P (2009) Respective roles of culturable and viable-but-nonculturable cells in the heterogeneity of *Salmonella enterica* serovar *typhimurium* invasiveness. Appl Environ Microbiol 75:5179–5185

Pava-Ripoll M, Goeriz Pearson RE, Miller AK, Ziobro GC (2012) Prevalence and relative risk of *Cronobacter* spp., *Salmonella* spp., and *Listeria monocytogenes* associated with the body surfaces and guts of individual filth flies. Appl Env Microbiol 78:7891–7902

Podolak R, Enache E, Stone W, Black DG, Elliott PH (2010) Sources and risk factors for contamination, survival, persistence, and heat resistance of *Salmonella* in low-moisture foods. J Food Protection 73:1919–1936

Poirel L, Nordmann P, De Champs C, Eloy C (2007) Nosocomial spread of QnrA-mediated quinolone resistance in *Enterobacter sakazakii*. Intl J Antimicrob Agents 29:223–224

Power KA, Yan Q, Fox EM, Cooney S, Fanning S (2013) Genome sequence of *Cronobacter sakazakii* SP291, a persistent thermotolerant isolate derived from a factory producing powdered infant formula. Genome Announc 1:e0008213

Proudy L, Bougle D, Leclercq R, Vergnaud M (2008) Tracing of *Enterobacter sakazakii* isolates in infant milk formula processing by BOX-PCR genotyping. J Appl Microbiol 105:550–558

Ray B, Jezeski JJ, Busta FF (1971) Isolation of salmonellae from naturally contaminated dried milk products. II. Influence of storage time on the isolation of salmonellae. J Milk Food Tech 34:423–427

Restaino L, Frampton EW, Lionberg WC, Becker RJ (2006) A chromogenic plating medium for the isolation and identification of *Enterobacter sakazakii* from foods, food ingredients, and environmental sources. J Food Prot 69:315–322

Riedel K, Lehner A (2007) Identification of proteins involved in osmotic stress response in *Enterobacter sakazakii* by proteomics. Proteomics 7:1217–1231

Rushdy A, Stuart JM, Ward LR, Bruce J, Threlfall EJ, Punia P, Bailey JR (1998) National outbreak of *Salmonella* Senftenberg associated with infant food. Epidemiol Infect 120:125–128

Reich F, König R, von Wiese W, Klein G (2010) Prevalence of *Cronobacter* spp. in a powdered infant formula processing environment. Intl J Food Microbiol 140:214–217

Reij MW, Aantrekker ED, ILSI Europe Risk Analysis in Microbiology Task Force (2004) Recontamination as a source of pathogens in processed foods. Intl J Food Microbiol 91:1–11

Rowe B, Hutchinson DN, Gilbert RJ, Hales BH, Beg NT, Dawkins HC, Jacob M, Rae FA, Jepson M (1987) *Salmonella ealing* infections associated with consumption of infant dried milk. Lancet 17:900–903

Santos RFS, da Silva N, Junqueira VCA, Kajsik M, Forsythe S, Pereira JL (2013) Screening for *Cronobacter* species in powdered and reconstituted infant formulas and from equipment used in formula preparation in maternity hospitals. Ann Nutr Metab 63:62–68

Scheepe-Leberkühne M, Wagner F (1986) Optimization and preliminary characterization of an exopolysaccharide synthesized by *Enterobacter sakazakii*. Biotech Lett 8:695–700

Shaker RR, Osaili TM, Abu Al-Hasan AS, Ayyash MM, Forsythe SJ (2008) Effect of desiccation, starvation, heat, and cold stresses on the thermal resistance of *Enterobacter sakazakii* in rehydrated infant milk formula. J Food Sci 73:M354–9.

Sheth AN, Hoekstra M, Patel N, Ewald G, Lord C, Clarke C, Villamil E, Niksich K, Bopp C, Nguyen TA et al (2011) A national outbreak of *Salmonella* serotype Tennessee infections from contaminated peanut butter: a new food vehicle for salmonellosis in the United States. Clin Infect Dis 53:356–362

Sonbol H, Joseph S, McAuley C, Craven H, Forsythe SJ (2013) Multilocus sequence typing of *Cronobacter* spp. from powdered infant formula and milk powder production factories. Intl Dairy J 30:1–7

Sotir MJ, Ewald G, Kimura AC, Higa JI, Sheth A, Troppy S, Meyer S, Hoekstra RM, Austin J, Archer J, Spayne M, Daly ER, Griffin PM (2009) Outbreak of *Salmonella* Wandsworth and Typhimurium infections in infants and toddlers traced to a commercial vegetable-coated snack food. Pediatr Infect Dis J 28:1041–1046

Stojanović MM, Katić V, Kuzmanović J (2011) Isolation of *Cronobacter sakazakii* from different herbal teas. Vojnosanit Pregl Mil Pharm Rev 68:837–841

Stoll BJ, Hansen N, Fanaroff AA, Lemons JA (2003) *Enterobacter sakazakii* is a rare cause of neonatal septicaemia or meningitis in VLBW infants. J Pediatrics 144:821–823

Szafranek J, Czerwicka M, Kumirska J, Paszkiewicz M, Łojkowska E (2005) Repeating unit structure of *Enterobacter sakazakii* ZORB A 741 O-polysaccharide. Pol J Chem 79:287–295

Tamura A, Kato M, Omori M, Nanba A, Miyagawa K, Wang CR, Zhou WH (1995) Flavor components and microorganisms isolated from Suancha (sour tea). Nippon Kasei Gakkaishi 46:759–764

Threlfall EF, Ward L, Hampton M et al (1998) Molecular fingerprinting defines a strain of *Salmonella enterica* serotype Anatum responsible for an international outbreak associated with formula-dried milk. Epidemiol Infect 121:289–293

Townsend SM, Hurrell E, Gonzalez-Gomez I, Lowe L, Frye JG, Forsythe S, Badger JL (2007) *Enterobacter sakazakii* invades brain capillary endothelial cells, persists in human macrophages influencing cytokine secretion and induces severe brain pathology in the neonatal rat. Microbiology 153:3538–3547

Townsend SM, Hurrell E, Caubilla-Barron J, Loc-Carrillo C, Forsythe SJ (2008) Characterization of an extended-spectrum beta-lactamase *Enterobacter hormaechei* nosocomial outbreak, and other *Enterobacter hormaechei* misidentified as *Cronobacter (Enterobacter) sakazakii*. Microbiology 154:3659–3667

Turcovský I, Kuniková K, Drahovská H, Kaclíková E (2011) Biochemical and molecular characterization of *Cronobacter* spp. (formerly *Enterobacter sakazakii*) isolated from foods. Ant Van Leeuwenhoek 99:257–269

Usera MA, Echeita A, Aladuen A et al (1996) Interregional foodborne salmonellosis outbreak due to powdered infant formula contaminated with lactose-fermenting *Salmonella virchow*. Eur J Epidemiol 12:377–381

Usera MA, Rodriguez A, Echeita A, Cano R (1998) Multiple analysis of a foodborne outbreak caused by infant formula contaminated by a atypical *Salmonella virchow* strain. Eur J Clin Microbiol Infect Dis 17:551–555

Wang X, Meng J, Zhang J, Zhou T, Zhang Y, Yang B, Xi M, Xia X (2012) Characterization of *Staphylococcus aureus* isolated from powdered infant formula milk and infant rice cereal in China. Intl J Food Microbiol 153:142–147

Weissman JB, Deen RMAD, Williams M, Swanston N, Ali S (1977) An island-wide epidemic of salmonellosis in Trinidad traced to contaminated powdered milk. West Indian Med J 26:135–143

Werber D, Dreesman J, Feil F et al (2005) International outbreak of *Salmonella* Oranienburg due to German chocolate. BMC Microbiol 5:7

Williams TL, Monday SR, Edelson-Mammel S, Buchanan R, Musser SM (2005) A top-down proteomics approach for differentiating thermal resistant strains of *Enterobacter sakazakii*. Proteomics 5:4161–4169

World Health Organization (WHO) (2007) Safe preparation, storage and handling of powdered infant formula guidelines. http://www.who.int/foodsafety/publications/micro/pif2007/en/index.html

Nuts and Nut Pastes

John C. Frelka and Linda J. Harris

Abstract Worldwide nut production has expanded rapidly in recent years with a corresponding increase in consumption. Large outbreaks of salmonellosis have been associated with nuts and their products during this same time period, which has resulted in a major shift in the approach used to process these products. A brief overview of the history and use of nuts and differences in production and harvest practices of several major nuts are presented in this chapter. The association of foodborne pathogens with nuts is discussed in the context of outbreaks, recalls, and surveys. Potential routes of contamination of nuts with foodborne pathogens are presented along with an overview of current thermal and nonthermal methods for the reduction of pathogens and the factors affecting their efficacy.

Keywords Almond • Butter • *E. coli* • Paste • Pathogen • Peanut • Nut • Safety • *Salmonella* • Seed

Introduction

Nuts are important agricultural commodities and have been a significant part of the human diet for a purported 780,000 years (Goren-Inbar et al. 2002). Nuts are defined botanically as "a hard, indehiscent, one-seeded pericarp generally resulting from a compound ovary, as the chestnut or acorn" or filbert (Rosengarten 1984). However, the word "nut" is commonly used in a much broader sense to include drupes (almonds, pecans, pistachios, and walnuts), legumes (peanuts), and seeds (Brazil nuts, cashews, flax, sunflower seeds, sesame seeds, and pine nuts), which have a similar composition and structure to botanical nuts, but are not nuts in the strictest sense (Rosengarten 1984) (Table 1). Though botanically diverse, nuts generally have shared characteristics, namely, a relatively hard, inedible outer shell with a softer,

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Table 1 Classification of common nuts by category and botanical family

Category	Common name	Scientific name	Botanical family
Achene	Sunflower seed	*Helianthus annuus*	Compositae
Capsule	Brazil nut	*Bertholletia excelsa*	Lecythidaceae
	Sesame seed	*Sesamum indicum*	Pedaliaceae
Drupes	Almond	*Prunus dulcis*	Rosaceae
	Cashew	*Anacardium occidentale*	Anacardiaceae
	Coconut	*Cocos nucifera*	Palmae
	Hickory	*Carya* spp.	Juglandaceae
	Macadamia	*Macadamia integrifolia*	Proteaceae
	Pecan	*Carya illinoinensis*	Juglandaceae
	Pistachio	*Pistacia vera*	Anacardiaceae
	Walnut	*Juglans regia*	Juglandaceae
Nuts	Acorn	*Quercus alba*	Fagaceae
	Chestnut	*Castanea dentata*	Fagaceae
	Filbert (hazelnut)	*Corylus avellana*	Corylaceae
Legumes	Peanut	*Arachis hypogaea*	Leguminosae

edible inner kernel (also called the meat) that is referred to as the nut. Drupes also have a fleshy outer coating, often called the hull, which is removed in processing. The hull corresponds to the flesh of other drupes, like nectarines and peaches, and the nut corresponds to the stone of these fruits. The hull may be discarded as waste or composted (e.g., walnuts and pistachios) or may be used as animal feed (e.g., almonds); shells may also be used in a wide range of products or purposes from animal bedding to biofuel. Peanuts are legumes, like peas and other beans, in which the nut consists of a pod made of a single folded carpel surrounding two seeds. The pod of a peanut corresponds to the shell of a true nut. Seeds, in the sense used here, represent a variety of different plant seeds, which are consumed as nuts but do not fall into a single group.

Nuts are grown in a wide variety of climates and in virtually every region of the world. The top three producing countries in 2011 for various nuts are summarized in Table 2. The dynamics of production have changed rapidly in the past decade with a trend of increased nut production worldwide. Since nuts are a diverse group of agricultural products encompassing a variety of assorted plants, they have different requirements for growth. Almonds are grown most extensively in moderate climates, predominantly in the Central Valley of California in the USA, with other significant production in Australia, Spain, Iran, and several countries on the Mediterranean coast. Walnuts also are adapted to Mediterranean-like climate zones (California Walnut Board 2012) and are grown widely in China, Iran, and the USA. About 99 % of the US walnut supply is produced in orchards within the Central Valley of California. Other nuts are adapted to grow in more tropical climates: macadamia nuts are grown in Australia and Hawaii, Brazil nuts are grown in Bolivia and Brazil, and cashews are grown in Africa (Nigeria, Ivory Coast), India, Asia (Vietnam, Indonesia), and Brazil. Hazelnuts are widely grown in Turkey,

Table 2 Top nut-producing countries in 2011 (FAO, 2012)

	First		Second		Third	
Nut	Country	Production (1,000 tonnes)[a]	Country	Production (1,000 tonnes)	Country	Production (1,000 tonnes)
Almond, with shell	USA	731	Spain	212	Iran	168
Brazil nut, with shell	Bolivia	48.5	Brazil	40.4	Ivory Coast	16.6
Cashew, with shell	Vietnam	1,270	Nigeria	813	India	675
Coconut	Indonesia	17,500	Philippines	15,200	India	11,200
Hazelnut, with shell	Turkey	430	Italy	129	USA	34.9
Peanut, with shell	China	16,100	India	6,930	Nigeria	2,960
Pistachio	Iran	472	USA	201	Turkey	112
Sesame seed	Myanmar	862	India	769	China	606
Sunflower seed	Russia	9,700	Ukraine	8,670	Argentina	3,670
Walnuts, with shell	China	1,660	Iran	485	USA	418
Other nuts[b]	China	132	USA	122	Indonesia	107

[a]Tonne refers to metric ton (1,000 kg; 2,200 lb)
[b]Includes pecan, butternut, pili nut, Java almond, Chinese olives, paradise nut, macadamia nut, and pignolia (pine) nut

Europe, Caucasus, the USA, and Iran. The top two producing countries for pistachios are Iran and the USA. Differences in climate, local production practices, harvest conditions, and postharvest handling create unique challenges for microbial contamination of nuts grown in each distinct region of the world.

Many nuts are mechanically harvested; others are harvested by hand or collected from the ground after the ripened product naturally drops. After harvest, all nuts go through various initial postharvest mechanical sorting steps that facilitate removal of debris such as sticks, rocks, leaves, and loose dirt. Most tree nuts have an outer hull that is removed. Hulling may occur before or after drying, depending on the nut. For example, almonds grown in California are shaken to the ground where they dry to a kernel moisture level of <7 %; drying facilitates removal of the hull using a series of sheer rollers, and the equipment can be configured such that the shells are removed at the same time. Other tree nuts (e.g., pistachios and walnuts) are more typically hulled shortly after harvest (before drying), usually via mechanical abrasion combined with water sprays. In-shell nuts are subsequently dried with forced, heated air or, in some countries, by sun exposure and ambient conditions.

Nuts are sold both in-the-shell and shelled (kernel only), with the exception of cashews, which are always shelled because the double shell surrounding the kernel

contains toxic compounds (Menninger 1977). Nuts can be consumed out of hand as a snack, but they are also extensively used as food ingredients and in baked goods, confectionary products, and snack foods. Shelling is usually a dry mechanical process, but in some countries, nuts are still shelled by hand. Pecans are dried in-shell or are conditioned by soaking in hot water before shelling to make the kernels pliable and prevent breakage; the nutmeat is then dried (Beuchat and Mann 2011a). Nuts may be stored at ambient or under cool (<15 °C) dry conditions for a year or more before quality begins to degrade. Almonds are often stored after shelling, but most nuts are stored in-the-shell and are then shelled on an as-needed basis.

Shelled nuts may be further processed, including sorting for size and quality. The kernel pellicle (skin) may be removed by a dry process (e.g., dry blanching of peanuts) or by application of hot water or steam (e.g., wet blanching of almonds). Kernels can be used whole or transformed into many different forms: halved, chopped (into various sizes), sliced, slivered, or ground (into nut meals, flours, or pastes). Nuts can be roasted (e.g., by hot air or in oil) and some roasting methods may include introduction of salt or other flavorings. In some cases, treatments may be applied to specifically reduce microbial loads without changing sensory properties.

Nuts or seeds can be ground into fine particles to yield a paste-like consistency. These products are often called "butters." Nut pastes may be made solely of nuts or may have other ingredients added such as salt, sugar or other seasonings, and/or hydrogenated vegetable oils to prevent separation. Palm oil has also been recently added to some peanut butters, marketed as "no-stir," based on consumer demand for products free of hydrogenated oils. Peanut butter is one of the most common and easily recognizable nut pastes and accounted for about 60 % (~2.5 billion pounds [1.1 million metric tons]) of peanut use in the USA in 2010 (ERS 2012). Also available are butters made from almonds, cashews, hazelnuts, macadamias, pistachios, sunflower seeds, and sesame seeds (tahini) (Mangels 2001) or flavored spreads that combine nut butters with other ingredients such as chocolate (which are especially popular in Europe). Nuts ground into pastes with sweeteners may also be used in confectionary products such as marzipan (a mixture of ground almonds, sugar or honey, and flavoring) or halva (made by mixing tahini with sugar and other ingredients).

Worldwide nut production has expanded rapidly in recent years. In 2012, global production of peanuts and tree nuts was 36.5 and 3.4 million metric tons (80 and 7.5 billion pounds), respectively, an increase of ca. 12 % and 20 % from 2009, respectively (International Nut and Dried Fruit Council (INC) 2012). Over the past decade, there has also been an increase in reported outbreaks of foodborne illness linked to the consumption of nuts and nut products. It is crucial for the nut industry to implement food safety programs that are adequate to handle current production levels and anticipated increases in production. This chapter will discuss (1) how nuts may become contaminated with foodborne pathogenic bacteria, (2) the survival of these organisms in the product and the nut production and processing environments, and (3) strategies for their control.

Foodborne Pathogens

Nuts tend to be high in fat and low in moisture (Beuchat 1978). With the exception of seasonal specialty products, such as fresh undried almonds or pistachios, nuts are typically dried to a water activity (a_w) below 0.70 (target between 0.50 and 0.65) (Beuchat 1978; Kader and Thompson 2002), which is lower than the minimum required for bacteria and most fungi to grow. For this reason, nuts and nut products have long been considered low risks for microbial food safety. However, foodborne pathogens can survive for long periods of time at low population levels in low-a_w foods; these products, including nuts and nut-based foods, are increasingly recognized for their contribution to outbreaks of foodborne illness (Beuchat et al. 2013a; Podolak et al. 2010; Scott et al. 2009).

Outbreaks of Foodborne Illness

Salmonella accounted for 18 of the 23 (78 %) reported outbreaks that have been associated with consumption of nuts and nut butters (Table 3). Other outbreaks have been linked to *E. coli* O157:H7 gastroenteritis (in-shell hazelnuts [Miller et al. 2012] and walnut kernels) and, in very unusual cases, *Clostridium botulinum* intoxication (peanut butter and canned peanuts). As with other low-a_w foods, outbreaks linked to nuts tend to be spread over many months and over wide geographic areas and as such they are challenging to investigate. It is possible that some nut-associated outbreaks, especially those involving strains with common serotypes or fingerprints, have gone unrecognized.

Consumption of raw almonds was associated with North American outbreaks in 2000/2001 and 2003/2004. A total of 168 cases reported in the USA and Canada from October 2000 to July 2001 were epidemiologically linked to the consumption of raw California almonds that were sold in bulk (Isaacs et al. 2005). The outbreak was identified, in part, by association with a very rare strain of *Salmonella* Enteritidis, phage type (PT) 30. Ultimately, the same microbe was isolated from case patients and almond samples collected from homes, retail outlets, distributors, and warehouses implicated in the outbreak. Traceback investigations led to a processing facility where the nuts were packed and to the hulling and shelling facility where the implicated lots of almonds were handled. The same strain of *Salmonella* Enteritidis PT 30 was isolated from environmental swabs collected at both the huller and processing facility (Isaacs et al. 2005). This was significant because at the time of the investigation the huller had not been in operation for several months. Likewise, drag swabs of the implicated orchards collected nearly 9 months after the outbreak-associated almonds had been harvested were positive for *Salmonella* Enteritidis PT 30 (Isaacs et al. 2005). This strain continued to be isolated from the implicated orchards for an additional 5 years (Uesugi et al. 2007). Although many potential sources were investigated, the ultimate origin of the orchard contamination was

Table 3 Outbreaks of foodborne illness associated with the consumption of nuts and nut pastes[a]

Type	Product	Pathogen	Year	Number of confirmed cases	Outbreak location(s)	Source
Nuts						
Almond	Raw whole	*S.* Enteritidis PT 30	2000–2001	168	Canada, USA	CDPH (2002), Chan et al. (2002), Isaacs et al. (2005)
	Raw whole	*S.* Enteritidis PT 9c	2003–2004	47	Canada, USA	CDPH (2004), Keady et al. (2004)
	Raw whole	*S.* Enteritidis NST3+ (aka PT 30)	2005–2006	15	Sweden	Ledet Müller et al. (2007)
	Raw whole	(*Salmonella* serovar not given)	2012	27	Australia	FSANZ (2012)
Coconut	Desiccated	*S. typhi*, *S.* Senftenberg and possibly others	1953	>50 (est. from epi curve)	Australia	Wilson and Mackenzie (1955)
	Desiccated	*S.* Java PT Dundee	1999	18	United Kingdom	Ward et al. (1999)
Hazelnut	In-shell	*E. coli* O157:H7	2011	7	USA	CDC (2011a), Miller et al. (2012)
Peanut	Canned	*C. botulinum* (type A)	1986	9	Taiwan	Chou et al. (1988)
	Savory snack	*S.* Agona PT 15	1994–1995	71	Israel, United Kingdom, USA	Killalea et al. (1996), Shohat et al. (1996), Threlfall et al. (1996)
	Flavored or roasted in-shell	*S.* Stanley and *S.* Newport	2001	97 Stanley 12 Newport	Australia, Canada, United Kingdom	Kirk et al. (2004)
	Boiled	*S.* Thompson	2006	100	USA	Marler Clark LLP (2006), ProMED-mail (2006)
Pine nut	Whole, bulk	*S.* Enteritidis	2011	43	USA	CDC (2011b)

Walnut	Raw shelled halves, pieces, walnut crumbs	*E. coli* O157:H7	2011	14	Canada	CFIA (2011), Health and Safety Watch (2011), PHAC (2011)
Nut pastes						
Hazelnut	Yogurt	*C. botulinum* (type B)	1989	27	United Kingdom	O'Mahony et al. (1990)
Peanut	Butter	*S.* Mbandaka	1996	15	Australia	Scheil et al. (1998)
	Butter	*S.* Tennessee	2006–2007	715	USA	CDC (2007a, b), Sheth et al. (2011)
	Butter	*C. botulinum* (types A and B)[b]	2006–2008	5	Canada	Sheppard et al. (2012)
	Butter, butter-containing products	*S.* Typhimurium	2008–2009	714	USA, one case in Canada	CDC (2009, 2010), Cavallaro et al. (2011)
	Butter	*S.* Bredeney	2012	30	USA	CDC (2012)
Sesame seed	Halva	*S.* Typhimurium DT 104	2001	17 (Australia), 18 (Norway), 27 (Sweden)	Australia, Germany, Norway, Sweden, United Kingdom	Brockmann et al. (2004)
	Tahini	*S.* Montevideo	2002	55	Australia	Unicomb et al. (2005)
	Tahini	*S.* Montevideo	2003	3	Australia	Unicomb et al. (2005)
	Tahini and halva	*S.* Montevideo	2003	10	New Zealand	Unicomb et al. (2005)

[a]Adapted from Harris et al. (2014)

[b]Pathogen caused intestinal toxemia botulism, which is very rare; two of three patients studied had a history of Crohn's disease and bowel surgery

never determined. However, it was concluded that the almonds most likely acquired *Salmonella* in the orchard during harvest and that this contamination was spread during postharvest handling.

The 2003/2004 outbreak linked to almonds was associated with equally rare *Salmonella* Enteritidis PT 9c, with 47 cases reported in the USA and Canada from September 2003 to April 2004. Raw almond kernels recovered from a consumer's house and samples collected at the almond processor were negative for *Salmonella*; however, the outbreak strain was isolated from one environmental sample collected at the processor and from three samples obtained at two huller-shellers that supplied almonds to the primary implicated processor (Keady et al. 2004). The source of the *Salmonella* was not identified.

Peanut butter was first linked to an outbreak of salmonellosis in 1996 in Australia (Scheil et al. 1998). Fifteen cases were identified; the outbreak strain was isolated in peanut butter from consumer households and from unopened jars collected at retail outlets and the processor. The source was ultimately determined to be contaminated roasted peanuts received from a peanut roasting facility.

A decade later, two large outbreaks (715 and 714 confirmed cases) in Canada and the USA (2006/2007 and 2008/2009) were linked to consumption of peanut butter; nine deaths were associated with the 2008/2009 outbreak (Cavallaro et al. 2011; Sheth et al. 2011). Both outbreaks were widespread and prolonged, with cases reported in 48 and 46 states over 12 and 7 months, respectively. The peanut butter associated with the 2008/2009 outbreak was sold in bulk for use in institutions and as an ingredient in multiple foods. The company had routinely tested the final product for *Salmonella* and had occasionally shipped lots of product that originally tested positive, although were negative in a second test. As a consequence, several thousand different products containing peanut butter were recalled (FDA 2009b). Despite the widespread media attention given to the 2008/2009 outbreak, a third US peanut butter outbreak occurred in 2012 (CDC 2012). This company also shipped product after repeatedly isolating *Salmonella* from nut butters and the production environment over a 3-year period (FDA 2012), leading to recall of hundreds of nut butters and nut products and temporary (from November 2012 to May 2013) suspension of the facility's registration (FDA 2013).

Clostridium botulinum is not considered an issue in low-a_W foods because the organism cannot grow and produce toxin below an a_W of 0.93 (Baird-Parker and Freame 1967). However, three unusual outbreaks of botulism in nut products have been reported (Chou et al. 1988; O'Mahony et al. 1990; Sheppard et al. 2012). Canned peanuts processed in an unlicensed facility were implicated in a botulism outbreak in Taiwan in 1986 among workers who ate in their factory cafeteria (Chou et al. 1988). The dried, shelled peanuts were boiled, placed into glass jars with the cooking liquid, and the jars were steamed for about an hour. This unvalidated process was insufficient to eliminate *C. botulinum* spores, and the high moisture levels in the product were sufficient to support growth and toxin production. Achieving an equilibrium a_W of $\leq$0.94 but not $\geq$0.96 in peanut spreads prevents *C. botulinum* toxin production (Clavero et al. 2000).

A large outbreak (27 cases and one death) of botulism in June 1989 was linked to consumption of hazelnut yogurt in the UK. The toxin was detected in opened and

unopened containers of yogurt and in a single sealed but swollen can of the low-acid hazelnut preserve used to flavor the yogurt (O'Mahony et al. 1990). The preserve processor had prepared the product from a mixture of roasted hazelnuts, water, starch, and other ingredients; the bulk mixture was briefly heated before being pumped into metal cans, which were then sealed and processed in boiling water for 20 min. The pH of the majority of preserve available at the processor was between 5.0 and 5.5. While most of the preserve lots were formulated with sugar, for some lots, including those received by the yogurt processor associated with the most cases, the sugar was replaced with aspartame. The processor had noted some blown cans among the stored aspartame-sweetened product. The cause of the outbreak was likely insufficient thermal treatment, possibly coupled with a higher water activity in the aspartame-sweetened product (Brett 1999), thus allowing *C. botulinum* to survive, grow, and produce toxin during several months of ambient storage before the preserve was added to the yogurt.

Under very different circumstances, immune-compromised adult patients experienced intestinal toxemia after ingestion of peanut butter containing *C. botulinum* spores (Sheppard et al. 2012). As with infant botulism, spores of *C. botulinum* can grow in the intestinal tract of persons with Crohn's disease or other intestinal complications (Sobel 2005).

Recalls of Nuts

Nuts and nut products are often recalled for undeclared allergens, presence of foreign material, and elevated levels of aflatoxin. Nuts (almonds, hazelnuts, macadamias, peanuts, pine nuts, pistachios, and walnuts) and nut pastes (peanut butter, cashew butter, and tahini) have been associated with a number of Class I recalls in the USA and Canada due to isolation of *Salmonella*. Between 2004 and 2011, nuts, seeds, and their products were the predominant low-a_W food category implicated in recalls and market withdrawals in the USA and Canada associated with *Salmonella* (Beuchat et al. 2013a). To a lesser extent, nuts have been recalled for isolation of or association with *E. coli* O157:H7 or *Listeria monocytogenes* (Palumbo et al. 2014b).

Prevalence and Levels of Foodborne Pathogens

A limited number of retail surveys have been done to screen nuts and edible seeds for the presence of *Bacillus cereus*, *E. coli* O157:H7, *L. monocytogenes*, and *Staphylococcus aureus* (Palumbo et al. 2014a). Most of the surveys have focused exclusively on *Salmonella* due to the association of this organism with outbreaks in nuts, seeds, and their products. Most of the published surveys have detected *Salmonella* in a small proportion of samples; however, many of these surveys have collected samples at retail where product age and handling are unknown and have evaluated a small number of samples of individual nut types and analyzed small

(10- or 25-g) units (Brockmann et al. 2004; NSW Food Authority 2012; Willis et al. 2009). Some of the collected samples had been roasted, and thus results are not directly comparable in surveys of raw product (Little et al. 2009, 2010).

A survey of nut products (915 samples) collected from retailers, manufacturers, and growers was performed in Australia by the New South Wales (NSW) Food Authority in 2011 (NSW Food Authority 2012). Almonds, Brazil nuts, cashews, hazelnuts, macadamias, mixed nuts, peanuts, pecans, pistachios, and walnuts were examined. A single sample (macadamias; one out of 76, 25-g samples) was positive for *Salmonella*. Other retail surveys have been performed in the UK and Brazil (Freire and Offord 2002; Kajs et al. 1976; Little et al. 2009, 2010; Willis et al. 2009); *Salmonella* was isolated in two out of 469, 25-g samples of Brazil nuts (Little et al. 2010) and in an unreported number of subsamples from a 2-kg sample of Brazil nuts (Freire and Offord 2002).

Several large surveys have determined *Salmonella* prevalence in raw nuts collected from processors shortly after harvest (Table 4). An 8-year survey in California analyzed 13,972, 100-g samples from individual lots of raw almond kernels that revealed a prevalence of 0.98 % ± 0.32 % for *Salmonella* (Bansal et al. 2010; Danyluk et al. 2007; Lambertini et al. 2012). *Salmonella* was detected with similar frequency in in-shell almonds sampled over 2 years (1.5 %; seven positive out of 455, 100-g samples) (Bansal et al. 2010). In contrast, the prevalence of *Salmonella* in four lots of almonds associated with an outbreak was 65 % (Danyluk et al. 2007).

The prevalence of *Salmonella* in California in-shell walnuts was 0.16 % (three of 1,904 375-g samples), whereas *E. coli* O157:H7 was not detected in any sample (Frelka 2013). Twenty-two of 944, 375-g samples were positive for *Salmonella* (2.3 % prevalence) in raw peanut kernels sampled over 3 years (2008–2010) (Calhoun et al. 2013). Sesame seeds, collected from imported shipments entering the USA, were contaminated with *Salmonella* at 11 % of 750-g samples from 177 shipments (Van Doren et al. 2013a) and 9.9 % of 1,500-g samples from 233 shipments (Van Doren et al. 2013b).

Estimating levels of *Salmonella* in positive lots is challenging, given the generally low prevalence of this organism. The most probable number (MPN) of *Salmonella* in 99 initially positive raw almond samples was estimated between 0.0044 and 0.15 per gram (Lambertini et al. 2012). Quantifiable levels of *Salmonella* (0.09 and 0.23 MPN/g) were reported in two positive samples of Brazil nuts (Little et al. 2010). Levels of *Salmonella* were estimated to be 6×10^{-4} to 0.04 MPN/g in 22 samples of sesame seeds (Van Doren et al. 2013b). When data are available, levels of *Salmonella* in outbreak-associated product have also been low. *Salmonella* at levels of <0.03–2 MPN/g, <3 MPN/g, and 1.5 MPN/g were determined in outbreak-associated in-shell peanuts (Kirk et al. 2004), peanut butter (Scheil et al. 1998), and peanut butter (Scott et al. 2009), respectively. Levels of 0.061 and 0.091 MPN/g were determined in recalled outbreak-associated almonds (Danyluk et al. 2007) but were estimated to be 1.2 MPN/g at the peak of the outbreak (Lambertini et al. 2012). The relatively low levels of *Salmonella* detected in outbreak-associated samples reflect the difficulty in detecting the pathogen in nuts. Routine testing is not likely to detect all or even most contaminated lots.

Table 4 *Salmonella* prevalence in naturally contaminated nuts sampled after harvest (before retail distribution)[a]

Nut type	Where collected	Sample size (g)	No. of samples tested (n)	No. positive for *Salmonella*	Percent positive (if $n > 50$)	References
Almond, raw kernel	Receiving, California	100	13,972	137	0.98 ± 0.32 (for 2001–2007 and 2010)	Bansal et al. (2010), Danyluk et al. (2007), Lambertini et al. (2012)
Almond, raw in-shell	Receiving, California	100	455	7	1.5 (for 2006–2007)	Bansal et al. (2010)
Almond, raw kernel	Receiving, Australia	25	60	1	1.7	Eglezos et al. (2008)
Almond, treated	RTE packages, Australia	25	42	0		Eglezos (2010)
Brazil nut, shelled and whole in-shell	Processor	50	20	0		Arrus et al. (2005)
Brazil nut	Receiving, Australia	25	60	0	0	Eglezos et al. (2008)
Brazil nut	RTE packages, Australia	25	40	0		Eglezos (2010)
Cashew	Receiving, Australia	25	100	0	0	Eglezos et al. (2008)
Cashew	RTE packages, Australia	25	45	0		Eglezos (2010)
Hazelnut	Receiving, Australia	25	48	0		Eglezos et al. (2008)
Hazelnut	RTE packages, Australia	25	51	0		Eglezos (2010)
Peanut	Receiving, Australia	25	653	0	0	Eglezos et al. (2008)
Peanut	RTE packages, Australia	25	343	0	0	Eglezos (2010)
Peanut	Processor, USA	375	944	22	2.3	Calhoun et al. (2013)
Sesame seed	Importer, USA	375	177	20	11	Van Doren et al. (2013a)
Sesame seed	Importer, USA	1,500 (composite samples)	233	23	9.9	Van Doren et al. (2013b)
Walnut, raw in-shell	Processor, California	100	935	0	0 (2010)	Frelka (2013)
Walnut, raw in-shell	Processor, California	375	1,904	3	0.16 (average 2011–2012)	Frelka (2013)

[a]Adapted from Palumbo et al. (2014a, b)

Potential Routes of Contamination

Surveys conducted over eight separate harvests revealed that a total of 151 *Salmonella* isolates representing 49 different serovars were recovered from almonds (Bansal et al. 2010; Danyluk et al. 2007; Bansal and Harris, unpublished). Thirteen different serovars were identified from the *Salmonella* isolates from peanuts over 3 years (Calhoun et al. 2013). A survey of imported spices, including sesame seeds, revealed 94 different serotypes in 187 *Salmonella*-positive samples (Van Doren et al. 2013a); 18 different serotypes of *Salmonella* were present in the 20 positive samples of sesame seeds. The diversity of *Salmonella* serotypes that have been identified in nut and seed surveys is suggestive of a wide range of environmental contamination sources.

Various steps in the harvesting and processing of nuts provide various opportunities for contamination of the nutmeat. Wet or dry contamination may occur in the field before or during harvest, during postharvest processing, or in the post-processing environment. While there are many similarities, each nut type and production region employs different harvest and processing methods. The harvest and processing steps that are typical for almonds, walnuts, pistachios, and peanuts grown in the USA, where tree nuts and peanuts are almost exclusively mechanically harvested, are outlined in Fig. 1.

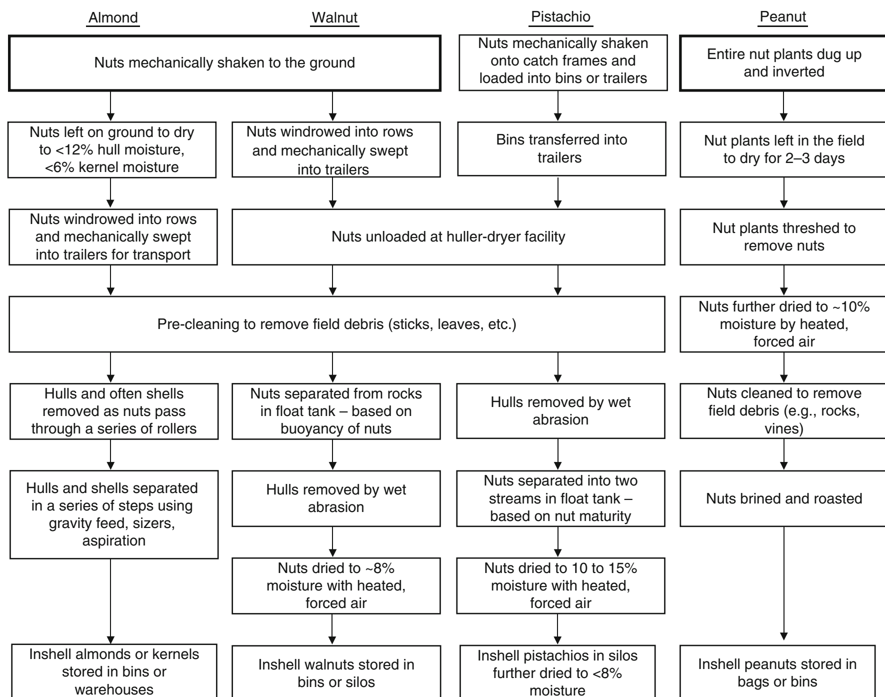

Fig. 1 Process flow diagram for almonds, walnuts, pistachios, and peanuts from harvest through storage

Trees are typically shaken to release the nuts either to the ground (e.g., almonds, walnuts, hazelnuts) or onto catch frames (pistachios), or they may fall naturally to the ground (macadamias). Nuts may dry on the ground after being shaken from the tree, or they may be harvested shortly after shaking by mechanically sweeping into windrows and then into harvest bins or trailers. Nuts may be partially processed (e.g., hulled, shelled) shortly after harvesting and then dried at relatively low (40 °C) to moderate (75–85 °C) temperatures, if necessary, prior to storage. Some nuts (e.g., almonds) are dried before collecting from the orchard and thus may be stockpiled for days to months before hulling and shelling. Stockpiled almonds are covered with tarps and fumigated to control insects; most nuts require fumigation or some other treatment to reduce the potential for damage from orchard and storage insect pests.

Peanuts grow in the soil and are mechanically harvested by lifting the plant out of the ground so that the pods are exposed to the air where the pods dry in windrows for 2–8 days depending upon weather conditions. The peanuts are then threshed from the vines and delivered to buying stations for further curing (forced-air drying to about 10 % moisture), cleaning, and grading before storage in warehouses. At peanut-shelling facilities, harvested product is cleaned to remove dirt, rocks, and other foreign material, followed by shelling and gravity separation that removes all but the peanut kernels, which are sorted, sized, packaged, graded, and stored.

Most nuts are harvested from the ground along with soil and other debris. Activities that have the potential to introduce pathogens onto the orchard floor (e.g., raw manure, contaminated irrigation water, wildlife, and grazing animals) may impact contamination of the product. Almonds harvested onto canvas tarps had lower aerobic plate counts than almonds harvested from the ground (King et al. 1970). Grazing domestic animals on grass or other cover crops in pecan orchards increased the risk of nut contamination by fecal microorganisms (Marcus and Amling 1973). In some regions of the world, there may be a greater reliance on manual labor to collect nuts from the trees or the ground. In these instances, worker hygiene training and the availability and maintenance of adequate sanitary facilities are essential in mitigating contamination by humans.

Commodity-specific guidance documents for Good Agricultural Practices (GAPs) have been published for some nuts (Almond Board of California (ABC) 2009; American Peanut Council (APC) 2011; California Pistachio Research Board (CPRB) 2009; Hazelnut Marketing Board (HMB) 2012), but data to quantify the food safety risk of common production practices for most nuts are generally lacking. Uesugi et al. (2007) determined that a single outbreak-associated *Salmonella* strain persisted in an almond orchard for at least 6 years. *Salmonella* multiply readily on wet almond hulls and shells (Uesugi and Harris 2006) and in soil in the presence of almond hull nutrients (Danyluk et al. 2008). Survival in the soil is enhanced when temperatures are cool (Beuchat and Mann 2010b; Danyluk et al. 2008). When wetted, *Salmonella* can move through intact almond hulls and shells to the kernel (Danyluk et al. 2008). Almonds that drop prematurely may come into contact with irrigation water, rainfall occasionally occurs during harvest when nuts are on the ground, and almonds that are missed during harvest may remain on the ground over the winter months. Growers and processors should be aware of the increased contamination potential

associated with wet product so that preharvest (e.g., orchard sanitation to remove nuts that remain after the previous harvest) and postharvest procedures (e.g., product segregation and testing) to control risks can be implemented.

Aqueous sources of contamination can also occur during postharvest handling. For many nuts (e.g., pistachios and walnuts), the outer hull is removed using a combination of physical abrasion and water sprays. Nuts may be submerged in water to separate the nuts from rocks and other orchard debris (e.g., walnuts and pecans), to separate well-formed heavy nuts that sink from those that may be damaged and float (e.g., pistachios), or to soften the shell before cracking (e.g., cashews and pecans). Whenever water is used, there is the potential to spread contamination from one lot to another and throughout the facility. Sanitizer-free walnut float tank water had aerobic plate counts of 6.5–7 log CFU/ml (Frelka 2013), and *Salmonella* survived for more than 2 weeks at ambient temperatures in float tank water in the presence of high microbial loads (Blessington 2011).

The use of antimicrobials in either float tanks of water or as sprays has been explored, but high organic loads in float tanks make this approach challenging because the activity of many antimicrobials is mitigated by organic matter (Beuchat and Mann 2011a; Beuchat et al. 2012, 2013b). In a commercial facility, there was no difference in microbial populations on in-shell walnuts that were sprayed with water and various peroxyacetic acid formulations (100 and 200 ppm, four different formulations) (Frelka 2013). Pecans may be soaked for a few minutes in hot water or for several hours in cold or ambient water to hydrate the kernel and reduce damage during cracking. Under laboratory conditions, significant decreases of 0.41–0.98 log CFU *Salmonella*/g on the shell surface occurred within 1 h after immersion of in-shell pecans in water containing 100 ppm of chlorine; increasing chlorine concentrations to 400 ppm did not impact the results (Beuchat and Mann 2011a; Beuchat et al. 2012).

Almond hulls and shells are removed by sheer rollers in a manner that allows mixing of the kernels with the hulls and shells, increasing the potential for cross-contamination (Du et al. 2007). During this process, large volumes of dust are generated, further increasing the potential for the spread of contaminants among lots, should they be present. *Salmonella* is capable of multiplying in almond dusts that are combined with small amounts of water or aqueous sanitizer (Du et al. 2010b); hence, efforts should be made to avoid introduction of water into almond huller-sheller facilities.

The shells of most tree nuts provide an important and effective barrier to microbial contamination, and the presence of a hull can further reduce the risk. The kernel inside an intact shell was once thought to be virtually sterile (Chipley and Heaton 1971; Kajs et al. 1976; Meyer and Vaughn 1969); however, there is substantial evidence that the shell provides variable levels of protection from contamination. For example, walnut kernels have low populations of bacteria when aseptically extracted from in-shell walnuts removed directly from the tree (Frelka 2013). Total aerobic plate and *E. coli*/coliform counts on kernels increase significantly as the walnuts move through the hulling and drying steps (Blessington 2011; Frelka 2013).

Shell thickness can vary significantly among different varieties of the same nut, and shell breakage can expose the kernel within and lead to contamination (Beuchat and Mann 2010a; Frelka 2013; King et al. 1970). The almond kernel can be partially or completely exposed at the time of harvest in soft-shell almond varieties. Wetting the shell suture also promotes microbial infiltration of the nut shell and subsequent contamination of the kernel (Beuchat and Heaton 1975; Beuchat and Mann 2010a; Marcus and Amling 1973). Walnut kernels extracted from nuts with broken shells have significantly higher microbial populations than kernels extracted from walnuts with visibly intact shells (Frelka 2013). Drying may influence shell integrity (Frelka 2013; King et al. 1970; Meyer and Vaughn 1969).

Contamination of nuts also may occur during or after further processing. In more than one peanut butter outbreak (Table 3), investigators concluded that contamination of the product with *Salmonella* most likely occurred within the processing environment. In some cases, ingress of water into the facility (e.g., water leakage through roof and skylights in poor repair) may have contributed to the contamination by providing an opportunity for the organism to multiply in the production environment. Potential for cross-contamination between treated and untreated product, poor ventilation that increased the possibility for contamination of food and food-contact surfaces, lack of documented cleaning and sanitation of equipment, and lack of a validated process that would ensure adequate reduction of *Salmonella* were also cited as contributing factors (Cavallaro et al. 2011; FDA 2009c, 2013; Sheth et al. 2011).

Survival of Pathogens in Nuts and Nut-Processing Environments

Once *Salmonella* is introduced into nuts, nut pastes, or the environment in which these products are processed, the organism may persist for extended periods of time (Beuchat and Heaton 1975; Beuchat and Mann 2010a; Blessington et al. 2012, 2013; Burnett et al. 2000; Kimber et al. 2012; Uesugi et al. 2006). In several cases, outbreak strains have been isolated from production facilities months after the affiliated product was processed (CDC 2012; FDA 2013; Isaacs et al. 2005; Sheth et al. 2011).

Lower storage temperatures reduce the rate of oxidation of the fats within nuts, and nuts are often held commercially or by consumers at cooler temperatures (Lee et al. 2011). Levels of *E. coli* O157:H7, *L. monocytogenes*, and *Salmonella* in nuts are usually stable at freezing or refrigeration temperatures with little to no decline observed in months to over a year of storage (Beuchat and Heaton 1975; Beuchat and Mann 2010a; Blessington et al. 2012, 2013; Burnett et al. 2000; Frelka 2013; Kimber et al. 2012; Uesugi et al. 2006). As temperatures increase, rates of reduction of inoculated organisms increase (Beuchat and Heaton 1975; Beuchat and Mann 2010a; Uesugi et al. 2006), but even at ambient temperatures, the declines

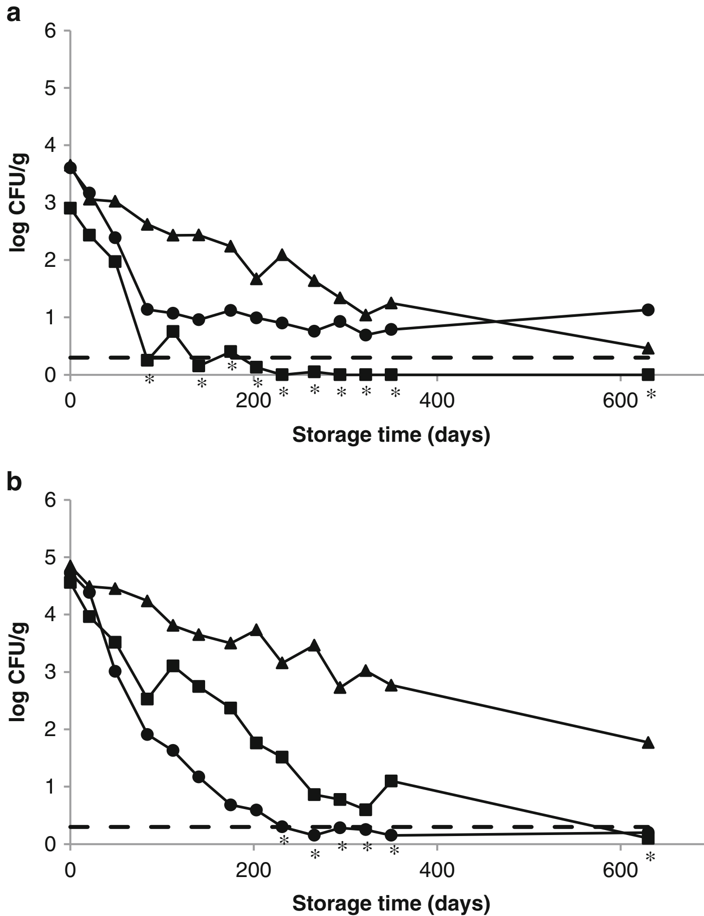

Fig. 2 Survival of *Salmonella* (*triangles*), *E. coli* O157:H7 (*squares*), and *Listeria monocytogenes* (*circles*) on inoculated almonds (**a**) and pistachios (**b**) stored at 23 °C; *asterisk* (*) indicates six of six replicates were positive via enrichment of 10-g samples (modified from Kimber et al. 2012)

are slow (Fig. 2). Linear reduction in *Salmonella* populations on almonds stored under ambient conditions ranged from 0.16 to 0.32 log CFU/g/month (Lambertini et al. 2012). Inoculated *E. coli* O157:H7 and *L. monocytogenes* populations generally declined more rapidly than *Salmonella* (Blessington et al. 2012, 2013; Kimber et al. 2012).

In some cases, the inoculation level of *Salmonella* does not influence the rate of decline (Uesugi et al. 2006), whereas in other cases, faster rates of decline are observed at lower inoculation levels (Beuchat and Mann 2010a; Blessington et al. 2012, 2013). A relatively rapid die-off after inoculation is sometimes followed by long-term persistence characterized by little to no measureable decline (Fig. 2). The practical significance of this observed “tailing” is currently unknown.

Control of Foodborne Pathogens

Long-term persistence and enhanced thermal resistance on tree nuts make control of foodborne pathogens in nuts and nut products a challenge. The appropriate level of control in these products has been debated (Schaffner et al. 2013). In the absence of extensive data a 5-log reduction is often proposed as long as appropriate procedures to ensure adequate control of the introduction of pathogens into the raw nuts and additional controls to prevent posttreatment recontamination are in place (FDA 2009a, 2011; Schaffner et al. 2013).

The almond industry in California reacted to outbreaks of salmonellosis associated with their product (Table 3) and implemented a food safety action plan that included the promulgation of regulations through the Almond Board of California (a US Department of Food and Agriculture Agricultural Marketing Service Federal Marketing Order). Since September 2007, these regulations require all almonds grown in California and sold in North America (Canada, Mexico, and the USA) to undergo a treatment capable of reducing *Salmonella* by at least 4 log (Federal Register 2007). The process criterion was based on an initial risk assessment evaluating the risk of salmonellosis from consumption of almonds (Danyluk et al. 2006). A subsequent risk assessment evaluated the implementation of the 2007 rule and concluded that the current production system was capable of preventing illness even if higher than typical levels of *Salmonella* were occasionally present (Lambertini et al. 2012). The Almond Board of California established a system to validate and verify that processes used by the almond industry were consistently meeting the mandate (ABC 2014b). These documents were used in the development of validation guidance for evaluating thermal treatments for low-a_W foods (Anderson and Lucore 2012).

Because sensory attributes and quality of nuts can be markedly altered with thermal treatment, several nonthermal methods of pathogen reduction also have been explored (Table 5; Pan et al. 2012). The response of *Salmonella* to desiccation and to inactivation treatments can differ significantly depending on the strain used, growth conditions (including growth on solid or in liquid medium and incubation temperature), method of inoculation, drying protocols and storage conditions between time of inoculation and treatment, product and product composition, treatment conditions, and recovery methods including media selected to recover potentially injured cells (Abd et al. 2012; Beuchat and Mann 2011a; Goodridge et al. 2006; Keller et al. 2012; Ma et al. 2009; Scott et al. 2009; Uesugi et al. 2006). Thus, care should be taken when conducting and interpreting these types of studies and when applying them to commercial settings.

Thermal Treatments

Nuts often are heat treated (e.g., oil or dry roasting or blanching) to change the flavor, texture, or characteristic of the product. These treatments have only recently been examined as a means to control foodborne pathogens. Thermal treatments are not

Table 5 Published treatments for potential control of microorganisms in nuts and nut pastes

Process type	Treatment	Product tested	References
Chemical	Acid solutions or sprays	Almonds, pecans	Beuchat et al. (2013b), Pao et al. (2006)
	Chlorine (bleach, sodium hypochlorite)	Coconut, hazelnuts, pecans, walnuts	Beuchat and Mann (2011a), Beuchat et al. (2012, 2013b), Blessington et al. (2013), Walter et al. (2009), Weller et al. (2013)
	Chlorine dioxide gas	Almonds	Wihodo et al. (2005)
	Methyl bromide	Almonds, walnuts	Schade and King (1977)
	Ozone	Pistachios	Akbas and Ozdemir (2006)
	Peracetic acid	Coconut, hazelnuts, pecans, walnuts	Beuchat et al. (2012, 2013b), Frelka and Harris (unpublished), Walter et al. (2009), Weller et al. (2013)
	Propylene oxide	Almonds, pecans	Beuchat (1973), Blanchard and Hanlin (1973), Danyluk et al. (2005)
Thermal	Dry (hot air) roasting	Almonds, pecans, sesame seeds	Beuchat and Mann (2011b), Torlak et al. (2013), Yang et al. (2010)
	Hot water	Almonds, pecans	Beuchat and Mann (2011a), Harris et al. (2012)
	Chlorine + hot water	Pecans	Beuchat and Mann (2011a)
	Indirect heating (water or silicon oil bath)	Peanut butter	He et al. (2011), Keller et al. (2012), Ma et al. (2009), Shachar and Yaron (2006)
	Infrared heating (gas catalytic IR)	Almonds	Bingol et al. (2011), Brandl et al. (2008), Yang et al. (2010)
	Distilled water + gas catalytic IR	Almonds	Bari et al. (2009)
	Dry roasting + gas catalytic IR	Almonds	Bari et al. (2009), Yang et al. (2010)

	Electrolyzed water + gas catalytic IR	Almonds	Bari et al. (2009)
	Hot water + gas catalytic IR	Almonds	Bari et al. (2009)
	Ozonated water + gas catalytic IR	Almonds	Bari et al. (2009)
	Superheated steam + gas catalytic IR	Almonds	Bari et al. (2010)
	Moist air convection heating	Almonds	Jeong et al. (2009), Jeong et al. (2011)
	Oil roasting	Almonds, pecans, walnuts	Abd et al. (2012), Beuchat and Mann (2011b), Du et al. (2010a, b), Meyer and Vaughn (1969)
	Radio-frequency heating	Almonds	Gao et al. (2011)
	Steam	Almonds	Chang et al. (2010), Lee et al. (2006)
Nonthermal	Electron beam irradiation	Almonds, peanut butter	Duong and Foley (2006), Hvizdzak et al. (2010)
	Ionizing irradiation (gamma rays)	Almonds, walnuts	Prakash et al. (2010), Wilson-Kakashita et al. (1995)
	High pressure processing	Almonds, peanut butter, sesame seeds	D'Souza et al. (2012), Goodridge et al. (2006), Grasso et al. (2010), Willford et al. (2008), Wuytack et al. (2003)
	Nonthermal plasma	Almonds	Deng et al. (2007), Niemira (2012)
	X-ray irradiation	Almonds, walnuts	Jeong et al. (2012)

universally applicable, as they may impact the quality of some nuts, particularly those with a high level of unsaturated fat, like walnuts (Rosengarten 1984), by increasing the rate of oxidative rancidity. Thus, thermal treatments capable of reducing foodborne pathogens while preserving the sensory characteristics of the nut have also been investigated, and several technologies are commercially available.

Similar to other low-a_w foods, drying foodborne pathogens on nuts confers significant additional resistance of the organisms to thermal destruction (Burnett et al. 2000; He et al. 2011; Horner and Anagnostopoulos 1972; Ma et al. 2009; Podolak et al. 2010). Nuts may be stored for long periods before processing. Storage of inoculated peanut butter for 2 weeks or almonds for 12 weeks did not significantly alter the thermal sensitivity of *Salmonella* (Abd et al. 2012; Keller et al. 2012). Increases in moisture or water activity of the nut or relative humidity of the heating environment increased the sensitivity of pathogens to heat (Brandl et al. 2008; Beuchat and Mann 2011b; He et al. 2011, 2013; Jeong et al. 2011; Mattick et al. 2000; Villa-Rojas et al. 2013) and have been used as strategies to improve process efficacy. The size of pecan pieces (Beuchat and Mann 2011b) and method of inoculation (Beuchat and Mann 2011a) significantly impacted the efficacy of thermal treatments.

Reported inactivation curves for *Salmonella* are sometimes linear (Harris et al. 2012; Keller et al. 2012) but are often nonlinear (Abd et al. 2012; Beuchat and Mann 2011b; Du et al. 2010a; He et al. 2011; Keller et al. 2012; Ma et al. 2009; Shachar and Yaron 2006); the Weibull model has been used to fit nonlinear curves. Inactivation curves for two strains of *Salmonella* in peanut butter were linear when cells were cultured on solid media and upwardly concave when cells were grown in broth (Keller et al. 2012).

Dry heat roasting methods, such as oil immersion and forced air, are common processes used for nuts. Under laboratory conditions, a 5-log reduction of *Salmonella* on almond kernels was achieved in hot oil in ca. 1.5 min at 121 °C (Abd et al. 2012; Du et al. 2010a); a 4-log reduction of *Salmonella* on pecan pieces and halves was achieved in ca. 1.5 and 2 min, respectively (Beuchat and Mann 2011b). Heating at 127 °C for 2 min is a recognized process for achieving a 5-log reduction of *Salmonella* on almonds (ABC 2007c). Peanuts often are dry roasted to facilitate removal of the skin prior to grinding and milling into peanut butter. The grinding process of about 20 min can generate temperatures of 71–77 °C. Under laboratory conditions, these temperatures and times result in reductions of *Salmonella* in peanut butter that are <1–1.5 log CFU/g (He et al. 2011; Shachar and Yaron 2006) or less than 3 log CFU/g (Ma et al. 2009). Heating for more than 20 min at 90 °C was necessary to achieve a 5-log or greater reduction in *Salmonella* in peanut butter (Ma et al. 2009).

In dry roasting, heat transfer is much less efficient (Beuchat and Mann 2011b). The efficacy of dry roasting can be improved by adding moisture to either the product or the process (Jeong et al. 2012), and processing equipment that introduce steam prior to or during heating of nuts are commercially available.

Because the dry roasting process is dynamic and there is a wide range of available equipment, validation of dry roasters is usually accomplished onsite with a

surrogate bacterium inoculated onto product and introduced into the product stream (ABC 2007b, 2014a; Anderson and Lucore 2012; Jeong et al. 2011). For these and other thermal processes, *Enterococcus faecium* NRRL B-2354 has been a widely accepted surrogate for *Salmonella* in almonds (ABC 2014a; Jeong et al. 2011). Use of this surrogate to validate processes in other nuts or low-a_W foods should be supported with additional data, using the food and process in question, and laboratory evaluation against the appropriate target pathogen.

Application of hot water or steam to remove almond skins (blanching) is used in almond processing (Harris et al. 2012). Almond kernels are submerged in hot water or steam to break down the pectin connecting the skin to the kernel; a set of rollers is used to facilitate removal of the loosened skins, and the resulting skin-free almonds are then quickly dried using forced hot air (Harris et al. 2012; ABC 2007a). Significant initial reductions of *Salmonella* were observed upon exposure of inoculated almonds to hot water (Harris et al. 2012). *D*-values of 1.2, 0.75, and 0.39 min were determined from the linear portion of the curve for temperatures of 70, 80, and 88 °C, respectively, and a *z*-value of 35 °C. Blanching almonds at 88 °C for 2 min is a recognized process for achieving a 5-log reduction of *Salmonella* (ABC 2007a). Hot water treatment of in-shell pecans at 75–95 °C during the conditioning step preceding shelling operations reduced *Salmonella* to various degrees depending on the methodology used for inoculation (Beuchat and Mann 2011a).

Radio-frequency (Gao et al. 2011) and infrared heating have been evaluated as alternatives to roasting to reduce *Salmonella* on almonds (Brandl et al. 2008; Bingol et al. 2011; Yang et al. 2010). Infrared radiation causes rapid increases in heat on the surface of the almond and, when combined with a short warm temperature hold time, significantly reduced populations of inoculated *Salmonella*. The process is typically much shorter than traditional thermal methods and can result in a product quality that is similar to untreated product (Brandl et al. 2008).

Nonthermal Treatments

Nonthermal treatments include use of gas fumigation, application of antimicrobial chemicals, and physical treatments. Gaseous ozone reduced by 2–3 logs *E. coli* and *Bacillus cereus* on pistachio kernels (Akbas and Ozdemir 2006). Propylene oxide (PPO) gas treatments are permitted in the USA and Canada for tree nuts but not peanuts (Environmental Protection Agency (EPA) 2006). Commercial PPO processes achieve a 5-log or greater reduction of *Salmonella* on almonds (ABC 2008; Danyluk et al. 2005) and have been evaluated for pecans under laboratory conditions (Beuchat 1973). Although effective, PPO is a batch process limited by the size of the PPO chamber. Bulk nuts (usually in bins, boxes, or totes) must be warmed to at least 30 °C, which can take one or more days; operational parameters include chamber temperature, PPO concentration and time, vacuum after gas injection, and the number of aeration cycles at the end of the PPO exposure time. To achieve the full 5-log reduction and to reduce PPO concentrations to below

300 ppm (a requirement of the US Environmental Protection Agency), nuts must be held for a further 2–5 days before shipping.

High hydrostatic pressure (HHP) has been evaluated for reduction of *Salmonella* on almonds (Goodridge et al. 2006; Willford et al. 2008), in peanut butter (D'Souza et al. 2012), and on sesame seeds (Wuytack et al. 2003). A 3- to 4-log reduction of *Salmonella* was achieved when inoculated almonds were suspended in water during HPP at 414 MPa for 6 min and dried at ambient temperature for 25 min after treatment (Willford et al. 2008). Greater reductions were achieved when higher temperatures were used to dry the almonds after HPP. In contrast, less than 2-log reductions of *Salmonella* were observed in peanut butter treated with high pressure at 600 MPa for 5 min (Grasso et al. 2010) or 400 or 600 MPa for 4–18 min (D'Souza et al. 2012). This is likely due to the low moisture and high oil content of the peanut butter matrix, compared with whole nuts that are suspended in water for HPP processing.

Before shelling, in-shell pecans are commercially cleaned and conditioned in water that is often chlorinated (Beuchat and Mann 2011a). Chorine is effective in maintaining water quality; reductions of inoculated *Salmonella* are minimal. Free chlorine levels can decline rapidly if nuts are not cleaned prior to conditioning (Beuchat et al. 2012). A 20-min exposure to lactic acid (2 %) or levulinic acid (2 %), in combination with sodium dodecyl sulfate (0.05 %), reduced *Salmonella* inoculated onto in-shell pecans by 3.4–3.7 log CFU/g. Although this type of treatment would be possible for in-shell nuts with smooth hard shells that are commonly soaked for long periods (e.g., pecans or cashews prior to shelling), it would not be widely applicable to many nuts especially those with porous shells (e.g., almonds or peanuts). Application of 10 % citric or lactic acids to in-shell almonds or almond kernels and holding for 1–5 min reduced *Salmonella* populations by 0.2–1.4 log CFU/g. The impact of these levels of acids on sensory characteristics of the nuts was not evaluated. Prior to roasting, almonds and pistachios are sometimes sprayed or dipped into saturated salt solutions or flavoring mixtures that may contain citric acid (Kim and Harris 2006). Significantly greater reductions in *Salmonella* were observed when almond kernels were exposed to water or citric or lactic acids prior to heating.

In-shell walnuts are sometimes exposed to very high concentrations of sodium hypochlorite to lighten the shell (common for North American markets where light shells are traditional). Walnuts with cracked or damaged shells are removed prior to treating, as the treatment would adversely affect the exposed kernels. Treating in-shell walnuts for 2 min in 3 % (30,000 ppm) sodium hypochlorite reduced populations of inoculated *Salmonella* by 2.5 log; further reductions were observed when the treated nuts were subsequently dried and stored (Blessington et al. 2013). This treatment is probably limited to in-shell walnuts and is becoming less common as the North American in-shell walnut market continues to decrease in favor of shelled product.

X-ray irradiation (Jeong et al. 2012), ionizing radiation (Prakash et al. 2010; Wilson-Kakashita et al. 1995), electron beam radiation (Duong and Foley 2006; Hvizdzak et al. 2010), and nonthermal plasma (Deng et al. 2007; Niemira 2012) have also been evaluated. A 4-log reduction in *Salmonella* on whole almonds using ionizing radiation required a dose of 5 kGy (Prakash et al. 2010); however, at this

level of treatment, significant negative sensory changes were detected by a trained panel, and the nut samples were rejected by a consumer panel. In peanut butter, a 4-log reduction in *Salmonella* was achieved with 3 kGy (Hvizdzak et al. 2010), but sensory effects were not evaluated.

Sanitation

Regardless of the method used to control pathogens in nuts and nut pastes, it is important to prevent the recontamination of treated product via robust sanitation and environmental monitoring programs (GMA 2010). These include taking precautions when designing equipment that can be easily cleaned and keeping the infrastructure in good repair to avoid ingress of water and development of niches that collect debris. Establishing a Primary *Salmonella* Control Area (generally the processing area after the inactivation step) is usually recommended (GMA 2010).

Cleaning and sanitizing within nut-processing environments are complicated by the significant amounts of dust generated during the postharvest handling of nuts, which consists of residual orchard soil as well as nut hull and shell particulates. Regular dry cleaning to reduce dust buildup and using appropriate air filters to prevent movement of microbial contaminants into the Primary *Salmonella* Control Area are important. Oil residues from some nuts (e.g., walnuts) also can coat equipment surfaces during operation.

Introduction of moisture can promote growth and survival of microorganisms in nut particulates (Beuchat and Mann 2010b; Du et al. 2010b; Uesugi and Harris 2006). Almond dust inactivated water-based quaternary ammonium sanitizers (Du et al. 2010b); therefore, using water or water-based sanitizers is not typically recommended unless equipment can be disassembled, dust is completely removed, and reassembly takes place after thorough drying. For wet-cleaning activities, separate cleaning rooms are sometimes used to clean and sanitize equipment, thereby reducing the opportunity for introduction of water into the facility. When disassembly or removal of equipment is not possible, a common practice is to control dusts by physical removal along with application of an isopropyl alcohol-based sanitizer on product-contact surfaces (Du et al. 2007, 2010b). Isopropyl alcohol-based sanitizers also reduce microbial loads on shoes when used in footbaths (Burnett et al. 2013). No significant reductions of microbial levels on the shoes over untreated controls were observed when water-based or dry quaternary ammonium sanitizers were used.

Conclusions

Nuts and nut pastes have long been a significant part of the human diet. Recent increases in production and consumption have been coupled with an increased awareness of microbial food safety issues associated with these products. Substantial

research has been devoted to understanding pathogen behavior and control on nuts since 2001; data from these studies have directly enhanced food safety guidance and have led to improvements to sanitation programs for these products (e.g., GMA 2010; Scott et al. 2009). Various thermal and nonthermal control methods and the means to appropriately validate these systems have been and continue to be developed and implemented (ABC 2014b; Anderson and Lucore 2012).

References

Abd SJ, McCarthy KL, Harris LJ (2012) Impact of storage time and temperature on thermal inactivation of *Salmonella* Enteritidis PT 30 on oil-roasted almonds. J Food Sci 71:M42–M47

Akbas MY, Ozdemir M (2006) Effectiveness of ozone for inactivation of *Escherichia coli* and *Bacillus cereus* in pistachios. Int J Food Sci Technol 41:513–519

Almond Board of California (ABC) (2007a) Guidelines for validation of blanching processes, v1.0, April 13, 2007. Available at: http://www.almonds.com/processors/processing-safe-product#pasteurization. Accessed 1 Sept 2014

Almond Board of California (ABC) (2007b) Guidelines for validation of dry roasting processes, v1.2, October 23, 2007. Available at: http://www.almonds.com/processors/processing-safe-product#pasteurization. Accessed 1 Sept 2014

Almond Board of California (ABC) (2007c) Guidelines for validation of oil roasting processes, v1.0, April 13, 2007. Available at: http://www.almonds.com/processors/processing-safe-product#pasteurization. Accessed 1 Sept 2014

Almond Board of California (ABC) (2008) Guidelines for validation of propylene oxide pasteurization, v3.0, October 1, 2008. Available at: http://www.almonds.com/processors/processing-safe-product#pasteurization. Accessed 1 Sept 2014

Almond Board of California (ABC) (2009) Good agricultural practices. Available at: http://www.almonds.com/growers/growing-safe-product/gaps. Accessed 1 Sept 2013

Almond Board of California (ABC) (2014a) Guidelines for using *Enterrococcus faecium* NRRL B-2354 as a surrogate microorganism in almond process validation. Available at: Accessed 1 Sept 2014

Almond Board of California (ABC) (2014b) Validation guidelines. Available at: http://www.almonds.com/processors/processing-safe-product#tc-pasteurization. Accessed 1 Sept 2014

American Peanut Council (APC) (2011) Good Agricultural Practices for safe growing and harvesting of peanuts. Available at: https://www.peanutsusa.com/phocadownload/GMPs/2011%20Final%20Chapter%201%20Good%20Agricultural%20Practices%20for%20Growers.pdf. Accessed 1 Sept 2013

Anderson DG, Lucore LA (2012) Validating the reduction of *Salmonella* and other pathogens in heat processed low-moisture foods. Alliance for Innovation & Operational Excellence, Alexandria, VA, Published online at http://community.pmmi.org/Alliance/Home/

Arrus K, Blank G, Clear R, Holley RA, Abramson D (2005) Microbiological and aflatoxin evaluation of Brazil nut pods and the effects of unit processing operations. J Food Prot 68:1060–1065

Baird-Parker AC, Freame B (1967) Combined effect of water activity, pH and temperature on the growth of *Clostridium botulinum* from spore and vegetative cell inocula. J Appl Microbiol 30:420–429

Bansal A, Jones TM, Abd SJ, Danyluk MD, Harris LJ (2010) Most-probable-number determination of *Salmonella* levels in naturally contaminated raw almonds using two sample preparation methods. J Food Prot 73:1986–1992

Bari ML, Nei D, Sotome I, Nishina I, Isobe S, Kawamoto S (2009) Effectiveness of sanitizers, dry heat, hot water, and gas catalytic infrared heat treatments to inactivate *Salmonella* on almonds. Foodborne Pathog Dis 6:953–958

Bari ML, Nei D, Sotome I, Nishina IY, Hayakawa F, Isobe S, Kawamoto S (2010) Effectiveness of superheated steam and gas catalytic infrared heat treatments to inactivate *Salmonella* on raw almonds. Foodborne Pathog Dis 7:845–850

Beuchat LR (1973) *Escherichia coli* on pecans: survival under various storage conditions and disinfection with propylene oxide. J Food Sci 38:1063–1066

Beuchat LR (1978) Relationship of water activity to moisture content in tree nuts. J Food Sci 43:754–755, 758

Beuchat LR, Heaton EK (1975) *Salmonella* survival on pecans as influenced by processing and storage conditions. Appl Microbiol 29:795–801

Beuchat LR, Mann DA (2010a) Factors affecting infiltration and survival of *Salmonella* on in-shell pecans and pecan nutmeats. J Food Prot 73:1257–1268

Beuchat LR, Mann DA (2010b) Survival and growth of *Salmonella* in high-moisture pecan nutmeats, in-shell pecans, inedible nut components, and orchard soil. J Food Prot 73:1975–1985

Beuchat LR, Mann DA (2011a) Inactivation of *Salmonella* on in-shell pecans during conditioning treatments preceding cracking and shelling. J Food Prot 74:588–602

Beuchat LR, Mann DA (2011b) Inactivation of *Salmonella* on pecan nutmeats by hot air treatment and oil roasting. J Food Prot 74:1441–1450

Beuchat LR, Mann DA, Alali WQ (2012) Evaluation of sanitizers for inactivating *Salmonella* on in-shell pecans and pecan nutmeats. J Food Prot 75:1930–1938

Beuchat LR, Komitopoulou E, Beckers H, Betts RP, Bourdichon F, Fanning S, Joosten HM, Ter Kuile BH (2013a) Low-water activity foods: increased concern as vehicles of foodborne pathogens. J Food Prot 76:150–172

Beuchat LR, Mann DA, Alali WQ (2013b) Efficacy of sanitizers in reducing *Salmonella* on pecan nutmeats during cracking and shelling. J Food Prot 76:770–778

Bingol G, Yang J, Brandl MT, Pan Z, Wang H, McHugh TH (2011) Infrared pasteurization of raw almonds. J Food Eng 104:387–393

Blanchard RO, Hanlin RT (1973) Effect of propylene oxide treatment on the microflora of pecans. Appl Microbiol 26:768–722

Blessington T (2011) A microbiological hazard analysis of California walnut production and handling. Ph.D. Dissertation, University of California, Davis

Blessington T, Mitcham EJ, Harris LJ (2012) Survival of *Salmonella enterica*, *Escherichia coli* O157:H7 and *Listeria monocytogenes* on inoculated walnut kernels during storage. J Food Prot 75:245–254

Blessington T, Theofel CG, Mitcham EJ, Harris LJ (2013) Survival of foodborne pathogens on inshell walnuts. Int J Food Microbiol 166:341–348

Brandl MT, Pan Z, Huynh S, Zhu Y, McHugh TH (2008) Reduction of *Salmonella* Enteritidis population sizes on almond kernels with infrared heat. J Food Prot 71:897–902

Brett M (1999) Botulism in the United Kingdom. Eurosurveillance 4(1):45

Brockmann SO, Piechotowski I, Kimmig P (2004) *Salmonella* in sesame seed products. J Food Prot 67:178–180

Burnett SL, Gehm ER, Weissinger WR, Beuchat LR (2000) Survival of *Salmonella* in peanut butter and peanut butter spread. J Appl Microbiol 89:472–477

Burnett SL, Egland SJ, McKelvey PJ, Cook FK (2013) Chemical decontamination of footwear soles to limit microbial transfer in a dry environment. Food Prot Trends 33(2):74–81

Calhoun S, Post L, Warren B, Thompson S, Bontempo AR (2013) Prevalence and concentration of *Salmonella* on raw shelled peanuts in the United States. J Food Prot 76:575–579

California Department of Public Health (CDPH) (2002) Environmental investigation of *Salmonella* Enteritidis Phage Type 30 outbreak associated with consumption of raw almonds. Available at: http://www.cdph.ca.gov/pubsforms/Documents/fdb%20eru%20Almond%20SE%20Rpt%202001-2002.pdf. Accessed 18 July 2013

California Department of Public Health (CDPH) (2004) Environmental investigation of *Salmonella* Enteritidis Phage Type 9c outbreak associated with consumption of raw almonds. Available at:

http://www.cdph.ca.gov/pubsforms/Documents/fdb%20eru%20Almond%20SE2004.pdf. Accessed 18 July 2013
California Pistachio Research Board (CPRB) (2009) Good agricultural practices manual: guidelines for California pistachio growers. Available at: http://www.gmaonline.org/downloads/technical-guidance-and-tools/Addendum_2_GAP_for_Pistachio_Growers.pdf. Accessed 11 June 2013
California Walnut Board (CWB) (2012) Walnut history & cultivation. Available at: http://www.walnuts.org/about-walnuts/walnut-history/. Accessed 18 July 2013
Canadian Food Inspection Agency (CFIA) (2011) Certain bulk and prepackaged bulk raw shelled walnuts may contain *E. coli* O157:H7 bacteria. Available at: http://www.inspection.gc.ca/english/corpaffr/recarapp/2011/20110404e.shtml. Accessed 6 Aug 2013
Cavallaro E, Date K, Medus C, Meyer S, Miller B, Kim C, Nowicki S et al (2011) *Salmonella* Typhimurium infections associated with peanut products. N Engl J Med 365:601–610
Centers for Disease Control and Prevention (CDC) (2007a) Multistate outbreak of *Salmonella* serotype Tennessee infections associated with peanut butter—United States, 2006–2007. MMWR Wkly 56(21):521–524
Centers for Disease Control and Prevention (CDC) (2007b) Salmonellosis—outbreak investigation, February 2007. Available at: http://www.cdc.gov/ncidod/dbmd/diseaseinfo/salmonellosis_2007/outbreak_notice.htm. Accessed 6 Aug 2013
Centers for Disease Control and Prevention (CDC) (2009) Multistate outbreak of *Salmonella* infections associated with peanut butter and peanut butter-containing products—United States, 2008–2009. MMWR Wkly 58(4):85–90
Centers for Disease Control and Prevention (CDC) (2010) Multistate outbreak of *Salmonella* Typhimurium infections linked to peanut butter, 2008–2009 (final update). Available at: http://www.cdc.gov/salmonella/typhimurium/update.html. Accessed 6 Aug 2013
Centers for Disease Control and Prevention (CDC) (2011a) Investigation update: multistate outbreak of *E. coli* O157:H7 infections associated with in-shell hazelnuts. Available at: http://www.cdc.gov/ecoli/2011/hazelnuts0157/index.html. Accessed 6 Aug 2013
Centers for Disease Control and Prevention (CDC) (2011b) Multistate outbreak of human *Salmonella* Enteritidis infections linked to Turkish pine nuts, November 17, 2011 (final update). Available at: http://www.cdc.gov/Salmonella/pinenuts-enteriditis/111711/index.html. Accessed 6 Aug 2013
Centers for Disease Control and Prevention (CDC) (2012) Multistate outbreak of *Salmonella* Bredeney infections linked to peanut butter manufactured by Sunland, Inc. (final update). Available at: http://www.cdc.gov/Salmonella/bredeney-09-12/index.html. Accessed 6 Aug 2013
Chan ES, Aramini J, Ciebin B, Middleton D, Ahmed R, Howes M, Brophy I, Mentis I, Jamieson F, Rodgers F, Nazarowec-White M, Pichette SC, Farrar J, Gutierrez M, Weis WJ, Lior L, Ellis A, Isaacs S (2002) Natural or raw almonds and an outbreak of a rare phage type of *Salmonella* Enteritidis infection. Can Commun Dis Rep 28:97–99
Chang S-S, Han AR, Reyes-De-Corcuera JI, Powers JR, Kang D-H (2010) Evaluation of steam pasteurization in controlling *Salmonella* serotype Enteritidis on raw almond surfaces. Lett Appl Microbiol 50:393–398
Chipley JR, Heaton EK (1971) Microbial flora of pecan meat. Appl Microbiol 22:252–253
Chou JH, Hwang PH, Malison MD (1988) An outbreak of type A foodborne botulism in Taiwan due to commercially preserved peanuts. Int J Epidemiol 17:899–902
Clavero MRS, Brackett RE, Beuchat LR, Doyle MP (2000) Influence of water activity and storage conditions on survival and growth of proteolytic *Clostridium botulinum* in peanut spread. Food Microbiol 17:53–61
D'Souza T, Karwe M, Schaffner DW (2012) Effect of high hydrostatic pressure and pressure cycling on a pathogenic *Salmonella enterica* serovar cocktail inoculated into creamy peanut butter. J Food Prot 75:169–173
Danyluk MD, Uesugi AR, Harris LJ (2005) Survival of *Salmonella* Enteritidis PT 30 on inoculated almonds after commercial fumigation with propylene oxide. J Food Prot 68:1613–1622

Danyluk MD, Harris LJ, Schaffner DW (2006) Monte Carlo simulations assessing the risk of salmonellosis from consumption of almonds. J Food Prot 69:1594–1599

Danyluk MD, Jones TM, Abd SJ, Schlitt-Dittrich F, Jacobs M, Harris LJ (2007) Prevalence and amounts of *Salmonella* found on raw California almonds. J Food Prot 70:820–827

Danyluk MD, Brandl MT, Harris LJ (2008) Migration of *Salmonella* Enteritidis phage type 30 through almond hulls and shells. J Food Prot 71:397–401

Deng S, Ruan R, Mok CK, Huang G, Lin X, Chen P (2007) Inactivation of *Escherichia coli* on almonds using nonthermal plasma. J Food Sci 72(2):M62–M66

Du W-X, Danyluk MD, Harris LJ (2007) Evaluation of cleaning treatments for almond-contact surfaces in hulling and shelling facilities. Food Prot Trends 27:678–683

Du W-X, Abd SJ, McCarthy KL, Harris LJ (2010a) Reduction of *Salmonella* on inoculated almonds exposed to hot oil. J Food Prot 73:1238–1246

Du W-X, Danyluk MD, Harris LJ (2010b) Efficacy of aqueous and alcohol-based quaternary ammonium sanitizers for reducing *Salmonella* in dusts generated in almond hulling and shelling facilities. J Food Sci 75(1):M7–M13

Duong C, Foley D (2006) The effect of electron beam radiation on raw almonds contaminated with different *Salmonella* strains. In: Abstracts of the 106th general meeting of the American Society for Microbiology, Orlando, FL, 21–25 May 2006. Available at: http://ieg.ou.edu/ASM2006/data/papers/P_101.htm. Accessed 19 Sept 2013

Economic Research Service (ERS), U.S. Department of Agriculture (2012) Fruit and tree nut yearbook spreadsheet files (89022). Available at: http://usda01.library.cornell.edu/usda/ers/89022/Table-A1.xlsx. Accessed 1 Nov 2012

Eglezos S (2010) The bacteriological quality of retail-level peanut, almond, cashew, hazelnut, Brazil, and mixed nut kernels produced in two Australian nut-processing facilities over a period of 3 years. Foodborne Pathog Dis 7:863–866

Eglezos S, Huang B, Stuttard E (2008) A survey of the bacteriological quality of pre roasted peanut, almond, cashew, hazelnut and Brazil nut kernels received into three Australian nut-processing facilities over a period of 3 years. J Food Prot 71:402–404

Federal Register (2007) Almonds grown in California: outgoing quality control requirements. Fed Reg 72:15021–15036 (C.F.R. part 981)

Food and Agricultural Organization (FAO) (2012) FAOSTAT agricultural production data. Food and Agricultural Organization of the United Nations, Rome. Available at: http://faostat3.fao.org/home/index.html#DOWNLOAD. Accessed 6 May 2013

NSW Food Authority (2012) Report on the prevalence of *Salmonella* and *E. coli* in ready to eat nuts and nut products sold in Australia. Available at: http://www.foodauthority.nsw.gov.au/science/market-analysis/ready-to-eat-nuts/. Accessed 31 May 2013

Food Standards Australia New Zealand (FSANZ) (2012) Warning and advice on raw almonds and possible *Salmonella* contamination. Available at: http://www.foodstandards.gov.au/media/pages/mediareleases/mediareleases2012/23october2012warning5699.aspx. Accessed 31 May 2013

Freire FCO, Offord L (2002) Bacterial and yeast counts in Brazilian commodities and spices. Braz J Microbiol 33:145–148

Frelka J (2013) The influence of postharvest handling practices on the microbiota of English walnuts (*Juglans regia* L.). M.S. Thesis, University of California, Davis

Gao M, Tang J, Villa-Rojas R, Wang Y, Wang S (2011) Pasteurization process development for controlling *Salmonella* in in-shell almonds using radio frequency energy. J Food Eng 104:299–306

Goodridge LD, Willford J, Kalchayanand N (2006) Destruction of *Salmonella* Enteritidis inoculated onto raw almonds by high hydrostatic pressure. Food Res Int 39:408–412

Goren-Inbar N, Sharon G, Melamed Y, Kislev M (2002) Nuts, nut cracking, and pitted stones at Gesher Benot Ya'aqov, Israel. Proc Natl Acad Sci USA 99:2455–2460

Grasso EM, Somerville JA, Balasubramaniam VM, Lee K (2010) Minimal effects of high-pressure treatment on *Salmonella enterica* serovar Typhimurium inoculated into peanut butter and peanut products. J Food Sci 75(8):E522–E526

Grocery Manufacturers Association (GMA) (2010) Industry handbook for safe processing of nuts. GMA Nut Safety Task Force, Grocery Manufacturers Association, Washington, DC. Available at: http://www.gmaonline.org/downloads/wygwam/Industry_Handbook_for_Safe_Processing_of_Nuts_1st_Edition_22Feb10.pdf. Accessed 1 June 2013

Harris LJ, Uesugi AR, Abd SJ, McCarthy KL (2012) Survival of *Salmonella* Enteritidis PT 30 on inoculated almond kernels in hot water treatments. Food Res Int 45:1093–1098

Harris LJ, Beuchat LR, Danyluk MD, Palumbo M (2014) Outbreaks of foodborne illness associated with the consumption of tree nuts, peanuts, and sesame seeds [table and references]. In: Outbreaks from tree nuts, peanuts, and sesame seeds. Available at: http://ucfoodsafety.ucdavis.edu/Nuts_and_Nut_Pastes. Accessed 1 Sept 2014

Hazelnut Marketing Board (HMB) (2012) Hazelnut industry Good Agricultural Practices manual. Oregon Hazelnut Commission, Aurora, OR. Available at: http://www.oregonhazelnuts.org/growers-corner/food-safety-update/gaps/. Accessed 18 Sept 2013

He Y, Guo D, Yang J, Tortorello ML, Zhang W (2011) Survival and heat resistance of *Salmonella enterica* and *Escherichia coli* O157:H7 in peanut butter. Appl Environ Microbiol 77:8434–8438

He Y, Li Y, Salazar JK, Yang J, Tortorello ML, Zhang W (2013) Increased water activity reduces thermal resistance of *Salmonella enterica* in peanut butter. Appl Environ Microbiol 79: 4763–4767

Horner KJ, Anagnostopoulos GD (1972) Viability of *Staphylococcus aureus*, *Salmonella* Typhimurium and *Salmonella* Senftenberg heated and recovered on a solid medium of controlled water activity. J Sci Food Agric 23:543–544

Hvizdzak AL, Beamer S, Jaczynski J, Matak KE (2010) Use of electron beam radiation for the reduction of *Salmonella enterica* serovars Typhimurium and Tennessee in peanut butter. J Food Prot 73:353–357

International Nut and Dried Fruit Council (INC) (2012) Global statistical review 2007–2012. INC, Reus, Spain

Isaacs S, Aramini J, Ceibin B, Farrar JA, Ahmed R, Middleton D, Chandran AU, Harris LJ, Howes M, Chan E, Pichette AS, Campbell K, Gupta A, Lior LJ, Pearce M, Clark C, Rodgers F, Jamieson F, Brophy I, Ellis A (2005) An international outbreak of salmonellosis associated with raw almonds contaminated with a rare phage type of *Salmonella* Enteritidis. J Food Prot 68:191–198

Jeong S, Marks BP, Orta-Ramirez A (2009) Thermal inactivation kinetics for *Salmonella* Enteriditis PT30 on almonds subjected to moist-air convection heating. J Food Prot 72:1602–1609

Jeong S, Marks BP, Ryser ET (2011) Quantifying the performance of *Pediococcus* sp. (NRRL B-2354: *Enterococcus faecium*) as a nonpathogenic surrogate for *Salmonella* Enteritidis PT30 during moist-air convection heating of almonds. J Food Prot 74:603–609

Jeong S, Marks BP, Ryser ET, Harte JB (2012) The effect of X-ray irradiation on *Salmonella* inactivation and sensory quality of almonds and walnuts as a function of water activity. Int J Food Microbiol 153:365–371

Kader AA, Thompson JF (2002) Postharvest handling systems: tree nuts. In: Kader AA (ed) Postharvest technology of horticultural crops, 3rd edn. Division of Agriculture and Natural Resources, University of California, Oakland, pp 399–406

Kajs TM, Hagenmaier R, Vanderzant C, Mattil KF (1976) Microbiological evaluation of coconut and coconut products. J Food Sci 41:352–356

Keady S, Briggs G, Farrar J, Mohle-Boetani JC, O'Connell J, Werner SB, Anderson D, Tenglesen L, Bidols S, Albanese B, Gordan C, Debess E, Hatch J, Keene WE, Plantenga M, Tierheimer J, Hackman AL, Rinehardt CE, Sandt CH, Ingram A, Hansen S, Hurt S, Poulson M, Pallipamu R, Wicklund J, Braden C, Lockett J, Van Duyne S, Dechet A, Smesler C (2004) Outbreak of *Salmonella* serotype Enteritidis infections associated with raw almonds—United States and Canada, 2003–2004. MMWR Wkly 53(22):484–487

Keller SE, Grasso EM, Halik LA, Fleischman GJ, Chirtel SJ, Grove SF (2012) Effect of growth on the thermal resistance and survival of *Salmonella* Tennessee and Oranienburg in peanut butter, measured by a new thin-layer thermal death time device. J Food Prot 75:1125–1130

Killalea D, Ward LR, Roberts D, de Louvois J, Sufi F, Stuart JM, Wall PG, Susman M, Schwieger M, Sanderson PJ, Fisher IST, Mead PS, Gill ON, Bartlett CLR, Rowe B (1996) International epidemiological and microbiological study of outbreak of *Salmonella* Agona infection from a ready to eat savoury snack—I: England and Wales and the United States. Br Med J 313: 1105–1107

Kim BU, Harris LJ (2006) The effect of pre-treatments on the reduction of *Salmonella* Enteritidis PT 30 on almonds during dry roasting (Abstract P4-17). In: International Association for Food Protection Annual Meeting, Calgary, Alberta, August 13–16

Kimber MA, Kaur H, Wang L, Danyluk MD, Harris LJ (2012) Survival of *Salmonella*, *Escherichia coli* O157:H7, and *Listeria monocytogenes* on inoculated almonds and pistachios stored at −19, 4, and 24 °C. J Food Prot 75:1394–1403

King AD Jr, Miller MJ, Eldridge LC (1970) Almond harvesting, processing, and microbial flora. Appl Microbiol 20:208–214

Kirk MD, Little CL, Lem M, Fyfe M, Genobile D, Tan A, Threlfall J, Paccagenella A, Lightfoot D, Lyi H, McIntyre L, Ward L, Brown DJ, Surnam S, Fisher IST (2004) An outbreak due to peanuts in their shell caused by *Salmonella* enterica serotypes Stanley and Newport—sharing molecular information to solve international outbreaks. Epidemiol Infect 132:571–577

Lambertini E, Danyluk MD, Schaffner DW, Winter CK, Harris LJ (2012) Risk of salmonellosis from consumption of almonds in the North American market. Food Res Int 45:1166–1174

Ledet Müller L, Hjertqvist M, Payne L, Pettersson H, Olsson A, Plym Forshell L, Andersson Y (2007) Cluster of *Salmonella* Enteritidis in Sweden 2005–2006—suspected source: almonds. Eurosurveillance 12(6):718

Lee S-Y, Oh S-W, Chung H-J, Reyes-De-Corcuera JI, Powers JR, Kang DH (2006) Reduction of *Salmonella* enterica serovar Enteritidis on the surface of raw shelled almonds by exposure to steam. J Food Prot 69:591–595

Lee LE, Metz D, Giovanni M, Bruhn CM (2011) Consumer knowledge and handling of tree nuts: food safety implications. Food Prot Trends 31:18–27

Little CL, Jemmott W, Surman-Lee S, Hucklesby L, de Pinna E (2009) Assessment of microbiological safety of edible roasted nut kernels on retail sale in England, with a focus on *Salmonella*. J Food Prot 72:853–855

Little CL, Rawal N, de Pinna E, McLauchlin J (2010) Survey of *Salmonella* contamination of edible nut kernels on retail sale in the UK. Food Microbiol 27:171–174

Ma L, Zhang G, Gerner-Smidt P, Mantripragada V, Ezeoke I, Doyle MP (2009) Thermal inactivation of *Salmonella* in peanut butter. J Food Prot 72:1596–1601

Mangels R (2001) Vegetarian journal's guide to nuts and nut butters. Veg J. Available at: http://www.vrg.org/journal/vj2001nov/2001novnuts.htm. Accessed 1 Nov 2012

Marcus KA, Amling HJ (1973) *Escherichia coli* field contamination of pecan nuts. Appl Microbiol 26:279–281

Marler Clark LLP (2006) Pumpkin festival boiled peanuts 2006. Available at: http://outbreakdatabase.com/details/pumpkin-festival-boiled-peanuts-2006/. Accessed 31 May 2013

Mattick KL, Jørgensen F, Legan JD, Lappin-Scott HM, Humphrey TJ (2000) Habituation of *Salmonella* spp. at reduced water activity and its effect on heat tolerance. Appl Environ Microbiol 66:4921–4925

Menninger EA (1977) Edible nuts of the world. Horticultural Books, Inc., Stuart, FL

Meyer MT, Vaughn RH (1969) Incidence of *Escherichia coli* in black walnut meats. Appl Microbiol 18:925–931

Miller BD, Rigdon CE, Ball J, Rounds JM, Klos RF, Brennan BM, Arends KD, Kennelly P, Hedberg C, Smith KE (2012) Use of traceback methods to confirm the source of a multistate *Escherichia coli* O157:H7 outbreak due to in-shell hazelnuts. J Food Prot 75:320–327

Niemira BA (2012) Cold plasma reduction of *Salmonella* and *Escherichia coli* O157:H7 on almonds using ambient pressure gases. J Food Sci 77(3):M171–M175

O'Mahony M, Mitchell E, Gilbert RJ, Hutchinson DN, Begg NT, Rodhouse JC, Morris JE (1990) An outbreak of foodborne botulism associated with contaminated hazelnut yoghurt. Epidemiol Infect 104:389–395

Palumbo M, Beuchat LR, Danyluk MD, Harris LJ (2014a) Prevalence and levels of foodborne pathogens on naturally-contaminated nuts and edible seeds [Tables 1–4 and references]. In: Surveys for foodborne pathogens in nuts. Available at: http://ucfoodsafety.ucdavis.edu/Nuts_and_Nut_Pastes. Accessed 1 Oct 2014

Palumbo M, Beuchat LR, Danyluk MD, Harris LJ (2014b) Recalls of tree nuts and peanuts in the U.S., 2001 to present [table and references]. In: U.S. recalls of nuts. Available at: http://ucfoodsafety.ucdavis.edu/Nuts_and_Nut_Pastes. Accessed 1 Sept 2014

Pan Z, Bingol G, Brandl MT, McHugh TH (2012) Review of current technologies for reduction of *Salmonella* populations on almonds. Food Bioprocess Technol 5:2046–2057

Pao S, Kalantari A, Huang G (2006) Utilizing acidic sprays for eliminating *Salmonella enterica* on raw almonds. J Food Sci 71:M14–M19

Podolak R, Enache E, Stone W, Black DG, Elliott PH (2010) Sources and risk factors for contamination, survival, persistence, and heat resistance of *Salmonella* in low-moisture foods. J Food Prot 73:1919–1936

Prakash A, Lim FT, Duong C, Caporaso F, Foley D (2010) The effects of ionizing irradiation on *Salmonella* inoculated on almonds and changes in sensory properties. Radiat Phys Chem 79:502–506

ProMED-mail (2006) Salmonellosis, boiled peanuts—USA (South Carolina). Archive No. 20061102.3135. Available at: http://www.promedmail.org. Accessed 6 Nov 2012

Public Health Agency of Canada (PHAC) (2011) Public health advisory: *E. coli* outbreak. Public Health Agency of Canada press release, April 4. Available at: http://www.marketwire.com/press-release/Public-Advisory-E-coli-Outbreak-1422244.htm. Accessed 1 Aug 2012

Rosengarten F Jr (1984) The book of edible nuts. Dover, Mineola, NY

Health and Safety Watch (2011) June 21, 2011 update: *E. coli* outbreak involving bulk and pre-packaged walnuts appears to be over. http://www.healthandsafetywatch.com/HSWEvents.aspx?EventID=cb38ec9b-8396-4861-9ab2-a048f8359393. Accessed 8 Aug 2012

Schade JE, King AD Jr (1977) Methyl bromide as a microbicidal fumigant for tree nuts. Appl Environ Microbiol 33:1184–1191

Schaffner DW, Buchanan R, Calhoun S, Danyluk MD, Harris LJ, Djordjevic D, Whiting R, Kottapalli B, Wiedmann M (2013) Issues to consider when setting intervention targets with limited data for low-moisture food commodities: a peanut case study. J Food Prot 2:360–369

Scheil W, Cameron S, Dalton C, Murray C, Wilson D (1998) A South Australian *Salmonella* Mbandaka outbreak investigation using a database to select controls. Aust N Z J Public Health 22:536–539

Scott VN, Chen Y, Freier TA, Kuehm J, Moorman M, Meyer J, Morille-Hinds T, Post L, Smoot L, Hood S, Shebuski J, Banks J (2009) Control of *Salmonella* in low-moisture foods. I: Minimizing entry of *Salmonella* into a processing facility. Food Prot Trends 29:342–353

Shachar D, Yaron S (2006) Heat tolerance of *Salmonella enterica* serovars Agona, Enteritidis, and Typhimurium in peanut butter. J Food Prot 69:2687–2691

Sheppard YD, Middleton D, Whitfield Y, Tyndel F, Haider S, Spiegelman J, Swartz RH, Nelder MP, Baker SL, Landry L, MacEachern R, Deamond S, Ross L, Peters G, Baird M, Rose D, Sanders G, Austin JW (2012) Intestinal toxemia botulism in 3 adults, Ontario, Canada, 2006–2008. Emerg Infect Dis 18:1–6

Sheth AN, Hoekstra M, Patel N, Ewald G, Lord C, Clarke C, Villamil E, Niksich K, Bopp C, Nguyen T-A, Zink D, Lynch M (2011) A national outbreak of *Salmonella* serotype Tennessee infections from contaminated peanut butter: a new food vehicle for salmonellosis in the United States. Clin Infect Dis 53:356–362

Shohat T, Green MS, Merom D, Gill ON, Reisfeld A, Matas A, Blau D, Gal N, Slater PE (1996) International epidemiological and microbiological study of outbreak of *Salmonella* Agona infection from a ready to eat savoury snack—II: Israel. Br Med J 313:1107–1109

Sobel J (2005) Botulism. Clin Infect Dis 41:1167–1173

Threlfall EJ, Hampton MD, Ward LR, Rowe B (1996) Application of pulsed-field gel electrophoresis to an international outbreak of *Salmonella agona*. Emerg Infect Dis 2:130–132

Torlak E, Sert D, Serin P (2013) Fate of *Salmonella* during sesame seeds roasting and storage of tahini. Int J Food Microbiol 163:214–217

U.S. Environmental Protection Agency (EPA) (2006) Reregistration eligibility decision for propylene oxide. Available at: http://www.epa.gov/oppsrrd1/REDs/propylene_oxide_red.pdf. Accessed 1 June 2013

U.S. Food and Drug Administration (FDA) (2009a) Guidance for industry: measures to address the risk for contamination by *Salmonella* species in food containing a peanut-derived product as an ingredient. Available at: http://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/ProducePlantProducts/ucm115386.htm. Accessed 3 Jan 2012

U.S. Food and Drug Administration (FDA) (2009b) Peanut butter and other peanut containing products recall list. Available at: http://www.accessdata.fda.gov/scripts/peanutbutterrecall/index.cfm. Accessed 20 June 2013

U.S. Food and Drug Administration (FDA) (2009c) Peanut Corporation of America, FDA Form 483. Available at: http://www.fda.gov/downloads/AboutFDA/CentersOffices/OfficeofGlobalRegulatoryOperationsandPolicy/ORA/ORAElectronicReadingRoom/UCM109843.pdf. Accessed 1 Dec 2012

U.S. Food and Drug Administration (FDA) (2011) Guidance for industry: measures to address the risk for contamination by *Salmonella* species in food containing a pistachio-derived product as an ingredient. Available at: http://www.fda.gov/downloads/Food/GuidanceRegulation/UCM273568.pdf. Accessed 3 Jan 2012

U.S. Food and Drug Administration (FDA) (2012). Letter to Sunland Inc. concerning suspension of food facility registration; notice of opportunity for hearing. Available at: http://www.fda.gov/AboutFDA/CentersOffices/OfficeofFoods/CFSAN/CFSANFOIAElectronicReadingRoom/ucm329370.htm. Accessed 1 Sept 2013

U.S. Food and Drug Administration (FDA) (2013). FDA authorizes Sunland to resume preparing and distributing food at peanut butter plant. http://www.fda.gov/food/recallsoutbreaksemergencies/outbreaks/ucm320413.htm. Accessed Dec 8 2013

Uesugi AR, Harris LJ (2006) Growth of *Salmonella* Enteritidis phage type 30 in almond hull and shell slurries and survival in drying almond hulls. J Food Prot 69:712–718

Uesugi AR, Danyluk MD, Harris LJ (2006) Survival of *Salmonella* Enteritidis phage type 30 on inoculated almonds stored at −20, 4, 23, and 35 °C. J Food Prot 69:1851–1857

Uesugi AR, Danyluk MD, Mandrell RE, Harris LJ (2007) Isolation of *Salmonella Enteritidis* phage type 30 from a single almond orchard over a 5-year period. J Food Prot 70:1784–1789

Unicomb LE, Simmons G, Merritt T, Gregory J, Nicol C, Jelfs P, Kirk M, Tan A, Thomson R, Adamopoulos J, Little CL, Currie A, Dalton CB (2005) Sesame seed products contaminated with *Salmonella*: three outbreaks associated with tahini. Epidemiol Infect 133:1065–1072

Van Doren JM, Kleinmeier D, Hammack TS, Westerman A (2013a) Prevalence, serotype diversity, and antimicrobial resistance of *Salmonella* in imported shipments of spice offered for entry to the United States, FY2007–FY2009. Food Microbiol 34:239–251

Van Doren JM, Blodgett RJ, Pouillot R, Westerman A, Kleinmeier D, Ziobro GC, Ma Y, Hammack TS, Gill V, Muckenfuss MF, Fabbri L (2013b) Prevalence, level and distribution of *Salmonella* in shipments of imported capsicum and sesame seed spice offered for entry to the United States: observations and modeling results. Food Microbiol 36:149–160

Villa-Rojas R, Tang J, Wang S, Gao M, Kang D-H, Mah J-H, Gray P, Sosa-Morales ME, López-Malo A (2013) Thermal inactivation of *Salmonella* Enteritidis PT 30 in almond kernels as influenced by water activity. J Food Prot 76:26–32

Walter EHM, Nascimento MS, Kuaye AY (2009) Efficacy of sodium hypochlorite and peracetic acid in sanitizing green coconuts. Lett Appl Microbiol 49:366–371

Ward L, Brusin S, Duckworth G, O'Brien S (1999) *Salmonella java* phage type Dundee—rise in cases in England: update. Eurosurveillance 3(12):1435

Weller LD, Daeschel MA, Durham CA, Morrissey MT (2013) Effects of water, sodium hypochlorite, peroxyacetic acid, and acidified sodium chlorite on in-shell hazelnuts inoculated with *Salmonella Enterica* serovar Panama. J Food Sci. doi:10.1111/1750-3841.12294

Wihodo M, Han Y, Selby TL, Lorcheim P, Czarneski M, Huang G, Linton RH (2005) Decontamination of raw almonds using chlorine dioxide gas (Abstract 99E-12). In: Institute of Food Technologists Annual Meeting 2005, New Orleans, LA, July 15–20. Available at: http://ift.confex.com/ift/2005/techprogram/paper_30566.htm. Accessed 29 Sept 2013

Willford J, Mendonca A, Goodridge LD (2008) Water pressure effectively reduces *Salmonella enterica* serovar Enteritidis on the surface of raw almonds. J Food Prot 71:825–829

Willis C, Little CL, Sagoo S, de Pinna E, Threlfall J (2009) Assessment of the microbiological safety of edible dried seeds from retail premises in the United Kingdom with a focus on *Salmonella* spp. Food Microbiol 26:847–852

Wilson MM, Mackenzie EF (1955) Typhoid fever and salmonellosis due to the consumption of infected desiccated coconut. J Appl Bacteriol 18:510–521

Wilson-Kakashita G, Geredes DL, Hall WR (1995) The effect of gamma irradiation on the quality of English walnuts (*Juglans regia*). Lebensm Wiss Technol 28:17–20

Wuytack EY, Diels AMJ, Meersseman K, Michiels CW (2003) Decontamination of seeds for seed sprout production by high hydrostatic pressure. J Food Prot 66:918–923

Yang J, Bingol G, Pan Z, Brandl MT, McHugh TH, Wang H (2010) Infrared heating for dry-roasting and pasteurization of almonds. J Food Eng 101:273–280

Flour and Meal

Deann Akins-Lewenthal

Abstract Flour and meal play a large role in our day-to-day lives as a basic source of human nutrition since they are the principal part of the diet for a large part of the world's population. Most cereal grains are milled into flour or meal and then mixed into dough for baking into breads, biscuits, cakes, or pastries or for drying as pasta. There are also circumstances where they are used as carriers for nutraceuticals, pharmaceuticals, spices, and flavors or as bulking/caloric agents in dried mixes and as an ingredient in ready-to-cook or ready-to-eat products. The principal microorganisms of concern are *Salmonella* and mycotoxigenic fungi. This chapter will discuss the microorganisms of interest, provide an overview of flour- and meal-related outbreaks, review sources of microbial contamination prior to milling, and discuss the management and mitigation of microbiological risks during the milling process.

Keywords Cereal grains • Flour • *Salmonella* • Mycotoxins • Milling

Introduction

Flour and meal play a large role in our day-to-day lives as a basic source of human nutrition. Since flour and other cereal products are the principal part of the diet for a large proportion of the world's population, their safety and quality for human consumption are a major concern for both food manufacturers and regulatory organizations. There are many different types of flour available made from a variety of sources such as wheat, seeds, corn, rice, coconuts, soybeans, and potatoes. However, this chapter will focus primarily on the microbiological food safety risks associated with flour and meal produced from cereal grains.

Most cereal grains are milled into flour or meal and then mixed into dough for baking into breads, biscuits, cakes, or pastries or for drying as pasta. Milled products can also go into breakfast cereals, snacks, malt beverages, pancakes, muffins, corn bread, baby foods, and meat products. Flour can also serve as the base for a

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wide variety of sauces, gravies, sausages, meat loaves, canned foods, and confections. Along with these foods, flour and flour by-products produced from the milling process can be sold as animal feed.

The principal microorganisms of concern associated with flour and meal are *Salmonella* and mycotoxigenic fungi. The microbial ecology and distribution of mycotoxigenic fungi and mycotoxins, as well as *Salmonella*, have been described previously (International Commission on Microbiological Specification for Foods 2005). Although *Salmonella* will not grow in flour and meal due to their low water activity (a_w 0.70), the pathogen can remain viable for months in the raw product (Gilbert et al. 2010). Mycotoxins are produced by fungi in the grain when the water activity is sufficient for mold growth; however, the toxic metabolites (mycotoxins) are stable in flour and meal and are not completely eliminated by processing and cooking procedures. Therefore they have the potential to be a prevalent health problem around the world if not controlled.

Microorganisms of Interest

Salmonella

The presence of *Salmonella* in low-moisture products is a concern because even small numbers of *Salmonella* in foods can cause illness in humans. In some instances, infection has occurred from consuming low-moisture products contaminated with less than 1CFU of *Salmonella* per gram depending on the host, the product, and the *Salmonella* strain (Table 1) (D'Aoust et al. 2001). The primary habitat of *Salmonella* is the intestinal tract of animals such as birds, reptiles, farm animals, wild animals, humans, and occasionally insects (Jay 2000). Salmonellosis usually presents symptoms of illness at 12–36 h after ingestion of a contaminated food and can last for 2–5 days (International Commission on Microbiological Specification for Foods 2005). Typical symptoms include diarrhea, nausea, abdominal pain, mild fever, and chills.

Table 1 Human infectious dose of *Salmonella* in low-moisture foods

Food	*Salmonella* serovar	Infectious dose
Chocolate	Eastbourne	10^2
Peanut butter	Mbandaka	$<10^1$
Chocolate	Napoli	10^1–10^2
Infant dried milk	Ealing	$<10^1$
Chocolate	Typhimurium	$<10^1$
Paprika potato chips	Saintpaul Javiana Rubislaw	$<4.5\times10^1$

Generally, flour and meal are not considered a high-risk ingredient for *Salmonella* contamination because they are intended to be cooked before consumption; however, there are circumstances in which raw flour and meal are used as carriers for nutraceuticals, pharmaceuticals, spices, or flavorings or as a bulking/caloric agent in dried mixes and as ingredients in ready-to-cook or ready-to-eat products. In applications such as these, flour and meal should be treated by a validated microbiological lethality process before use.

The availability of data is limited on the prevalence of *Salmonella* in flour or meal. The low water activity of flour and meal (a_w ~0.7) ensures that the growth of *Salmonella* will not occur, but the bacteria can remain viable for months, determined by laboratory experiments (Dack 1961). Microbiological surveys conducted in the USA have focused on milled flour, whereas an Australian study included incoming wheat, intermediate milling fractions, and finished flour. A bacteriological survey of sixty healthy foods conducted in 1978 detected *Salmonella* (*S*. Molade) in one rye flour sample and three soy flour samples (*S*. Cubana) (Andrews et al. 1979). A 1993 study of more than 3,000 US wheat flour samples revealed that 1.3 % of the samples were contaminated with *Salmonella* (Richter et al. 1993). A second study in 2007, of ca. 4,000 US wheat flour samples, revealed that 0.14 % were *Salmonella* positive (Sperber 2007). An Australian study detected *S*. Chester and *S*. Hvittingfoss in the environment of two mills (<0.5 % of 412 samples) (Berghofer et al. 2003) and another study found *Salmonella* in 2 % of unscreened wheat (Eglezos 2010).

Introduction of *Salmonella* onto wheat by fecal contamination of human or animal carriers would be diluted by the sheer volume of flour processed through flour mills; hence, the cell populations of *Salmonella* in contaminated flour are typically very small. For example, assays of *Salmonella*-positive unopened retail flour packs and complaint samples associated with a New Zealand flour-associated outbreak of salmonellosis revealed low levels of *Salmonella* of between 0.0036 and 0.024 MPN/g (Eglezos 2010).

Thermal Resistance of Salmonella in Flour and Meal

The amount of water in flour and meal during heating will affect the heat resistance of *Salmonella* within these foods. *Salmonella* inactivation in dry foods of low water activity requires considerably higher temperatures and longer exposure times (e.g., hours to days) than in moist foods of high water activity (Archer et al. 1998). *Salmonella* cells are purported to be inactivated during heating by the vibration of water molecules that cause disulfide bonds and hydrogen bonds in the proteins such as critical enzymes to weaken and break. When less water is present, the vibration of water molecules is reduced, resulting in less protein denaturation (Hiramatsu et al. 2005; Laroche et al. 2005; Mattick et al. 2001). Hiramatsu et al. (2005) also discuss the water replacement hypothesis as a possible explanation for an underlying mechanism for increased heat resistance of *Salmonella* in low water activity environments and products. The structure of the protein and the function of the

membranes are hypothesized to be preserved by sucrose and trehalose through the inhibition of structural damage by replacing bacterial membranous water in desiccated conditions. Different resistances to dryness among bacterial strains in the presence of sucrose might be related to the abilities of bacteria to accumulate intracellular sucrose and/or trehalose by de novo synthesis and/or translocation (Hiramatsu et al. 2005). The effects of heat stress are also thought to be from the damage to the permeabilization of membranes, particularly the plasma membrane. When temperatures exceed the physiological range, the plasma membranes become hyperfluid and destabilization of the lamellar phase occurs, producing a drastic leakage of ions from the cells and a rapid cell death (Laroche et al. 2005).

Two studies have determined the heat sensitivity of *Salmonella* in flour. Archer et al. (1998) determined that the initial water activity of the flour before heating was a significant factor affecting the heat resistance of *Salmonella* Weltevreden. The relative humidity during storage of the flour before heating did not have a significant effect on the heat resistance. The second study investigated the effect of dry heat on inactivation of *Salmonella* Newington, *S.* Typhimurium, *S.* Anatum, *S.* Kentucky, *S.* Cubana, *S.* Senftenberg, *S.* Thompson, and *S.* Tennessee in maize flour at 10 and 15 % moisture content. The thermal inactivation times were greater in flour with 10 % moisture content than 15 %, with a 99 % reduction of *S.* Senftenberg occurring in 2.2 h in 15 % moisture flour and in 5.8 h in the 10 % moisture product (Van Cauwengerge et al. 1981). *Salmonella* Thompson and *S.* Tennessee were more resistant to heat inactivation than the other *Salmonella* evaluated serotypes, indicating variation in heat tolerance among serotypes or strains.

Mycotoxins

Certain fungi are able to grow in grains and produce metabolites toxic to animals and humans (Bittencourt et al. 2005). Such metabolites are known as mycotoxins and can produce carcinogenic, mutagenic, and teratogenic effects in humans and animals. The mycotoxins of principal concern in cereal grains are summarized in Table 2. For many years mycotoxicosis was not considered a serious disease.

Table 2 Mycotoxins of concern in cereal grain commodities

Mycotoxin	Food/commodity	Fungal genus and species
Aflatoxins	Maize	*Aspergillus flavus*, *A. parasiticus*
Ochratoxin	Barley, wheat	*Aspergillus ochraceus*, *A. carbonarius*, *Penicillium verrucosum*
Zearalenone	Maize, wheat	*Fusarium graminearum*, *F. culmorum*, *F. crookwellense*
Deoxynivalenol	Barley, maize, wheat	*Fusarium graminearum*, *F. culmorum*, *F. crookwellense*
Fumonisins	Maize	*Fusarium verticillioides*, *F. proliferatum*

However, since 1970, cases of mycotoxicosis have increased in many countries, likely due to better surveillance and reporting practices. Mycotoxigenic fungi can invade grains before or after harvest and produce toxins. Many mycotoxigenic-field fungi pose a major threat to the world food supplies because their mycotoxins cannot be completely controlled by postharvest handling, storage techniques, processing, or cooking procedures. Fungi that contaminate grain in the field can survive drying after harvest or can recontaminate grain after drying and may remain viable for many months during storage. Survival is greatest at low temperatures and in low-moisture grains (Christensen 1987). While mycotoxin-producing field fungi can grow at high moisture levels (a_w >0.90), storage fungi that are capable of growth at lower water activities also can rapidly contaminate grains after harvest. Once mycotoxins are formed in the grain, they can be carried into the flour or meal during milling, surviving the heat treatment or other procedures applied to kill fungi. There are currently no commercially applied methods for removing mycotoxins in flour and meal during the milling process, but there are management strategies (discussed later in the chapter) that can mitigate mycotoxin production.

Deoxynivalenol

Deoxynivalenol, also known as DON or vomitoxin, is produced by *Fusarium graminearum* and *F. culmorum* (FDA 2012a; Prelusky et al. 1994; Richard 2007). DON belongs to the class of mycotoxins called trichothecenes of the type B group. Outbreaks of swine refusal in the central USA during the 1970s led to the discovery of DON. *F. graminearum* is common in wheat produced in North America and China, whereas *F. culmorum* is the dominant species of *Fusarium* in cooler wheat-growing areas such as Finland, France, Poland, and the Netherlands (Miller 1994).

F. graminearum is a pathogen of wheat, barley, oats, rye, and corn and causes head scab or head blight in wheat, rye, and triticale and stalk and ear rot of maize, leading to the contamination of these crops with DON (Haschek et al. 2002; Hocking 2003; Vincelli and Parker 2012; Yuen and Schoneweis 2007). The microorganisms survive on plant residue left on the field from the previous season's crop, thereby providing an inoculum for the new crop (Richard 2007). It has been reported that spores of *F. graminearum* can be produced on infested residue 1–3 years following harvest (Vincelli and Parker 2012). *Fusarium* can be present under normal growing conditions, with contamination of the crop occurring when conidia of the microorganism are windblown to the corn silks or in grains to small anthers which emerge outside the floret during anthesis (Miller 1994).

Derivatives of DON also occur, including nivalenol, 3-acetyldeoxynivalenol (3-ADON), and 15-acetyldeoxynivalenol (15-ADON). Nivalenol is similar to deoxynivalenol in structure but is more toxic. The 3-ADON is more commonly found in Europe, Asia, Australia, and New Zealand, whereas 15-ADON is more common in North America. Although vomitoxin can be lethal if ingested in sufficient quantities, the major problem for North American livestock producers with contaminated feeds is subacute toxicosis arising from chronic, low-level

consumption of these toxins, subsequently leading to poor growth performance. Vomitoxin can cause gastroenteritis, feed refusal, necrosis, hemorrhage in the digestive tract, destruction of bone marrow, and suppression of blood cell formation (Bennett and Klich 2003; Boermans and Leung 2007). Animals exhibit signs of gastrointestinal problems, vomiting, loss of appetite, poor feed utilization and efficiency, bloody diarrhea, reproductive problems, abortions, and death (Bennett and Klich 2003; Boermans and Leung 2007; Rotter et al. 1996). In swine, diets containing 5 ppm can reduce feed intake by 30–50 % (Vincelli and Parker 2012).

Consumption of DON at high enough concentrations can cause gastroenteritis with vomiting in humans and is believed to be responsible for a number of gastrointestinal syndromes reported in different parts of the world, including the former Soviet Union, China, Korea, Japan, and India (Bhat et al. 1989). Short-term feeding trials with animals revealed low acute toxicity, and additional results revealed that DON may have teratogenic potential. Since 1991, *F. graminearum* head blight reemerged as a problem in the USA and Canada. The prevalence and severity of *F. graminearum* epidemics on wheat and barley have increased, especially in the eastern portion of the USA (Miller 1994). The US Food and Drug Administration has issued advisory levels for the industry (Table 3); however, an advisory level for raw grain intended for milling has not been established because normal manufacturing practices and additional treatment can reduce DON levels in finished products (Chesemore 1993; FDA 2012a).

Fumonisins

Fumonisins are produced primarily by *Fusarium verticillioides* and *F. proliferatum* and are common in maize. *F. verticillioides* is a soilborne pathogen that can cause symptomless infections in maize plants and invade the grain. Infection of the kernel may occur by invasion through the silk, fissures in the kernel pericarp, and/or through systemic infection of the plant. Kernel infection can occur in 100 % of shelled maize and have no visible sign of mold damage or deterioration. This is important because contaminated maize may be interpreted as good quality.

Table 3 US Food and Drug Administration guidelines for deoxynivalenol in wheat and derived products and other grains

Product	Concentration (ppm)
Finished wheat products for human consumption, such as flour, bran, and germ	1
Grain and grain by-products destined for swine, not to exceed 20 % of diet	5
Grain and grain by-products destined for all other animals, providing that these ingredients do not exceed 40 % of the diet	5
Grain and grain by-products for beef, feedlot cattle older than 4 months, and chickens; not to exceed 50 % of the diet	10

Fumonisin B1 is the most common form and has been detected in finished processed maize-based foods, such as maize meal and maize flour (Reddy et al. 2010). Fumonisins interfere with sphingolipid metabolism and cause leukoencephalomalacia in horses and rabbits, pulmonary edema in swine, and liver cancer in rats (Haschek et al. 2002; Miller 1994; Vincelli and Parker 2012; Wang et al. 1991). They may also play a part in human esophageal cancer in South Africa, Northeastern Italy, and Northern China, as well as cases of neural tube defects in the USA (Marasas et al. 2004; Martins et al. 2008; Vincelli and Parker 2012; Zain 2011). In addition to their adverse effect on the brain, liver and lungs, fumonisins also affect the kidneys, pancreas, testes, thymus, gastrointestinal tract, and blood cells (Vincelli and Parker 2012). The FDA has established industry guidelines for acceptable levels of fumonisin in human food and animal feed (Table 4) (FDA 2012b).

Ochratoxin A

Ochratoxin A (OA) was first isolated from *Aspergillus ochraceus* in 1965, shortly before Scandinavian research revealed that kidney disease in pigs resulting from consumption of moldy feed was due to ochratoxin A (Bennett and Klich 2003; Van der Merwe et al. 1965). Ochratoxin A has been detected in most European countries and in northern North America, primarily at low concentrations in raisins, barley,

Table 4 US Food and Drug Administration guidelines for total fumonisins (FB1 + FB2 + FB3) in maize products intended for human and animal consumption

Product	Concentration (ppm)
Human	
Degermed dry-milled maize products (e.g., flaking grits, corn grits, corn meal, corn flour with fat content of <2.25 %, dry weight basis)	2
Whole or partially degermed dry-milled maize products (e.g., flaking grits, corn grits, corn meal, corn flour with fat content of >2.25 %, dry weight basis)	4
Dry-milled maize bran	4
Cleaned maize intended for mass production	4
Cleaned maize intended for popcorn	3
Animal feed	
Equids and rabbits (no more than 20 % of diet)	5
Swine and catfish (no more than 50 % of diet)	20
Breeding ruminants, breeding poultry, and breeding mink (no more than 50 % of diet)	30
Ruminants >3 months old being raised for slaughter and mink being raised for pelt production (no more than 50 % of diet)	60
Poultry being raised for slaughter (no more than 50 % of diet)	100
All other species or classes of livestock and pet animals (no more than 50 % of diet)	10

soy products, oats, rye, and coffee with barley having a particularly high likelihood of contamination (Bennett and Klich 2003; CAST 2003; Wagacha and Muthomi 2008; Zain 2011). The source of ochratoxin A in cooler climates is *Penicillium verrucosum*, a common contaminant of barley and the only confirmed ochratoxin A producer in this genus (Chu 1974). *P. verrucosum* is xerophilic, capable of growth at a water activity of 0.80. However, ochratoxin A production ceases below a water activity of 0.86 (Northolt et al. 1979), so most ochratoxin A contamination occurs immediately after harvest when the grain has not yet dried to a safe moisture content. Any time the integrity of the seed coat has been compromised, there is potential for invasion by ochratoxin-producing fungi.

Ochratoxin A levels can accumulate in body tissues and fluids of either humans or animals consuming contaminated food because ochratoxin A is slowly eliminated from the body (Creppy 1999). It is primarily a kidney toxin, but in high enough concentrations can damage the liver as well. Ochratoxin A is a carcinogen in rats and mice and is a suspected carcinogen in humans (Beardall and Miller 1994; Bennett and Klich 2003; Miller 1994). Balkan endemic nephropathy is a kidney disease associated with tumors in humans that may be caused by ochratoxin A (Pfohl-Leszkowicz et al. 2002). It has also been hypothesized that the gene for phenylketonuria occurs in high frequency to provide a heterozygous advantage against ochratoxin poisoning (Vincelli and Parker 2012) and that ochratoxin A might be a risk factor for testicular cancer (Schwartz 2002).

Aflatoxins

Aflatoxins are primarily produced by some strains of *Aspergillus flavus* and most, if not all, strains of *A. parasiticus*. There are four major aflatoxins, B1, B2, G1, and G2, plus two additional toxins that are significant, M1 and M2 (Peraica et al. 1999; Richard 2007). The M toxins were first isolated from milk of lactating animals fed with aflatoxin-contaminated feed. Mold growth and aflatoxin production are favored by warm temperatures, high humidity, and drought conditions. Aflatoxins may be found in maize, oil seeds, tree nuts, and dried fruits. The saprophytic organism is disseminated via their conidia, carried by wind or insects to the growing crop where it can enter and grow on the host including the ears or kernels in the developing grain. Insects can also provide transmission and portals of entry into the host plant (Richard 2007).

Aflatoxins are toxic and can cause chronic toxicity and immune system suppression. They can damage the liver in animals and are carcinogenic to some species. B1 is the most potent of the aflatoxins (Bennett and Klich 2003; Zain 2011). Effects of aflatoxins depend on breed, age, dose, species, length of exposure, and nutritional status. The toxins may be lethal when consumed in large amounts. Sublethal doses produce chronic toxicity and low levels of chronic exposure result in cancers, primarily liver cancer (Bennett and Klich 2003). Clinical studies on animals have shown decreased production of milk, diminished weight gains, less egg production, as well as gastrointestinal problems, reproductive problems, anemia, and jaundice.

Table 5 US Food and Drug Administration action levels for total aflatoxins in food and feed

Commodity	Concentration (ppb)
Cottonseed meal as a feed ingredient	300
Maize and peanut products for finishing beef cattle	300
Maize and peanut products for finishing swine	200
Maize and peanut products for breeding beef cattle, swine, and mature poultry	100
Maize for immature animals and dairy cattle	20
All products, except milk, designated for humans	20
All other feedstuffs	20
Milk	0.5

Human consumption of aflatoxins has resulted in edema, liver damage, and death. Aflatoxins have also been associated with liver cancer; the International Agency for Research on Cancer has classified aflatoxin B1 as a human carcinogen (International Agency for Research on Cancer 1982). Worldwide liver cancer incidence rates are 2–10 times higher in developing countries than in developed countries (Henry et al. 1999). The FDA has established action levels for total aflatoxins in food and feed (Table 5) (FDA 2012c).

Zearalenone

Zearalenone is a toxin produced by *Fusarium* species and is an estrogenic compound known as F-2 toxin (Bennett and Klich 2003; Christensen 1987). *Fusarium* species that produce zearalenone include *F. graminearum*, *F. culmorum*, and *F. crookwellense*. Zearalenone may coexist with vomitoxin due to the same microorganisms possibly producing both compounds (Richard 2007). Zearalenone occurs naturally in high-moisture maize in late autumn and winter, primarily from *F. graminearum* in North America and *F. culmorum* in Northern Europe. The formation of the toxin is favored by high humidity and cool temperatures. The ability to produce toxin in cool temperatures has led to increased levels of this mycotoxin during storage where conditions for fungal growth and mycotoxin formation are favorable. Zearalenone has been detected in maize and other crops such as wheat, barley, sorghum, and rye throughout various countries in the world (CAST 2003). In wheat, sorghum, and maize, zearalenone occurs in preharvest grain, but in other commodities there is insufficient information to determine if zearalenone occurs pre- or postharvest (World Health Organization 2000).

Zearalenone and related metabolites possess strong estrogenic activity (Prelusky et al. 1994). Hence, when fed to domestic animals in sufficient amounts, they can cause severe reproductive and infertility problems (Zain 2011). Swine are most significantly affected and are considerably more sensitive to zearalenone than other

animals (Prelusky et al. 1994; Zain 2011). Although the compound is not especially toxic, 1–5 ppm is sufficient to cause physiological responses in swine (Bennett and Klich 2003; Vincelli and Parker 2012). Zearalenone is also an endocrine disrupter and has been implicated in an outbreak of precocious pubertal changes in thousands of young children in Puerto Rico (Schoental 1983). Zearalenone is speculated to play a role in human breast cancer, although the FDA does not support this hypothesis (Kuiper-Goodman et al. 1987). While the involvement of zearalenone in human toxicosis has not been confirmed, it is considered to be a potential hazard.

Overview of Flour and Meal-Related Outbreaks

A decade ago raw flour was considered to be of low risk in causing foodborne illness because it was intended to be cooked before consumption. However, more recently several outbreaks of salmonellosis have been associated with uncooked flour consumption, and when contamination occurs, a large number of people can be affected.

Salmonella

The first indication that flour may have been the vehicle of a foodborne outbreak was in 1952, when a series of eight outbreaks of *Salmonella* paratyphi B phage type I occurred in New South Wales, Australia. Although flour was suspected to be the cause of the outbreak, however, the microorganism was not isolated from the flour (Dack 1961).

In 2005, 25 people in the USA acquired salmonellosis after consuming contaminated cake batter ice cream. In this outbreak, a dry cake mix that was designed to be baked was added to a pasteurized sweet cream base. Although flour was not confirmed to be the ingredient of contamination, the Food and Drug Administration (FDA) warned food manufacturers and retail and foodservice industries that *Salmonella* is known to be present in flour and other ingredients that might be listed on the dry cake mix label. The FDA also commented that dry cake mix should not be considered ready-to-eat if it has not been processed to ensure it is safe to consume without further cooking (FDA 2005).

The first confirmed outbreak of salmonellosis attributed to flour occurred in 2008, in New Zealand. Sixty-seven cases of illness were reported, of which twelve patients were hospitalized (McCallum et al. 2013). The outbreak strain was detected in opened flour from the patients' homes as well as flour obtained from retail shelves. Results of the investigation revealed that individuals with the infection were more likely to have consumed contaminated flour in the form of raw baking mixture.

Human and Animal Mycotoxicosis

Since 1970, there has been an increase in the number of cases of mycotoxicosis in many countries, increasing awareness of the mycotoxicosis disease. The Food and Agriculture Organization of the United Nations estimates that 25 % of the world's food crops are affected by mycotoxins every year (Mannon and Johnson 1985). In developed countries, improved standards of crop management and storage, along with public education, extensive surveillance, and stringent regulations limit human exposure to mycotoxins. In many less-developed countries, public awareness of food contamination with mycotoxins is low by contrast. Mycotoxin surveillance programs in less-developed countries often are limited or focused on export commodities because of limited resources. There is little doubt that populations in these less-developed countries are exposed to significant levels of mycotoxins in maize, wheat, barley, and other cereal grains.

In 1933, Japanese scientists associated sporadic outbreaks of human toxicoses with the consumption of *Fusarium*-contaminated breads produced from wheat, barley, and other grains contaminated and discolored by red mold disease, akakabi-byo (Desjardins 2006). Several *Fusarium* species were isolated, but the most frequent contaminant was *F. graminearum*. Patients experienced nausea, vomiting, and diarrhea, often accompanied by headache, dizziness, trembling, euphoria, and visual hallucinations. Russia, China, and Korea had patients with similar symptoms that were associated with bread that was also produced from red mold-contaminated flour.

During World War II, a severe foodborne illness outbreak occurred in rural areas of Russia and Kazakhstan. The disease, known as alimentary toxic aleukia (ATA), was believed to have been caused by T-2 toxins produced by *Fusarium* (Desjardins 2006). Due to war, there was a shortage of farm workers, so very little of the grain was harvested in the fall and it overwintered in the fields. By spring, near-famine conditions occurred and people were forced to eat the overwintered grain that was milled into flour and baked into bread (Khattak 1988). The toxin caused a burning sensation in the mouth and throat, vomiting, diarrhea, abdominal pain, bone marrow destruction, hemorrhaging, and death (Bullerman and Bianchini 2007). Also during this time period, another outbreak of human mycotoxicosis occurred in the former Soviet Union, known as "drunken bread." This syndrome was caused by the consumption of bread made from rye flour infected with *Fusarium graminearum*. The illness was milder than ATA and was a nonfatal self-limiting disorder. Symptoms included headaches, dizziness, tinnitus, trembling, and shaking of the extremities, with an unsteady or stumbling gait. Gastrointestinal symptoms, including abdominal pain, nausea, and diarrhea, were also reported, with the duration of illness lasting 1–2 days (Bullerman 2001).

An outbreak of gastrointestinal disorders affecting several thousands of people occurred in India from July to September 1987. *Fusarium* species were isolated from the implicated wheat and flour, as were mycotoxins that included deoxynivalenol, acetyldeoxynivalenol, nivalenol, and T-2 toxin. The disease was characterized by mild to moderate abdominal pain or the feeling of fullness of the abdomen within

15 min to 1 h after consuming products made with refined wheat flour. Symptoms also included irritation of the throat, vomiting, diarrhea, and some with blood in the stool (Bullerman and Bianchini 2007; Bullerman 2001). Children who consumed large amounts of wheat preparations over periods of more than a week experienced secondary infections such as upper respiratory tract infections. Unseasonal rain at harvest resulted in standing crops of wheat becoming rain damaged and moldy. Farmers sold the wheat on the open market at a nearly 50 % discount. Flour millers then mixed infected wheat with sound wheat and milled it into flour for distribution.

Mycotoxins do not only affect humans, as animals are also susceptible when they consume contaminated feed. In 1928, swine were made ill from *F. graminearum-contaminated* grain produced for feed. The feed caused the swine to vomit upon consumption, and they often refused to eat it. Later experiments revealed that the ingestion of as little as 13 g of heavily blighted barley could induce vomiting in a 132-pound swine (Christensen and Kernkamp 1936). Cooking the feed did not reduce its toxicity, indicating the toxin was heat stable. *F. graminearum* also caused swine feed refusal in the USA in 1965 and 1972. It was during these outbreaks that deoxynivalenol (DON) was subsequently isolated from naturally infected maize.

An outbreak of leukoencephalomalacia during the winter of 1934 and 1935 in the USA caused the deaths of approximately 5,000 horses in central Illinois. *Fusarium verticillioides*-contaminated animal feed was the cause of the deaths. *F. verticillioides* was first isolated by Sheldon in the USA in 1904 from maize associated with leukoencephalomalacia (Sheldon 1904) and later in South Africa (Kellerman et al. 1990).

Sources of Contamination Prior to Milling

A variety of environmental conditions (e.g., rainfall, sunlight, temperature, and soil conditions) can influence the composition of grain microflora (Deibel and Swanson 2001), as can agricultural practices (e.g., application of chemicals, organic farming, and type of harvest equipment) and the presence of insects, birds, and animals. Grains generally present few microbiological risks when they are harvested in good condition, dried rapidly to a low moisture content, and stored under conditions that prevent water ingress (International Commission on Microbiological Specifications for Foods 2011). However, contamination with *Salmonella* and mycotoxigenic fungi may occur under unfavorable conditions and once present can also contaminate flour and flour-based products.

Preharvest Contamination

Field crops have the potential to be contaminated before harvest in the field with mycotoxigenic fungi and/or pathogenic bacteria. Field fungi invade seeds and grains before harvest and require for growth a water activity level of greater than 0.90,

equivalent to 20–25 % moisture content (Tagne et al. 2003). Field fungi that survive drying or recontaminate grain after drying cannot grow; however, they can remain viable for many months during storage. Survival is greatest at low temperatures and low moisture contents (Christensen 1987). When grain is processed into flour or meal, fungi are important because they are capable of growth in products produced from flour and meal. Once toxins are produced, mycotoxins are not completely inactivated or eliminated by processing and cooking procedures used for cereal products.

Prevention of fungal growth in crops is achieved by harvesting in areas with minimal crop stress, visually checking for fungal growth and insect infestation, and drying crops to a safe moisture level (≤14 %). Current research is investigating the ability to breed cultivars resistant to fungal infection, develop transgenic cultivars capable of interfering with toxin production, develop crops genetically engineered to resist insect damage, and exclude toxigenic fungi by pre-infection of plants with biocompetitive, non-toxigenic fungal strains (Choudhary and Kumari 2010).

Pathogenic bacteria, such as *Salmonella,* are primarily transmitted through human and animal fecal matter. Application of contaminated irrigation water, runoff water, or raw or inadequately composted manure to production systems may introduce pathogens to cereal crops. By application of contaminated water or soil, the plant itself can become contaminated with human pathogens. *Salmonella* species have been identified in soils that were treated with cattle manure used to grow grain such as spring wheat and barley (De Freitas et al. 2003). Baloda et al. (2001) determined that when a *Salmonella*-contaminated manure slurry was deposited on agricultural soil, the pathogen could be isolated for up to 14 days after the manure was applied, indicating a potentially high risk of transmission of the pathogen to the environment, animals, and humans. De Freitas et al. (De Freitas et al. 2003) investigated the dynamics of soil microbial populations affected by repeated applications of hog and cattle manure. *Enterobacteriaceae* populations were determined to be 10 times higher in soils that received cattle manure than in soils receiving hog manure.

Research has also revealed possible contamination routes via internalization into edible parts of the plant by different routes such as through the roots or the plant's vascular system. *Salmonella enterica* reportedly can internalize lettuce (Bernstein et al. 2007) and tomatoes (Guo et al. 2002), specifically when exceptionally large populations of the pathogen are present. Current research focuses on dicots (e.g., spinach, lettuce, alfalfa, and radishes), with little research on monocots (e.g., wheat, maize, rice, and millet) (Kutter et al. 2006). Monocot plants could pose a food safety hazard if the kernel is contaminated or by reintroduction of pathogens into the food supply by contaminated feed. Contaminated kernels could cycle pathogens into animals and thus into animal waste which could then be used on plants. *Salmonella* can systemically, through the plant shoot, colonize and spread within the barley plant (Kutter et al. 2006). This systemic spreading of *Salmonella* may occur in the vascular system through water transport but may also have been actively supported in an unknown way (Dong et al. 2003). A similar invasion process for colonizing plants is known for plant pathogens, where colonization of the root surface

is followed by infection of the vascular parenchyma and invasion of the xylem (Vasse et al. 1995). Penetration into the xylem leads to systemic colonization of the plants. Further research is needed in monocot plants to determine if pathogenic bacteria can become internalized into edible tissue or phylloplane, by way of contaminated soil, seed, or water.

Postharvest

In addition to contamination in the field, foodborne pathogen and mycotoxin contamination can also occur postharvest during transportation or storage. The most likely points of contamination are from storage silos, transport vehicles, and dust. *Salmonella* has been isolated from mice, rats, insects, and wild birds (Gilbert et al. 2010), all of which can come in contact with grains postharvest. Insects including the rice weevil, the lesser grain borer, and the red flour beetle have been shown to carry *Salmonella* Montevideo after being placed in *S.* Montevideo-contaminated wheat environment for 21 days. Progeny of the insects reared in the same wheat samples was also carrying *S.* Montevideo. An expanded study revealed that the rice weevil, the saw-toothed grain beetle, and the red flour beetle were able to transmit *S.* Montevideo from contaminated wheat to clean wheat (Crumrine et al. 1971).

Transportation vehicles should be clean with no water leakages and free from birds, insects, or vermin as part of good agricultural practices. Antifungal agents such as propionic acid and acetic acid can be used with short-term storage (Choudhary and Kumari 2010). Storage areas should also be clean and closed to prevent entry of birds, insects, and vermin. Handling equipment used postharvest should follow these same guidelines.

Grains are usually stored at low moisture content. Recommended maximum moisture levels for 1 year of storage are 14 % for rice, 13–14 % for wheat (Atwell 2001), 13 % for maize and barley, and 11 % for oats (International Commission on Microbiological Specification for Foods 2005). If the relative humidity remains below 0.60, fungi are not able to grow (Hocking 2003). However, if the temperature and moisture gradient develops during storage, localized pockets of higher moisture may develop, allowing fungal growth (Birck et al.; Hocking 2003; Wicklow 1995). Adequate ventilation in storage bins will remove moisture, prevent condensation, lower and equilibrate temperatures, and prevent heating. CO_2 sensors can be effectively used to monitor early detection of fungal spoilage during storage. They can detect stored-product insect infestation and grain spoilage due to fungi before spoilage detection by traditional methods such as visual inspection, smell, and temperature cables. Such an early warning system can provide more timely information to make correct management decisions to avoid the cost of spoilage mitigation measures such as turning, aeration, and fumigation (Christensen 1987).

Managing and Mitigating Microbiological Risk During the Milling Process

Grain Receiving and Cleaning

Mycotoxins are a primary concern in raw grains, hence, routine tests for appropriate mycotoxins are recommended before production of flour or meal. Rapid screening tests, such as enzyme-linked immunosorbent assay, can be used to determine the contamination level. Once the grain is received into the mill, the cleaning house removes foreign material (stones, husks, etc.) and poor quality or mold-infested kernels. Cleaning is performed with an assortment of machines that utilize air currents, magnets, and screens to separate the grain from the chaff and other undesirable material and foreign substances. The cleaning process may lower mycotoxin concentrations by removing contaminated material; however, these operations do not destroy mycotoxins. Cleaning removes kernels with extensive fungal growth, broken kernels, and fine materials, which help to reduce mycotoxin levels. Sydenham et al. (1994) determined that cleaning reduced fumonisin concentrations in maize by 26–29 %. Cleaning can also remove scab-infested wheat and barley kernels, which can reduce DON levels by 5.5–19 % in wheat before milling (Abbas et al. 1993). In contrast, Scudamore et al. (2003) reported that cleaning only reduced ochratoxin in barley by 2–3 %.

Currently, high-speed optical sorting is being investigated to reduce DON concentrations of intact wheat kernels. Image analysis and near-infrared spectroscopy have also been utilized. In imaging studies, kernel morphology and color are the primary features used to detect damaged kernels, because *Fusarium*-damaged kernels have a white or pinkish color. Studies using near-IR spectroscopic measurement determined that with as few as two wavelengths, 95–97 % of *Fusarium*-damaged kernels of hard red winter wheat could be detected (Delwiche et al. 2005). This technology has also been used to remove *Fusarium*-damaged maize kernels, resulting in an 81 % reduction of aflatoxin and 85 % of fumonisin (Pearson et al. 2004). Ongoing research is examining the potential to achieve even greater removal of mycotoxin-contaminated grains under conditions appropriate for speeds required for commercial processing.

Tempering

After cleaning, most grains are tempered for ease of processing. Maize, however, can be milled without tempering. Tempering is the adjustment of the moisture level in grain to facilitate milling and to help obtain maximum separation of the bran from the endosperm. For example, with wheat flour, the moisture content during tempering is increased to 13.5–16.5 % (Atwell 2001). Grain is held in temper for as little as a few hours and up to 24–36 h, depending on the type of grain and initial

moisture content. The tempering equipment, like the mill, runs continuously and may not be cleaned frequently. Grain could become lodged in dead spaces and may remain at high moisture content for a prolonged period of time, thereby promoting microbial growth and even grain sprouting. This can contribute to increased mold, yeast, and bacterial counts. During tempering, antimicrobials such as aqueous or gaseous chlorine and ozone or acetic acid can be used to suppress microbial growth in the tempering equipment. Dhillon et al. (2010) evaluated a fluidized bed system for grain disinfection and determined that the combined treatment of gaseous ozone plus acetic acid and ozonated water reduced aerobic plate counts and yeast and mold counts by 1.7 and 3.3 log CFU/g, respectively. Another study determined the feasibility of using ozone as a substitute for chlorinated tempering water to control bacteria and fungi in a wheat mill. Results revealed a 75–80 % reduction in aerobic plate counts compared to the conventional treatment of chlorinated water (Gwirtz 2009). Kurtzman and Hesseltine (1970) addressed the chlorine tolerance of microorganisms found in wheat and flour. The study determined that while chlorine levels used in tempering wheat should be effective in reducing bacteria and fungi, carry-over of microorganisms from wheat to flour occurs as a result of contaminants being protected from the action of chlorine. For example, microorganisms can be protected by being located within the crease of the kernel of the wheat. In cases where the microorganisms are not within the kernel's crease, protection could occur as a result of the chlorine reacting with other organic material in the tempering bin. Thus, the concentration of chlorine at the surface of the wheat would be negligible. A study in Australia revealed higher mesophilic aerobic bacterial counts after tempering; however, it is not clear as to whether an antimicrobial was being used (Berghofer et al. 2003).

Milling

After tempering, the grain is milled into the final product through grinding and separating steps. During milling, the endosperm is gradually reduced in particle size by running it between a series of steel rollers and is then separated from the bran and germ by running it over sieves. Flour and meal with 14 % or less moisture content (a_w 0.68–0.7) will not support microbial growth (Atwell 2001). However, these operations create a considerable amount of heat, so moisture condensation in the break rolls, reduction rolls, and sifters can lead to a buildup of flour residues inside the equipment and encourage microbial growth. Microorganisms, particularly fungi, may become established in these higher moisture residues, causing contamination of the milled products (Berghofer et al. 2003).

Surface-adhering contaminants concentrate in the bran and germ as the wheat layers are separated, resulting in lower microbial counts in the endosperm, which is the "cleanest" refined flour-end product (Berghofer et al. 2003; Sperber 2007). Mycotoxin contamination is also redistributed and concentrated in certain milled fractions, including the germ and bran (Bullerman and Bianchini 2007; Magan and

Aldred 2007). Katta et al. (1997) determined that during milling of maize, fumonisin B1 was in greatest concentrations in the bran fraction, which is used as animal feed. Fractions used for food production, including flakes, grits, and flour, had the lowest concentrations of fumonisin. Scudamore et al. (2003) determined that milling of wheat and barley concentrated DON, zearalenone, aflatoxins, and ochratoxin A in the germ and bran fractions. Importantly although the bran and germ are not used in refined white flour, both are required to be included in whole wheat flours.

Water Control

The critical moisture content target for flour and meal is 14 % or less, at which point it is stable because *Salmonella* and mycotoxigenic fungal growth is not supported (Atwell 2001; Gilbert et al. 2010). *Salmonella* growth is repressed when water is not present so it is important to control water leaks, condensation, and leaking roofs and windows. The milling environment should be kept as dry as possible, and dry cleaning methods should be used for the equipment and the environment to prevent the establishment of harborage sites (International Commission on Microbiological Specifications for Foods 2011). Routine dry cleaning of the milling equipment will help avoid accumulation of static material, where molds and insects thrive (International Commission on Microbiological Specification for Foods 2005). Dragging or cleaning of spouts will help prevent accumulation of moist product in the system. Dragging is accomplished by propelling brushes through the spouts.

Pathogen Environmental Monitoring Program (PEM)

A PEM program is important for pathogen risk mitigation because it monitors potential pathogens in the manufacturing environment under normal operating conditions. The monitoring program should be thoughtful and aggressively applied. An effective pathogen monitoring program is a critical component to measuring the overall effectiveness of the microbiological controls that are in place and as a root-cause investigational tool. The program should be used to enhance practices, eliminate sites of contamination, and correct potential equipment design problems before they pose a risk to the product. Environmental monitoring testing allows for targeted and actionable information to reduce the risk of cross contamination. The use of proper target pathogens should be decided based upon the product, the process, and past history of pathogen contamination. A PEM program is specific to the individual facility under consideration and specific to the individual operations within the facility. There is no one-size-fits-all program. It is also important that employees never be discouraged from finding a pathogen-positive result. If pathogens are present in the manufacturing environment, finding it through an aggressive PEM program can allow for corrective actions to occur to mitigate the risk of product contamination.

Integrated Pest Management Program (IPM)

An integrated pest management program is another key component for controlling microbiological contamination in the milling environment. The program should control birds, rodents, and insects. If fumigation is used for insect control, boots and elevators should be cleaned immediately after fumigation to remove dead insects. These insects will decompose and can contribute large numbers of microorganisms to the flour.

Validated Lethality Processes

Raw agricultural commodities such as flour and meal are being used in ready-to-eat and ready-to-cook applications. With current food trends, people are consuming raw foods, and not following proper cooking methods. A recent national survey of 1,032 individuals in the USA revealed that 58 % consumed refrigerated cookie dough before baking and 80 % licked beaters after mixing batters for cakes, brownies, and muffins. The survey also revealed that 24 % of consumers consumed raw biscuits, 22 % consumed raw pie crusts, and 11 % consumed raw pizza crust (Rose et al. 2012).

Flour and meal used in these types of situations should be treated with a validated lethality process to reduce the risk of *Salmonella* contamination. Heat treatments are often used to reduce or eliminate microorganisms in foods, and the application of heat treatments for wheat flour has been investigated (Upreti et al. 2010; Wiseblatt 1967). Laroche et al. (2005) determined that the thermal inactivation of microorganisms in food powders was affected by the water activity at the time of treatment. Heating flour can affect the functionality of the product by denaturing gluten or gelatinizing starch. Balancing the heat required for a specified microbiological reduction as well as maintaining gluten functionality is a challenge. There are several different commercial products that have been heat-treated for different levels of microbiological reduction and gluten functionality. Other treatment technologies such as irradiation and ozone have also been studied. While these approaches can be effective for reducing microbial loads, the quality and functionality of the flour can be adversely affected.

Data Gaps

There are many data gaps when addressing the microbiological safety of flour and meal. With flour and meal being used in ready-to-cook and ready-to-eat applications, the risk to public health needs to be fully vetted. Such data gaps include:

- Elucidation of the routes of introduction of *Salmonella* into grain, flour, and meal.
  - Additional research is needed on monocot plants to determine if foodborne pathogens can become internalized into edible tissue or the phylloplane, by way of contaminated soil, seed, or water.

- Data on the prevalence and cell populations of *Salmonella* in flour and meal.
- Data on other foodborne pathogens as potential hazards in flour and meal.
  - In 2009, prepackaged, ready-to-bake cookie dough was recalled due to *E. coli* O157:H7. The outbreak resulted in 77 ill consumers, with 35 patients hospitalized and 10 developing life-threatening hemolytic-uremic syndrome (Neil et al. 2012). Flour tainted with *E. coli* O157:H7 that was used in the cookie dough remains the prime suspect as the source of this foodborne illness event. Typically, flour is purchased in very large quantities for commercial use in food products. If the flour contains pathogens, a single purchase can contaminate multiple lots of finished product, which could have happened in the cookie dough situation.
- Development of grain varieties that are resistant to fungal infection and subsequent mycotoxin formation.

Conclusion

Flour and meal will continue to be an important component of nutrition to people all over the world; therefore, *Salmonella* and mycotoxigenic fungi need to be managed and mitigated to prevent public health problems. Food manufacturers must analyze the food safety risk of using these ingredients in ready-to-cook or ready-to-eat applications, especially for those applications not treated with a validated lethality step. Equally important is to continue research on grains related to pathogen contamination and mycotoxin risk. It is imperative that all measures to protect public health are pursued. Further research to fill in data gaps will be a key to our success in providing safe flour and meal ingredients to the population.

References

Abbas HK, Mirocha CJ, Pawlosky RJ, Pusch DJ (1993) Effect of cleaning, milling and baking on deoxynivalenol in wheat. Appl Environ Microbiol 50:482–486

Andrews HW, Wilson CR, Poelma PL, Romero A, Mislivec PB (1979) Bacteriological survey of sixty health foods. Appl Environ Microbiol 37:559–566

Archer J, Jervis ET, Bird J, Gaze JE (1998) Heat resistance of *Salmonella weltevreden* in low-moisture environments. J Food Prot 61(8):969–973

Atwell WA (2001) Wheat flour. Practical guides for the food industry. Eagan Press, St. Paul, MN

Baloda SB, Christensen L, Trajcevska S (2001) Persistence of a *Salmonella enterica* serovar Typhimurium DT12 clone in piggery and in agricultural soil amended with *Salmonella-contaminated* slurry. Appl Environ Microbiol 67:2859–2862

Beardall JM, Miller JD (1994) Disease in humans with mycotoxins as possible causes. In: Miller JD, Trenholm HL (eds) Mycotoxins in grains. Compounds other than aflatoxin. Eagan Press, St. Paul, MN, pp 487–539

Bennett JW, Klich M (2003) Mycotoxins. Clin Microbiol Rev 16(3):497–516

Berghofer L, Hocking AD, Miskelly D, Jansson E (2003) Microbiology of wheat and flour milling in Australia. Int J Food Microbiol 85:137–149

Bernstein N, Sela S, Neder-Lavon S (2007) Assessment of contamination potential of lettuce by *Salmonella enterica* serovar Newport added to the plant growing medium. J Food Prot 70:1717–1722

Bhat RW, Ramakrishna Y, Beedu SR, Munshi KL (1989) Outbreak of trichothecene mycotoxicosis association with consumption of mould-damaged wheat products in Kasmir Valley. India Lancet 333:35–37

Birck NMM, Lorini I, Scussel VM. Fungus and mycotoxins in wheat grain at post harvest. 9th international working conference on stored product protection PS2-12-6281:198–205

Bittencourt ABF, Oliveria CAF, Dilkin P, Correa B (2005) Mycotoxin occurrence in corn meal and flour traded in São Paulo, Brazil. Food Control 16:117–120

Boermans HJ, Leung MCK (2007) Mycotoxins and the pet food industry: toxicological evidence and risk assessment. Int J Food Microbiol 119:95–102

Bullerman LB, Bianchini A (2007) Stability of mycotoxins during food processing. Int J Food Microbiol 119:140–146

Bullerman LB (2001) Fusaria and toxigenic molds other than aspergilli and penicillia. In: Doyle MP, Beuchat LR, Montville TJ (eds) Food microbiology: fundamentals and frontiers. ASM Press, Washington, DC, pp 481–497

CAST (2003) Mycotoxins-risks in plant, animal and human systems. Task force report. Counc Agric Sci Technol 139:1–191

Chesemore RG (1993) Letter to state agricultural directors, state feed control officials and food, feed and grain trade organizations, FDA Associate Commissioner for Regulatory Affairs. U.S. Food and Drug Administration, College Park, MD

Choudhary AK, Kumari P (2010) Management of mycotoxin contamination in preharvest and post harvest crops: present status and future prospects. J Phytol 2(7):37–52

Christensen CM (1987) Field and storage fungi. In: Beuchat LR (ed) Food and beverage mycology. Van Nostrand Reinhold, New York, pp 211–231

Christensen JJ, Kernkamp HCH (1936) Studies on the toxicity of blighted barley to swine. Minnesota Agric Expt Stat Bull 113:111–113

Chu FS (1974) Studies on ochratoxins. Crit Rev Toxicol 2:499–524

Creppy EE (1999) Human ochratoxicosis. J Toxicol Toxin Rev 18:277–293

Crumrine MH, Foltz VD, Harris JO (1971) Transmission of *Salmonella montevideo* in wheat by stored-product insects. Appl Microbiol 22:578–580

Dack GM (1961) Public health significance of flour bacteriology. Cereal Sci Today 6:9–10

D'Aoust JY, Maurer J, Bailey JS (2001) *Salmonella* species. In: Doyle M, Beuchat L, Montville T (eds) Food microbiology: fundamentals and frontiers. ASM Press. Washington, DC, pp 141–178

De Freitas JR, Schoenau JJ, Boyetchko SM, Cyrenne SA (2003) Soil microbial populations, community composition, and activity as affected by repeated applications of hog and cattle manure in eastern Saskatchewan. Can J Microbiol 49:538–548

Deibel KE, Swanson KMJ (2001) Cereal and cereal products. In: Downes FP, Ito K (eds) Compendium of methods for the microbiological examination of foods. American Public Health Association, Washington, DC, pp 549–553

Delwiche SR, Pearson TC, Brabec DL (2005) High-speed optical sorting of soft wheat for reduction of deoxynivalenol. Plant Dis 89:1214–1219

Desjardins AE (ed) (2006) Trichothecenes. In: *Fusarium* mycotoxins-chemistry, genetics and biology. The American Phytopathological Society, St. Paul, MN, pp 13–14

Dhillon B, Wiesenborn D, Dhillon H, Wolf-Hall C (2010) Development and evaluation of a fluidized bed system for wheat grain disinfection. J Food Sci 75:E372–378

Dong Y, Iniguez AL, Ahmer BM, Triplett EW (2003) Kinetics and strain specific of rhizosphere and endophytic colonization by enteric bacteria on seedlings of Medicago sativa and Medicago truncatula. Appl Environ Microbiol 69:1783–1790

Eglezos S (2010) Microbiological quality of wheat grain and flour from two mills in Queensland, Australia. J Food Prot 73:1533–1536

Food & Drug Administration (FDA) (2005) Bulletin to the food service and retail food store industry regarding cake batter ice cream and similar products. http://www.fda.gov/Food/FoodSafety/RetailFoodProtection/IndustryandRegulatoryAssistanceandTrainingResources/ucm114975.htm. Accessed 10 June 2012

Food & Drug Administration (FDA) (2012a) Guidance for industry and FDA: advisory levels for deoxynivalenol (DON) in finished wheat products for human consumption and grains and grain by-products used for animal feed. http://www.fda.gov/Food/GuidanceComplianceRegulatoryInformation/GuidanceDocuments/NaturalToxins/ucm120184.htm. Accessed 20 July 2012

Food & Drug Administration (FDA) (2012b) Guidance for industry: fumonisin levels in human foods and animal feeds; final guidance contains nonbinding recommendations. http://www.fda.gov/Food/GuidanceComplianceRegulatoryInformation/GuidanceDocuments/ChemicalContaminantsandPesticides/ucm109231.htm. Accessed 12 Nov 2012

Food & Drug Administration (FDA) (2012c) Guidance for industry: action levels for poisonous or deleterious substances in human food and animal feed. http://www.fda.gov/Food/GuidanceComplianceRegulatoryInformation/GuidanceDocuments/ChemicalContaminantsandPesticides/ucm077969.htm. Accessed 15 Nov 2012

Gilbert S, Lake F, Cressey P, King N (2010) Risk profile: *Salmonella* (non typhoidal) in cereal grains. Institute of Environmental Science & Research Limited, Christchurch, New Zealand

Gwirtz J (2009) Ozone use in flour milling. World grain. http://www.world-grain.com/Departments/Milling%20Operations/2009/4/Ozone%20use%20in%20flour%20milling.aspx. Accessed 21 Sept 2012

Guo X, van Lersel MW, Chen J, Brackett RE, Beuchat LR (2002) Evidence of association of *Salmonella* with tomato plants grown hydroponically in inoculated nutrient solution. Appl Environ Microbiol 68:3639–3643

Haschek WM, Rousseaux CG, Wallig MA (2002) Selected mycotoxins affecting animal and human health. In: Haschek WM, Rousseaux CG, Walley MA (eds) Handbook of toxicological pathology. Academic, New York, NY, pp 645–698

Henry SH, Bosch FX, Troxell TC, Bolger PM (1999) Reducing liver cancer-global control of aflatoxin. Science 286:2453–2454

Hiramatsu R, Matsumota M, Sakae K, Miyazaki Y (2005) Ability of Shiga toxin-producing Escherichia coli and Salmonella spp. to survive in a desiccation model system and in dry foods. Appl Environ Microbiol 71:6657–6663

Hocking AD (2003) Microbiological facts and fiction in grain storage. Proceedings of the Australian Postharvest Technical Conference. June 25–27. Canberra, Australia

International Agency for Research on Cancer (IARC) (1982) The evaluation of the carcinogenic risk of chemicals to humans. IARC Monograph Supplement 4, 1982. International Agency for Research on Cancer, Lyon, France

International Commission on Microbiological Specification for Foods (ICMSF) (2005) Cereals and cereal products. In: Microorganisms in foods 6: microbial ecology of food commodities. Kluwer Academic/Plenum, New York, NY, pp 313–355

International Commission on Microbiological Specifications for Foods (ICMSF) (2011) Cereal and cereal products. In: Microorganisms in foods 8: use of data for assessing process control and product acceptance. Springer Science, New York, NY, pp 209–225

Jay JM (ed) (2000) Foodborne gastroenteritis caused by *Salmonella* and *Shigella*. In: Modern food microbiology. Aspen Publishers, Gaithersburg, MD, pp 511–530

Kata SK, Cagampang AE, Jackson LS, Bullerman LB (1997) Distribution of *Fusarium* molds and fumonisins in dry-milled corn fractions. Cereal Chem 74:858–863

Kellerman TS, Marasas WF, Thiel PG, Gelderblom WC, Cawood M, Coetzer JA (1990) Leukoencephalomalacia in two horses induced by oral dosing of fumonisin B_1. Onderstepoort J Vet Res 57:269–275

Khattak S (1988) Mycotoxins in food cereal grains and their products. Pak J Pharm 1:67–74

Kuiper-Goodman TP, Scott PM, Watanabe H (1987) Risk assessment of the mycotoxin zearalenone. Regul Toxicol Pharmacol 7:253–306

Kurtzman CP, Hesseltine CW (1970) Chlorine tolerance of microorganisms found in wheat and flour. Cereal Chem 47:244–246

Kutter S, Hartmann A, Schmid M (2006) Colonization of barley (Hordeum vulgare) with *Salmonella enterica* and *Listeria* spp. FEMS Microbiol Ecol 56:262–271

Laroche C, Fine F, Gervais P (2005) Water activity affects heat resistance of microorganisms in food powders. Int J Food Microbiol 97:307–315

Magan N, Aldred D (2007) Post-harvest control strategies: minimizing mycotoxins in the food chain. Int J Food Microbiol 119:131–139

Mannon J, Johnson E (1985) Fungi down on the farm. New Sci 105(1446):12–16

Marasas WFO, Riley RT, Hendricks KA, Sadler TW, Gelineau-van J, Missmer SA, Cabrera J, Torres O, Gelderblom WC, Allegood J, Martinez C, Maddox J, Miller JD, Starr L, Sullards MC, Roman AV, Voss KA, Wang E, Merrill AH Jr (2004) Fumonisins disrupt sphingolipid metabolism, folate transport, and neural tube development in embryo culture and in vivo: a potential risk factor for human neural tube defects among populations consuming fumonisin contaminated maize. J Nutr 134:711–716

Martins H, Almeida I, Marques MF, Guerra MM (2008) Fumonisins and deoxynivalenol in corn-based food products in Portugal. Food Chem Toxicol 46:2585–2587

Mattick KL, Jorgensen F, Wang P, Pound J, Vandeven MH, Ward LR, Legan JD, Lappin-Scott HM, Humphrey TJ (2001) Effect of challenge temperature and solute type on heat tolerance of Salmonella serovars at low water activity. Appl Environ Microbiol 67:4128–4136

McCallum L, Paine S, Sexton K, Dufour M, Dyet K, Wilson M, Campbell D, Bandaranayake D, Hope V (2013) An outbreak of Salmonella Typhimurium phage type 42 associated with the consumption of raw flour. Foodborne Pathg Dis 10:159–164

Miller JD (1994) Epidemiology of fusarium ear diseases of cereals. In: Miller JD, Trenholm HL (eds) Mycotoxins in grain-compounds other than aflatoxin. Eagan Press, St. Paul, MN, pp 19–36

Neil KP, Biggerstaff G, MacDonald JK, Trees E, Medus C, Musser KA, Stroika SG, Zink D, Sotir MJ (2012) A novel vehicle for transmission of *Escherichia coli* O157:H7 infections associated with consumption of ready-to-bake commercial prepackaged cookie dough – United States, 2009. Clin Infect Dis 54:511–518

Northolt JD, van Egmond HP, Paulsch WE (1979) Ochratoxin A production by some fungal species in relation to water activity and temperature. J Food Prot 42:485–490

Peraica M, Radic B, Lucic A, Pavlovic M (1999) Toxic effects of mycotoxins in humans. Bull World Health Organ 77:754–766

Pearson TC, Wicklow DT, Pasikatan MC (2004) Reduction of aflatoxin and fumonisin contamination in yellow corn by high-speed dual wavelength sorting. Cereal Chem 81:490–498

Pfohl-Leszkowicz A, Petkova-Bocharova T, Chernozemsky IN, Castegnaro M (2002) Balkan endemic nephropathy and associated urinary tract tumours: a review on aetiological causes and the potential role of mycotoxins. Food Add Contam 19:282–302

Prelusky DB, Rotter BA, Rotter RG (1994) Toxicology of mycotoxins. In: Miller JD, Trenholm HL (eds) Mycotoxins in grain-compounds other than aflatoxin. Eagan Press, St. Paul, MN, pp 359–403

Reddy KRN, Farhana NI, Salleh B, Olliveira CAF (2010) Microbiological control of mycotoxins: present status and future concerns. In: Mendez-Vilas A (ed) Current research, technology, and education topics in applied microbiology and microbial biotechnology. Formatex, pp 1078–1086

Richard JL (2007) Some major mycotoxins and their mycotoxicoses – an overview. Int J Food Microbiol 119:3–10

Richter KS, Dorneanu E, Eskridge KM, Rao CS (1993) Microbiological quality of flours. Cereal Foods World 38(5):367–369

Rose DJ, Bianchini A, Martinez B, Flores RA (2012) Methods for reducing microbial contamination of wheat flour and effects on functionality. Cereal World Foods 57(3):104–109

Rotter BA, Prelusky DB, Pestka JJ (1996) Toxicology of deoxynivalenol (vomitoxin). J Toxicol Environ Health 48:1–34

Schoental R (1983) Precocious sexual development in Puerto Rico and oestrogenic mycotoxins (zearalenone). Lancet 537
Schwartz GG (2002) Hypothesis: does ochratoxin A cause testicular cancer? Cancer Causes Control 13:91–100
Scudamore KA, Banks J, MacDonald SJ (2003) Fate of Ochratoxin A in the processing of whole wheat grains during milling and bread production. Food Add Contam 20:1153–1163
Sheldon JL (1904) A corn mold. Agric Exp Stn Nebr Annu 17:23–43
Sperber WH, The North American Millers' Association Microbiology Working Group (2007) Role of microbiological guidelines in the production and commercial use of milled cereal grains: a practical approach for the 21st century. J Food Prot 70:1041–1053
Sydenham EW, Van der Westhuizen L, Stockenstrom S, Shepard GS, Thiel PG (1994) Fumonisin-contaminated maize: physical treatment for the partial decontamination of bulk shipments. Food Add Contam 11:25–32
Tagne A, Kongsdal O, Ngoko Z, The C, Mathur SB (2003) *Fusarium pallidoroseum* in maize samples of three agro-ecological zones of Cameroon. J Stored Prod Res 39:367–374
Upreti P, Roberts JS, Jalali R (2010) Heat-treated flour. U.S. patent application 20100092639
Van Cauwengerge J, Bothast RJ, Kwolek WF (1981) Thermal inactivation of eight *Salmonella* serotypes on dry corn flour. Appl Environ Microbiol 42:688–691
Van der Merwe KJ, Steyn PS, Fourie L, Scott DB, Theron JJ (1965) Ochratoxin A, a toxic metabolite produced by *Aspergillus ochraceus*. Nature 205:1112–1113
Vasse J, Frey P, Trigalet A (1995) Microscopic studies of intercellular infection and protoxylem invasion of tomato roots by *Pseudomonas solanacearum*. Mol Plant Microbe Interact 8:241–151
Vincelli P, Parker G (2012) Fumonisin, vomitoxin, and other mycotoxins in corn produced by *Fusarium* fungi. http://www.ca.uky.edu/agc/pubs/id/id121/id121.htm. Accessed 10 June 2012
Wagacha JM, Muthomi JW (2008) Mycotoxins problem in Africa: current status, implications to food safety and health and possible management strategies. Int J Food Microbiol 124:1–12
Wang E, Norred WP, Bacon CW, Riley RT, Merrill AH Jr (1991) Inhibition of sphingolipid biosynthesis by fumonisins. J Biol Chem 266:14486–14490
Wicklow DT (1995) The mycology of stored grain: an ecological perspective, Ch. 7. In: Jayas DS, White NDG, Muir WE (eds) Stored grain ecosystems. Marcel Dekker, New York, NY, pp 197–249
Wiseblatt L (1967) Reduction of the microbial populations in flour incorporated into refrigerated foods. Cereal Chem 44:269
World Health Organization (2000) Zearalenone. Food additives series: 44. Geneva, Switzerland
Yuen GY, Schoneweis SD (2007) Strategies for managing *Fusarium* head blight and deoxynivalenol accumulation in wheat. Int J Food Microbiol 119:126–130
Zain M (2011) Impact of mycotoxins on humans and animals. J Saudi Chem Soc 15:129–144

Chocolate and Confectionary

David C. Bean and Laurie S. Post

Abstract Confectionary products are often considered relatively inert from a microbiological perspective. Nevertheless, several *Salmonella* outbreaks have been attributed to confectionary, particularly chocolate products. The cause of these outbreaks was generally traced back to lapses in GMP, particularly cross contact issues and water ingress. Managing *Salmonella* in chocolate manufacture begins with a validated cocoa bean-roasting process. However, the potential for pathogen recontamination exists with the addition of ingredients and inclusions post process. This risk can be managed by a stringent supplier assurance program including prerelease microbiological testing of these materials. In addition to assured ingredients, the manufacturing environment must include a strict containment policy for raw and finished goods, control of water use including the prevention of water leaks, and ongoing microbial surveillance. Manufacturing equipment needs to be hygienically designed and amenable to sanitation processes, should a contamination event occur. Lastly, an effective microbiological verification program is essential to ensure all described processes are in control.

Keywords Chocolate • Cocoa • Cacao • Halva • Roasting • Low-moisture foods • Confectionary

Introduction

Confectionary products are widely consumed and enjoyed throughout the world. Confectionary constitutes a broad range of product types, and the nature of confectionary products varies considerably with geography. While there are many different confectionary products and methods of manufacture, this chapter will focus

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predominantly on product types which have documented microbiological food safety risks—most notably chocolate. Most sugar confectionary, such as boiled sweets, toffees, fudge, fondants, jellies, and pastilles, and other confectionary products involve processes lethal to most vegetative bacteria and tend to be microbiologically inert.

Descriptions and definitions of the most important chocolate products are provided by the Codex Standards 87-1981 (chocolate; Codex Alimentarius Commission 1981), 142-1983 (composite and filled chocolate; Codex Alimentarius Commission 1983), and 147-1985 (cocoa butter confectionary; Codex Alimentarius Commission 1985). The main microbiological hazard in chocolate confectionaries is *Salmonella* (International Commission on Microbiological Specifications for Foods (ICMSF) 2005). Chocolate products including bars, blocks, and bonbons have been implicated in outbreaks of *Salmonella* gastroenteritis.

In addition to chocolate, outbreaks of salmonellosis have also been attributed to consumption of regional specialty confections, the most notable being halva. Halva is a Turkish candy made with ground sesame seeds and honey, often with fruit and nuts added. Halva shares some physical properties with chocolate, an a_w of 0.18 and a fat content of approximately 32 % (Kotzekidou 1998).

Overview of Confectionary-Related Outbreaks

Chocolate

Compared to many other food types with high intrinsic *Salmonella* risks, the risk of acquiring salmonellosis from chocolate products is comparatively low. Nevertheless, there have been several high-profile outbreaks attributed to chocolate since the 1960s. These are summarized in Table 1.

The first well-documented outbreaks of salmonellosis attributed to chocolate occurred in 1970. Cocoa powder contaminated with *Salmonella* serotype Durham was used in confectionary products and subsequently caused an outbreak that affected 110 people in Sweden (Gastrin et al. 1972). In 1973–1974, 95 cases of salmonellosis were reported in Canada (D'Aoust et al. 1975) and an additional 30 in the United States (Craven et al. 1975) acquired from chocolate contaminated with *S.* Eastbourne. Christmas-wrapped chocolate balls manufactured by a Canadian company were implicated as the vehicle of transmission. In 1982–1983, an outbreak involving 245 people in the UK was traced to two types of chocolate bars produced in Italy and contaminated with *S.* Napoli (Gill et al. 1983). In 1986, 33 cases of gastroenteritis due to *S.* Nima were reported in Canada and the United States. These were eventually traced back to chocolate coins imported from Belgium (Hockin et al. 2009). In 1987, 349 confirmed cases of salmonellosis in Norway were part of an outbreak linked to chocolate contaminated with *S.* Typhimurium, in which an estimated 20,000–40,000 persons became ill (Kapperud et al. 1989, 1990). In 2001,

Table 1 Published salmonellosis outbreaks associated with chocolate

Year	Country	Serovar	Vehicle	Source of contamination	*Salmonella* CFU/g	No. of cases	Age of cases	Reference
1970	Sweden	Durham	Chocolate products	Cocoa powder	–	110	53 % ≤15 years	(Gastrin et al. 1972; WHO 1973)
1973–1974	Canada and USA	Eastbourne	Chocolate balls from Canada	Cocoa beans	2.5	95 (Canada) 30 (USA)	3 years (median)	(Craven et al. 1975; D'Aoust et al. 1975)
1982	England and Wales	Napoli	Chocolate bars from Italy	Contaminated water speculated	2–23	245	58 % ≤15 years	(Gill et al. 1983)
1985–1986	Canada and USA	Nima	Chocolate coins from Belgium	Unknown	0.04–0.24	29 (Canada) 4 (USA)	62 % 1–4 years	(Anon 1986; Hockin et al. 2009; WHO 1986a, b)
1987	Norway and Finland	Typhimurium	Chocolate products from Norway	Avian contamination speculated	≤1	349 (361)	6 years (median)	(Kapperud et al. 1989, 1998)
2001–2002	Germany/Europe	Oranienburg	Two chocolate brands from Germany	Unknown	1.1–2.8	>439	15 years (median)	(Werber et al. 2005)
2006	United Kingdom	Montevideo	Chocolate tablets	Contaminated water ingress	–	56	4 (median)	(Elson 2006b)

an outbreak of *S.* Oranienburg occurred in Germany, resulting in 373 reports of infection. Those individuals affected had consumed a specific brand of chocolate distributed exclusively through a single supermarket chain (Ethelberg 2002; Fisher et al. 2002; Werber et al. 2002, 2005). Most recently, in 2006, an outbreak of *S.* Montevideo in the United Kingdom was traced to chocolate (Elson 2006a). Thirty-seven cases of *S.* Montevideo infection were also reported to have the same pulsed-field gel electrophoresis profile (Elson 2006b).

It should be noted that outbreaks of salmonellosis, such as those described above, are very likely an underestimate of the actual occurrence of *Salmonella* in chocolate. A study in Mexico revealed that two of 44 (4.5 %) wrapped chocolate products were contaminated with *Salmonella* (serotypes Agona and Derby) (Torres-Vitela et al. 1995). While this may not be a reflection on the chocolate industry as a whole, it certainly does highlight the necessity of thorough microbiological verification of products made by chocolate manufacturers.

Halva

In early June 2001, at least 10 people, predominantly children, were infected with *S.* Typhimurium definitive phage type (DT)104 in the south of Sweden (de Jong et al. 2001). Further investigation revealed that 27 individuals had become infected after consuming halva. The age of the patients ranged from 5 months to 50 years old, with 15 of the cases being younger than 10 years. *Salmonella* of the same type was also isolated from five jars of halva, four with pistachio and one with cocoa flavoring.

Subsequently, 29 potential cases were detected in Norway (Aavitsland et al. 2001), and at least 22 people became ill in Australia. A public health warning was issued in Germany after *Salmonella* was obtained from 11 of 117 (9.4 %) ready-to-eat food items containing sesame (Brockmann 2001). This included not only the *S.* Typhimurium DT 104 outbreak strain but also *S.* Offa, *S.* Tennessee, and *S.* Poona isolated from halva (Brockmann et al. 2004). Another study of Turkish halva revealed that 120 samples were *Salmonella* negative but exceeded the recommended quality criteria for *S. aureus*, aerobic plate count, and yeast and mold counts in 3, 20, and 32 % of samples, respectively (Kahraman et al. 2010).

Like chocolate, *Salmonella* may persist in halva for long time periods. In one artificial contamination trial, *Salmonella* was recovered from the product after 8 months of storage (Kotzekidou 1998).

Other Confections

In Italy, *Salmonella* was recovered from torrone, a traditional nougat confectionary (De Grandi et al. 1987). It is believed the contamination was introduced from eggs used in the recipe. Nougat typically has an a_w range of 0.4–0.7 (ICMSF 2005).

In the United Kingdom, 36 cases of *Salmonella* Enteritidis phage type (PT) 4 infection were attributed to marshmallow (Lewis et al. 1996). The outbreak occurred in October 1995 and mainly affected children. *S*. Enteritidis PT4 was also isolated from samples of the marshmallow, and all isolates had indistinguishable pulsed-field gel electrophoresis profiles. Contamination was attributed to raw eggs used in the recipe without a subsequent cooking step. Marshmallow has an a_w range of 0.6–0.75 (ICMSF 2005).

Salmonella Risks in Chocolate Manufacture

The production of chocolate requires a long and well-managed supply chain. Chocolate carries inherent risks due to the nature of the raw materials and the manufacturing process involved. An overview of the chocolate-making process is provided in Fig. 1. The major areas of concern are:

- *Salmonella* contamination of raw cocoa beans.
- *Salmonella* risk profile of chocolate-making ingredients.

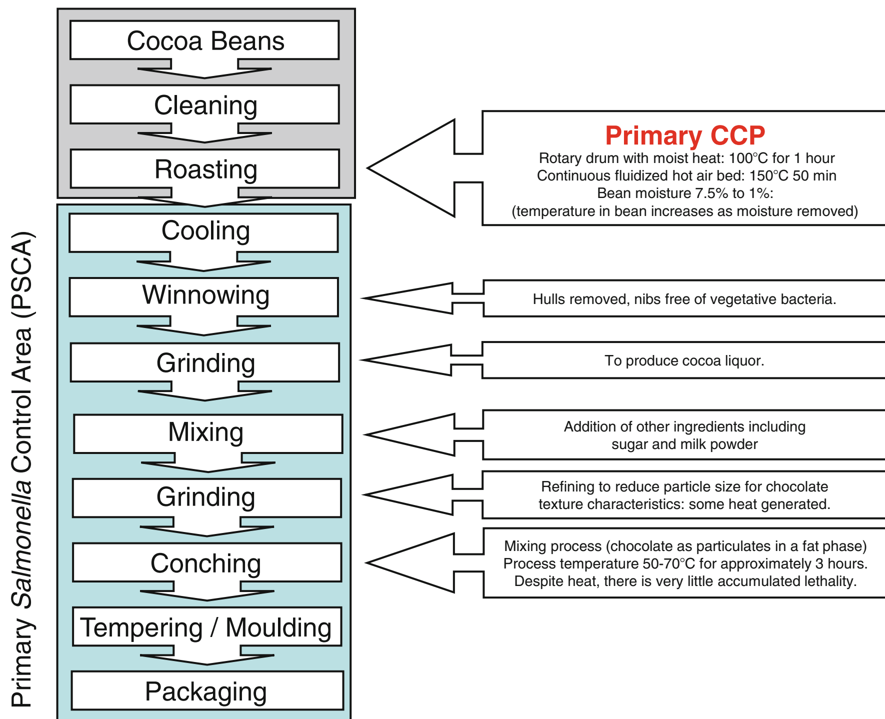

Fig. 1 An overview of the chocolate-making process highlighting the sole thermal kill step in the process

  - Chocolate liquor.
  - Other essential components of chocolate.
  - Inclusions used in chocolate making.

- Physiology of *Salmonella* in chocolate.
  - Increased thermal resistance of *Salmonella* in chocolate.
  - Low infectious dose of *Salmonella* in chocolate.
- Epidemiology of chocolate-related outbreaks (geographically widespread and age related).

Salmonella Contamination of Raw Cocoa Beans

Cocoa beans, the principle raw material in chocolate manufacture, are the seeds of the tree *Theobroma cacao*. The production and harvesting of cocoa beans has been well described elsewhere (Afoakwa 2010; Thompson et al. 2001; Wood and Lass 2008) but is worth revisiting to understand the potential opportunities for *Salmonella* contamination.

Processing begins with the harvesting of healthy ripe fruit of the cacao tree: the cocoa seed pod. The pods are usually collected in piles in the field and broken open on site or at a processing plant. Each pod contains about 30 beans surrounded by sterile pulp. Processing begins by removing the beans from the pods and collecting in heaps or boxes for fermentation. Fermentation is a spontaneous 2- to 8-day process which involves a succession of microbial populations, including yeasts (Nielsen et al. 2005), lactic acid bacteria (Kostinek et al. 2008), and acetic acid bacteria (Nielsen et al. 2007), which lead to the development of a characteristic flavor and color of fermented cocoa beans. The fermentation process produces ethanol and acetic acid as well as considerable heat (Schwan and Wheals 2004). These compounds diffuse into the beans and cause the death of the cocoa seed embryo and, in turn, initiate the physical and biochemical changes in the beans that form the development of cocoa's characteristic flavor and color.

The conditions during fermentation, which lead to curing of the beans, also lead to the reduction of other microorganisms. The initial cocoa bean microbial population is variable in number and type; however, the key groups active during fermentation are yeasts, lactic acid bacteria, and acetic acid bacteria. The ongoing fermentation process creates an adverse microenvironment within the fermentation heap, and typically only sporeforming bacteria survive this process. The beans should be free of viable pathogenic bacteria, although a recent metagenomic study of cocoa fermentation did detect *Salmonella* sequences (Illeghems et al. 2012).

While the fermentation process leaves the beans relatively free from vegetative bacteria, the beans still have a high moisture level and must be promptly dried to ensure microbiological stability. The drying process is typically performed in an open environment, and often beans are dried directly on the ground with minimal separation from the soil. *Salmonella* can persist indefinitely in the field, and there is

Fig. 2 Potential sources of microbial contamination in the processing of cocoa beans: harvested cocoa beans heaped for fermentation (*top left*); post-fermentation beans are dried in the open and susceptible to environment contamination, including birds (*top right*) and other animals (*bottom left*); and fermented beans in storage are also susceptible to contamination from animals (*bottom right*). Images courtesy of Martin Gilmour and Rodney Snyder

considerable opportunity for beans to become contaminated with *Salmonella*. For example, a survey of environmental *Salmonella* in an almond orchard revealed the same strain persisted over the 5-year duration of the study (Uesugi et al. 2007). Figure 2 shows cocoa beans in various stages of processing prior to roasting and illustrates the numerous opportunities for contamination. Managing *Salmonella* contamination of the cocoa beans relies upon good agricultural practices (GAPs) at the farm level and GMPs during storage.

Salmonella may persist on the cocoa bean postharvest. *S.* Oranienburg and *S.* Enteritidis inoculated onto cocoa beans were still viable after 21 days at either 4 °C or 21 °C, although survival was affected by the experimental method used to harvest the cells (Komitopoulou and Penaloza 2009).

Salmonella contamination of cocoa beans may serve as a mechanism for subsequent introduction of the bacteria into the manufacturing environment. For example, investigation into the Norwegian *S.* Typhimurium outbreak revealed a number of *Salmonella* serovars isolated from dust samples collected in rooms in which cocoa beans were stored or rinsed (*S*. Brancaster, *S*. Onireke, and *S*. Stockholm) (Kapperud et al. 1990). A study of cocoa beans from three Brazilian producers found one of 119 samples was positive for *Salmonella* (da Silva do Nascimento et al. 2010). At least one entirely new serovar, *Salmonella* Sherbrooke, has been isolated from cocoa (Gosselin et al. 1979). Furthermore, the Canadian and US *S.* Eastbourne outbreak of 1974 is believed to have been initiated from contaminated cocoa beans (D'Aoust et al. 1975).

Roasting of cocoa beans is necessary for their flavor development but is also the primary *Salmonella* control step in chocolate-making processes (Grocery Manufacturers Association 2009). Two common designs of roasters are employed: a rotary drum roaster with moist heat (100 °C for 1 h) and a continuous fluidized hot air bed (150 °C for 50 min). From the authors' experience, the moist heat roaster provides greater heat penetration allowing the beans to be processed at a lower temperature while achieving a comparable lethal effect. This has been shown experimentally with cocoa and hazelnut shells (Izurieta and Komitopoulou 2012) and almonds (Jeong et al. 2009). Beans can also be processed using an initial steam treatment to provide a validated microbial reduction followed by roasting to dry the beans. This process allows for an efficient kill step and the ability to roast at low temperatures that encourage flavor creation. Systems are commercially available which deliver this process (e.g., see http://www.buhlergroup.com/global/en/process-technologies/roasting-debacterizing.htm).

It is essential that the raw beans are strictly segregated and contained prior to roasting. This is critical to avoid recontamination of processed beans, the processing environment, and the chocolate subsequently manufactured from them (Carrasco et al. 2012). It is extremely difficult, if not impossible, to eradicate *Salmonella* from a chocolate-making process post-roasting, once it has become contaminated (Bell and Kyriakides 2002).

Salmonella Risk Profile of Chocolate Making

Chocolate Liquor

Roasted cocoa beans are cooled and winnowed to remove the hulls or shells. Alternatively, dehulled cocoa beans or nibs may also be roasted directly. From either process, the resulting nibs should be free of *Salmonella* and other vegetative microorganisms due to the lethal effect of the roasting and physical reduction of residual microflora, which are removed with the hulls.

The cocoa nibs are ground to make chocolate liquor, which contains only 1–2 % moisture. The effectiveness of the cocoa bean roast step and the degree of sanitary handling of the roasted beans post process will ultimately impact the resultant

microbiological safety of the chocolate liquor. The low water activity of chocolate liquor ensures vegetative bacteria will not grow, although they may persist at very low cell numbers.

Other Essential Components of Chocolate

In addition to cocoa and its derivatives, there are other essential ingredients required in chocolate making. Any of these ingredients have the potential to introduce *Salmonella*, particularly as they are added after the primary control roasting step. The following ingredients are added to chocolate liquor prior to or during the mixing, grinding, and conching steps (see Fig. 1). The relative amounts of each of these ingredients depends on the type of chocolate being produced and is defined in Codex Alimentarius Standard 87-1981 (Codex Alimentarius Commission 1981).

- Sugar: Generally not considered to be a high *Salmonella* risk. It does not support bacterial growth and is often used in the food industry to reduce water activity levels in food products as a means to inhibit bacterial growth.
- Milk: Milk powders are microbiologically critical raw ingredients. Dairy ingredients have historically been documented to be subject to cross-contamination following thermal heat processing (Olsen et al. 2004; Rowe et al. 1987; Ryan et al. 1987). Milk powders have been suspected or confirmed as vehicles for *Salmonella* spp. transmission in several outbreaks particularly in powdered infant formula (Cahill et al. 2007), and the pathogen can survive in milk powder for prolonged periods of storage (D'Aoust 1977).
- Cocoa butter: Roasted beans or dehulled nibs are pressed to produce cocoa butter. The residual cocoa cake is used to make cocoa powder. This processing step often occurs in the regions where cocoa is farmed and deficient hygienic practices may allow the cocoa butter to be contaminated with debris (e.g., wood, metal, stones). Cocoa butter is approximately 90 % fat and, therefore, does not support bacterial growth. However, if cocoa butter gets wet (during storage or transportation), mold can grow in the cracks and crevices of the cocoa butter block within damaged packaging. Cocoa butter could, therefore, potentially serve as a vector for the introduction of *Salmonella* into a process if the outside of packaging is contaminated or *Salmonella* is carried on debris.
- Lecithin: Soy lecithin is used as an emulsifier in chocolate making, to adjust and maintain the viscosity and flow properties of chocolate (Afoakwa 2010), and often regarded as being of low pathogen risk. However, soy lecithin contaminated with *Salmonella* was the cause of a precautionary voluntary recall of chocolate products and a plant closure in Canada (there were no reported illnesses associated with the consumption of the products) (Public Health Agency of Canada 2006; Reynolds 2006).

Minor ingredients such as salt and vanilla are not generally considered to be high risk sources of contamination (D'Aoust 1977), although carmine red (a food additive) was identified as a vehicle for *S*. Cubana in candy coatings (Lennington 1967).

Inclusions Used in Chocolate Making

Ingredients such as coconut, nuts, egg albumin, and spices are often added to chocolate to create new products. All such inclusions are added after the microbiological kill step and, therefore, also pose a potential microbiological risk. It is crucial that these ingredients receive an appropriate process to inactivate microbiological pathogens (where appropriate) before use and are subject to microbiological verification testing.

- Nuts: Nuts and materials derived from nuts (e.g., peanut butter) are commonly included in many chocolate and confectionary products. Nuts and nut-derived products have been the root cause of several outbreaks (Cavallaro et al. 2011; Scheil et al. 1997; Sheth et al. 2011), and *Salmonella* is readily recovered from retail nuts (Little et al. 2010). See Chap. C7 for more information on *Salmonella* in nut products.
- Coconut: Raw coconut has a long history of association with *Salmonella* contamination (Schaffner et al. 1967). More recently, an outbreak of 167 cases of paratyphoidal *Salmonella* in Singapore was attributed to imported coconut (Teoh et al. 1997).
- Egg albumin: Nougat and fondant are components of some confectionary products, and both are manufactured with egg albumin (Nummer et al. 2012). Eggs represent a potential source of contamination and have been implicated in contamination of a nougat product (De Grandi et al. 1987). Dried egg products have been recognized as a potential source of *Salmonella* contamination for some time (Solowey et al. 1947; Solowey and Rosenstadt 1947), and the Code of Federal Regulations has provided guidelines for dry heat disinfection of *Salmonella*-contaminated egg whites (Code of Federal Regulations 2005; National Advisory Committee on Microbiological Criteria for Foods 2006).
- Extruded grains: Extruded rice, wheat, and soy products may be included in chocolates to provide texture and enhance nutritional profiles. Each of these carries an inherent risk of *Salmonella* contamination. This is especially so for soya products, which were the cause of a 2010 recall of granola bars in the United States (US Food and Drug Administration 2010). See Chap. C5 for more information.
- Spices: Some chocolate manufacturers include exotic ingredients in their products, such as chili and other types of spices or herbs. Spices are a notorious potential source of *Salmonella* (Zweifel and Stephan 2012) and must be screened carefully if to be introduced into chocolate. See Chap. C1 for more information on spice contamination.

It is therefore vitally important that all ingredients are subjected to a robust raw material specification process (Williams et al. 2006).

Cocoa Powder

Cocoa powder is produced as a by-product of cocoa butter making. While cocoa powder has uses other than chocolate making, such as flavoring chocolate milk, it is also used in recipes for chocolate confections (e.g., fondant fillings or a powdered topping for truffles). The microbiota of cocoa powder generally consists of Gram-positive sporeformers (Gabis et al. 1970) but occasionally has been identified as containing *Salmonella* (Lima et al. 2011).

Increased Thermal Resistance

A greater heat resistance is conferred on bacteria when in chocolate. Chocolate is a low-moisture matrix (water activity: 0.3–0.4), which is sufficient to inhibit growth of all microorganisms. However, while the low water activity does not favor bacterial growth, it does enhance heat resistance of organisms in chocolate. Moreover, the high-fat and high-sugar content of chocolate does provide a stabilizing effect on *Salmonella* (Aviles et al. 2013; Barrile et al. 1970). It has been demonstrated that a decreased water activity will increase heat resistance in *Salmonella*, particularly when sucrose is responsible for the a_w decrease (Goepfert et al. 1970). The practical result of this is a significant increase in the thermal resistance of *Salmonella* in chocolate (Table 2) (Barrile and Cone 1970). This increase in thermal resistance is significant: after the beans are roasted, there is no further significant thermal processing.

While temperatures of 70–80 °C are reached during the milling, refining, and conching of chocolate, these do not effectively kill *Salmonella* populations (da Silva do Nascimento et al. 2012; Krapf and Gantenbein-Demarchi 2010). Once contaminated, elimination of *Salmonella* or other pathogens in chocolate is a difficult, if not impossible, task, owing to the low water activity of the product (D'Aoust 1977; de Smedt 1989). The low water activity and high-fat content in chocolate increase thermal resistance of *Salmonella* so that temperatures reached during chocolate production (even after considerable overheating) do not completely inactivate populations of *Salmonella* (de Smedt 1989). The temperatures required to pasteurize chocolate are not achievable without destroying the product.

Several studies on the heat resistance of *Salmonella* in chocolate were conducted to assess the potential for the application of a heat process to eliminate the pathogen (Barrile and Cone 1970; Goepfert and Biggie 1968; Lee et al. 1989). In molten chocolate, *S.* Typhimurium had a *D*-value of 396 min (6.6 h) and 816 min (13.6 h) at 71.1 °C and 65.6 °C, respectively (Goepfert and Biggie 1968). Similar heat resistance was observed for *Salmonella* in milk chocolate (Lee et al. 1989), in which the *D*-values were 4.5, 4.6, and 6.6 h at 71 °C for *S.* Eastbourne, *S.* Senftenberg, and *S.* Typhimurium, respectively. Interestingly, the results from both of these studies revealed that *S.* Typhimurium was more heat resistant than *S.* Senftenberg 775 W

Table 2 *D*-values for *Salmonella* in chocolate and chocolate components (table adapted from Doyle and Mazzotta 2000)

Salmonella serovar	Moisture content	*D*-value (minutes)						Reference
		50 °C	60 °C	70 °C	71 °C	80 °C	90 °C	
Milk chocolate								
Anatum	–	–	180–270	60–90	–	–	6–18	(Barrile et al. 1970)
Anatum	0 %	–	–	1,200	–	–	–	(Barrile and Cone 1970)
Anatum	1 %	–	–	510	–	–	–	(Barrile and Cone 1970)
Anatum	2 %	–	–	240	–	–	–	(Barrile and Cone 1970)
Anatum	4 %	–	–	210	–	–	–	(Barrile and Cone 1970)
Eastbourne	–	–	–	270	–	–	–	(Lee et al. 1989)
Senftenberg 775 W	–	–	–	360–480	–	96–144	36–42	(Goepfert and Biggie 1968)
Senftenberg 775 W	–	–	–	–	276	–	–	(Lee et al. 1989)
Typhimurium	–	–	–	678–1,050	–	222	72–78	(Goepfert and Biggie 1968)
Typhimurium	–	–	–	–	396	–	–	(Lee et al. 1989)
Salmonella spp.[a] (first 3 h)	–	217	102	51	–	–	–	(da Silva do Nascimento et al. 2012)
Salmonella spp.[a] (after 3 h)	–	1,077	482	702	–	–	–	(da Silva do Nascimento et al. 2012)
Dark chocolate								
Salmonella spp.	–	1,570	1,008	600	–	142	25	(Krapf and Gantenbein-Demarchi 2010)
Cocoa butter								
Salmonella spp.	–	245	306	–	–	–	–	(Krapf and Gantenbein-Demarchi 2010)
Cocoa liquor								
Salmonella spp.	–	999	760	248	–	70	26	(Krapf and Gantenbein-Demarchi 2010)

[a]Cocktail of five *Salmonella* serovars, including: *S*. Typhimurium, *S*. Oranienburg, *S*. Senftenberg, *S*. Eastbourne, and *S*. Enteritidis

(typically considered an unusually heat-resistant strain) in milk chocolate (Goepfert and Biggie 1968; Lee et al. 1989).

Chocolate and chocolate candies have such low-moisture content that microbes heated in them are subjected to dry, rather than moist, heat. *Salmonella* cells are much more susceptible to inactivation by heat (71 °C) when traces of water are added to the chocolate mass (Barrile and Cone 1970). A dramatic decrease in the *D*-value was evidenced with 2.0 % added moisture, reducing the *D*-values from 20 h to 4 h. *D*- and *z*-values for different *Salmonella* serotypes in chocolate are presented in Table 2.

The cellular mechanism for this enhanced heat resistance in *Salmonella* is presumably an interplay between the chocolate matrix and cellular functions of the bacteria. The *rpoS* gene is induced during many processes in food production and is responsible for increased resistance (Dodd and Aldsworth 2002). This is certainly the case for *Salmonella,* as *rpoS*-deficient mutants of *S.* Typhimurium are more susceptible to heating in chocolate than their wild-type counterparts (Haxgart et al. 2010).

Low Infectious Dose

Chocolate was one of the first products that provided strong evidence that ingestion of large numbers of salmonellae is not a prerequisite for human infection (D'Aoust and Pivnick 1976) and that food ingredients may protect *Salmonella* against the acidic conditions of the stomach (D'Aoust 1977; Waterman and Small 1998). Even small numbers of *Salmonella* present in the product could colonize the lower gastrointestinal tract and produce clinical symptoms.

Retrospective quantitative analysis of outbreak-associated contaminated material has revealed that very low *Salmonella* cell numbers were present (Table 1). For example, *S.* Nima was present in cell concentrations as low as 4.3–24 CFU per 100 g, with approximately 25 g (1.1–6 *S.* Nima CFU) of chocolate consumed by patients (Hockin et al. 2009). An infectious dose of 50 CFU of *S.* Napoli was calculated as sufficient to cause illness in a 10-year-old boy (Greenwood and Hooper 1983).

These retrospective attempts at enumeration of *Salmonella* in outbreak-associated products to determine the infectious dose in chocolate have led to a reevaluation of the infectious dose data generated by direct challenge testing of healthy volunteers (McCullough 1951). Outbreak investigations have shown that the dose required to cause disease may be considerably lower than previously thought (Blaser and Newman 1982).

It is believed that the high-fat food matrix protects *Salmonella* against the acidic conditions of the stomach (D'Aoust 1977) and may explain why ingestion of only a few salmonellae is necessary to cause illness in chocolate. The protracted nature of chocolate-associated outbreaks reflects both the long shelf life of chocolate (Tamminga et al. 1976) and the long survival of *Salmonella* in these products (Barrile et al. 1970). *Salmonella* Oranienburg was isolated from chocolates 5

months after manufacture (Werber et al. 2005). In an *S.* Napoli outbreak in England and Wales, this interval was 12 months (Gill et al. 1983). *Salmonella* has been shown to persist for years in inoculated chocolate samples (de Smedt 1989).

Epidemiology

Chocolate products are commonly consumed by children, and, therefore, this age group is often overrepresented in chocolate *Salmonella* outbreaks. Common to all reported chocolate-associated outbreaks is that the epidemics were prolonged in time, widely disseminated geographically, and affected a large number of people, predominantly children (Anon 1986; Craven et al. 1975; D'Aoust et al. 1975; Gill et al. 1983; Kapperud et al. 1990).

During the Canadian *S.* Eastbourne outbreak, 46 % of all cases were children under 4 years of age (D'Aoust et al. 1975). In the subsequent *S.* Nima outbreak, the index case was always a child 2–4 years of age (Anon 1986).

Children also tend to be a group more susceptible to *Salmonella* infection. While most will recover from the gastroenteritis, in others, particularly those with underlying complications, there may also be additional potentially life-threatening complications (Dickinson 1974).

Managing and Mitigating Salmonella Risk

Controlling *Salmonella* in chocolate manufacture is not dissimilar to other low-moisture products described in this book. Collectively, industry guidance and best practices have been reviewed and compiled, and these recommendations are very applicable to chocolate (Grocery Manufacturers Association 2009).

When a root cause was identified for the major chocolate *Salmonella* outbreaks of the last four decades, it was either cross-contamination from raw cocoa bean handling (Craven et al. 1975; Gastrin et al. 1972) or failings in GMPs (Gill et al. 1983; Kapperud et al. 1998). The principal means used to control *Salmonella* in chocolate products is a strong preventative control program, including HACCP (Cordier 1994; International Office of Cocoa Chocolate and Sugar Confectionery 1991), Good Manufacturing Practices (de Smedt 1989), and appropriate microbiological hold and test programs for ingredients and finished products to verify control programs are effectively managing risks.

In the manufacture of chocolate, *Salmonella* can be introduced into the product from a number of different sources. Subsequent to cocoa bean roasting, there are no additional critical control points in the process to control microbiological hazards. Indeed, downstream of roasting, the chocolate manufacturing process can essentially be thought of as a blending operation. *Salmonella* is known to persist in manufacturing environments and can be a source of cross-contamination (Podolak et al. 2010).

Prerequisite programs focusing on the process environment such as water control, containment, traffic flow, and sanitation take on a greater significance, as they are the only effective means of reducing post-process environmental contamination risk.

If adequate control measures are not in place and chocolate does get contaminated, no heating step will be able to destroy the contamination without destroying the product. Once contaminated, *Salmonella* can survive in chocolate for many months (Gill et al. 1983; Tamminga et al. 1976, 1977; Werber et al. 2005). It should also be noted that *E. coli*, specifically Shiga toxin-producing strains, have similar longevity in chocolate (Baylis et al. 2004).

Risk in chocolate manufacturing is managed by effective Food Safety Control Programs in the following key areas:

- Process validation and monitoring.
- Containment/traffic flow programs.
- Water control.
- Raw material control.
- Equipment design.
- Cleaning, sanitation, and lot definition.

The effectiveness of these programs to control pathogen risk is monitored and measured by finished product testing and environmental monitoring programs.

Process Validation and Monitoring

The primary control step in chocolate making is cocoa bean roasting. Vegetative bacteria, including *Salmonella,* will not survive an effective roasting step. It is essential that the roasting step is validated to ensure it is delivering the appropriate degree of lethality (US Food and Drug Administration 2009). A validation study should be performed on each roasting line process as results obtained from one specific manufacturing plant will vary from another's, because each manufacturer's process will be different.

Ideally, a validation study would be performed in a laboratory; however, where thermal processes cannot be authentically replicated in a laboratory setting, an in-plant validation using a nonpathogenic surrogate for *Salmonella* can be performed (Grocery Manufacturers Association 2009). Neither *Salmonella* nor any other food-borne pathogen should ever be used for in-plant validation studies. In-plant validation studies apply the surrogate bacteria directly to the actual process to gauge the lethality of times and temperatures used. The ability to recover and quantify the level of the test organism at the end of the process reflects its effectiveness. It is critical that validation includes calibrated measuring devices to record the actual temperature and time process conditions during cocoa bean roasting in order to assure that the specified temperature is achieved. The location and monitoring frequency of temperature monitoring devices should be specified for each roasting operation. Belt speed and oven length are also critical process parameters. In-plant validations should be performed only by experts qualified to conduct process validation studies.

The selection of a surrogate organism must be based on the surrogate's thermal resistance in chocolate: a surrogate successfully utilized in a different matrix may not be appropriate for chocolate. Furthermore, not all members of a bacterial species are created equal, and different bacterial strains might behave quite differently from one another. Specific strains of bacterial species should be identified as surrogates.

Each time a surrogate is selected and used, it should be verified in a laboratory that its heat resistance equals or exceeds the heat resistance of *Salmonella* in the matrix at the temperature range associated with the process. The physiological state (e.g., whether the cell is stressed) of the surrogate organism will also affect its resistance to heat (Wesche et al. 2009). For example, cells in the log phase of growth are more susceptible to heat damage than cells in stationary phase (Rees et al. 1995). Cells grown at higher temperatures or those exposed to a sublethal heat shock (Mackey and Derrick 1990), those grown in growth limiting concentrations of carbohydrates (Tolker-Nielsen and Molin 1996), and those growing at a reduced water activity (dependent on the agent used to control the a_w) (Goepfert et al. 1970) are typically more heat resistant.

While it has been shown that *Salmonella* can be readily recovered from unroasted cocoa beans, there are no published data quantifying the population. Therefore, each processor must make a judgment call on what roasting conditions to use in their process based upon knowledge of their materials and process variables. In the absence of accurate data quantifying pathogen burden in cocoa, a default 5-log minimum kill of *Salmonella* is recommended (Schaffner et al. 2013), as suggested by regulatory agencies (US Food and Drug Administration 2009). However, the only assurance that a process can achieve the desired log reduction is from a well-conducted validation study.

Regardless of the cocoa bean roast conditions selected by a processor, it is important to consider process verification through an effective microbiological testing program on roasted cocoa beans.

Containment and Traffic Flow

Raw cocoa beans are potentially contaminated with *Salmonella* (da Silva do Nascimento et al. 2010; Kapperud et al. 1990) and must be kept in a segregated area or in dedicated storage silos. Conversely, roasted beans are pathogen-free and must be protected from recontamination. If a plant has poor segregation and traffic control between raw ingredients and processed product, there is a high risk of cross-contamination.

There are several steps that the plant can take to prevent cross-contamination between raw materials and finished product (Grocery Manufacturers Association 2009). Raw material receiving and processing areas must be physically segregated from finished product processing and packaging areas. The manufacturing process should be linear; raw materials should enter on one end of the line, and finished product should exit from the other side. The same concept applies for air flow: to

avoid cross-contaminating the finished product, appropriately filtered air should move from processed material toward the raw areas. Sections of the line that carry raw materials should not pass finished product sections of the line.

Personnel should be well educated to appreciate the risk of cross-contamination. Staff assigned to working in raw material areas must not be allowed access to post-process areas. Foot traffic into post-process areas must be controlled. Often, the installation of a hygiene juncture is used to create a transition area where protective clothing can be put on prior to entry into the post-roasting area.

Cleaning and maintenance equipment must be dedicated to raw material handling and post-process areas and must not be interchanged. Color coding of the equipment can help maintain this separation.

Water Control

The growth of *Salmonella* in the plant is restricted by controlling moisture and water use. Bacteria cannot multiply in a dry environment, and, therefore, it is crucial to keep the plant as dry as is feasibly possible. Potential water sources such as leaking water and steam lines, condensation, leaking roofs, and improper wet cleaning practices must be eliminated. Removal of standing water restricts the spread of *Salmonella* across surfaces. When wet cleaning occurs, especially during shutdowns, water must be restricted and controlled to assure product safety.

Water can also serve as a vehicle for contamination, e.g., tainted water was identified as a possible source of an outbreak due to chocolate bars manufactured in Italy (Gill et al. 1983). Similarly, a leaking pipe was reported to be the cause of contamination in the 2006 UK *S.* Montevideo outbreak (Das 2006). Microscopic leaks from water-jacketed storage tanks or water used in cleaning can lead to product contamination.

Raw Material Control

In the absence of a post-roaster kill step, supplier assurance programs are essential to protect chocolate from *Salmonella* contamination entering the process through raw materials. It is important to only obtain raw materials from trusted suppliers who only use high-quality ingredients. Suppliers are controlled through audits and specifications. Raw materials are controlled within the plant by microbiological testing programs and inspections upon arrival prior to release into production processes.

If rework is used in the manufacture of chocolate, it must be stored and transported in a sanitary way. If mishandled, rework can serve as a cause for contamination entering into the production lines.

Equipment Design

Pathogens can also be introduced into the finished product from the plant environment. Even in a plant that receives good routine cleaning, there is still the potential for microbial harborage areas. This must be a factor when considering plant layout and equipment design. Enrobers and tempering units, for example, are particularly difficult to clean thoroughly. Poorly designed equipment can contain niches where sanitizers are unable to penetrate and pathogens are protected. As the machines operate and parts move and vibrate, pathogens in these harborage spots can find their way back into the product.

Common equipment defects can lead to formation of microbial growth niches. For example, improperly welded metal surfaces may have gaps that can trap food residue and water. Metal to metal surfaces that are bolted together, as well as drilled or poorly installed stainless steel cladding, can also have similar types of gaps. Unsealed hollow-tubed structural components can collect food residue and moisture inside the tube and, likewise, serve as growth niches. Any area that allows water and product to collect and is inaccessible to sanitizers will provide a growth niche for microbes. All equipment that requires cleaning with water needs to be disassembled, and all parts must be thoroughly dried before reassembly. It is also important to reassemble the dry equipment as hygienically as possible.

Cleaning, Sanitation, and Lot Definition

Chocolate-making processes tend to run for long periods of time and utilize equipment and storage vessels that are not designed to be wet-cleaned (e.g., holding tanks and enrobers), making it extremely difficult to define microbiological clean breaks in chocolate-making systems. This in turn makes "lot" definition difficult: a "lot" could potentially consist of several months of continuous production.

The use of wet sanitation procedures in areas where water cannot be effectively dried creates a greater food safety risk than that incurred if the equipment had only been dry cleaned (e.g., scraped, flushed).

Scheduled dry cleaning breaks in a process operating at steady state may serve as evidence for lot designation. Such a break could rely on "cleaning" with product flushes and would consist of:

- Disassembly of equipment and, where possible, removal of parts that could be cleaned, sanitized, and dried in a segregated wet-wash area.
- A wipe-down of belts using sanitizing cloths.
- Physical removal of chocolate from piping and enclosed parts of the system by flushing with a product paste or cocoa butter and/or use of a sanitary "pig" (often a snug fitted premolded solid projectile that is propelled by air pressure through a processing line to flush product residue) where applicable.

- And/or circulation of hot cocoa butter using a process validated for effective temperature and time at equipment surfaces. The treatment parameters will be specific for the given process. It is important to note that *Salmonella* attached to surfaces are generally more heat resistant than nonadherent bacteria. For example, *S.* Enteritidis attached to glass and stainless steel exhibited about a twofold increase in *D*-values at 52 °C, compared to unattached cells (Dhir and Dodd 1995; Humphrey et al. 1995).

The use of product flushing to clean the process requires validation that the flushing protocol is as effective as traditional wet cleaning and sanitizing protocols in the reduction of a population of *Salmonella* to a target.

Lastly, an ongoing microbiological testing and data analysis program can verify the absence of *Salmonella* in chocolate, chocolate liquor storage tanks, and the process environment. While validated preventative procedures, especially Critical Control Points (CCPs), are preferred to microbiological testing as evidence that a food safety risk has been mitigated, a robust track record of testing results also provides strong evidence that *Salmonella* control programs are in place and effective. Coupled with *Salmonella* mitigation activities such as: scheduled breaks in the rework stream; application of discrete validated wet cleaning protocols (e.g., on belts); validated cleaning with product flushes; raw material screening; and, validation of cocoa bean roasting; continuous testing can help verify control as well as pinpoint the entry point of potential contamination.

Emerging Challenges

Raw Cocoa

There is a current consumer trend to consume raw foods for a perceived nutritional benefit. Cocoa is no exception. Raw chocolate products do not receive a validated thermal kill step. Manufacturers of raw cocoa products claim their products are not exposed to temperatures that exceed 42 °C (National Confectioners Association Chocolate 2011). This process is well-below standard roasting temperatures, and therefore, raw chocolate is likely to contain the same level of microbial loading it acquired from the farm.

This was demonstrated during May 2012 when a California producer recalled organic cacao nibs because they were potentially contaminated with *Escherichia coli* O157:H7 (US Food and Drug Administration 2012).

New Salmonella Detection Methods

Food manufacturers are under immense pressure to increase the speed of their microbiological testing programs. Manufacturers of test kits have responded to this need and have produced a number of products with a decreased result time (Jasson et al. 2010). It should be noted that chocolate is a notoriously difficult matrix to work with, especially when using techniques based on nucleic acid amplification. Cocoa contains polyphenols which can be inhibitory to PCR and may result in false negative results (Gryson et al. 2004, 2007; Jasson et al. 2011). It is recommended that such methods be subjected to a robust validation using one's own matrices before utilizing them.

Conclusion

The manufacture of confectionary, like other low a_w products, is not without its challenges. Future changes in the wake of consumer demands are likely to bring new problems for product developers and food microbiologists alike. It is, however, very possible to manufacture safe, quality confectionary product provided some basic concepts are applied. These center around sound GMPs, validated microbiological kill steps, quality raw materials, and microbiological verification of the product and environment. Following these simple tenants will ensure that manufacturers can confidently offer microbiologically safe products and consumers can continue to indulge their sweet tooth.

References

http://www.buhlergroup.com/global/en/process-technologies/roasting-debacterizing.htm

Aavitsland P, Alvseike O, Guérin PJ, Stavnes TL (2001) International outbreak of *Salmonella* typhimurium DT104 – update from Norway. Eurosurveillance 5(33):1701

Afoakwa EO (2010) Chocolate science and technology. Wiley-Blackwell, Hoboken

Anon (1986) *Salmonella nima* in British Columbia. Can Med Assoc J 135(11):1286

Aviles B, Klotz C, Smith T, Williams R, Ponder M (2013) Survival of *Salmonella enterica* serotype Tennessee during simulated gastric passage is improved by low water activity and high fat content. J Food Prot 76(2):333–337

Barrile J, Cone J, Keeney P (1970) A study of *Salmonellae* survival in milk chocolate. Manuf Confect 50:34–39

Barrile JC, Cone JF (1970) Effect of added moisture on the heat resistance of *Salmonella anatum* in milk chocolate. Appl Microbiol 19(1):177–178

Baylis CL, MacPhee S, Robinson AJ, Griffiths R, Lilley K, Betts RP (2004) Survival of Escherichia coli O157:H7, O111:H- and O26:H11 in artificially contaminated chocolate and confectionery products. Int J Food Microbiol 96(1):35–48

Bell C, Kyriakides A (2002) *Salmonella*: a practical approach to the organism and its control in foods. Blackwell, Oxford, UK

Blaser MJ, Newman LS (1982) A review of human salmonellosis: I. Infective dose. Rev Infect Dis 4(6):1096–1106
Brockmann S (2001) International outbreak of *Salmonella* typhimurium DT104 due to contaminated sesame seed products – update from Germany (Baden-Württemberg). Eurosurveillance 5(33):1699
Brockmann SO, Piechotowski I, Kimmig P (2004) *Salmonella* in sesame seed products. J Food Protect 67(1):178–180
Cahill SA, Wachsmuth IK, Costarrica M, Embarek PKB (2007) Powdered infant formula as a source of *Salmonella* infections in infants. Clin Infect Dis 46:268–273
Carrasco E, Morales-Rueda A, García-Gimeno RM (2012) Cross-contamination and recontamination by *Salmonella* in foods: a review. Food Res Int 45(2):545–556
Cavallaro E, Date K, Medus C, Meyer S, Miller B, Kim C, Nowicki S, Cosgrove S, Sweat D, Phan Q, Flint J, Daly ER, Adams J, Hyytia-Trees E, Gerner-Smidt P, Hoekstra RM, Schwensohn C, Langer A, Sodha SV, Rogers MC, Angulo FJ, Tauxe RV, Williams IT, Behravesh C (2011) *Salmonella* typhimurium infections associated with peanut products. New Engl J Med 365(7):601–610
Code of Federal Regulations (2005) 9 CFR 590.575 – heat treatment of dried egg whites
Codex Alimentarius Commission (1981) Codex standard for chocolate and chocolate products. Codex Alimentarius Commission
Codex Alimentarius Commission (1983) Codex standard for composite and filled chocolate. Codex Alimentarius Commission
Codex Alimentarius Commission (1985) Codex standard for cocoa butter confectionery. Codex Alimentarius Commission
Cordier JL (1994) HACCP in the chocolate industry. Food Control 5(3):171–175
Craven PC, Mackel DC, Baine WB, Barker WH, Gangarosa EJ, Goldfield M, Rosenfeld H, Altman R, Lachapelle G, Davies JW, Swanson RC (1975) International outbreak of *Salmonella* eastbourne infection traced to contaminated chocolate. Lancet 1(7910):788–792
D'Aoust J, Aris BJ, Thisdele P, Durante A, Brisson N, Dragon D, Lachapelle G, Johnston M, Laidley R (1975) *Salmonella* eastbourne outbreak associated with chocolate. Can Inst Food Sci Technol J 8(4):181–184
D'Aoust JY, Pivnick H (1976) Small infectious doses of *Salmonella*. Lancet 1(7964):866
D'Aoust JY (1977) *Salmonella* and the chocolate industry. J Food Prot 40:718–727
da Silva do Nascimento M, da Silva N, da Silva IF, de Cássia da Silva J, Marques ER, Santos ARB (2010) Enteropathogens in cocoa pre-processing. Food Control 21:408–411
da Silva do Nascimento M, Brum DM, Pena PO, Berto MI, Efraim P (2012) Inactivation of *Salmonella* during cocoa roasting and chocolate conching. Int J Food Microbiol 159(3):225–229
Das P (2006) Infectious disease surveillance update. Lancet Infect Dis 6(8):474
De Grandi DM, Mistretta A, Lelo S (1987) Contaminazione dei torroncici con salmonelle. Pasticcetia Internazionale 58:139
de Jong B, Andersson Y, Giesecke J, Hellström L, Stamer U, Wollin R (2001) *Salmonella* typhimurium outbreak in Sweden from contaminated jars of helva (or halva). Eurosurveillance 5(29):1715
de Smedt J (1989) Avoiding *Salmonella* problems: microbial GMPs. Manuf Confect 69:72–75
Dhir VK, Dodd CE (1995) Susceptibility of suspended and surface-attached *Salmonella* enteritidis to biocides and elevated temperatures. Appl Environ Microbiol 61(5):1731–1738
Dickinson PC (1974) Contaminated chocolate balls. Can Med Assoc J 110(9):1018–1019
Dodd CE, Aldsworth TG (2002) The importance of RpoS in the survival of bacteria through food processing. Int J Food Microbiol 74(3):189–194
Doyle ME, Mazzotta AS (2000) Review of studies on the thermal resistance of *Salmonellae*. J Food Prot 63(6):779–795
Elson R, Outbreak Control Team (2006a) UK Health Protection Agency view on food alert announcing the recall of a number of confectionery products. Eurosurveillance 11(26). Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=2986

Elson R, Outbreak Control Team (2006b) National increase in human *Salmonella* Montevideo infections in England and Wales: March to July 2006. Eurosurveillance 11(26). Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=2985

Ethelberg S (2002) International outbreak of *Salmonella Oranienburg*, October–December 2001, part 2: Denmark. Eurosurveillance 6(3). Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=2006

Fisher IS, de Jong B, van Pelt W, Aramini J, Berghold C, Matthys F, Powling J, Siitonen A (2002) International outbreak of *Salmonella Oranienburg*, October–December 2001, part 3: other countries. Eurosurveillance 6(3). Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=2007

Gabis DA, Langlois BE, Rudnick AW (1970) Microbiological examination of cocoa powder. Appl Microbiol 20(4):644–645

Gastrin B, Kampe A, Nystrom KG, Oden-Johanson B, Wessel G, Zetterberg B (1972) Salmonella durham epidemic caused by contaminated cocoa. Lakartidningen 69(46):5335–5338

Gill ON, Sockett PN, Bartlett CL, Vaile MS, Rowe B, Gilbert RJ, Dulake C, Murrell HC, Salmaso S (1983) Outbreak of Salmonella napoli infection caused by contaminated chocolate bars. Lancet 1(8324):574–577

Goepfert JM, Biggie RA (1968) Heat resistance of Salmonella typhimurium and Salmonella senftenberg 775W in milk chocolate. Appl Microbiol 16(12):1939–1940

Goepfert JM, Iskander IK, Amundson CH (1970) Relation of the heat resistance of salmonellae to the water activity of the environment. Appl Microbiol 19(3):429–433

Gosselin FM, Basu AK, Caprioli TM, Kasatiya SS (1979) A new Salmonella serotype isolated in Canada: Salmonella sherbrooke (16:d:1,6). Can J Microbiol 25(11):1328–1329

Greenwood MH, Hooper WL (1983) Chocolate bars contaminated with Salmonella napoli: an infectivity study. Br Med J 286(6375):1394

Grocery Manufacturers Association (2009) Control of Salmonella in low-moisture foods. Washington, DC

Gryson N, Messens K, Dewettinck K (2004) Evaluation and optimisation of five different extraction methods for soy DNA in chocolate and biscuits. Extraction of DNA as a first step in GMO analysis. J Sci Food Agric 84:1357–1363

Gryson N, Dewettinck K, Messens K (2007) Influence of cocoa components on the PCR detection of soy lecithin DNA. Eur Food Res Technol 226:247–254

Haxgart SN, Rønsbo MH, Christensen N, Nielsen DS, Knøchel S (2010) Inactivation of salmonella during conching and storage of different types of chocolate, in food microbiology. Copenhagen

Hockin JC, D'Aoust J-Y, Bowering D, Jessop JH, Khanna B, Lior H, Milling ME (2009) An international outbreak of Salmonella nima from imported chocolate. J Food Prot 52(1):51–54

Humphrey TJ, Slater E, McAlpine K, Rowbury RJ, Gilbert RJ (1995) Salmonella enteritidis phage type 4 isolates more tolerant of heat, acid, or hydrogen peroxide also survive longer on surfaces. Appl Environ Microbiol 61(8):3161–3164

ICMSF (International Commission on Microbiological Specifications for Foods) (2005) Chapter 10: cocoa, chocolate, and confectionery, in microorganisms in foods 6: microbial ecology of food commodities. Kluwer Academic/Plenum Publishers, New York, pp 467–479

Illeghems K, De Vuyst L, Papalexandratou Z, Weckx S (2012) Phylogenetic analysis of a spontaneous cocoa bean fermentation metagenome reveals new insights into its bacterial and fungal community diversity. PLoS One 7(5):e38040

International Office of Cocoa Chocolate and Sugar Confectionery (1991) The IOCCC code of hygienic practice based on HACCP for the prevention of Salmonella contamination in cocoa, chocolate and confectionery products. IOCCC, Brussels

Izurieta WP, Komitopoulou E (2012) Effect of moisture on Salmonella spp. heat resistance in cocoa and hazelnut shells. Food Res Int 45:1087–1092

Jasson V, Jacxsens L, Luning P, Rajkovic A, Uyttendaele M (2010) Alternative microbial methods: an overview and selection criteria. Food Microbiol 27:710–730

Jasson V, Baert L, Uyttendaele M (2011) Detection of low numbers of healthy and sub-lethally injured *Salmonella enterica* in chocolate. Int J Food Microbiol 145(2–3):488–491

Jeong S, Marks BP, Orta-Ramirez A (2009) Thermal inactivation kinetics for salmonella enteritidis PT30 on almonds subjected to moist-air convection heating. J Food Prot 72(8):1602–1609

Kahraman T, Issa G, Ozmen G, Buyukunal S (2010) Microbiological and chemical quality of tahini halva. Br Food J 112(6):608–616

Kapperud G, Lassen J, Aasen S, Gustavsen S, Hellesnes I (1989) The contaminated chocolate epidemic of 1987. Tidsskr Nor Laegeforen 109(19–21):1982–1985

Kapperud G, Gustavsen S, Hellesnes I, Hansen AH, Lassen J, Hirn J, Jahkola M, Montenegro MA, Helmuth R (1990) Outbreak of Salmonella typhimurium infection traced to contaminated chocolate and caused by a strain lacking the 60-megadalton virulence plasmid. J Clin Microbiol 28(12):2597–2601

Kapperud G, Stenwig H, Lassen J (1998) Epidemiology of Salmonella typhimurium O:4-12 infection in Norway: evidence of transmission from an avian wildlife reservoir. Am J Epidemiol 147(8):774–782

Komitopoulou E, Penaloza W (2009) Fate of Salmonella in dry confectionery raw materials. J Appl Microbiol 106(6):1892–1900

Kostinek M, Ban-Koffi L, Ottah-Atikpo M, Teniola D, Schillinger U, Holzapfel WH, Franz CM (2008) Diversity of predominant lactic acid bacteria associated with cocoa fermentation in Nigeria. Curr Microbiol 56(4):306–314

Kotzekidou P (1998) Microbial stability and fate of Salmonella enteritidis in halva, a low-moisture confection. J Food Prot 61(2):181–185

Krapf T, Gantenbein-Demarchi C (2010) Thermal inactivation of Salmonella spp. during conching. LWT Food Sci Technol 43(4):720–723

Lee BH, Kermasha S, Baker BE (1989) Thermal, ultrasonic and ultraviolet inactivation of Salmonella in thin films of aqueous media and chocolate. Food Microbiol 6(3):143–152

Lennington K (1967) FDA looks at the Salmonella problem – ever-present threat to the industry. Manuf Confect 47:31–33

Lewis DA, Paramathasan R, White DG, Neil LS, Tanner AC, Hill SD, Bruce JC, Stuart JM, Ridley AM, Threlfall EJ (1996) Marshmallows cause an outbreak of infection with Salmonella enteritidis phage type 4. Commun Dis Rep CDR Rev 6(13):183–186

Lima LJR, Kamphuis HJ, Nout MJR, Zwietering MH (2011) Microbiota of cocoa powder with particular reference to aerobic thermoresistant spore-formers. Food Microbiol 28(3):573–582

Little CL, Rawal N, de Pinna E, McLauchlin J (2010) Survey of Salmonella contamination of edible nut kernels on retail sale in the UK. Food Microbiol 27:171–174

Mackey BM, Derrick C (1990) Heat shock protein synthesis and thermotolerance in Salmonella typhimurium. J Appl Bacteriol 69(3):373–383

McCullough NB (1951) Relative pathogenicity of certain Salmonella strains for man and mice. Public Health Rep 66(47):1538–1540

National Advisory Committee on Microbiological Criteria for Foods (2006) Requisite scientific parameters for establishing the equivalence of alternative methods of pasteurization. J Food Prot 69(5):1190–1216

National Confectioners Association Chocolate Council (2011) Raw chocolate: risks and recommendations. Manuf Confect 57–59

Nielsen DS, Honholt S, Tano-Debrah K, Jespersen L (2005) Yeast populations associated with Ghanaian cocoa fermentations analysed using denaturing gradient gel electrophoresis (DGGE). Yeast 22(4):271–284

Nielsen DS, Teniola OD, Ban-Koffi L, Owusu M, Andersson TS, Holzapfel WH (2007) The microbiology of Ghanaian cocoa fermentations analysed using culture-dependent and culture-independent methods. Int J Food Microbiol 114(2):168–186

Nummer BA, Shrestha S, Smith JV (2012) Survival of Salmonella in a high sugar, low water-activity, peanut butter flavored candy fondant. Food Control 27(1):184–187

Olsen SJ, Ying M, Davis MF, Deasy M, Holland B, Iampietro L, Baysinger M, Sassano F, Polk LD, Gormley B, Hung MJ, Pilot K, Orsini M, van Duyne S, Rankin S, Genese C, Bresnitz EA,

Smucker J, Moll M, Sobel J (2004) Multidrug-resistant Salmonella typhimurium infection from milk contaminated after pasteurization. Emerg Infect Dis 10(5):932–934
Podolak R, Enache E, Stone W, Black DG, Elliott PH (2010) Sources and risk factors for contamination, survival, persistence, and heat resistance of Salmonella in low-moisture foods. J Food Prot 73(10):1919–1936
Public Health Agency of Canada (2006) Possible *Salmonella* illness. Canada
Rees CED, Dodd CER, Gibson PT, Booth IR, Stewart GSAB (1995) The significance of bacteria in stationary phase to food microbiology. Int J Food Microbiol 28(2):263–275
Reynolds G (2006) Soy lecithen blamed for Hershey's *Salmonella* scare. www.foodqualitynews.com
Rowe B, Begg NT, Hutchinson DN, Dawkins HC, Gilbert RJ, Jacob M, Hales BH, Rae FA, Jepson M (1987) Salmonella Ealing infections associated with consumption of infant dried milk. Lancet 2(8564):900–903
Ryan CA, Nickels MK, Hargrett-Bean NT, Potter ME, Endo T, Mayer L, Langkop CW, Gibson C, McDonald RC, Kenney RT, Puhr ND, McDonnell PJ, Martin RJ, Cohen ML, Blake PA (1987) Massive outbreak of antimicrobial-resistant *Salmonellosis* traced to pasteurized milk. J Am Med Assoc 258(22):3269–3274
Schaffner CP, Mosbach K, Bibit VC, Watson CH (1967) Coconut and *Salmonella* infection. Appl Microbiol 15(3):471–475
Schaffner DW, Buchanan RL, Calhoun S, Danyluk MD, Harris LJ, Djordjevic D, Whiting RC, Kottapalli B, Wiedmann M (2013) Issues to consider when setting intervention targets with limited data for low-moisture food commodities: a peanut case study. J Food Prot 76(2): 360–369
Scheil W, Dalton C, Cameron S, Murray C (1997) A multi-state Salmonella Mbandaka outbreak associated with peanut butter: the South Australian experience. J Clin Epidemiol 50(S1):18
Schwan RF, Wheals AE (2004) The microbiology of cocoa fermentation and its role in chocolate quality. Crit Rev Food Sci Nutr 44(4):205–221
Sheth AN, Hoekstra M, Patel N, Ewald G, Lord C, Clarke C, Villamil E, Niksich K, Bopp C, Nguyen T, Zink D, Lynch M (2011) A national outbreak of salmonella serotype tennessee infections from contaminated peanut butter: a new food vehicle for *Salmonellosis* in the United States. Clin Infect Dis 53(4):356–362
Solowey M, McFarlane VH, Spaulding EH, Chemerda C (1947) Microbiology of spray-dried whole egg: II incidence and types of *Salmonella*. Am J Publ Health 37(8):971–982
Solowey M, Rosenstadt A (1947) Occurrence of multiple Salmonella types in spray-dried whole egg powder. J Bacteriol 54(1):48
Tamminga SK, Beumer RR, Kampelmacher EH, van Leusden FM (1976) Survival of Salmonella eastbourne and Salmonella typhimurium in chocolate. J Hyg 76(1):41–47
Tamminga SK, Beumer RR, Kampelmacher EH, van Leusden FM (1977) Survival of Salmonella eastbourne and Salmonella typhimurium in milk chocolate prepared with artificially contaminated milk powder. J Hyg 79(3):333–337
Teoh YL, Goh KT, Neo KS, Yeo M (1997) A nationwide outbreak of coconut-associated paratyphoid A fever in Singapore. Ann Acad Med Singapore 26(5):544–548
Thompson SS, Miller KB, Lopez AS (2001) Cocoa and coffee, in food microbiology: fundamentals and frontiers. In: Doyle MP, Beuchat LR, Montville TJ (eds) ASM Press, Washington DC, pp 721–733
Tolker-Nielsen T, Molin S (1996) Role of ribosome degradation in the death of heat-stressed Salmonella typhimurium. FEMS Microbiol Lett 142(2–3):155–160
Torres-Vitela MR, Escartin EF, Castillo A (1995) Risk of Salmonellosis associated with consumption of chocolate in Mexico. J Food Prot 58(5):478–481
Uesugi AR, Danyluk MD, Mandrell RE, Harris LJ (2007) Isolation of Salmonella enteritidis phage type 30 from a single almond orchard over a 5-year period. J Food Prot 70(8):1784–1789
US Food and Drug Administration (2009) Guidance for industry: measures to address the risk for contamination by Salmonella species in food containing a peanut-derived product as an ingredient. http://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/ProducePlantProducts/ucm115386.htm

US Food and Drug Administration (2010) Lovin oven LLC Voluntarily Recalls Certain Health Valley® Organic Peanut Crunch, Dutch Apple and Wildberry Chewy Granola Bars. http://www.fda.gov/Safety/Recalls/ucm201351.htm

US Food and Drug Administration (2012) Funfresh Foods, Inc. Voluntarily Recalls World Berries™ Organic "Cacao Nibs" Because of possible health risk. http://www.fda.gov/Safety/Recalls/ucm303012.htm

Waterman SR, Small PL (1998) Acid-sensitive enteric pathogens are protected from killing under extremely acidic conditions of pH 2.5 when they are inoculated onto certain solid food sources. Appl Environ Microbiol 64(10):3882–3886

Werber D, Ammon A, Dreesman J, Feil F, van Treeck U, Fell G, Hauri AM, Tschäpe H, Roggentin P, Weise E, Bräunig J (2002) International outbreak of Salmonella Oranienburg, October–December 2001, part 1: Germany. Eurosurveillance 6(3)

Werber D, Dreesman J, Feil F, van Treeck U, Fell G, Ethelberg S, Hauri AM, Roggentin P, Prager R, Fisher IS, Behnke SC, Bartelt E, Weise E, Ellis A, Siitonen A, Andersson Y, Tschape H, Kramer MH, Ammon A (2005) International outbreak of *Salmonella Oranienburg* due to German chocolate. BMC Infect Dis 5:7

Wesche AM, Gurtler JB, Marks BP, Ryser ET (2009) Stress, sublethal injury, resuscitation, and virulence of bacterial foodborne pathogens. J Food Prot 72(5):1121–1138

WHO (1973) Salmonella surveillance. Wkly Epidemiol Rec 48(39):377–381

WHO (1986a) Salmonella nima infection from chocolate. Wkly Epidemiol Rec 48:371

WHO (1986b) Salmonella nima infection. Wkly Epidemiol Rec 31:242–243

Williams Jr J, Clavero R, Silliker JH, Flowers RS (2006) Microbial control for confectionery plants. Manuf Confect

Wood GAR, Lass RA (2008) Cocoa. Wiley-Blackwell, Hoboken

Zweifel C, Stephan R (2012) Spices and herbs as source of Salmonella-related foodborne diseases. Food Res Int 45:765–769

Salty Snack Foods

Jeff Kuehm and Diana Casas

Abstract The salty snack food category includes products such as potato chips, tortilla chips, corn puffs, popcorn, and pretzels. Although rare, salty snack products have been involved in microbiological contaminant-related recalls and outbreaks due to *Salmonella*. A food safety framework of risk assessment, risk management, and hazard control and monitoring can be used to design and implement targeted microbiological control strategies. Salty snack manufacturing involves thermal processes, such as frying, baking, popping, and/or extrusion, which are capable of dramatically reducing the microbial load associated with the raw materials; therefore, the key food safety risk for savory snacks would be from infectious gastrointestinal pathogen contamination following the cook step. Hence, risk mitigation strategies would focus on the two main sources of contamination which are ingredients added post-cook (i.e., topical seasonings) and the process environment. Programs to monitor the effectiveness of these controls would include a sensitive ingredient program, sanitation verification, and environmental monitoring for pathogens.

Introduction

Low-moisture, ready-to-eat, salted, savory snacks made from a variety of base raw materials will be addressed in this chapter. These types of foods will be referred to as salty snacks, for simplicity, a term widely recognized by the industry. Salty snack foods generally include products such as potato chips, potato crisps, tortilla chips, corn chips, corn puffs, popped chips, multigrain chips, popcorn, pretzels, rice cakes, crackers, pita/bagel chips, and fried pork rinds. Examples of some of the most popular salty snacks are depicted in Fig. 1. This chapter will address all these products in a generic manner and call out specific product and process attributes where needed.

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Fig. 1 Example of popular salty snacks

Fig. 2 Entire aisle at local supermarket dedicated to salty snacks

Salty Snack Food Industry and History

Have you been down the salty snack aisle at your local grocery store lately (Fig. 2)? The salty snack food industry continues to enjoy popularity and sales growth as consumers seek convenient, fun, great-tasting foods to be eaten as a midday snack or to complement their meal. In addition, salty snacks have had a relatively good track record in food safety. However, this history is not totally without blemish. Outbreaks and recalls associated with low-moisture foods have included some salty snack products.

Before delving into the microbiology of these products, here are a few historical facts about the origin of several salty snacks taken from the book *50 Years: A Foundation for the Future*, published by the Snack Food Association in 1987 (Snack Food 1987).

Corn Chips: During the US Depression era, there were two men who commercially manufactured corn chips. The snack evolved from fried tortillas, a common food in Mexican and Mexican-American diets. The first was I. J. Filler who conceived the idea of manufacturing a rectangular-shaped corn chip. Filler got help from his father who designed a rudimentary grinder and rolling machine for grinding and forming the masa (maize dough). At the same time, Elmer Doolin bought from a small Mexican entrepreneur the recipe for ribbon-shaped corn chips and a handheld potato ricer. Elmer and his mother set up operation in her kitchen and began producing "Fritos" and selling them from the back of his Model-T Ford. Within a year, he moved his business from his garage into a manufacturing plant.

Extruded Puffs: Even though the "corn curl" or "collet" had been created before the 1950s, it wasn't until then that the products were distributed by snack food salesmen. Edward Wilson first discovered that when equipment processing moistened, corn kernels ran continuously for extended periods, it would become very hot, and the extruded ribbon of corn would puff up as it came out. The puffed corn would then harden as it hit the cool air. These puffed ribbons were then fried and salted and became known as "Korn Kurls."

Popped Corn: Popped corn dates back to the 1600s. It is even said that the Pilgrims enjoyed this snack at the first Thanksgiving. However, it wasn't until later in 1885, *when* Charles Cretor was credited with developing a gasoline-powered corn popping machine that made popped corn into a viable commercial product. This machine became the forerunner of the corn poppers found in movie theaters.

Potato Chips: This snack traces its roots to a Native American chef in Saratoga, New York. In 1853, railroad magnate Commodore Cornelius Vanderbilt sent his fried potatoes back to the kitchen saying that they were too thick. George Crum, the cook, decided that Vanderbilt needed a sarcastic reply, so he cooked up paper-thin potatoes and fried them to a crisp and salted them. What was meant as a stunt turned into an instant hit. The commodore loved the crunchy potato slices and so did his friends. "Saratoga Chips" became the fad at the resort.

Pretzels: Pretzels were first made sometime after 610 A.D. when monks in France offered them as a reward to children for learning their prayers. Originally, pretzels were called "pretiola" (little reward), but the name evolved to "bretzel" when the baked snack became popular in Austria and Germany. The immigrants later brought the "bretzel" to the New World. The first commercial pretzel bakery was established in the United States in 1861 by Julius Sturgis in Lancaster County, Pennsylvania.

Hence, salty snacks have a long and colorful history. However, the move from small-scale batch to industrialized processing, local to national and even international distribution, and the use of an expanded array of globally sourced raw materials and ingredients have had a tremendous impact on changing the food safety landscape from these early days.

Salty Snack Processing

Like the other products addressed in this book, the preservation of salty snacks is attributed to their low water activities. The basic design for processing salty snacks is to either remove water from an existing food (e.g., a potato with natural moisture content of ca. 80 % or pork skins at ca. 50 %) or rehydrate an already low-moisture raw material (e.g., wheat flour, corn meal, rice flour, potato flakes, etc.) and then dehydrate it to create a shelf-stable product. The main exception is popcorn, which simply uses the existing moisture in the popcorn kernel to steam puff the inner starch to form the well-recognized popcorn butterfly and mushroom shapes.

Typical Hazard Analysis Critical Control Point (HACCP) style flow diagrams outlining the basic process steps involved in the production of the most common salty snacks are found in Figs. 3, 4, 5, 6, and 7. The process steps shaded in blue depict the high-moisture points of the process, whereas the non-shaded steps are low moisture.

Potato chip processing (Fig. 3) involves washing, peeling, and slicing whole potatoes. The slices are then washed and flumed to the fryer (Fig. 8), salted and/or seasoned, and packaged. For fried corn tortilla chips (Fig. 4), whole corn is cooked and soaked in an alkaline environment to soften the kernels and loosen the outer pericarp (Fig. 9). The corn is then washed, milled, sheeted, and cut into the characteristic shape of tortilla chips which are then toasted, fried, seasoned, and packaged. Pretzels (Fig. 5) are manufactured by mixing flour with leavening agents and flavorings, extruding and cutting to shape (Fig. 10), and cooking in caustic solution to give the pretzels their brown outer skin which are then salted, baked, and packaged. Fried corn puff production (Fig. 6) involves hydration of cornmeal to a moisture content between 12 and 18 % which is then passed through an extruder applying high shear and pressure resulting in high temperatures, so when the meal is forced

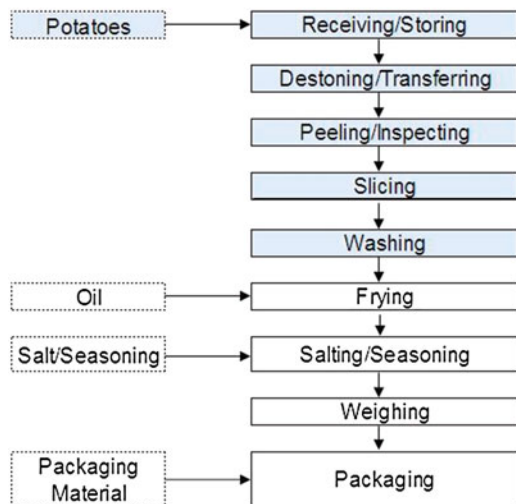

Fig. 3 Fried potato chip HACCP flow diagram

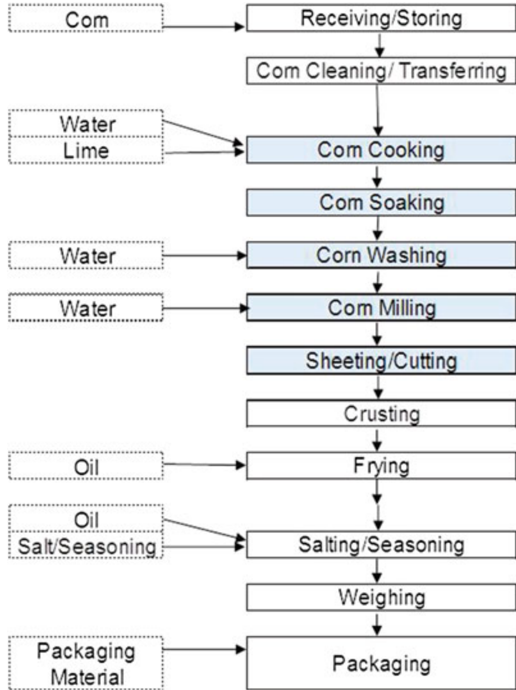

Fig. 4 Fried tortilla chip HACCP flow diagram

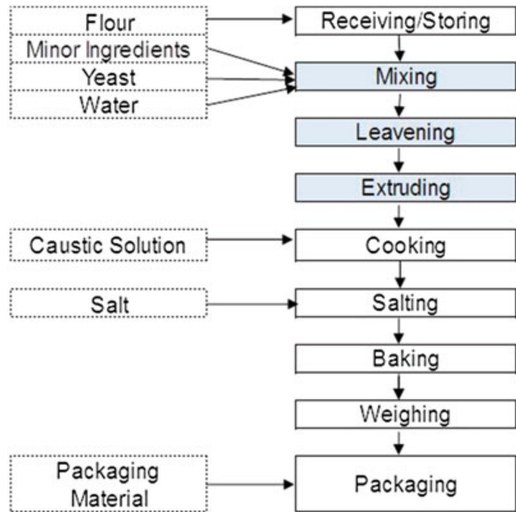

Fig. 5 Pretzel HACCP flow diagram

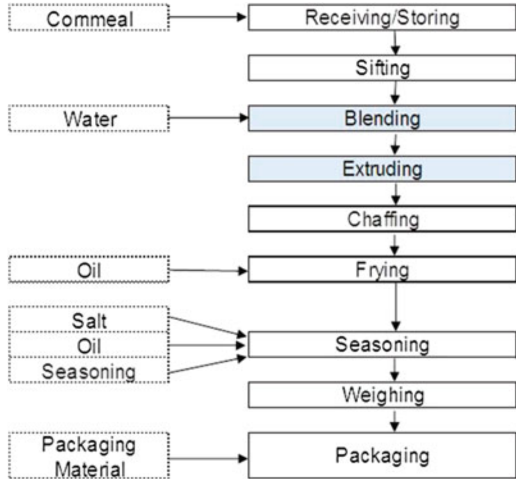

Fig. 6 Fried corn puffs HACCP flow diagram

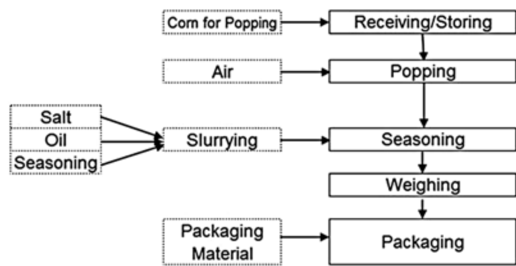

Fig. 7 Popcorn HACCP flow diagram

Fig. 8 Potato slices being washed and flumed to the fryer

Fig. 9 Corn alkaline soak tanks for fried tortilla chip process

Fig. 10 Pretzel dough extruding and cutting

through a die head aperture, it rapidly expands to form the puff or collet. The collets are then fried or baked, seasoned (Fig. 11), and packaged. Finally, popcorn (Fig. 7) is simply made by passing popcorn kernels through a hot air popper followed by seasoning and packaging (Fig. 12). Most of these salty snacks are packaged in flex bags using films with strong oxygen/moisture barriers and optional nitrogen flushing to protect the product's quality and avoid oxidation and premature staling. Finished product moistures are typically below 2 %.

Fig. 11 Fried corn puffs seasoning tumbler

Fig. 12 Popcorn being conveyed to packaging

Risk Assessment and Risk Management

Conducting a microbiological risk assessment on any product or process must begin with the product concept and evolve as the product design is further defined, prototypes are created, production process is specified, the production site is identified, equipment is purchased, and engineering test runs are scheduled and continue through full-scale production. From the risk assessment, risk management strategies are developed and implemented.

Outbreaks/Recalls

Although rare, salty snack products have been involved in microbiological contaminant-related recalls and have posed a risk to public health as evidenced by recent outbreaks. The most notable outbreaks involving salty snacks have been:

- Paprika-powdered potato chips, Germany 1993.
- Peanut-flavored corn puffs, Israel, England, Wales, and United States 1996.
- Vegetable seasoned puffs, United States 2007.

In Germany, an outbreak of human salmonellosis was traced to contaminated paprika and paprika-powdered potato chips. The outbreak occurred between April and September of 1993. There were an estimated 1,000 cases, with children below 14 years principally affected. About 42 % of the cases were caused by three serovars (*S.* Saintpaul, *S.* Rubislaw, and *S.* Javiana); however, altogether 94 different serovars were reported as being isolated from both patients and paprika-containing products during the outbreak period. Besides the high number of serovars isolated, this outbreak was particularly alarming due to the low levels of *Salmonella* present and the assumed low infectious dose. Levels of 0.04–0.45 *Salmonella* cells per gram were found in the snacks, with an estimated infectious dose of 4–45 organisms (assuming 100 g consumption) and an attack rate of 1 in 10,000 exposed persons (Lehmacher et al. 1995).

In a British/Israel outbreak, between December 1994 and January 1995, 27 isolates of *Salmonella* Agona were identified in England and Wales. During a similar timeframe, a large outbreak of *S.* Agona infections was reported in Israel and, subsequently, several cases in the United States. The implicated snack was identified as a kosher, peanut-flavored, ready-to-eat savory maize-based snack, manufactured and imported from Israel. Over half of the cases were children aged 7 years and younger. In eight of the packages of product tested for quantitative studies, the estimated *Salmonella* count ranged from 2 to 45 organisms per package (25 g). The cause of the product contamination remains unknown. *S.* Agona was not isolated from the production line, the ingredients, or any employee stool samples during the investigation (Killalea et al. 1996; Shohat et al. 1996; Threlfall et al. 1996).

During 2007, in the United Sates, PulseNet identified an outbreak of *Salmonella* Wandsworth infection, eventually involving 23 states. A multistate case–control study revealed a strong association between illness and consumption of a puffed rice and corn snack with a vegetable coating. Ninety-one percent of cases occurred in children aged 10 months to 3 years. Among the patients for whom clinical information was available, all had diarrhea, 76 % had bloody stools, and six patients were hospitalized. No deaths were attributed to these infections. During this time, the outbreak strain of *S.* Wandsworth was isolated from sealed product bags obtained from retail stores. A number of *Salmonella* serotypes were subsequently associated with this outbreak, including *S.* Typhimurium, *S.* Mbandaka, *S.* Kentucky, *S.* Haifa, and *S.* Saintpaul. The FDA determined that a spice utilized in making the product was the likely source of the *Salmonella* contamination. A sample from a mixed batch

of spice blend tested positive for *S.* Wandsworth. A sample from a lot of parsley powder, an ingredient in the spice blend, also tested positive for *S.* Wandsworth. The FDA inspection further determined that the supplier of the spices failed to inspect and handle raw materials to ascertain that they were clean and suitable for processing into food (CDC 2007; Safety Recalls 2007; Marler 2008).

In two of these outbreaks, a topical seasoning/spice blend was identified as the source of product contamination. In the third outbreak, the source of contamination was never determined; however, the peanut butter flavoring or the process environment could have been the culprit.

Pathogen of Concern

To date and to our knowledge, all outbreaks associated with salty snacks, such as the three described above, have involved products contaminated with *Salmonella*. In fact, almost all microbiologically related recalls involving salty snacks are due to *Salmonella*. The only other pathogen associated with a salty snack recall has been *Listeria monocytogenes,* which occurred in 2012. This recent recall was for ready-to-eat (RTE) popcorn (Safety Recalls 2012). There were no confirmed illnesses reported, and the cause of the recall was the detection of *Listeria monocytogenes* on product contact surfaces, indicating a direct risk of cross-contamination. This limited outbreak and recall information would point to *Salmonella* as THE pathogen of concern for salty snacks. A science-based risk assessment would reinforce this conclusion.

As previously discussed, almost all salty snacks involve a thermal process, such as frying, baking, popping, and/or extrusion, which is capable of dramatically reducing the microbial load associated with the raw materials used for these products. Typical aerobic plate counts for unseasoned (base) finished products are <100–1,000 CFU/g. Organisms that may survive these processes are typically sporeformers. The sporeformers capable of causing food poisoning (i.e., *Bacillus cereus* and a few clostridial species) would not be a concern because they must be able to multiply to high numbers to cause illness. Their growth would not be possible in finished products that have water activities less than 0.3. This would also be true for the vegetative bacterial toxin-producing strains of *Staphylococcus* (e.g., *Staphylococcus aureus*), which could recontaminate the product after cooking. *S. aureus* cannot grow in the finished product, so any post-cook exposure of product to *S. aureus* would not present a health risk.

Therefore, the key food safety risk for savory snacks would be from infectious gastrointestinal pathogen contamination following the cook step. Of the vegetative, infectious pathogens, *Salmonella* predominates as the most likely to occur. This pathogen, which has a history of causing illness in low-moisture foods, has been isolated from raw materials used to make salty snacks, has also been isolated from and known to take up residence in food processing environments, and has been shown to remain viable in a dry environment for extended periods of time (Manufacturers Association 2009a). When considering other infectious pathogens

(including protozoans and other parasites) during the risk assessment, they would not be included on the "likely to occur" list because they are not known to be present in the raw materials/ingredients used for salty snacks (with the potential exception of potatoes, since they are grown in soil) and are not known to persist or proliferate in a food processing environment. In the case of potatoes, the washing and peeling process would remove surface/soil contaminants prior to slicing and frying. Only *Listeria monocytogenes* would warrant some consideration, because it is a known environmental contaminant and currently, in the United States, there remains a zero tolerance for its presence in an RTE food product. However, upon further assessment, *L. monocytogenes* is unlikely to present a public health risk because consumption of large cell numbers of *L. monocytogenes* per serving is often needed to cause disease (when compared with *Salmonella*) (Consumer Protection Directorate-General 1999) which are unlikely to occur with a salty snack, listeria would not be capable of growth on salty snacks, and there is no history of listeriosis outbreaks associated with salty snack consumption.

Microbiological control programs for low-moisture salty snacks should primarily focus on preventing post-cook contamination by *Salmonella*. The two main sources of post-cook contamination are ingredients (especially topical seasonings) and the process environment, which will be discussed in the following sections.

Sources of Salmonella Contamination

Ingredients

The production of salty snacks involves the use of several raw commodities that may be a source of *Salmonella*, such as potatoes, pork skins, wheat flour, whole corn, corn meal, popcorn kernels, and rice flour. Because these are considered "raw" commodities (and have no validated pathogen inactivation step within their harvesting or processing), *Salmonella*-negative specifications are typically not accepted as appropriate or will not be agreed upon by the suppliers, so microbiological control prior to receipt is impractical. Fortunately, thermal processing steps used in the manufacture of salty snacks help to mitigate the risk from incoming pathogens. However, these raw materials do present a source of contamination to the process environment where they are used and require appropriate control measures for containment (i.e., sanitation practices and line segregation). There are limited data available on *Salmonella* prevalence and levels in most of these commodities, but one must assume this pathogen could be present but likely at low levels. Doan and Davidson's (2000) review on the microbiology of potatoes reported *Salmonella* as one of many microorganisms that can be isolated from potatoes since they are grown in soil and thus exposed to a wide variety of environmental microorganisms. Sperber et al. (2007) in 2007 reported 0 *Salmonella* positive out of 1,772 samples of whole corn. This same publication reported 6 *Salmonella* positives out of 4,358 wheat flour samples (0.14 %), whereas Richter et al. (1993) in 1993 reported a

Salmonella-positive rate of 1.3 %. Regarding corn meal, in 1994, Bothast et al. (1973) reported 0 *Salmonella* positives out of 24 samples tested. Anaya et al. (2008) reported a *Salmonella*-positive rate of 8–13 % (reported as unpublished data) for popcorn kernels. Unfortunately, no quantitative data for the *Salmonella* levels in these commodities are available.

For salty snacks, the greatest food safety concern comes from the topical seasonings that are typically applied after the thermal processing steps, resulting in little to no opportunity for pathogen reduction. If *Salmonella* were present, it could pose a risk to consumer health, as evidenced by the outbreaks previously discussed. Most salty snacks are seasoned shortly after the cook step via a tumbler where seasoning and possibly oil are sprayed or drizzled onto the product. At this step, the product is cooling rapidly. There are a few applications where seasoning is applied to the product shortly after exiting the fryer, and there is a brief period of exposure to high temperatures. However, due to the low level of moisture at this point, a pathogen reduction of one log or less could be expected.

The manufacturing of topical seasonings used on salty snacks involves either a dry-blending or a spray-drying process. Most topical seasonings are dry blended, but there are a few high-volume products that are seasoned with spray-dried dairy powders, such as cheese-flavored corn puffs and cheddar popcorn.

With dry blending, a wide variety of functional components are mixed to create a relatively homogenous blend. The blending process, which occurs at ambient temperature, provides no pathogen control or inactivation. Contaminants coming in on one of the seasoning's individual components will be present in the finished seasoning blend. Risk management rests upon the microbiological controls utilized by the suppliers of the spices, dairy powders, flavoring ingredients, and other microbiologically sensitive ingredients used in the seasoning blend. It is the seasoning supplier's responsibility to understand these controls and monitor the microbiological quality of these materials as necessary to minimize the potential of *Salmonella* being present. A risk assessment must be conducted on each component in the formulation. Some questions to ask may include:

- Is this component considered a microbiologically sensitive ingredient? (In other words, has it been known to or does it have the potential to be contaminated with *Salmonella*?)
- Does this component receive a validated thermal inactivation treatment during its processing? If not, is an inactivation step available (e.g., steam, irradiation, or fumigation) that would not compromise quality or regulatory compliance?
- What microbiological testing is conducted by the supplier? Is the *Salmonella* sample size adequate for detecting *Salmonella* with an appropriate level of confidence?
- Does the supplier have an adequate environmental pathogen monitoring program?

Risk management would then involve setting appropriate microbiological specifications for each component, defining supplier audits, and establishing lot verification testing. This would apply to both the seasoning blend and the salty snack manufacturer.

A risk assessment for spray-dried seasonings would be slightly different in that most of these products receive a pasteurization treatment prior to spray drying. For the spray-drying process, the components are blended into a slurry, homogenized, thermally processed, and pumped to the spray dryer. The primary risk for spray-dried seasonings is from post-process contamination. There have been several recalls of spray-dried materials due to the presence of *Salmonella* (e.g., nonfat dried milk and infant formula) (Manufacturers Association 2010b). Spray-dried seasonings would be equally susceptible to environmental contamination as other dried ingredients. The design of spray-drying operations presents opportunities for microbial niches/contamination such as baghouse filters, points of elevated moisture from wet downs or condensation, or the possibility of pulling environmental air through large pneumatic conveyance systems. Risk management for spray-dried seasonings would include: validating the pre-dryer thermal processing treatment, establishing environmental controls (i.e., eliminating sources of moisture), implementing environmental monitoring in the spray dryer areas, developing dryer entry SOPs, identifying and inspecting dryer risk points, validating and verifying wet sanitation SOPs, and conducting microbiological testing of the finished powder and possibly sifter tailings.

In summary, salty snack manufacturers should perform a thorough risk assessment of all ingredients, with a focus on topical seasonings, understand all suppliers' control programs, implement appropriate preventive controls (such as validated thermal processes where needed), and establish adequate microbiological verification testing internally and by suppliers.

Processing

With *Salmonella* being the primary pathogen of concern, the two key points of emphasis during processing are the cook step and the exposure of the post-cooked product to the processing environment.

Many salty snacks have the benefit of a frying step, which provides a robust thermal inactivation treatment. The frying temperature profile needed to achieve the moisture levels required to produce high-quality snacks and to avoid premature product staling will easily provide a greater than 5–7 log inactivation of *Salmonella*. This applies to high-moisture intermediate products (e.g., corn masa and fresh potato slices) and lower-moisture intermediate products (e.g., corn puff collets). The fryer should be able to mitigate any risk of low-level contamination of *Salmonella* that may be present in the raw materials. Unpublished results of studies conducted by manufacturers and contract laboratories have revealed that even low frying temperatures to the point where the product would be unacceptable for sale will provide the same level of pathogen inactivation. This is why the frying step for most salty snacks is unlikely to be considered a critical control point within an HACCP plan.

Oven-baking processes can be more challenging and are more dependent upon the matrix of the product being baked. Like frying, baking parameters for salty snacks

are designed to deliver the desired end-product quality with pathogen inactivation as a side benefit. The baking step should be validated to determine the level of *Salmonella* inactivation achieved for risk assessment and risk management purposes. Fortunately, most salty snacks are in a high-moisture intermediate form when entering the oven, which should enhance pathogen inactivation. Pretzels and potato crisps are formed and cut from high-moisture dough; likewise, pita chips enter the oven as sliced high-moisture pita bread. The need for establishing critical limits and monitoring of a baking process should be determined based on hazard analysis and thermal validation studies' results.

After the cook step, the finished product is exposed to the processing environment for varying lengthens of time, depending on the size and design of the process. Here, the risk of cross-contamination must be considered and addressed. This subject will be covered by many chapters in this book and has been the focus of recent articles and guidance documents (e.g., Control of *Salmonella* in Low-Moisture Foods [Manufacturers Association 2009a]) since it is a common theme for low-moisture, RTE foods. The common risk management tools to avoid cross-contamination include:

- Perform a risk assessment of the process environment and implement corrective actions where needed.
- Keep dry areas dry!
- Eliminate activities that can create dust or aerosols from contacting the floor or adjacent equipment (e.g., use of high pressure sprays or compressed air).
- Provide barriers, including employee practices and traffic patterns, to separate raw areas from finished product areas.
- Implement effective product zone specific sanitation practices.

Another important preventive control to minimize the opportunity for cross-contamination relates to wet sanitation practices. The ability to quickly dry equipment that has undergone wet cleaning is critical. Unfortunately, there are numerous pieces of equipment used in the industry that are not of optimal sanitary design and present niches for food debris and moisture to collect, which can lead to microbial growth if not identified and eliminated. Once identified, sanitation SOPs should include steps to dismantle, inspect, and thoroughly dry these critical post-thermal process product contact surfaces.

As mentioned, some seasonings are applied using a spray. The seasonings are often mixed with oil to create a slurry that is then pumped to the spray applicator. These systems incorporate kettles, pumps, and tubing to deliver the seasoning slurry. Moisture in these systems will allow microbial growth and lead to product contamination upon start-up of the process line. After wet cleaning, these systems must be thoroughly drained, dismantled, inspected, and allowed to air-dry or be placed in a "hot room" to facilitate drying. Alternatively, some companies pump hot oil through the system to flush out any remaining moisture and then leave oil in the system until start-up. A sanitation verification step for wet sanitation of this equipment can include collecting a "purge" sample of the first seasoning slurry exiting at the final spray application point at start-up of production for microbiological analysis.

Testing for indicator organisms such as aerobic plate counts, coliforms, or *Enterobacteriaceae* can alert the sanitation team to deficiencies in their practices. Systems that only spray oil should not be overlooked if they also receive a wet cleaning and sanitation.

Although most seasoning slurry applications are oil based (a mixture of powdered flavoring and oil) with no risk of microbial growth during processing, there are occasions in which a water-based slurry may be used for a specific quality or nutritional requirement. This would present a potential opportunity for growth of toxin-producing pathogens and require additional preventive controls. Depending upon the pH, salt concentration, and water activity of the slurry, *Staphylococcus aureus* and/or *Bacillus cereus* would be the most likely target organisms for validating control from a food safety perspective. The most reliable control is to formulate the seasoning powder with antimicrobial agents to preserve the slurry during processing. If this is not possible, strict frequencies for applying cleaning and sanitation for the slurry would need to be implemented. Microbial validation and verification would be required to establish the sanitation frequency and compliance monitoring.

Finished Product

Due to their low water activity (generally less than 0.3), salty snacks are not a risk for microbial growth or spoilage in their finished state. Maintaining its microbiological stability depends upon keeping the product free of excess moisture. The following are examples of opportunities for water exposure: under-dehydrated "lumps" (cluster of chips or clumps of corn masa), moisture residue after a wet sanitation, or condensation (e.g., leakage from water-cooled equipment). Another source of excess moisture can be the bulk packaging of product that is too warm, resulting in "dew point" condensation within the container. When salty snack products are exposed to excess moisture, localized areas of elevated water activity can be created. This would normally result in visible mold growth on the surface of the product. Fortunately, this is extremely rare and not considered likely to occur. In addition, since product staling is often the most common consumer complaint received by the salty snack industry, extra measures are already in place to minimize product exposure to moisture, which contributes to product staling.

Abuse by consumers is also unlikely to occur. In general, consumers recognize salty snacks as a food to be consumed "as is." Recipes do exist where salty snacks may be used as a base (e.g., *Fritos* Chili Pie) or as a topping (e.g., toppings for salads or casseroles). However, in most of these cases, the opportunities for abuse would be unlikely since the salty snack appeal is lost quickly when it becomes soggy and loses its naturally crispy texture. In addition, the other components are often perishable and require refrigeration.

Overall, the risk assessment for the finished salty snack product reveals little practical concern, so additional controls beyond keeping the product dry are not required.

Hazard Control and Monitoring

Once risk assessment and risk management procedures have been utilized to design food safety into the manufacture of salty snacks, means of verification are needed to monitor effectiveness of the preventive controls. Based on our previous discussion, three critical verification tools can be highlighted:

- Sensitive ingredient program (SIP).
- Sanitation verification.
- Environmental monitoring for pathogens.

These programs are important to the manufacture of any low-moisture, RTE food but will be addressed emphasizing their relevance to salty snacks.

Sensitive Ingredient Program

This program begins with the risk assessment process in which all ingredients used in the manufacture of the end product are reviewed and categorized. Food manufacturers have various means and terminology for categorizing and prioritizing ingredients, and most have developed their own internal list of ingredients they consider microbiologically sensitive. No matter what terminology is used, most use similar questions to assist their assessment, such as:

– Has the ingredient been involved in any past outbreaks or recalls?
– Does this ingredient contain a sensitive ingredient?
– Is there a treatment to kill pathogens in the manufacture of this ingredient?
– Is there a treatment to kill pathogens in the manufacture of the salty snack?

Once the ingredients are categorized, verification testing schemes can be established to focus efforts on the most critical ingredients. Blended or spray-dried topical seasonings are the most critical ingredients for salty snack foods and would be the central focus of a company's risk management strategies. Microbiological specifications should clearly define the microbiological criteria which include the amount tested. A common mistake for pathogen specifications is use of the simple term "negative" or "not detected" without regard to the sample size to be tested. A sample size that is in accordance with a recognized sampling plan such as those described in the FDA Bacteriological Analytical Manual (e.g., 375 g, 750 g, or 1,500 g) (FDA 2013) or an ICMSF 2-class attribute plan (ICMSF 2002) should be specified. At a minimum, the seasoning supplier should test the appropriate sample size taken from every lot for *Salmonella* using an officially recognized, validated detection method. A best practice for the snack manufacturer is to conduct at some defined frequency verification testing of the seasonings. This can be done using either pre-shipment samples or sampling upon receipt.

Sanitation Verification

The foundation for the sanitary manufacture of any food product, including salty snack foods, is assuring that the manufacturing equipment is effectively cleaned and sanitized. This means that the direct and indirect (e.g., utensils, overhead lines, etc.) product contact surfaces and adjacent equipment should be free of pathogens, have minimal residual soils and very low levels of surviving microorganisms, and be free of residual moisture. Verification of these cleaning and sanitation factors requires diligent inspection and applying the appropriate tools and methods.

The first step in verification of cleaning and sanitation operations is visual inspection. Proper visual cleaning inspection requires a bright flashlight and possibly an inspection "mirror" for the more difficult to access areas. Inspections should be performed during and/or immediately after cleaning to ensure that visible soils are being removed and allow for timely re-cleaning if needed. ATP hygiene testing can also be used to provide a real-time measure of surface cleanliness. A failed ATP test may indicate excessive residual organic material is present which then requires re-cleaning and retesting. Only after inspection and acceptable ATP results should the sanitizer be applied to the surfaces. Maximum sanitizer activity can only be achieved on adequately cleaned surfaces. Visual inspection for residual moisture that may promote microbial regrowth on the surfaces should also be conducted a few hours after sanitation.

While visual inspection and ATP testing provide simple, practical, and real-time verification measures for soil removal, the gold standard for sanitation validation and verification is microbiological testing. Microbiological sampling should be conducted after completion of the sanitation process for the best measure of sanitation effectiveness. If the processing line is scheduled to be down for an extended period of time, such as weekend downtime, microbiological testing prior to start-up can also be used as an indicator of post-sanitation recontamination or to verify that microbial regrowth did not occur. This is especially critical for pumps or other pieces of equipment that require disassembly for drying. Based on personal experience, we have observed visible and odorous microbial growth in a "cleaned" slurry pump. Ensuring that all product contact surfaces, including pipes, pumps, and other enclosed equipment, are thoroughly dry will help to avoid such sanitation failures.

Environmental Monitoring for Pathogens

Due to the nature of salty snacks and their processing, *Salmonella* spp. should be the main target organism for an environmental monitoring program. Sampling should be focused on the high-hygiene, post-cook areas where product is exposed prior to packaging. This sampling is to verify that sanitary controls in these areas have been effective and that salmonellae are unlikely to present a direct risk of contamination of the product stream. Sampling should be conducted on non-product

contact surfaces where *Salmonella* would be more likely to be found and could eventually contaminate the product stream.

A secondary area of focus for sampling should be in locations in the manufacturing facility where "raw commodities" are stored or processed. This sampling is also used to verify sanitary controls, but, as opposed to sampling the high-hygiene, post-cook areas, this sampling is done to ensure that pathogens that may have been brought in with the raw commodities are being contained and not allowed to spread or grow. Traffic paths used by production and maintenance employees, carts, and fork trucks exiting these areas should be sampled. The number and frequency of samples collected should be determined by a risk assessment, considering the raw materials used, plant and process line layout, traffic patterns, plant size, the number of process lines, and history of positive findings. The final element is a sound corrective action plan to address positive results, which includes an investigative review and additional sampling as needed to help identify potential sources, sanitation followed by resampling for verification of effectiveness, and then subsequent and frequent resampling of the positive and surrounding sites during production, to determine if contamination has been properly controlled. Practices in this area should also be reviewed for any additional actions such as a change in traffic patterns or enhanced modifications to existing sanitation SOPs.

Summary

Utilizing the food safety framework of risk assessment, risk management, and hazard control and monitoring for salty snacks, we see that:

- Salmonella is the pathogen of concern.
- After confirmation of pathogen reduction using process validation studies, microbiological control programs should focus on preventing post-cook contamination from ingredients (especially topical seasonings) and the process environment.

  - Risk management for ingredient control should involve setting appropriate microbiological specifications, conducting supplier audits, and establishing lot verification testing.
  - Control strategies for avoiding cross-contamination in the processing environment include:

    Performing a risk assessment of the process environment and implementing corrective actions where needed.
    Keeping dry areas and equipment dry.
    Eliminating activities that can create dust or aerosols (e.g., use of high pressure sprays or compressed air).
    Providing barriers, including employee practices and traffic patterns, to separate raw areas from finished product areas.
    Implementing effective, validated sanitation practices.

– Three important means for verifying these controls are:

  - Sensitive ingredient program.
  - Sanitation verification.
  - Environmental monitoring for pathogens.

Aggressive implementation of this framework should contribute to the continued safe production and consumption of salty snack.

Disclaimer The contents of this chapter reflect the views of the authors and do not represent the official views of PepsiCo.

References

CDC (2007) *Salmonella* wandsworth outbreak investigation. June–July 2007. In: Salmonella. Centers for Disease Control and Prevention. Available via DIALOG. http://www.cdc.gov/salmonella/wandsworth.htm. Accessed 05 Apr 2013

FDA (2013) Food Bacteriological Analytical Manual (BAM). http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalManualBAM/default.htm. Accessed 26 Apr 2013

FDA Safety Recalls (2007) Veggie booty snack food identified in product recall. FDA. http://www.fda.gov/Safety/Recalls/ArchiveRecalls/2007

FDA Safety Recalls (2012) Dale and Thomas popcorn issues voluntary recall of certain popcorn products due to possible health risk. FDA. http://www.fda.gov/Safety/Recalls/ucm321955.htm

GMA (Grocery Manufacturers Association) (2009a) Control of Salmonella in low-moisture foods. Washington, DC. www.gmaonline.org

GMA (Grocery Manufacturers Association) (2010b) Annex to control of Salmonella in low-moisture foods. Washington, DC. www.gmaonline.org

Health & Consumer Protection Directorate-General (1999) European Commission. Opinion of the Scientific Committee on Veterinary Measures relating to public health on *Listeria monocytogenes*

ICMSF (International Commission on Microbiological Specifications for Foods) (2002) Microorganisms in foods 7. Springer, New York, NY

Marler B (2008) Veggie booty *Salmonella* final report: New York Department of Health. Marler Blog. http://www.marlerblog.com/2008/02/articles/legal-cases/veggie-booty-*Salmonella-final*-report-new-york-department-of-health/index.html

Snack Food Association (1987) 50 years: a foundation for the future. 1987

Doan C, Davidson P (2000) Microbiology of potatoes and potato products: a review. J Food Prot 63:668–683

Sperber W, North American Millers' Association Microbiology Working Group (2007) Role of microbiological guidelines in the production and commercial use of milled cereal grains: a practical approach for the 21st century. J Food Prot 70:1041–1053

Anaya I, Aguirrezabal A, Ventura M et al (2008) Survivability of *Salmonella* cells in popcorn after microwave oven and conventional cooking. Microbiol Res 163:73–79

Bothast R, Rogers R, Hesseltine C (1973) Microbiology of corn and dry milled corn products. Paper presented at the Institute of Food Technologists' Meeting, 10–14 June 1973

Killalea D, Ward L, Roberts D et al (1996) International epidemiological and microbiological study of outbreak of *Salmonella* Agona infection from a ready to eat savoury snack: I—England and Wales and the United States. BMJ 313:1105–1107

Lehmacher A, Bockemühl J, Aleksic S (1995) Nationwide outbreak of human salmonellosis in Germany due to contaminated paprika and paprika-powdered potato chips. Epidemiol Infect 115:501–511

Richter K, Dorneanu E, Eskridge K et al (1993) Microbiological quality of flours. Cereal Food World 38(5):367–369

Shohat T, Green M, Merom D et al (1996) International epidemiological and microbiological study of outbreak of *Salmonella* Agona infection from a ready to eat savoury snack: II—Israel. BMJ 313:1107

Threlfall E, Hampton M, Ward L et al. (1996) Application of pulsed-field gel electrophoresis to an international outbreak of *Salmonella* Agona. Centers for Disease Control and Prevention. http://www.cdc.gov/eid/article/2/2/96-0209 article.htm. Accessed 05 Apr 2013

Pet Foods

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Abstract Food for pets and other animals may be prepared and processed in environments and from ingredients that result in *Salmonella* contamination. A number of ingredients used in dry pet foods and animal feed have a historic association with *Salmonella* contamination, including rendered animal proteins. Pets and animals that consume *Salmonella*-contaminated foods can become infected or carriers of the pathogen. The risk of salmonellosis to humans from pet food is greater than that in pets, given the relationships between humans and pets in the home and the low infective dose to humans of Salmonella compared to animals.

Introduction

There are approximately 63 million dogs and 81.4 million cats in the United States (Crane et al. 2010), making pets an important part of the lives of many Americans. These pets are fed in a number of ways. Globally, pet food production reached 20.5 million tons in 2012, and the United States accounted for 8 million tons (or 39 % of the total pet food produced) over the same period (Anonymous 2013). Prior to World War II, the primary pet food was canned, wet food, accounting for 91 % of pet food that was sold. The need for tin during the World War II effort, which was otherwise used for canning, resulted in the removal of canned product as the primary food source for pets. Subsequently, dry pet foods became much more popular and, by 1946, they accounted for 85 % of the pet food sold (Crane et al. 2010).

The US Federal Government's present philosophy is to regulate pet food in a manner consistent with human food. Under the new Food Safety Modernization Act (FSMA), the same standards that govern human food will also apply to pet foods (http://www.fda.gov/AnimalVeterinary/Products/AnimalFoodFeeds/ucm347941.htm). This includes aspects related to safety, sanitation, and labeling, which can ultimately impact the health and well-being of animals as well as the health of humans. People

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with companion animals often eat pet food, either intentionally or accidentally, and also may come into contact with an animal's mouth and/or fecal material. This contact can lead to potential zoonotic transmission of *Salmonella* from animals to humans.

The primary microbe of concern to humans in dry pet food is *Salmonella*, due to its ability to survive in dry environments and cause illness, if ingested. Product recall procedures for pet food are similar to those for human food. Several examples are available for recent foodborne illness outbreaks associated with pet food, mostly caused by *Salmonella*. In 2012, a 20-state foodborne illness outbreak involving 49 cases was reported by the US Centers for Disease Control and Prevention (CDC). *Salmonella* serovar Infantis was obtained from the dry dog food. Although no deaths were associated with this outbreak, ten people were hospitalized. *S*. Infantis was reported by the CDC to be the #11 serovar responsible for human foodborne illnesses in 2011 (CDC 2012). In 2013, there have been 20 pet food recalls because of *Salmonella* contamination, including 6 on pet treats for dogs and birds and 14 for pet food for dogs, cat, birds, and fish (FDA 2014).

Estimates of foodborne salmonellosis in humans in the United States are over one million cases per annum, with more than 48,000 individual cases reported in 2008, resulting in millions of dollars in costs and human suffering (Scallan et al. 2011a, b).

Salmonellosis in Humans

In a report from the CDC looking at the years 1998–2013, 48 million estimated cases of foodborne illness occur annually in the United States. Of those, 9.4 million are caused by foodborne pathogens. *Salmonella* is the second leading cause of foodborne illness, causing over 1,400 foodborne illness outbreaks and nearly 40,000 reported cases of illness over the 10-year period; however, the reported number of cases belies the number of actual cases. Lynch and Tauxe (2009) estimated that up to 1.4 million actual cases of salmonellosis may occur in the United States per annum. According to the Centers for Disease Control and Prevention's FoodNet 2011 Surveillance Report, over half of the human cases of salmonellosis in the United States were caused by four serovars, namely, Enteritidis (18.2 %), Typhimurium (12.6 %), Newport (12.3 %), and Javiana (9.6 %) (CDC 2012a) (Table 1).

Table 1 *Salmonella* outbreaks in humans from 1998 to 2008 by top serovar. A total of 1,291 outbreaks with confirmed cause serotype listed (CDC 62:2013)

Serovar	Number of cases	Percent of outbreaks (%)
Enteritidis	418	32
Typhimurium	170	13
Heidelberg	102	8
Newport	93	7

Pet Food Products

Approximately 8 million tons of pet foods are sold annually in the United States. While the microbiological risk from pet food may seem like a fairly straightforward topic, to the contrary, it is quite complex. Areas of microbiological concern, in relation to canned high-moisture pet food, include ingredients, canning, and whether the product is conducive to microbial growth. There are also microbiological challenges with dry pet food. Although it may seem that a dry product is inhospitable to microorganisms, there are significant microbial issues associated with such products.

The most common microbial concern associated with dry pet food and treats is *Salmonella*. Over the last several years, there have been multiple foodborne illness outbreaks and product recalls involving *Salmonella*-contaminated dry pet foods and pet treats.

Pet Food and Salmonella

Salmonella has been detected in numerous types of pet food products, including dry pet food, jerky treats, and pig ear dog treats. Another reason for concern regarding *Salmonella* in dry pet food is because it can survive for months to years in low water activity environments.

Dry pet food has a low water activity (a_w <0.60) and contains a mixture of cereals (e.g., wheat, white and brown rice, corn, and barley), soybean meal, and animal by-products, such as meat meal, meat and bone meal, fish meal, feather meal, or blood meal (ICMSF, Volume 6, 2005). All of these have been associated with *Salmonella* contamination. Pet food ingredients include protein, carbohydrate, fats, and other components, including vitamins and minerals, flavoring, and additives that enhance appearance, such as colors, as well as binders.

Salmonella Survival in Pet Foods

Like many foodborne pathogens, *Salmonella* requires a relatively high water activity for growth to occur. The lower limit of water activity for growth of *Salmonella* is 0.94 (ICMSF Volume 5, 1996, p.224). However, salmonellae can survive for a year in products with lower water activities (ICMSF Volume 5, 2005, 5 p. 224). Low water activity products allow better survival of *Salmonella* at 25 °C than elevated temperatures (D'Aoust 1989, p.339).

Rendered Animal Protein Ingredient

One of the main protein sources in many dry pet foods is rendered animal protein (RAP). RAPs start with animal by-products from the unused portion of animals (e.g., from slaughter or butcher shops in the case of human foods, road kill, decreased farm animals, etc.). RAP is made by first boiling or, more commonly, from

high-pressure steam treatment of miscellaneous by-products of animal material to remove the fat. Once the fat is removed, the protein phase is typically expelled from the cooking process under high pressure to remove moisture. The protein phase is dry and may be about 2.5–6.0 % moisture with an a_w of ca. 0.40 (Thompson 2008; Kinley et al. 2010). This material is then ground into a meal referred to as meat meal or meat and bone meal (Thompson 2008). The meal is a protein-rich component, which has been cooked thoroughly enough to kill most microorganisms, including vegetative bacteria, such as *Salmonella* (Kinley et al. 2010). The RAP is often shipped as an ingredient to pet food plants, where it is added to the other ingredients and combined in an extruder, which cooks the product further. Recontamination of RAP can occur at the rendering facility, in the transport vehicle, or in the pet food processing plant, resulting in a high prevalence of *Salmonella* in RAPs, relative to foods for direct human consumption (Anonymous 1990; Bisplinghoff 1992a, b). Rendering facilities receive large quantities of raw meat by-products, which are likely to be contaminated with a variety of pathogenic microorganisms, including *Salmonella*.

Contamination Levels of Salmonella in Rendered Animal Protein

Salmonella was first detected in animal by-product in the United States in 1948 when Edwards et al. (1948) obtained *Salmonella* Typhimurium and *Salmonella* Bareilly from chicken feed. An extensive review of information on the worldwide occurrence of *Salmonella* in animal feeds (not pet foods) from 1950 to 1979 was published by Williams (1981). *Salmonella* contamination was highest in animal protein (42 %), followed by meal (22 %) and mash feed (10 %), with the lowest prevalence in vegetable protein concentrate and pelleted feed (3 % each). Hence, RAPs are suspected of being major sources of *Salmonella* contamination in animal feeds (FDA 1990) and have been the most extensively studied of feed ingredients; however, at normal inclusion rates, RAPs may only comprise 2.5–5.0 % of the feed blend (Franco 2004). Studies conducted in the United Kingdom revealed that *Salmonella* was present in 0.9, 2.7, 6.5, and 13.2 % of cereals, soya meal, sunflower meal, and fish meal, respectively (Anonymous 1990). *Salmonella* has also been detected in the United Kingdom in pelleted samples of soybean meal, sunflower meal, groundnut meal, and tapioca meal (Anonymous 1990). All the aforementioned animal feed ingredients may also be components of pet foods.

Studies of the most probable number (MPN) levels of *Salmonella* in RAP samples, previously testing positive for *Salmonella* (Bisplinghoff 1992a; Smittle et al. 1992), have revealed a range of <0.3–11 MPN/g, with a geometric mean of 7 *Salmonella*/100 g. The median was 5–7 *Salmonella*/100 g in other studies (Smittle et al. 1992). No statistically significant relationship was observed between the MPN of *Salmonella* and the prevalence level in RAPs (Smittle et al. 1992). Another study revealed a median of 9 *Salmonella*/100 g (Franco 2004), with the highest level detected at 78 MPN/g. A more recent but limited study reported an average of 16 MPN/g, with a range of 0.2–78 MPN/g (Kinley et al. 2010).

Infected hens (or asymptomatic carriers) can transmit *Salmonella* Enteritidis into intact shell eggs by a transovarian mechanism (Bryan 1988) or by other means (McIlroy et al. 1989). It is interesting to note that in surveys which included 26,000 RAP samples, *Salmonella* Enteritidis was not detected, despite a *Salmonella* sp. prevalence of 25 and 61 % recovered from renderer/packer and protein blender plants, respectively (Smittle et al. 1992). Similar data were reported by Franco (2004). Published data on the worldwide prevalence of *Salmonella* in animal feeds and feed ingredients are shown in Table 2 (D'Aoust 1989). The reasons for the failure to recover *S*. Enteritidis could be related to methodology, competition with other strains of *Salmonella*, or the unique properties of RAPs which inhibit or kill *Salmonella* Enteritidis (Anonymous 1990; FDA 1990). However, the extensive research survey data (cited above) suggest that RAPs may not be a significant source of *Salmonella* Enteritidis infections in laying flocks.

Cross-Contamination of RAP with Salmonella spp.

Cross-contamination of RAP from the raw ingredients to the cooked product frequently occurs in RAP processing plants (Kinley et al. 2010). Salmonellae may become established in growth niches in the RAP plant environment, which receives continual introduction of raw and often spoiled animal waste contaminated with multiple serovars of *Salmonella* sp. These growth niches occur at locations where moisture is entrapped either by unhygienically designed equipment, the facility itself, improper maintenance or repair practices, or unsanitary operating practices, including misapplied sanitation and uncontrolled standing water in the plant. Salmonellae, which can grow to large populations in the environment, can contaminate large quantities of finished product under certain circumstances. For example, salmonellae can grow in condensate formed inside equipment used to transport warm finished product (e.g., bucket elevators and screw conveyors). This condensate then drips onto finished product, sporadically contaminating it with *Salmonella*. One drop of such condensate containing one million *Salmonella* would be adequate to contaminate 15 tons of RAPs at a mean contamination level in *Salmonella-positive* product of 7 CFU/100 g.

The RAP heat treatment, generally 100–121 °C, which is applied during processing, is sufficient to destroy *Salmonella* and many other microorganisms (Fuller and Wilder 1987; FDA 1990; Kinley et al. 2010). Subsequently, recontamination may occur, resulting in the high reported prevalence of *Salmonella* in RAPs relative to foods for direct human consumption (FDA 1990). Rendering facilities receive large quantities of raw meat by-products, likely to be contaminated with a variety of pathogenic microorganisms, including *Salmonella*. Additional foodborne pathogens of concern in raw meat products include *Listeria monocytogenes*, *Clostridium perfringens*, *Staphylococcus aureus*, *Yersinia enterocolitica*, *Campylobacter jejuni*, *e*nteropathogenic *Escherichia coli*, and others, including, in rare instances, *Bacillus anthracis* (Bryan 1988; Erickson and Kornacki 2002). Undercooked meat has been a source of gastrointestinal anthrax, occurring mainly in Africa, the Middle East, Southeastern Asia, and Central and Southern Africa. *B. anthracis* can be distributed

Table 2 *Salmonella* in feeds and feed[a]

	Percentage positive/number tested							
Region	Meat meal	Meat & bone meal	Blood meal	Bone meal	Feather meal	Fish meal	Others	Complete feed
Australia	72/100	69.5/164	22/100	[b]	78/250	–	6.1/1100	43.5/200
Europe	2.3/2260	22.8/786	120/1.7	33/6	3/1517	2/840	7/566	13/717
South America	39.3/300	–	22/68	–	37.5/8	0/4	–	–
North America	81/21	–	–	–	40/15	–	6.3/48	–
Middle East	9/152	53.8/13	0/10	38/129	–	21.2/99	5/400	5.4/484
Asia	68/25	22.7/132	–	5/20	0/18	22.2/379	0.8/236	43/21

[a]Adapted from D'Aoust, J-Y. 1989. *Salmonella*. pp. 378–445. In M.P. Doyle (ed.), Foodborne Bacteria Pathogens. Marcel Dekker, Inc. New York
[b]Not tested
[c]Various date ranges between 1968-1986

through contaminated feed additives, such as those from improperly rendered animals (Erickson and Kornacki 2002); however, properly applied rendering conditions will destroy *B. anthracis* (Erickson and Kornacki 2002). A 2001 study revealed that 84.5, 71.4, 8.3, and 29.8 % of raw material used for RAP product was contaminated with *Salmonella*, *C. perfringens*, *L. monocytogenes*, and *C. jejuni*, respectively (Kinley et al. 2010; Troutt et al. 2001).

It has been reported that as few as one CFU of *Salmonella* in a food product may cause human illness (D'Aoust 1985); however, data supporting such a low infective dose in animals are generally lacking (D'Aoust 1989). Reports on experimentally induced animal infection have generally revealed that ingestion of large numbers of *Salmonella* cells (e.g., greater than 10,000 CFU) is necessary to induce salmonellosis in most animals (D'Aoust 1989). It has been reported that inocula as low as one viable cell per gram can initiate infection in chickens (McIlroy et al. 1989), and the CDC has reported that a common vehicle for lateral transmission of *Salmonella* into poultry flocks is contaminated feed (McIlroy et al. 1989; Crump et al. 2002). However, it has not been conclusively established that ingestion of low populations (e.g., 7–9 *Salmonella*/100 g RAPs added to a feed blend) will result in an animal infection. In fact, it was once reported that "there is no direct evidence that Salmonella in meat and bone meal causes salmonellosis" (Sutton et al. 1992). In another study, *Salmonella choleraesuis* ser kunzendorf at an inoculum of 30 CFU/100 g was added to the rations of 24 weanling pigs (Sutton et al. 1992). Rectal swabs obtained over the course of 14 days were consistently negative for *Salmonella*. Swabs of the gastrointestinal tract of piglets killed after 14 days were also *Salmonella*-negative (Sutton et al. 1992).

A greater risk of *Salmonella* infection occurs when conditions are created that increase the moisture content of a feed to levels at which *Salmonella* can grow. Hence, it is important that renderers and those handling such ingredients strive to keep RAPs and animal feed dry. For example, Linton et al. (1970) determined that liquid feed containing one *Salmonella* CFU/mL grew to 200,000/mL after 48 h at 20 or 28 °C in a liquid feed pipeline. However, salmonellae did not grow at 20.8 °C in meat and bone meal with either 4 or 7.5 % moisture content, respectively (Bisplinghoff 1992a, b). The moisture content of animal by-products for use in feeds is commonly ca. 7 % (Fuller and Wilder 1987). As part of a study by Kinley (2010), it was shown that various types of meal had water activities of 0.41–0.49. *Salmonella* will not grow below an a_w of 0.93 (D'Aoust 1989). Hence, salmonellae should not grow in dry feed ingredients of animal origin but may survive in these low-moisture conditions for extended periods of time.

Other Risk Factors for Salmonellosis in Animals from Exposure to Contaminated Animal Feeds

There is evidence that animals can become infected from ingestion of dry feed naturally contaminated with *Salmonella*, which has been known to lead to human infection (Crump et al. 2002). It is also well known that the incidence of *Salmonella*

infection increases dramatically as meat animals move from the farm to the slaughter operation. In one study, the infection rate in calves was 0.5 % on the farm and 36 % after the animals were placed in holding pens for 2–5 days (CDC 1987). There are many potential sources of *Salmonella* infection in animals, but no single source has been implicated as the major contributor to disease transmission in animals (Silliker 1980). In addition, it has been observed that dogs and cats may carry *Salmonella* in their intestinal tracts and remain asymptomatic. It is estimated that *Salmonella* is found in feces of healthy dogs 1–36 % of the time and in cat feces 1–18 % of the time (Sanchez et al. 2002).

Other risk factors for animal-acquired salmonellosis include innate immunity, consumption of feed with mycotoxins (known to suppress the immune system and increase susceptibility to illness), age of the animal (young animals and aged animals are more susceptible to illness), stress (e.g., poultry in high-density cages and hens undergoing forced molting are more susceptible to *Salmonella* infection), and exposure of the animals to unsanitary and unhygienic conditions (Hird et al. 1984). Stress factors include transportation, starvation, early weaning, change of ration, overcrowding, age, pregnancy, giving birth, overwork, concurrent diseases, ingestion of oral antibiotics and antihelminthics, high gastrointestinal worm burden, trauma, fractures, hot and humid weather, surgery, and anesthesia (Hird et al. 1984).

Recent Foodborne Illness Outbreaks Associated with Pet Foods

Over the years, there has been a lingering question as to whether contaminated pet food can actually lead to human illness. In 2006–2007, a foodborne illness outbreak of human *salmonellosis* was caused by contaminated dry dog food, thus answering this long-standing question (CDC 2008a). The causative agent, *Salmonella enterica* serovar Schwarzengrund, was found in dry dog food produced at a pet food manufacturing facility in Pennsylvania. A CDC update revealed a total of 79 reported cases in 21 states (CDC 2008b). Interestingly, *S*. Schwarzengrund is rarely reported in human foodborne illness outbreaks, as, according to the CDC (2011), it is not in the top 20 *Salmonella* serovars associated with human illness.

In 2012, an outbreak of human salmonellosis in the United States and Canada was associated with *Salmonella* Infantis in dry dog food (CDC 2012b, c). A total of 49 cases and 10 hospitalizations were reported in 20 states; however, there were no deaths. A chicken jerky product for dogs was the vehicle for a *Salmonella* outbreak in New Hampshire in 2013 (FSN 9/10/2013). Also in 2013, a manufacturer voluntarily recalled five brands of pet food for cats, dogs, and ferrets, due to *Salmonella* contamination (FSN 6/18/2013).

Seafood and beef pet treats contaminated with *Salmonella* Thompson, distributed in Washington State and British Columbia in 2005, were produced using raw, frozen beef and salmon, cutting the formed product to shape, and then dehydrating them. The beef came directly from slaughter operations. The dehydration process did not produce temperatures high enough to kill *Salmonella* (CDC 2006).

Regulations Related to Salmonella *Contamination in Animal Feeds, Including Pet Food*

According to the US Food and Drug Administration, there is a distinction between "food for animals" and pet food. Pet food includes any food for pets, including treats and chews as described in section 201(f) of the Food, Drug, and Cosmetic Act (FD&C Act) (FDA (2013a) 690.800). The distinction is based on risk to humans. Since there are more dogs and cats in homes than cattle in the United States, for example, there is a different risk profile for owners of pets versus owners of cattle. According to a FDA Guidance Document (FDA (2013a) 690.800), pet food is adulterated when *Salmonella* is present in one or more subsamples of pet food or pet food ingredients, regardless of serovar. Animal feed, other than pet food, is considered adulterated if *Salmonella* is present in one or more subsamples or ingredients and it is a serovar pathogenic to the species for which the feed is intended. According to the FDA, *Salmonella* pathogenicity for a given animal species is dependent on the serovar (FDA (2013a) 690.800). Due to the proximity of pets to many humans, the risk of the presence of any serovar of *Salmonella* in pet foods is considered high. The concern of the presence of *Salmonella* in pet food is based more upon proximity and handling of pet food by humans than risk to the animal. The FDA's concern regarding the presence of *Salmonella* in animal feed, however, is based on the risk to the animal as well as the potential for such infected animals to result in human infection.

The FDA recognizes that there are differences in risk between human food and pet food and has plans to issue four foundational proposed rules, as part of the Food Safety Modernization Act, two of which are relevant to this book. The first rule is "Preventive Controls for Animal Foods" and the second is "Preventive Controls in Human Foods." Recent remarks by Michael R. Taylor (2012), Deputy Commissioner for Foods at FDA, included that "the general principles for the rules are the same." The separate rules allow for differences between types of food, and both will require preventive controls consistent with Hazard Analysis and Critical Control Points (HACCP). However, the scope of these controls may be greater for human food rather than food for animals and will include verification activities and monitoring applied to a wider variety of quality assurance items, as described below.

The FDA Food Safety Modernization Act and Salmonella Contamination in Pet Foods

The FDA has released draft rules that may be included in the Food Safety Modernization Act (FSMA), requiring current good manufacturing practices (cGMPs) and hazard analysis and risk-based preventive controls (FDA (2013b) 64735). At the time of this writing, these are only proposed rules and are subject to change before becoming final rules.

The proposed rules have two main subparts: (1) The cGMP section has provisions for personnel, plant and grounds cleaning and maintenance, sanitary operations, sanitary facilities and controls, equipment and utensils, processes and controls, and warehousing and distribution, and (2) the second subpart is related to hazard analysis and risk-based preventive controls. This part requires the manufacturer to perform a hazard analysis that includes biological, chemical, physical, and radiological hazards. Records of preventive controls are also required which are based upon the hazards that are reasonably likely to occur. The records would address process controls, sanitation controls, a recall plan, and any other controls found to be necessary.

Both subparts of the rule are intended to address the primary topic of this chapter (i.e., the presence of *Salmonella* in dry pet food). The cGMP section is similar to The Association of American Feed Control Officials' (AAFCO) "Model Good Manufacturing Practice Regulations for Feed and Feed Ingredients" of 2009 (FDA (2013b) 64735). The hazard analysis and risk-based preventive control section, while similar to Hazard Analysis and Critical Control Point (HACCP) concepts, differs in the draft rules "in that preventive controls may be required at points other than CCPs and critical limits would not be required for all preventive controls" (FDA (2013b) 64735). The difference is that the facility will have the opportunity to evaluate the hazard as well as its severity in determining if CCPs would be needed (FDA (2013b)64735).

Bactericidal Treatments as Intervention Measures for Salmonella in Pet Foods

The treatment of RAPs or feeds with short-chain free fatty acids has shown promise in reducing *Salmonella* contamination (Bisplinghoff 1992a, b; Hinton and Linton 1988; Khan and Katamay 1969). Bacteriostatic or bactericidal activity has been observed when propionic, acetic, sorbic, benzoic, fumaric, and formic acids have been applied to feeds under certain conditions (Bisplinghoff 1992a, b; Khan and Katamay 1969).

Pre-extrusion treatment of dry pet food as an enrobing component also shows promise. For example, acidic calcium sulfate was added prior to extrusion in one batch of test kibble, whereas acidic calcium sulfate was added as an enrobing component in another batch. *Salmonella* was recovered in 1.3 and 0 % of the first and second batches, respectively (Perkin et al. 2009).

Other Efforts to Control Salmonella in Pet Foods

There is considerable similarity in efforts to control *Salmonella* in pet food processing facilities compared to food processing facilities. Environmental controls are of great importance during pet food production. In addition, the use of high quality ingredients is critical, as is proper operation and sanitary control of processes.

Other approaches to *Salmonella* reduction in feed and feed ingredients include the application of a terminal thermal kill step to inactivate salmonellae in RAPs and cooling these rendered by-products, after they leave the expeller, to reduce condensate formation in equipment downstream (Bisplinghoff 1992a, b), including pelleting and irradiation (FDA 1990).

Conclusions

Since World War II, dry pet food has been the predominant type of pet food produced and purchased in the United States. The FDA currently regulates pet food in much the same manner as it does human food, due to (1) the close association that pets have with humans, particularly infants and young children who are more susceptible to salmonellosis than older children and healthy adult human populations, and (2) because of the low infectious dose of *Salmonella* in humans. However, the situation with animal feed is different in that the infectious dose of *Salmonella* may be higher in animals than in humans; hence, these products are regulated differently. Nevertheless, human foodborne illnesses may occur from infected animals who have consumed contaminated animal feed.

A historical cause of *Salmonella* contamination in pet food and animal feed is related to the inclusion of rendered animal proteins, which are recontaminated after the cooking step.

Controlling the recontamination of animal feed, RAPs (and other ingredients), and pet food during processing, transportation, and blending, along with maintaining these products in a dry state, is critical to reducing the risk of *Salmonella* contamination and infection of animals and humans.

References

Anonymous (1990) Industry testifies on *Salmonella* risk: rendered products not culprit experts say. Render Magazine. February, p. 10–11, 21

Anonymous (2013) 2013 Alltech global feed survey. http://www.alltech.com/sites/default/files/2013-feed-tonnage-report.pdf. Accessed 6 Jan 2014

Bisplinghoff FD (1992a) APPI business meeting. Sheraton O'Hare Hotel, Chicago, IL. March 26–27

Bisplinghoff FD (1992b) Antimicrobial properties of commercial organic acid compounds in meat and bone meal. In: Snoeyenbos GH (ed) Proceedings of the Symposium on the Diagnosis and Control of *Salmonella*. USAHA annual meeting San Diego, CA. October 29, 1991. Carter Printing Co., Richmond, VA

Bryan FL (1988) Risks associated with vehicles of foodborne pathogens and toxins. J Food Prot 51:498–508

Centers for Disease Control and Prevention (1987) Update: *Salmonella Enteritidis* infections in the northeastern United States. Morbidity and Mortality Weekly Report, April: 204–205/8a. Committee on *Salmonella*. 1966. An evaluation of the Salmonella problem. National Academy of Sciences, Washington, DC

Centers for Disease Control and Prevention (2006) Human salmonellosis associated with animal-derived pet treats: United States and Canada, 2005. MMWR Morb Mortal Wkly Rep 55(25): 702–705
Centers for Disease Control and Prevention (2008a) Multistate outbreak of human *Salmonella* infections caused by contaminated dry dog food: United States, 2006–2007. MMWR Morb Mortal Wkly Rep 57(19):521–524
Centers for Disease Control and Prevention (2008b) Update: recall of dry dog and cat food products associated with human *Salmonella* Schwarzengrund infections—United States, 2008. MMWR Morb Mortal Wkly Rep 57(14):1200–1202
Centers for Disease Control and Prevention (2012a) FoodNet 2011 surveillance report. US Department of Health & Human Services. http://www.cdc.gov/foodnet/PDFs/2011_annual_report_508c.pdf. Accessed 17 Jan 2014
Centers for Disease Control and Prevention (2012b) Notes from the field: human *Salmonella* infantis infections linked to dry dog food: United States and Canada, 2012. MMWR Morb Mortal Wkly Rep 61(23):436
Centers for Disease Control and Prevention (2012c) Multistate outbreak of human *Salmonella* Infantis infections linked to dry dog food (final update). http://www.cdc.gov/salmonella/dog-food-05-12. Accessed 20 Jun 2013
Centers for Disease Control and Prevention (2013) Surveillance for foodborne disease outbreaks: United States, 1998–2008. MMWR Morb Mortal Wkly Rep 62(SS02):1–34
Crane SW, Cowell CS, Stout NP, Moser EA, Millican J, Romano P Jr, Crane SE (2010) Commercial pet foods. In: Hand MS, Thatcher CD, Remillard RL, Roudebush P, Novotny BJ (eds) Small animal clinical nutrition, 5th edn. Mark Morris Institute, Topeka, KS
Crump JA, Griffin PM, Angulo FJ (2002) Bacterial contamination of animal feeds and its relationship to human foodborne illness. Clin Infect Dis 35:859–865
D'Aoust J-Y (1985) Infective dose of Salmonella Typhimurium in cheddar cheese. Am J Epidemiol 122:717–720
D'Aoust J-Y (1989) *Salmonella*. In: Doyle MP (ed) Foodborne bacterial pathogens. Marcel Dekker, Inc., New York, NY, pp 328–445
Edwards PR, Bruner DW, Moran AB (1948) The genus *Salmonella*: its occurrence and distribution in the United States. KY Agr Exp Stn Bull 525:530
Erickson MC, Kornacki JL (2002) *Bacillus anthracis*: current knowledge in relation to contamination of food. J Food Prot 66(4):691–699
Food and Drug Administration (1990) Working group on *Salmonella* in feeds. Center for Veterinary Medicine, Food and Drug Administration, Washington, DC
Food and Drug Administration (2013a) Guidance for FDA staff: compliance policy guide sec.690.800 *Salmonella* in food for animals. Food and Drug Administration, Washington, DC
Food and Drug Administration (2013b) Current good manufacturing practices and hazard analysis and risk-based preventive controls for food for animals. Fed Reg. 78(64735) https://www.federalregister.gov/articles/2013/10/29/2013-25126/current-good-manufacturing-practice-and-hazard-analysis-and-risk-based-preventive-controls-for-food
Food and Drug Administration (2014) Recalls and withdrawals. http://www.fda.gov/animalVeterinary/safetyhealth/recallswithdrawals/default.htm. Accessed 7 Feb 2014
Franco DA (2004) A survey of *Salmonella* serovars and most probable numbers (MPN) in rendered animal protein meals: inferences for animal and human health. In: Cruz Suárez LE, Ricque Márie F, Nieto López MG, Villarreal D, Scholz U, González M (eds) Avances en Nutrición Acuicola VII. Momorias del VII Simposium Internacioonal de Nutrición Acuicola November 16–19. Hermosillo, Sonora, Mexico
Fuller HL, Wilder OHM (1987) Meat animal byproducts and their utilization: Part 2. In: Price JF, Schweigert BS (eds) The science of meat and meat products, 3rd edn. Food & Nutrition, Westport, CT
Hinton M, Linton AH (1988) Control of *Salmonella* infections in broiler chickens by the acid treatment of their feed. Vet Rec 123:416–421

Hird DW, Pappaioanou M, Smith BP (1984) Case–control study of risk factors associated with isolation of *Salmonella saint-paul* in hospitalized horses. Am J Epidemiol 120:852–864

ICMSF (1996) Salmonellae. In: Microorganisms in foods 5: characteristics of microbial pathogens. Blackie Academic & Professional, London

ICMSF (2005) Feeds and pet foods. In: microorganisms in foods 6: microbial ecology of food commodities, 2nd ed. Kluwer Academic/Plenum Publishers, New York

Khan M, Katamay M (1969) Antagonistic effect of fatty acids against *Salmonella* in meat and bone meal. Appl Microbiol 17:402–404

Kinley B, Rieck J, Dawson P, Jiang X (2010) Analysis of *Salmonella* and enterococci isolated from rendered animal products. Can J Microbiol 56:65–73

Linton AH, Jennet NE, Heard TW (1970) Multiplication of *Salmonella* in liquid feed and its influence on the duration of excretion in pigs. Res Vet Sci 11(5):452–457

Lynch MF, Tauxe RV (2009) Salmonellosis: nontyphoidal. In: Evans AS, Brachman PS (eds) Bacterial infections of humans, Springer, New York, Chapter 32

McIlroy SG, McCracken RM, Neill SD, O'Brien JJ (1989) Control, prevention, and eradication of *Salmonella enteritidis* infection in broiler and broiler breeder flocks. Vet Rec 125(22): 545–548

News Desk (2013) Nutra recalls more pet food for potential Salmonella contamination. Food Safety News. June 18

News Desk (2013) NH Salmonella outbreak linked to chicken jerky for dogs. Food Safety News. September 10

Perkin DA, Phebus RK, Michael M, Thippareddi H (2009) Poster: control of post-extrusion *Salmonella* contamination in dry pet food utilizing pHresh 10™ (acid calcium sulfate). International Association for Food Protection Annual Meeting. July 12–15, Grapevine, TX

Sanchez S, Hofacre CL, Lee MD, Maurer JJ, Doyle MP (2002) Animal sources of salmonellosis in humans. J Am Vet Med Assoc 221(4):492–497

Scallan E, Hoekstra RM, Angulo FJ, Tauxe RV, Widdowson M-A, Roy SL et al (2011a) Foodborne illness acquired in the United States: major pathogens. Emerg Infect Dis 17(1):7–15, http://www.cdc.gov/EID/content/17/1/7.htm

Scallan E, Griffin PM, Angulo FJ, Tauxe RV, Hoekstra RM (2011b) Foodborne illness acquired in the United States: unspecified agents. Emerg Infect Dis 17(1):16–22, http://www.cdc.gov/EID/content/17/1/16.htm

Silliker JH (1980) Status of *Salmonella*: ten years later. J Food Prot 43:307–313

Smittle RB, Kornacki JL, Flowers RS (1992) *Salmonella* survey of rendered animal proteins in the U.S.A. and Canada. Abstract for the World Congress on Foodborne Infections and Intoxications, Berlin. June 16–19

Sutton AL, Scheidt AB, Patterson JA (1992) Studies with meat and bone meal inoculated with *Salmonella choleraesuis* on *Salmonella* colonization of the gastrointestinal tract of the weaning pig. Final Report submitted to Fats and Proteins Research Foundation, Inc. by Department of Animal Sciences and School of Veterinary Medicine of Purdue University, West Lafayette, IN

Taylor MR (2012) Role of the food safety modernization act in ensuring the safety of pet food. Remarks at the Pet Food Forum. April 4. Pet Food Forum, Schaumburg, IL

Thompson A (2008) Ingredients: Where pet food starts. Top Companion Anim Med 23:127–132

Troutt HF, Schaeffer D, Kakoma I, Pearl GG (2001) Prevalence of selected foodborne pathogens in final rendered products. FPRF Directors Digest No. 312. Alexandria, VA

Williams JE (1981) *Salmonellas* in poultry feeds: a worldwide review. World's Poultry Sci 37:6

Dried Teas and Herbs

Li Maria Ma, Shefali Dobhal, and Chris Timmons

Abstract Microbiological safety issues related to dried teas and culinary herbs are influenced by their particular production processes and consumption practices; therefore, a brief description of the production and consumption practices associated with these products, followed by a general review of related microbiological safety issues, is provided. Dried teas are relatively safe products, as evidenced by the lack of reported foodborne illnesses associated with tea consumption. This positive safety record is at least partially due to the heat treatments (steaming and drying) in tea production and during consumer preparation prior to consumption (i.e., by freshly boiled water infusion). In contrast, dried culinary herbs can be carriers of many different microorganisms of public health concern, including *Salmonella*, *Listeria monocytogenes*, *Bacillus cereus*, and *Clostridium perfringens*. The safety risk is even more pronounced when contaminated dried herbs are added directly to ready-to-eat foods. A systematic approach that includes the implementation of good agricultural practices and good manufacturing practices along the entire production chain, as well as a microbial reduction treatment for imported herbs, is essential in reducing such risk.

Keywords Dried teas • Dried culinary herbs • Foodborne human pathogens • *Salmonella* • *Listeria monocytogenes* • *Bacillus cereus* • *Clostridium perfringens* • Food safety

Introduction

The use of dried plant parts for medical and food purposes has been very important in many regions and cultures around the world; thus, the microbiological safety related to these products is also of priority. For the scope of this chapter, dried teas

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are defined as the cured leaves of the tea plant, *Camellia sinensis*, whereas dried herbs are considered the leafy parts of plants that are dried for culinary usages (Woodward 2003). The microbiological safety concerns associated with dried teas and herbs are influenced by their particular production processes and especially their end use (consumption practices); therefore, a brief description of the production and consumption practices of these products is described first in the chapter, followed by related microbiological safety issues and recommendations.

Production and Consumption Practices

Dried Teas

Origin

Cultivation of the tea plant, *C. sinensis*, an evergreen tree, originated in China during the time of Warring States (475 BC–221 BC) (Fischer 2010). Due to its specific requirements of climate and soil, tea cultivation is currently confined to tropical and subtropical regions of the world, with major production areas in Asia, including China, India, Sri Lanka, and Indonesia. On the African continent, major tea producers include Kenya, Malawi, Rwanda, Tanzania, and Uganda. Tea is also produced in South America (Argentina and Brazil) and the Near East (Iran and Turkey). Among tea-producing counties, China, India, Sri Lanka, Kenya, and Indonesia are the top five principal producers, accounting for 77 % of world tea production and 80 % of global exports (Saberi 2010; Majumder et al. 2011).

Cultivation

Tea plants are propagated either from seed or by cutting. It takes about 3 years before a new plant is ready for harvesting and 4–12 years for a tea plant to bear seed. A tea plant can grow up to 16 m (52 ft) if left undisturbed, but cultivated plants are pruned to waist height for ease of picking (Campbell 1966; Rolfe and Cave 2003; Panda 2011).

Harvesting

Only the terminal tea sprouts with 2–3 buds and leaves on the mature plant are picked. These buds and leaves are called “flushes.” During growing season, a plant will produce a new flush every 7–15 days and these are harvested at the same intervals. Most harvesting is by handpicking; however, mechanical harvesting has been used in large tea plantations (Campbell 1966; Panda 2011; Rolfe and Cave 2003).

Processing

After picking, the leaves of *C. sinensis* soon begin to wilt and oxidize unless they are immediately dried. Depending on the processing regimes, teas can generally be divided into four categories: white, green, oolong, and black. White tea is the least processed, and fresh tea leaves go through steaming and drying without prior withering. Green tea is produced when freshly harvested leaves are subjected to withering and steaming prior to rolling/shaping and drying. Oolong tea goes through withering, bruising, brief oxidation, and drying. Black tea, which represents >90 % of the total tea consumption worldwide, is the most heavily processed tea type. The traditional processing of black tea comprises withering, rolling, full oxidation, and drying. Withering is a process whereby the freshly plucked tea leaves are spread out on mats in a thin layer or in troughs in thick layers for 8–18 h to reduce the moisture content of the leaves to about 55–72 %. The withered leaves are then rolled (bruised) to break down the leaf cell structure and bring enzymes and the substrate polyphenols in the leaf into contact. The bruised leaves are spread out in a layer 5–8 cm thick for 45 min to 3 h, for oxidation, depending on the types of tea. During oxidation, simple flavonoids in the tea leaves are oxidized by endogenous enzymes into complex polyphenols that impart a bright red color and a characteristic astringent flavor to the oxidized teas (oolong and black tea). This enzymatic oxidation process is also known as fermentation in the tea industry, although no microorganisms are involved. The final step, drying, achieved by blowing hot air through tea leaves, arrests oxidation by inactivating enzymes, resulting in color enhancement and the final balance of tea aromas, and reduces the moisture content of the leaves to less than 5 %. Following drying, the leaves are sorted and graded to yield a commercial product (Campbell 1966; Heiss and Heiss 2007; Rolfe and Cave 2003; Campbell 1963; Anonymous 2003; Balentine et al. 1997). The manufacturing process of different types of tea is illustrated in Fig. 1.

In addition to these four types of tea, a great range of flavors have been added to these traditional teas. Among the best known are Chinese jasmine tea, with jasmine oil or dried flowers, and Earl Grey tea, which contains oil of bergamot.

Packaging and Marketing

Dried tea can be packaged as loose leaves in plastic or foil bags that are either vacuum sealed or closed with a twist tie, or packaged first in small tea bags made of a blend of wood and vegetable fibers (Anonymous 2013; Altman 2003) that are then packed into a large container. Packing is usually done by machine. Without careful moisture control (dry) and temperature control (cool) during packaging, storage, and distribution, tea may become unfit for consumption due to the growth of undesired molds and bacteria. Packaged tea is sold either as wholesale or retail. It usually requires between 20 and 30 weeks for newly harvested tea to reach the store shelf after it has been harvested from the tea plant (Anonymous 2003).

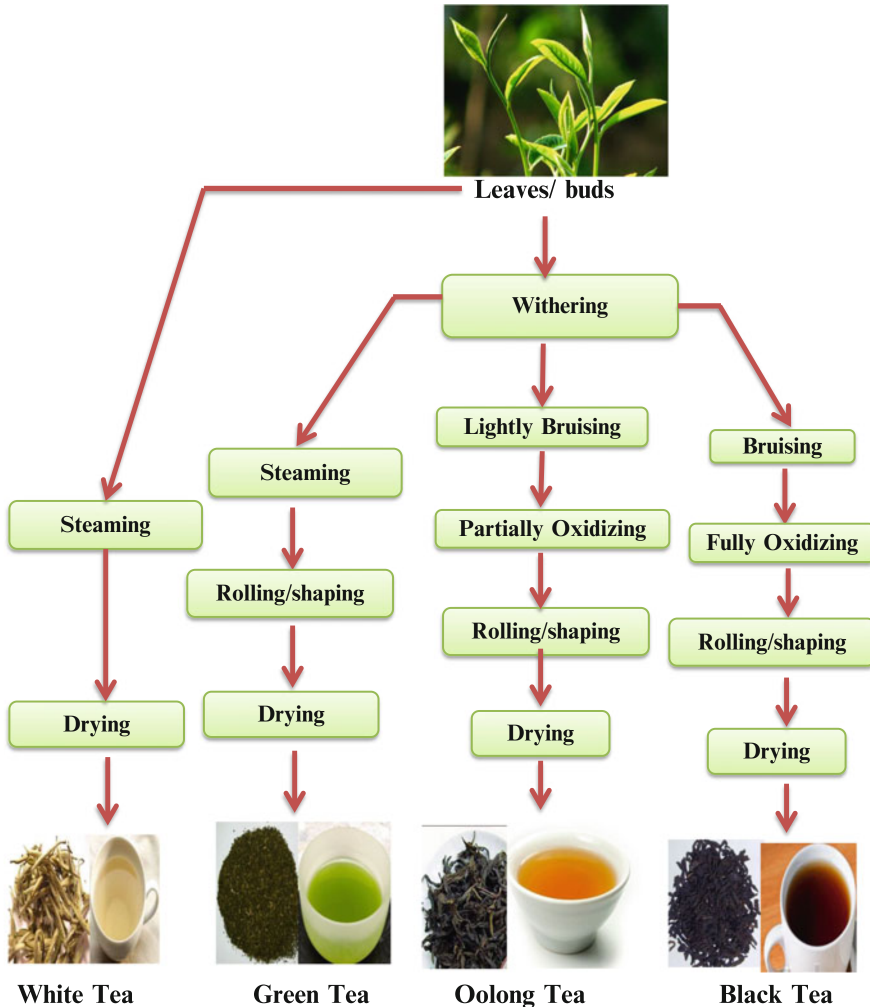

Fig. 1 Overview of tea manufacturing process

Consumption Practices

Drinking tea infusion originated in China during the Shang Dynasty (1500 BC–1046 BC) as a medicinal practice (Heiss and Heiss 2007), but the drink became common during the Qin Dynasty (third century BC). It gained wide popularity during the Tang Dynasty (618–906), when it spread to Korea, Japan, and Vietnam. Tea was first introduced to Portuguese priests and merchants in China during the sixteenth century. Drinking tea became popular in Britain during the seventeenth century, and the British introduced tea to India to compete with the Chinese monopoly. By the late nineteenth century, tea had become an everyday beverage for all levels of

society in most regions of the world. Now, tea is the second most consumed beverage in the world, after water (Saberi 2010).

Tea is generally prepared by brewing (infusing) dried tea leaves or tea bags in freshly boiled water for 3–5 min. The best temperature for brewing tea depends on its type. Teas that have little or no oxidation, such as green or white tea, are best brewed at lower temperatures, between 65 and 85 °C (149 and 185 °F), whereas teas having longer oxidation periods, such as black tea, should be brewed at higher temperatures, around 100 °C (212 °F). The higher temperatures are required to extract the large, complex, flavorful phenolic molecules in oxidized teas. In addition, boiling reduces the dissolved oxygen in the water that would otherwise react with phenolic molecules, thereby reducing their potency as antioxidants. Consequently, to preserve the antioxidant potency, especially for white and green teas brewed at a lower temperature, water should be boiled vigorously to boil off dissolved oxygen and then be allowed to cool to the appropriate temperature before being added to the tea (Fischer 2010; Rasmussen and Rhinehart 1999).

In many countries, especially in the United States, tea is commonly enjoyed as a cold beverage, iced tea. Iced tea may be freshly made using loose tea leaves or tea bags, or from an instant iced tea mix made by preparing infused liquid tea and then dehydrating it. Iced tea is also available in bottles and cans as a ready-to-drink beverage. Fountain iced tea, served commonly at restaurants, is prepared using tea concentrate, which is dispensed using the same methods as fountain drinks. The tea concentrates are usually produced from black tea by extraction and drying (freeze, spray, or vacuum) (Panda 2011).

Sun tea, which is popular in the southern United States, is brewed on hot days by placing tea and room-temperature water together in a glass jar left outdoors in direct sunlight for several hours (Clayton 2011). This often results in a mellower flavored tea.

Dried Herbs

Although the terms herbs and spices are often used interchangeably, in general, herbs are the green and leafy parts of plants that are most flavorsome when used fresh, whereas spices are derived from any part of a plant that is not a leaf and are best used dry (Davidson 2010; Kaefer and Milner 2008; Lai and Roy 2004; Suppakul et al. 2003). A single plant species can yield both herbs and spices; for example, dill leaves are an herb, whereas dill seeds are a spice. Depending on their use and properties, herbs may be divided into culinary herbs, medicinal herbs, and sacred herbs. This chapter focuses on the culinary herbs that are used in flavoring, seasoning, and garnishing foods. The Herb Society of America (http://www.herbsociety.org/herbs/top-ten-herb-seeds.html) lists sweet basil (*Ocimum basilicum*), common thyme (*Thymus vulgaris*), bay (*Laurus nobilis*), common sage (*Salvia officinalis*), Greek oregano (*Origanum vulgare*), chive (*Allium schoenoprasum*), dill (*Anethum*

Table 1 The origins, usages, and major areas of cultivation of the top ten herbs selected by The Herb Society of America (modified from Peter 2004; Kowalchik and Hylton 1998)

Botanical name	Family	Common name	Origin	Major areas (for cultivation)	Part used
Allium schoenoprasum	Liliaceae	Chive	Northern Europe	Austria, Canada, France, Germany, Italy, Netherlands, Switzerland, United Kingdom, and United States	Leaf
Anethum graveolens	Apiaceae	Dill	France, Spain, and Russia	Canada, Denmark, Egypt, Germany, Hungary, India, Netherlands, Mexico, Pakistan, Romania, United Kingdom, and United States	Fruit, leaf, and top
Laurus nobilis	Lauraceae	Bay (laurel)	Countries bordering the Mediterranean	Algeria, Belgium, Cyprus, France, Greece, Italy, Israel, Morocco, Mexico, Portugal, Spain, Turkey, Yugoslavia, the Canary Islands, Central America, and the southern United States	Leaves
Lavandula spp.	Labiatae (Lamiaceae)	Lavender	Native to western Mediterranean, mainly Pyrenees mountains in northern Spain	North Africa, Mediterranean, Europe, France, Western India, and United States (southern part)	Flowers, stem, and leaf
Ocimum basilicum	Labiatae (Lamiaceae)	Sweet basil	India, Iran, Africa	Belgium, Egypt, France, Bulgaria, Hungary, Indonesia, Morocco, Greece, Yugoslavia, India, Italy, Poland, Spain, and United States (particularly in California)	Leaf, terminal shoot
Origanum vulgare	Labiatae (Lamiaceae)	Greek oregano	Greece, Italy, and Spain	Albania, France, Greece, Italy, Mexico, Spain, Turkey, Yugoslavia, and eastern United States	Leaf and flower
Petroselinum crispum	Apiaceae	Parsley	Sardinia	Algeria, California, Louisiana, Belgium, France, Germany, Greece, Italy, Japan, Lebanon, Netherlands, Portugal, Spain, Turkey, and United Kingdom	Leaf and root
Rosmarinus officinalis	Labiatae (Lamiaceae)	Rosemary	Europe	Algeria, France, Germany, Italy, Morocco, Portugal, Romania, Russia, northwestern Spain, Tunisia, Turkey, Yugoslavia, and United States	Terminal shoot and leaf
Salvia officinalis	Labiatae (Lamiaceae)	Common sage	Albania and Greece	Cyprus, Albania, Dalmatian islands, Canada, southern France, Italy, Portugal, Spain, Turkey, Yugoslavia, United Kingdom, and United States	Terminal shoot and leaf
Thymus vulgaris	Labiatae (Lamiaceae)	Common thyme	China and East Indies	Bulgaria, Canada, France, Germany, Greece, Italy, Morocco, Portugal, Russia, Spain, Tunisia, Turkey, United Kingdom, and United States (Catskill Mountains of New York City and naturalized patches in western Massachusetts)	Leaf, terminal shoot

graveolens), parsley (*Petroselinum crispum*), rosemary (*Rosmarinus officinalis*), and lavender (*Lavandula* spp.) as the ten most popular culinary herbs. The origins, distributions, and usages of the top ten culinary herbs are summarized in Table 1.

Origin

Culinary herbs originated in different parts of the world but are now grown widely throughout the world. Most originated in Mediterranean countries, where they are still being produced, but others originated from the temperate climate countries such as Italy, France, and England. The United States is best known for its production of high-quality parsley, oregano, tarragon, and basil (Peter 2004).

Cultivation

Herbs require intensive care at all stages of their production, from land preparation, seeding, and transplanting to weed control and fertilizer application. They are usually planted in autumn or spring, depending on the type and if the weather and soil conditions favor crop establishment. They are either planted as seeds or vegetatively propagated from divisions, rhizomes, or cuttings. They can be cultivated in a greenhouse or on flat raised beds in the field, in either full sunlight or partial shade. To facilitate harvesting, they are planted in rows with optimum spacing depending on the scales of operations (Witten 2003).

Harvesting

With few exceptions, leaves and other aerial parts of herbal plants are harvested by cutting the stem at an appropriate level. On a small to medium scale, harvesting is done mainly by hand with a sickle, a reaping hook, or other types of cutting tools, depending on the type of herb being harvested and the site of production. To harvest by hand, a bunch of plants are grouped together by hand, the stalks are sliced, and a rubber band is slipped around the cut stalks to maintain bunch integrity. Bay leaves are generally cut using trimming shears, leaving approximately 2 cm of the stem connected. For large-scale commercial harvesting, the use of specialized equipment minimizes bruising or other damage to the herbs (Witten 2003).

Processing

After harvesting, herbs are processed by drying, cleaning, and sorting, and in a few circumstances, heat treatment or irradiation is included, depending on the end-user requirement. Drying methods vary according to the type of herb and the cost and scale of production. For small-scale and low-cost production, traditional

methods, such as sun or shade drying, are commonly used. Freshly harvested herbs are often spread out on a flat, clean, dry surface (such as concrete or a thoroughly swept floor with or without a fabric covering), either in a shed or directly under the sun to dry in the ambient air. In other cases, herbs are bundled together as three to four stems and suspended upside down, in a dark, dry place with good ventilation (e.g., oregano, sage, basil, dill, and parsley). For high-quality herbs, growers often spread individual stems and/or leaves on layered screens with airspaces in between them inside a shed, and drying air, either as ambient air or air heated by solar energy, wood, or fossil fuel, is forced into the shed by fans (Witten 2003). Herbs with high-volatile oils, such as dill and lavender, are best dried at temperatures between 35 °C and 38 °C (95 °F and 100 °F), but commercial herb processors often use higher temperatures <45 °C (113 °F). Freeze-drying, the best method for preservation of flavor and aroma of herbs, has been used for the production of chives (Peter 2004). Since it involves high technology and capital cost, this method is not practical for small producers. In the production of organic herbs, steam sterilization at 135 °C (275 °F) for 3 min is often used. Most herbs used in cooking are cut into small pieces (about 2.5 mm) free of stems, with the exception of bay leaves which are used whole. Stems are generally removed by sifting and shaking or rubbing the herbs through a fine wire screen. For manufacturer-grade and bulk operations, a chaffcutter or mechanized rubbing equipment is used. The processing of dried herbs is summarized in Fig. 2.

Packaging and Marketing

After processing, herbs are assessed for quality, according to the buyer's standards; tested according to the Codex Code of Hygienic Practice (CAC 1995) for microbial loads, chemical residues, and moisture content; and then graded accordingly. They are then packed in sacks or polythene plastic bags and stored in dry, dark, and cool areas protected from insects and vermin. Woven polypropylene outer bags, drums, cardboard boxes, or wool bales are used for storing and shipping large quantities. Some herbs are sterilized by gamma (Co-60 or Cs-137) irradiation (e.g., United States) and packed in special boxes according to the guidelines of the producer or the end-user countries (ASTM 2010).

The United States produces nearly 40 % of its annual spice (including dried herbs) needs and imports the rest (Buzzanell et al. 1995) from more than 50 countries; 5 of these countries (Indonesia, Mexico, India, Canada, and China) account for one-half of the annual value of spice imports.

Consumption Practices

Culinary herbs are used widely in food preparation due to their distinctive flavors, aromas, and beneficial effects on human health. They can be added to foods during cooking (hot application) or used as ingredients or garnishes in ready-to-eat dishes

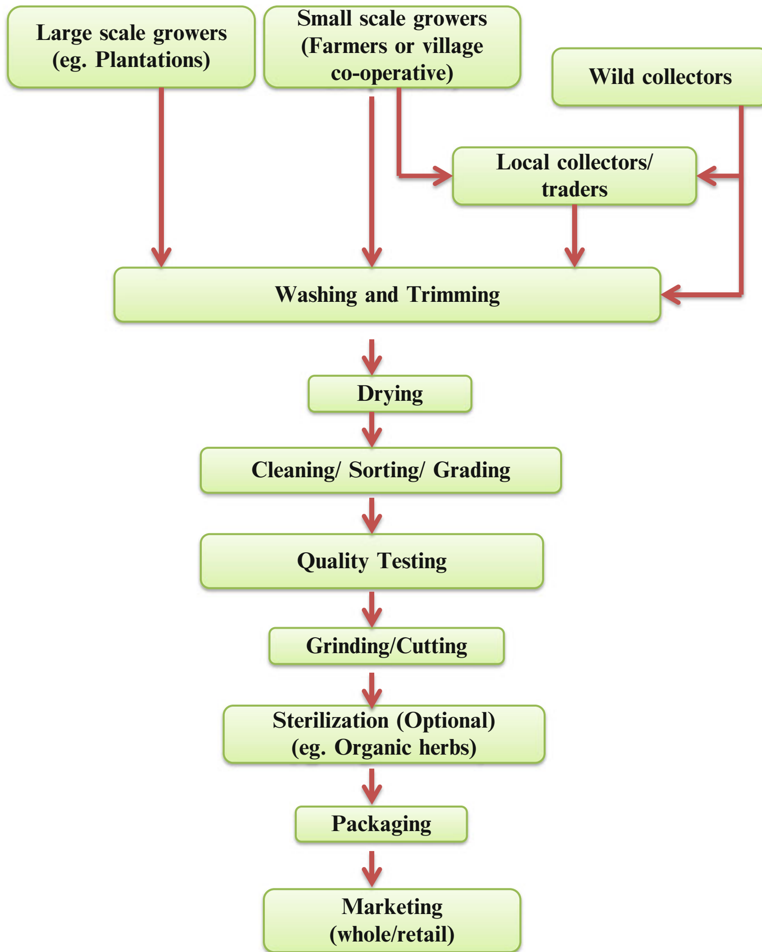

Fig. 2 Overview of postharvest processing of dried herbs

or drinks (cold application). In hot applications, dried herbs are usually added into stews, soups, and sauces during cooking or sprinkled on top of snacks or breads before baking, therefore exposing them to a heat treatment before consumption. In cold applications, dried herbs (flakes) are combined with other ingredients and chilled before being consumed with bread or crackers. For example, Mediterranean white bean hummus is prepared by mixing dried basil leaves with other ingredients and then refrigerated for 1 h prior to serving. Dried flakes are also used as a topping on cooked soup (e.g., chives over the potato leek soup) or sprinkled (raw) directly over bread, pizzas, and soups to enhance flavor.

Microbiological Safety Issues Related to Dried Teas and Herbs

Dried Teas

Dried tea leaves are not free of microorganisms. Any microorganisms in the tea production field could be present on fresh tea leaves. Fresh leaves are harvested either by hand or machine, and microorganisms from hands or harvesting equipment can be transferred to leaves. Without cleaning or washing, leaves are steamed, rolled, and dried, with temperatures above 80 °C (176 °F), which may substantially reduce microbial loads on tea leaves. However, subsequent handling after drying, such as grading and packaging, may result in microbial contamination of the processed dried tea (Wilson et al. 2004; Bouakline et al. 2000). Therefore, certain microbial loads are expected on the dried tea leaves. Reports of the microbiological quality of a variety of commercial tea leaves have revealed a wide range of results with total aerobic bacteria counts ranging from 10^3 to 10^7 CFU/g, sporeformers at 10^2 CFU/g, yeast and molds from 10^1 to 10^2 CFU/g, and coliforms from <3.0 to 10^2 CFU/g. The human foodborne pathogens *Bacillus cereus* and *Staphylococcus aureus* have also been detected in dried tea (Chukeatirote et al. 2004; Donia 2008; Mishra et al. 2006).

The general practices of using freshly boiled water, usually hot enough to kill vegetative foodborne pathogens, in tea preparation, and consuming tea in a relatively short time after preparation may play important roles in the safety record of dried tea. However, consumption of iced tea or sun tea has raised some safety concerns. In 1995, the Cincinnati, Ohio, health department found high total coliform counts and the presence of *E. coli* in a sample of iced tea obtained from a restaurant after a patron complained that the iced tea served there was "off odor" and cloudy (Mohommad and Reynolds 1996). High total coliform counts (ranging from 0 to >200,000 CFU/ml) were also detected in iced tea samples from 20 other area restaurants. Similar tests in at least three other states revealed similar total coliform counts in iced tea samples. In response to local and state health department requests for guidelines on preparing and storing iced tea in a manner to reduce bacterial contamination, the Centers for Disease Control and Prevention (CDC), in 1996, published a "Memo on Bacterial Contamination of Iced Tea" in which it stated "Tea leaves may be contaminated with coliform bacteria. If iced tea is brewed at inadequate temperatures or in an improperly-cleaned urn, or if it is stored for too long, it may grow coliform bacteria, most frequently *Klebsiella* and *Enterobacter*, and less-commonly *E. coli*. In particular, the faucet of iced tea urns may provide a nidus for bacterial contamination" (CDC 1996). The tea industry recommends that iced tea be brewed at 90.5 °C (195 °F) for 3–5 min, that tea be stored for no longer than 8 h, and that the tea brewer, storage dispenser, and faucet be cleaned daily (CDC 1996). In the same memo, the concern over possible inadequate brewing temperatures in the making of sun tea (steeping tea bags in a container of water in the sun) was mentioned, although there has never been any documented foodborne illnesses associated with sun tea consumption.

Dried Herbs

As with many other agricultural plant products, dried herbs are susceptible to foodborne human pathogen contamination at any point throughout their production chain: cultivation, harvesting, processing, packaging, storage, and marketing (McKee 1995). Early contamination may come from field production (cultivation), where contaminated soil or irrigation water, domestic and wild animals, and field workers could all serve as a source of human pathogen contamination. During harvesting and processing, worker hygiene and processing equipment and environmental conditions play important roles in contamination events. A number of outbreaks of foodborne illness have been traced back to fresh herbs. For example, seven outbreaks of *Shigella sonnei* infection associated with eating fresh parsley occurred in the United States and Canada during the summer of 1998 (CDC 1999). Trackback investigation conducted by state and local health departments, CDC, FDA, and Canadian Food Inspection Agency identified farms in Baja California, Mexico, as sources of the contaminated parsley. Field investigations revealed that workers had limited hygiene education and limited sanitary facilities available on the farm at the time of the outbreak and that non-chlorinated municipal water was used in the packing shed for chilling parsley in a hydrocooler immediately after harvest and for making ice with which the parsley was packed for transport (CDC 1999). In 1999, a *Salmonella* Thompson outbreak in California was associated with fresh cilantro (Campbell et al. 2001). *Salmonella* Senftenberg-contaminated fresh basil (grown in Israel and prepacked) was identified as the source of a 2007 outbreak affecting several countries, including the United Kingdom, Denmark, the Netherlands, and the United States (Pezzoli et al. 2008).

Interestingly, dried herbs have not been specifically identified as the sole source of foodborne illness. However, the presence of human pathogenic bacteria in dried herbs is well documented and a potential risk is evident. Surveys on the microbiological safety of dried herbs conducted in Japan (Hara-Kudo et al. 2006), Austria (Kniefel and Berger 1994), Australia (Pafumi 1986), the Netherlands (DeBoer et al. 1985; te Giffel et al. 1996), Mexico (Garcia et al. 2001), the United States (Satchell et al. 1989), the United Kingdom (Sagoo et al. 2009; Little et al. 2003), Spain (Sospedra et al. 2010), and Italy (Vitullo et al. 2011) have revealed the presence of foodborne human pathogens, including a great variety of *Salmonella* serotypes, *L. monocytogenes*, *Bacillus cereus*, *Clostridium perfringens*, *Staphylococcus aureus*, and *Shigella* spp., in these products at either production or retail. For example, in the United Kingdom, among 743 retail dried herb samples tested, 1.4 % were positive for *Salmonella*, 0.28 % contained high counts of *B. cereus* ($\geq 10^5$ CFU/g[1]), 0.7 % had high counts of *C. perfringens* ($\geq 10^3$ CFU/g), and 5.7 % contained high *E. coli* counts ($\geq 10^2$ CFU/g) (Sagoo et al. 2009). In Spain, *Shigella* spp. was detected in 9 % of 23 dried herb samples and 4 % were positive for *S. aureus* (Sospedra et al. 2010). In addition to these survey findings, dried herbs have been recalled at least nine times between 1973 and 2003 because of contamination with *Salmonella* (oregano, 3 recalls; sage, 2 recalls; thyme, 2 recalls; and basil leaves, 1 recall) or *L. monocytogenes* (bay leaves, 1 recall) (Vij et al. 2006).

The presence of *Salmonella* spp. in dried herbs is a major microbiological safety concern due to their high tolerance to desiccation stress, enabling them to survive for extended periods of time on these dried products (Hiramatsu et al. 2005; Lehmacher et al. 1995; Ristori et al. 2007). The risk of human infection is especially high when contaminated dried herbs are added to foods with minimal treatment prior to consumption. Sporeforming bacteria, such as *B. cereus* and *C. perfringens*, are natural inhabitants of the soil environment. Endospores of these species can withstand the drying step of herb processing and, therefore, survive indefinitely until the environmental temperatures are high enough to allow germination and growth. *Shigella* contamination is most likely from contact with human sewage or infected food handlers. Similarly, *S. aureus* is most likely transmitted by direct contact with food handlers during harvesting and processing. *E. coli* is commonly used as an indicator of fecal contamination and its presence on dried herbs may indicate contamination by human and/or domestic or wild animal feces.

Most importantly, the microbiological safety concerns associated with dried herbs are governed primarily by their end use (consumption practices) (Sagoo et al. 2009). Adding dried herbs to many foods before or during cooking presents the least health risk, as cooking heat would kill most of the human pathogenic bacteria, such as *Salmonella*, *Shigella*, and *L. monocytogenes*, although proper temperature and time controls should be in place for the cooked foods, as the spores of *B. cereus* and *C. perfringens* may germinate and cells may multiply. However, the consumption practices of directly adding dried herbs to ready-to-eat (RTE) foods without further heat treatment before consumption poses a risk, as occurred in a *Salmonella* Wandsworth outbreak associated with a snack of puffed rice and corn with a dried vegetable and herb coating (Veggie Booty) in 2007 (CDC 2007). A lot of parsley powder, used as an ingredient in the coating blend, tested positive for the pathogen (Flynn 2009).

Concluding Remarks

As with many agricultural commodities, dried teas and herbs are susceptible to microbial contamination at any point throughout their production chain. The microbial quality and safety of these products at the time of consumption is critically influenced by production conditions, processing treatment, and consumption practices. Dried teas are generally microbiologically safe products, as evidenced by the lack of reported foodborne illness associated with tea consumption and the absence of detectable human pathogens in tea samples in microbial survey data. This safety record is at least partially due to the heat treatments (steaming and drying) applied during tea processing and at the time of consumption (fresh boiled water infusion for several minutes) that can eliminate many vegetative human pathogens, if present. However, dried herbs can be carriers of many different microorganisms of public health concern. Survey results from different countries, and recalls of contaminated dried herbs have revealed the presence of human pathogens such as

Salmonella, *B. cereus*, *and C. perfringens*. The consumption practice of adding dried herbs during cooking should reduce this risk, but bacterial spores such as those of *B. cereus* and *C. perfringens* may survive cooking conditions, germinate due to heat shock, multiply, and produce toxins, if cooked dishes are held at room temperature for too long. However, any consumption practices in which the dried herbs are added directly to ready-to-eat (RTE) foods without further heat treatment before consumption pose risk. As dried herbs are usually consumed in small quantity, as ingredients in a variety of restaurant-prepared or homemade RTE dishes, they can be difficult to implicate in the event of foodborne illness. In more than half of the foodborne illnesses, either sporadic cases or outbreaks, the implicated vehicles remain unknown (Scallan et al. 2011). Nevertheless, the microbial safety issues related to dried herbs have to be addressed.

As the microbial quality of dried plant products is related directly to the conditions of their production at cultivation, harvest, postharvest processing, packaging, storage, and marketing, a systematic approach to control along the entire production chain is essential for reducing the microbial safety risk associated with these products. This approach includes (1) the implementation of good agricultural practices (GAPs) at cultivation, harvesting, and primary processing; (2) the application of good manufacturing practices (GMPs) during processing, packaging, storage, and transportation; and (3) the use of good hygienic practices, such as those recommended in Codex Alimentarius Commission (CAC) during handling (CAC, 1995). Finally, quality checking by the criteria established by either CAC or official agencies that have jurisdiction over the finished products is a critical component of a food safety plan. While few regulations exist currently with respect to acceptable levels of microorganism contamination in dried herbs, the Codex Code of Hygienic Practice for dried herbs and spices specifies that these products should be free from pathogenic microorganisms at levels that may represent a hazard to health (CAC 1995) and that *Salmonella* should be absent in treated ready-to-eat dried herbs and spices. Additionally, the European Spice Association (ESA) has specified that *Salmonella* should be absent in a 25-g sample of spice (ESA 2004), *E. coli* should be present at less than 10^2 CFU/g, and other bacteria should be present only at cell numbers agreed upon by the buyer and seller (Muggeridge and Clay 2001).

Given that most dried herbs are produced in countries where the infrastructure for hygiene is still in development, establishing a sound and systematic safety program can be challenging in the short term. Therefore, a microbial reduction treatment, such as irradiation, fumigation, or heat, which can mitigate the risk of contamination associated with imported dried herbs, is essential (Castro et al. 2011; Hsu et al. 2010; Rushing 2006). These final treatments could be applied only to dried herbs that are destined for RTE applications, thereby reducing potential negative impacts on the flavor, aromas, and antioxidant properties of untreated herbs, as well as on the cost of production (Polovka and Suhaj 2010).

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References

Altman R (2003) Good times for the tea-bagging business. http://www.teaandcoffee.net/0303/tea.htm#prof. Accessed 6 Mar 2013

Anonymous (2003) Tea plant: cultivation, processing, and classification. Imperial Tea Garden. http://www.imperialteagarden.com/CamelliaSinensis.html

Anonymous (2013) Tea packaging, distribution, and trading. United Kingdom Tea Council. http://www.tea.co.uk/tea-packaging-distribution-and-trading. Accessed 6 Mar 2013

ASTM (2010) Standard guide for irradiation of dried spices, herbs, and vegetable seasonings to control pathogens and other microorganisms, vol F1885–04. ASTM International, West Conshohocken, PA

Balentine DA, Wiseman SA, Bouwens LC (1997) The chemistry of tea flavonoids. Crit Rev Food Sci Nutr 37(8):693–704

Bouakline A, Lacroix C, Roux N, Gangneux JP, Derouin F (2000) Fungal contamination of food in hematology units. J Clin Microbiol 38(11):4272–4273

Buzzanell PJ, Dull R, Gray F (1995) The spice market in the United States: recent developments and prospects. Agriculture Information Bulletin No. (AIB-709)

CAC (1995) Code of hygienic practice for spices and dried aromatic plants, Codex Alimentarius Commission Available via http://www.greenfoodec.eu/documents/codeofhygienicpracticeforspicesanddriedaromaticplants.pdf. Accessed 6 Mar 2013

Campbell HR (1963) Tea manufacture. Oxford University Press, London, UK

Campbell HR (1966) Tea growing. Oxford University Press, London, UK

Campbell JV, Mohle-Boetani J, Reporter R, Abbott S, Farrar J, Brandl M, Mandrell R, Werner SB (2001) An outbreak of salmonella serotype Thompson associated with fresh cilantro. J Infect Dis 183(6):984

Castro MF, Rezende AC, Benato EA, Valentini SR, Furlani RP, Tfouni SA (2011) Studies on the effects of phosphine on Salmonella enterica serotype Enteritidis in culture medium and in black pepper (Piper nigrum). J Food Prot 74(4):665–671

CDC (1996) Memo on bacterial contamination of iced tea. Available via http://www.maricopa.gov/envsvc/AboutUs/pdf/Iced%20Tea.pdf. Accessed 6 Mar 2013

CDC (1999) Outbreaks of Shigella sonnei infection associated with eating fresh parsley: United States and Canada, July–August 1998. MMWR Morb Mortal Wkly Rep 48(4):285–289

CDC (2007) Salmonella wandsworth outbreak investigation, June–July 2007. Available via http://www.cdc.gov/salmonella/wandsworth_071107.htm. Accessed 6 Mar 2013

Chukeatirote E, Bankluay K, Kaprom A, Sampanvejsobha S, Winyayong P (2004) Microbiological quality of some tea products in Chiang Rai Province, Thailand. Chiang Mai J Sci 31(2):185–189

Clayton L (2011) How to brew the ultimate sun tea. http://drinks.seriouseats.com/2011/07/how-to-make-sun-tea.html. Accessed 6 Mar 2013

Davidson A (2010) The Oxford companion to food. Oxford University Press, London, UK

DeBoer E, Spielenberg WM, Janssen FW (1985) Microbiology of spices and herbs. Antonie Van Leeuwenhoek 51:435–438

Donia MAA (2008) Microbiological quality and aflatoxinogenesis of Egyptian spices and medicinal plants. Global Vet 2(4):175–181

ESA (2004) European spice association quality minima document. Available via http://www.ipcnet.org/images/ESA-QS191104.pdf Accessed 6 Mar 2013

Fischer A (2010) The way of tea: reflections of life with tea, 1st edn. Tuttle Publishing, North Clarendon, VT

Flynn D (2009) Veggie booty salmonella outbreak. Available via http://www.foodsafetynews.com/2009/09/meaningful-outbreak-9-veggie-booty-salmonella-outbreak/#.UtQGArT7s1I. Accessed 6 Mar 2013

Garcia S, Iracheta F, Galvan F, Heredia N (2001) Microbiological survey of retail herbs and spices from Mexican markets. J Food Prot 64(1):99–103

Hara-Kudo Y, Ohtsuka K, Onoue Y, Otomo Y, Furukawa I, Yamaji A, Segawa Y, Takatori K (2006) Salmonella prevalence and total microbial and spore populations in spices imported to Japan. J Food Prot 69(10):2519–2523
Heiss ML, Heiss RJ (2007) The story of tea: a cultural history and drinking guide. Ten Speed, Berkeley, CA
Hiramatsu R, Matsumoto M, Sakae K, Miyazaki Y (2005) Ability of shiga toxin-producing Escherichia coli and salmonella spp. To survive in a desiccation model system and in dry foods. Appl Environ Microbiol 71(11):6657–6663. doi:10.1128/aem.71.11.6657-6663.2005
Hsu W-Y, Simonne A, Jitareerat P, Marshall MR Jr (2010) Low-dose irradiation improves microbial quality and shelf life of fresh mint (Mentha piperita L.) without compromising visual quality. J Food Sci 75(4):M222–M230. doi:10.1111/j.1750-3841.2010.01568.x
Kaefer CM, Milner JA (2008) The role of herbs and spices in cancer prevention. J Nutr Biochem 19(6):347–361. doi:10.1016/j.jnutbio.2007.11.003
Kniefel W, Berger B (1994) Microbiological criteria of random samples of spices and herbs retailed on the Austrian Market. J Food Prot 57:893–901
Kowalchik C, Hylton WH (1998) Rodale's illustrated encyclopedia of herbs. Rodale, Emmaus, PA
Lai PK, Roy J (2004) Antimicrobial and chemopreventive properties of herbs and spices. Curr Med Chem 11(11):1451–1460
Lehmacher A, Bockemuhl J, Aleksic S (1995) Nationwide outbreak of human salmonellosis in Germany due to contaminated paprika and paprika-powdered potato chips. Epidemiol Infect 115(3):501–511
Little C, Omotoye R, Mitchell R (2003) The microbiological quality of ready-to-eat foods with added spices. Int J Environ Health Res 13(1):31–42. doi:10.1080/0960312021000063331
Majumder AB, Bera B, Rajan A (2011) Tea statistics: global scenario. Int J Tea Sci (IJTS) 8(1):121–124
McKee LH (1995) Microbial contamination of spices and herbs: a review. Food Sci Technol 28(1):1–11. doi:10.1016/S0023-6438(95)80004-2
Mishra BB, Gautam S, Sharma A (2006) Microbial decontamination of tea (Camellia sinensis) by gamma radiation. J Food Sci 71(6):M151–M156. doi:10.1111/j.1750-3841.2006.00057.x
Mohommad AI, Reynolds J (1996) A preliminary investigation report of iced tea contamination. J Environ Health 59(1):6
Muggeridge M, Clay M (2001) Quality specifications for herbs and spices. In: Peter VK (ed) Handbook of herbs and spices. Woodhead Publishing Ltd., Cambridge, UK, pp 13–22
Pafumi J (1986) Assessment of the microbiological quality of spices and herbs. J Food Prot 49: 958–963
Panda H (2011) The complete book on cultivation and manufacture of tea. Asia Pacific Business Press Inc., Kamla Nagar, Delhi
Peter KV (2004) Handbook of herbs and spices, vol 2. Woodhead Publishing Ltd., Cambridge, UK
Pezzoli L, Elson R, Little CL, Yip H, Fisher I, Yishai R, Anis E, Valinsky L, Biggerstaff M, Patel N, Mather H, Brown DJ, Coia JE, van Pelt W, Nielsen EM, Ethelberg S, de Pinna E, Hampton MD, Peters T, Threlfall J (2008) Packed with Salmonella: investigation of an international outbreak of Salmonella Senftenberg infection linked to contamination of prepacked basil in 2007. Foodborne Pathog Dis 5(5):661–668
Polovka M, Suhaj M (2010) The effect of irradiation and heat treatment on composition and antioxidant properties of culinary herbs and spices: a review. Food Rev Int 26(2):138–161. doi:10.1080/87559121003590227
Rasmussen W, Rhinehart R (1999) Tea basics: a quick and easy guide. John Wiley, New York, NY
Ristori CA, dos Santos Pereira MA, Gelli DS (2007) Behavior of Salmonella Rubislaw on ground black pepper (Piper nigrum L.). Food Control 18(3):268–272. doi:10.1016/j.foodcont.2005.10.015
Rolfe J, Cave Y (2003) Camellias: a practical gardening guide. Timber, Portlant, Oregon
Rushing JW (2006) Methods to ensure microbiological safety of organically produced medicinal plants: a review. Hort Sci 41(2):292–295

Saberi H (2010) Tea: a global history. Reaktion Books, London, UK

Sagoo SK, Little CL, Greenwood M, Mithani V, Grant KA, McLauchlin J, de Pinna E, Threlfall EJ (2009) Assessment of the microbiological safety of dried spices and herbs from production and retail premises in the United Kingdom. Food Microbiol 26(1):39–43. doi:10.1016/j.fm.2008.07.005

Satchell FB, Bruce VR, Allen G, Andrews WH, Gerber HR (1989) Microbiological survey of selected imported spices and associated fecal pellet specimens. J Assoc Off Anal Chem 72(4): 632–637

Scallan E, Griffin PM, Angulo FJ, Tauxe RV, Hoekstra RM (2011) Foodborne illness acquired in the United States: unspecified agents. Emerg Infect Dis 17(1):16–22

Sospedra I, Soriano J, Mañes J (2010) Assessment of the microbiological safety of dried spices and herbs commercialized in Spain. Plant Foods Hum Nutr 65(4):364–368. doi:10.1007/s11130-010-0186-0

Suppakul P, Miltz J, Sonneveld K, Bigger SW (2003) Antimicrobial properties of Basil and its possible application in food packaging. J Agric Food Chem 51(11):3197–3207. doi:10.1021/jf021038t

te Giffel MC, Beumer RR, Leijendekkers S, Rombouts FM (1996) Incidence of Bacillus cereus and Bacillus subtilis in foods in the Netherlands. Food Microbiol 13(1):53–58. doi:10.1006/fmic.1996.0007

Vij V, Ailes E, Wolyniak C, Angulo FJ, Klontz KC (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(1):233–237

Vitullo M, Ripabelli G, Fanelli I, Tamburro M, Delfine S, Sammarco ML (2011) Microbiological and toxicological quality of dried herbs. Lett Appl Microbiol 52(6):573–580. doi:10.1111/j.1472-765X.2011.03040.x

Wilson C, Dettenkofer M, Jonas D, Daschner FD (2004) Pathogen growth in herbal teas used in clinical settings: a possible source of nosocomial infection? Am J Infect Control 32(2):117–119. doi:10.1016/j.ajic.2003.09.004

Witten G (2003) Herbal harvest: commercial organic production of quality dried herbs, 3rd edn. Blooming Books, Burnley, VIC

Woodward P (2003) Herbs and spices. The Gale Group Inc. http://www.answers.com/topic/herbs-and-spices. Accessed 6 Mar 2013

Part IV
Product Testing

Regulatory Testing Guidelines and Recommendations

Warren E. Stone

Abstract End-product microbiological testing is rarely required in US food safety regulations. However, as foodborne illness headlines continue to proliferate so do calls from consumer advocacy groups and politicians for regulatory mandates requiring such testing. While end-product microbial testing may appear to be a prudent approach toward ensuring food safety, it is generally understood and accepted by food safety scientists that this alone is not a reliable means for assuring the absence of microbial pathogens due to statistical sampling and technical limitations that must be recognized. Presented here are details on those few regulations that do mandate testing regimens and those regulatory platforms that, while not mandating testing, are best fulfilled by specified uses of the practice.

Keywords Mandatory microbiological testing • Regulations • Verification

Introduction

Over the past decade, regulatory and consumer advocacy drivers have placed an increasing onus on food companies to expand their microbial testing activities, with the objective of a reduction in foodborne illness outbreaks, thus protecting the public health. In response to two major foodborne illness outbreaks associated with the state's marquis product, peanuts, the Georgia Department of Agriculture released new regulations mandating all food processing plants in the state to initiate end-product testing of all food products in 2010. The new regulations supported state legislation, which was passed that spring. The law states that all positive test results must be reported to the Georgia Department of Agriculture, even if the product was not distributed and the problem was corrected. In an effort described on her website as "To reduce the risk of *E. coli* ending up in the hamburgers and

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other food we eat …," Senator Kirsten Gillibrand (D-NY) attempted to author the *E. Coli Eradication Act*—new legislation that would require all plants that process ground beef to test their products regularly before it is ground and again before it is combined with other beef or ingredients (Gillibrand 2010). In January 2000, the Center for Science in the Public Interest petitioned the United States Department of Agriculture (USDA) "to require companies producing ready-to-eat meat and poultry products to test their… final products for the presence of *Listeria monocytogenes"* (CSPI 2000).

While microbial testing of finished product may, on the surface, appear to be a prudent approach toward ensuring food safety in a variety of products, the trained scientist and statistician would wholeheartedly disagree. It is generally understood and accepted by food safety scientists throughout the world that finished product testing alone will not deliver safe food; product testing alone is not a reliable means for assuring the absence of microbial pathogens (ICMSF 2002). Even the United States Department of Agriculture's Food Safety and Inspection Service (USDA, FSIS) in their Federal Register announcement regarding testing for six Shiga toxin-producing *Escherichia coli* (STEC), in addition to *E. coli* O157:H7, in raw beef manufacturing trimmings beginning June 4, 2012, stated, "FSIS acknowledges that the best approach to reducing STEC contamination lies not in comprehensive end-product testing but in the development and implementation of science-based preventive controls, with end-product testing to verify process control" (USGPO 2012).

The routes by which adulterants contaminate food are often complex and not well understood. Likewise, their distribution within foods is typically not homogeneous. Microbiological testing of finished food products has statistical sampling and technical limitations that must be recognized. As the amount of microbial contamination becomes lower, the chances of accurately identifying contaminated food through sampling decrease, especially when the contamination is not uniformly distributed throughout the food, as is typically the case (Moorman 2011). In addition, current testing methods may lack the sensitivity to find low-level contamination. As a result, negative test results do not prove that a food is not contaminated with pathogens. Based on these limitations, the margin of perceived safety derived from finished product testing results is often exaggerated, and the scientific limitations of the sampling plan are all but forgotten. When an inadequate sampling and testing scheme is applied to a food with limited understanding of the route(s) of contamination and distribution, the lot deemed to be "acceptable" may be proved "unacceptable" if more samples are analyzed.

Safety can neither be tested nor inspected into the finished product. Moreover, it is also understood that safety, quality, and effectiveness must be designed and built into the product and process; each step of the manufacturing process should be controlled to maximize the probability that the finished product meets all safety, quality, and design specifications. Food product safety is not a result of testing but rather is obtained through the implementation of robust preventive controls based on in-depth

hazard analyses of all components of the manufacturing process and supply chains (Moorman 2011). Consequently, finished product testing and inspection regimes, by themselves, are generally incompatible with assuring public health expectations for safe food. The sampling plans that underpin the testing and inspection regimens cannot prevent the release and acceptance of defective materials.

The reality of this situation was one of the reasons the Pillsbury Company, the US Army Natick Laboratories, and the National Aeronautics and Space Administration (NASA) developed the Hazard Analysis and Critical Control Point (HACCP) food safety system more than 45 years ago. Sampling, and subsequent microbial analysis, of finished product in order to establish microbiological safety of each batch of astronaut's food proved to be impractical, if not impossible. To help quantify the impracticality of attribute sampling and the resultant destructive testing of end product that would be necessary to assure microbiological safety, the following example is provided. Consider a batch of food in which *Salmonella* is present in one out of every 1,000 units (defect rate = 0.1 %). A sampling plan that analyzed 60 units from the batch and rejected the batch if one positive (*Salmonella* present) sample were discovered would have a greater than 94 % probability of acceptance; the *Salmonella* would not be detected 94 % of the time (Scott and Stevenson 2006).

In addition to the statistical evidence that this sampling plan would be ineffective in detecting the contaminated product, there is the practical and economic reality that many firms would not be able to destructively test 60 units out of every batch of product for the presence of pathogenic bacteria. Thus, an alternative approach had to be developed in order to obtain the level of assurance of product safety that NASA required for foods produced for the space program (Scott et al. 2006). The concept of HACCP was born and has become a mainstay of food safety systems worldwide. HACCP plans typically do not utilize end-product microbial testing as a control measure but rather as a verification practice to determine if the system is functioning properly (NACMCF 1997).

From a regulatory perspective, conditions whereby the food *could* have been contaminated are enough to deem the food to be adulterated according to the Federal Food, Drug, and Cosmetic Act (USGPO 1938). There is an inclination by many to utilize microbial analysis for the presence of an adulterant when an unexpected event occurs. Testing to reveal the absence of the adulterant will not change the fact that conditions existed whereby the food *may* have become adulterated. While testing in these circumstances can be informative in guiding and supporting decisions pertaining to the safety of the food, an acceptable test result does not mean the event did not occur and, consequently, cannot be used as the sole determinant of the acceptability of the food (Moorman 2011).

End-product microbiological testing is rarely required in US food safety regulations. Nevertheless, limited situations exist whereby explicit microbial evaluation is obligatory in US regulations; however, none of these mandates currently address low-moisture foods.

US Regulatory Requirements for Finished Product Microbiological Testing

While relatively scarce, there are limited codified federal requirements for microbiological testing found in various US food safety rules. Mandatory microbial testing requirements can be found in:

1. Title 9 Code of Federal Regulations (CFR) Part 430, United States Department of Agriculture Food Safety Inspection Service Requirements for Special Classes of Product (also known as "Control of *Listeria monocytogenes* in post-lethality exposed ready-to-eat products").
2. FSIS Process Control Verification Criteria and Performance Standard Regulations for Meat and Poultry Products, 9 CFR Parts 310.25, 381.94 (*Salmonella* and *Campylobacter* performance standards).
3. Title 21CFR Part 118, Production, Storage, and Transportation of Shell Eggs, http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm.
4. 21 CFR Part 120.25, Hazard Analysis and Critical Control Point (HACCP) Systems (Juice HACCP), Process verification for certain processors, http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=120.25.
5. 21 CFR Part 129, Processing and Bottling of Bottled Drinking Water, http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm.
6. Public water testing systems for food use: FDA 21 CFR Part 110.37, USDA 9 CFR 416. 2 (g)(1), and FDA Food Code 5-102.13, http://www.fda.gov/downloads/Food/GuidanceRegulation/RetailFoodProtection/FoodCode/UCM374510.pdf.

USDA FSIS Control of Listeria monocytogenes *in Post-lethality Exposed RTE Products*

One of the more routinely employed governmental mandated testing programs is the program found in Title 9 CFR Part 430—FSIS Requirements for Specific Classes of Product, also known as "Control *of Listeria monocytogenes* in post-lethality exposed ready-to-eat (RTE) products." To comply with the requirements of 9 CFR 430, FSIS developed corresponding compliance guidelines to help establishments producing RTE meat and poultry products, especially small and very small establishments, in their use of control methods for *L. monocytogenes.* The regulation requires producers of post-lethality exposed RTE products to conduct environmental monitoring of food-contact surfaces for the presence of *L. monocytogenes* and prescribes corrective action guidelines that must be followed in the event of positive findings. Depending on the product and the situation, some of these corrective actions may involve additional finished product testing for *L. monocytogenes.* Establishments are further required to maintain a record keeping system that can

match sample test results to finished products. The testing frequency and intensity of the corrective actions are delineated in the guidance and depend on the type of product being produced (FSIS 2006).

USDA FSIS Process Control Verification Criteria

Another example of mandatory microbiological testing in the food industry is FSIS' Pathogen Verification Criteria regulations, 9 CFR 310.25 and 9 CFR 381.94. These regulations address livestock and poultry slaughter, respectively. Part (a) of each regulation states, "Each official establishment that slaughters livestock (poultry) must test for *Escherichia coli* Biotype 1 (*E. coli*)." The regulations then proceed to delineate sampling requirements, sampling frequencies, and recording of test results. Also addressed in these regulations are pathogen reduction performance standards for *Salmonella*. These are performance standards based upon the microbial testing results from sampling performed by FSIS; testing to demonstrate compliance with the standards is not mandatory, as are the requirements of the two regulations discussed above. Testing to monitor performance standards will be discussed later in this chapter (FSIS 1996).

Prevention of **Salmonella** *Enteritidis in Shell Eggs During Production, Storage, and Transportation: 21 CFR Part 118*

Beginning in May 2010, the Center for Disease Control and Prevention (CDC) identified a nationwide, fourfold increase in the number of *Salmonella* Enteritidis isolates through PulseNet, the national subtyping network made up of state and local public health laboratories and federal food regulatory laboratories. CDC received reports of approximately 200*S*. Enteritidis cases every week during late June and early July. This compared to an average of approximately 50 reports of *S*. Enteritidis to the CDC each week over the previous 5 years. Many states also reported similar increases since May 2010. The subsequent investigation identified positive environmental samples, along with the DNA fingerprint, also known as pulsed-field gel electrophoresis typing, indistinguishable from the outbreak strain, indicating that two producers were likely sources of the contaminated shell eggs. CDC reported that from May 1 to November 30, 2010, over 1,900 illnesses were reported that were likely associated with this outbreak. The result of these events resulted in the promulgation of new regulations and guidance documents from FDA: Guidance for Industry: Prevention of *Salmonella* Enteritidis in Shell Eggs During Production, Storage, and Transportation and 21 CFR Part 118.

This December 2011 guidance provides recommendations for compliance with the regulation 21 CFR Part 118; Production, Storage, and Transportation of Shell Eggs

(http://www.fda.gov/Food/GuidanceComplianceRegulatoryInformation/GuidanceDocuments/FoodSafety/ucm285101.htm#B). The regulation mandates environmental testing for *Salmonella* Enteritidis in the pullet growing areas (118.4(a)(2)) and growing areas for mature hens (118.5). Additionally, the regulation requires egg testing for *Salmonella* Enteritidis (118.6). In authoring the regulation, FDA also spelled out required sampling methodology for *Salmonella* Enteritidis (118.7) as well as recordkeeping provisions for the *Salmonella* Enteritidis prevention plan (118.10) (FDA 2011).

The *Salmonella* quarterly and annual reports can be accessed at http://origin-www.FSIS.USDA.gov/Science/Quarterly_Salmonella_Results/index.asp.

21 CFR Part 120.25, Hazard Analysis and Critical Control Point (HACCP) Systems (Juice HACCP)

The FDA Juice HACCP regulation (21 CFR Part 120) requires in section 120.8(a) that "every juice processor have and implement a written HACCP plan whenever a hazard analysis reveals that one or more food hazards are reasonably likely to occur." Subpart B of the regulation further requires a performance standard of a five-logarithm (5-log) reduction in target microbial pathogens, termed "pertinent organism" in the regulatory language. FDA further defined the "pertinent microorganism" as "the most resistant microorganism of public health significance that is likely to occur in the juice (120.24(a))." Most processors comply with this requirement by employing validated pasteurization procedures (FDA 2002).

FDA did leave the door open to any juice producer who could meet the 5-log performance standard without using pasteurization, however. In doing so, they authored Part 120.25 of the regulation, which addressed those processors that rely on treatments that do not come into direct contact with all parts of the juice to achieve the 5-log reduction standard. For those processors that choose this option, FDA, in section 120.25, requires analysis of the finished product for biotype 1 *Escherichia coli*. As in Part 118, Shell Egg Rules, the agency prescribed sampling, testing, and corrective action protocols as well.

Bottled Water Regulations: 21 CFR Part 129, Processing and Bottling of Bottled Drinking Water

Under their statutory authority, FDA regulates bottled water as a food. The Federal Food, Drug, and Cosmetic Act (FD&C Act or the Act) provides FDA with broad regulatory authority over food that is introduced or delivered into interstate commerce. Under the FD&C Act, manufacturers are responsible for producing safe, wholesome, and truthfully labeled food products, including bottled water products.

FDA has established specific regulations for bottled water in 21 CFR. These regulations include standard of identity regulations in 21 CFR 165.110(a) that define different types of bottled water, such as spring water and mineral water, and standard of quality regulations in 21 CFR 165.110(b) that establish allowable levels for chemical, physical, microbial, and radiological contaminants in bottled water. FDA also has established current Good Manufacturing Practice (cGMP) regulations for the processing and bottling of bottled drinking water in 21 CFR Part 129.

In May of 2009, FDA published a final rule in the *Federal Register* (74 FR 25664, http://www.gpo.gov/fdsys/pkg/FR-2009-05-29/pdf/E9-12494.pdf) that amended part 129 to require bottled water manufacturers to test *source* water for total coliform on a weekly basis (129.35(a)(3)). If any coliform organisms are detected, manufacturers must determine whether any of the organisms are *Escherichia coli* (*E. coli*), regarded by FDA as an indicator of fecal contamination. FDA's final rule also amended its bottled water regulations to require, if any coliform organisms are detected in *finished bottled water products*, that manufacturers determine whether any of the coliform organisms are *E. coli*. Bottled water containing *E. coli* will be considered adulterated. Source water containing *E. coli* will not be considered to be of a safe, sanitary quality and will be prohibited from use in the production of bottled water. FDA also requires that before a bottler can use source water from a source that has tested positive for *E. coli*, the bottler must take appropriate measures to rectify or eliminate the cause of *E. coli* contamination of that source and that the bottler must keep records of such actions.

Water Testing

Purity of the water supply for food processing facilities is addressed by both the FDA and USDA FSIS. FDA regulation 21 CFR Part 110.37 states, "…The water supply … shall be derived from an adequate source. Any water that contacts food or food-contact surfaces shall be safe and of adequate sanitary quality. Running water … shall be provided … for the processing of food, for the cleaning of equipment, utensils, and food-packaging materials, or for employee sanitary facilities." Similarly, FSIS' Sanitation Performance Standard's 9 CFR 416.2 (g)(1) asserts "A supply of running water that complies with the National Primary Drinking Water regulations (40 CFR part 141), at a suitable temperature… must be provided in all areas where required...."

Processors using municipality-supplied water can comply with these regulations by having the municipality supply them with certificates of potability or similar documents as well as microbial test results. Operators in more rural areas, however, often supply their factories with water from wells. In these cases, various microbiological testing regimens may be required to satisfy FDA and FSIS regulations or other requirements of states, counties, or water districts. The above-referenced FSIS regulation goes on to declare "If an establishment uses a private well…, it must make available to FSIS … documentation certifying the potability of the water … at

least semi-annually." FDA's 2013 Food Code also states in section 5-102.13 that "… water from a nonpublic water system shall be sampled and tested at least annually and as required by state water quality regulations."

If a food processing facility is supplying water for consumption by over 25 people, customers, employees, and guests, they are operating a "public water system" (PWS) and are regulated by the Environmental Protection Agency's (EPA) requirements, 40 CFR Part 141. The (EPA) National Primary Drinking Water Regulations (http://water.epa.gov/drink/contaminants/index.cfm) are legally enforceable standards that apply to public water systems. While these standards contain an exhaustive list of potential contaminants, microbiological and otherwise, which operators must conform to, nowhere in the EPA regulations is mandatory testing spelled out. EPA Drinking Water Standards can be found at www.epa.gov/safewater/mcl.html Environmental Protection Agency (2012).

Regulatory Foundations for Common Microbiological Testing Regimens

There are several situations in food safety management where microbiological testing may satisfy a regulatory requirement even though the regulation does not direct testing for a specific organism with an explicit sampling plan such as those situations mentioned above. Some of these requirements can be found in regulations that have been with us for awhile, and others may manifest themselves in regulations that must be promulgated by legislative directive through the Food Safety Modernization Act (FSMA).

FSMA: A New World in Regulatory Relations

In January of 2011, President Obama signed the Food Safety Modernization Act (FSMA). FSMA represents the most significant expansion of food safety requirements and FDA food safety authorities since the original enactment of the Food, Drug, and Cosmetic Act in 1938. The legislation grants FDA a number of new powers, including mandatory recall authority. The legislation further requires FDA to undertake more than a dozen rulemakings and issue at least 10 guidance documents, as well as a host of reports, plans, strategies, standards, notices, and other tasks (FSMA 2011).

One of the foremost sections of FSMA is section 103, Hazard Analysis and Risk-based Preventive Controls. Section 103 states "The owner, operator, or agent in charge of a facility shall verify that the preventive controls are effectively and significantly minimizing or preventing the occurrence of identified hazards, including through the use of environmental and product testing programs and other appropriate means." Section 103 also mandates verification programs which, among other things, specify "the preventive controls implemented … are effectively and significantly minimizing or preventing the occurrence of identified hazards."

Microbiological testing, such as in an environmental monitoring program (EMP) or finished product microbial analysis, could become widespread verification activities throughout the industry. While not specifically directed verbatim through the legislation, these regimens may be used, at least in part, to satisfy FSMA regulatory requirements.

Preventive controls utilizing microbial testing that result from a hazard analysis, such as the ones that shall be mandated once FSMA regulations are solidified, may not all manifest themselves as critical control points (Scott 2012). Case in point: FSMA mentions environmental monitoring, training, and supplier verification activities as examples of preventive controls (FSMA). HACCP guidelines issued by the National Advisory Committee on the Microbiological Criteria for Foods (NACMCF) and Codex both designate validation as a verification activity (NACMCF 1996; Codex 2003). Historically, validation has yielded the science behind critical limits (CLs) employed at critical control points (CCPs) (e.g., 70 °C for 15 s). However, since the FSMA, as part of its verification requirements, directs processors to have validation for *all* preventive controls, validation requirements may need to address the entire food safety system, not just the CLs that support CCPs (Scott 2012). In our future regulatory environment, justification for why a hazard is not reasonably likely to occur may be viewed as a form of validation. Some of these preventive controls could be classified as prerequisite programs, the foundation for effective food safety plans, such as HACCP, to operate effectively. Prerequisite programs typically do not lend themselves to traditional validation protocols (Stone 2012).

Consider a thermal process designed to eliminate a pathogenic microbe. Traditional validation would include inoculating the target food matrix with the organism of concern, applying the thermal process mitigation, and quantitatively evaluating the resulting product for the presence of the organism. This approach would not be practical for something like an environmental monitoring program; one would not inoculate the processing environment with pathogenic bacteria and see if their EMP were able to detect and eliminate the organism (Stone and Meyer 2011). Such conundrums of microbial validation will have to be further defined as FSMA transitions from law to regulation.

FSMA Update: On January 16, 2013, FDA published two proposed regulations resulting from the FSMA legislation. The proposed rules establish standards for growing, harvesting, packing, and holding of produce for human consumption (the produce safety proposed rule) and current good manufacturing practice and hazard analysis and risk-based preventive controls for human food (the preventive controls proposed rule). These are the first of several proposed rules that would establish the foundation of, and central framework for, the modern food safety system envisioned by Congress in the FSMA.

The produce rule, formally known as “Standards for the Growing, Harvesting, Packing, and Holding of Produce for Human Consumption Proposed Rule,” contains two significant testing provisions. The proposed regulation would require that all agricultural water be safe and of adequate sanitary quality for its intended use. “Agricultural water” would be defined in part as water that is intended or likely to contact produce or food-contact surfaces. The proposed rule would require that, at the

beginning of the growing season, the agricultural water system components under a farm's control be inspected to identify conditions that are reasonably likely to introduce pathogens to produce or food-contact surfaces. FDA is proposing that specific criteria be established for water that is used for certain purposes, with proposed requirements for periodic analytical testing (proposed sections 112.44 and 112.45).

When agricultural water is used for sprout irrigation water, is applied in a manner that directly contacts sprouts during or after harvest, is used to make a treated agricultural tea (fertilizer), touches food-contact surfaces, or is used for hand washing during or after harvest, the water must be tested using an appropriate analytical method. If there is any detectable generic *E. coli* in a 100-ml sample, the water source must immediately be discontinued and specified follow-up actions must be taken. Follow-up actions may include making changes to the system and re-testing or treating the water.

When agricultural water directly contacts produce, other than sprouts, during growing activities, microbial testing must demonstrate that there are fewer than 235CFU generic *E. coli* per 100 ml for any single sample, or a rolling geometric mean of not more than 126CFU per 100 ml, or the source must be immediately discontinued and the processor must take specified follow-up actions (http://www.fda.gov/Food/FoodSafety/FSMA/ucm334552.htm#E).

On the same day, FDA released "Current Good Manufacturing Practice and Hazard Analysis and Risk-Based Preventive Controls for Human Food Proposed Rule" (USGPO 2013). Even though the FSMA statute mentions microbial testing, both environmental testing and product testing, in this proposed regulation, FDA did not delineate any specific requirements for microbial testing. Instead, they included an Appendix titled "The Role of Testing as a Verification Measure in a Modern Food Safety System" where they state, "Although the proposed rule that is the subject of this document does not include provisions for environmental monitoring or finished product testing, we believe that these regimes can play a critical role in a modern food safety system." This Appendix discusses potential roles of microbial testing in a food safety plan. In the publication of the proposed regulation, the agency asked for comments on such potential roles for microbial testing as part of a food safety program. FDA has further stated publicly that the final regulation will contain requirements for the use of microbial testing as part of an overall food safety plan (Scott and Kraemer 2013). Included in this Appendix is a discussion regarding the practice of testing for *Salmonella* in the facility environment for processors of low-moisture foods.

Environmental Monitoring

Environmental monitoring is a multifaceted process that can encompass testing for pathogens or indicator organisms (such as aerobic plate count, total coliforms, generic *E. coli*, etc.) in the factory surroundings. Machinery and equipment, utensils, personnel, buildings, floors, air (both passive and compressed), and other

features in the facility may be the target of such a plan. Environmental monitoring programs (EMPs) are not control programs per se but typically are used for verification of food safety activities in a manufacturing establishment. An EMP that includes all the necessary steps to be successful can aid in validating the effectiveness of the sanitation procedures, employee practices, overall GMPs, and other product protection programs (Stone and Meyer 2011).

EMPs can be valuable tools in the fight against foodborne disease in low-moisture food operations. Numerous foodborne illness outbreaks involving low-moisture foods in the last few decades have revealed (1) the ability of *Salmonella* to survive and cross-contaminate in manufacturing environments and (2) that prevention of cross-contamination is aided by the absence of water, even during cleaning and sanitation activities. Water in the dry-processing environment is one of the most significant risk factors for *Salmonella* contamination because the presence of water allows the pathogen to grow in the environment and significantly increases the risk for product contamination. In a low-moisture manufacturing facility, water is the enemy. This is contrary to wet food processors that typically stop all operations and clean and sanitize their equipment with validated water-based sanitation procedures on a routine basis, often daily. For the wet processor, when properly managed, this creates the separation of foods processed into cleanup to cleanup lots. Low-moisture food processors do not have this luxury of "lot separation" via daily wet sanitation. The combination of these risk factors demonstrates the need for an EMP in a low-moisture operation to verify that all food safety controls are working in harmony to deliver safe product to the consumer.

Certain parts of the food industry, such as ready-to-eat (RTE) meat and poultry and shell eggs, as mentioned earlier, have regulatory requirements for environmental monitoring. These regulations designate the actual tests to be conducted and the testing frequencies that must be adhered to. For most of the industry, including low-moisture foods, environmental monitoring is not a regulatory mandate but has gained popularity in many segments, most notably in the production of RTE foods. The current FSMA legislation requires FDA to promulgate regulations to enhance the protection of the nation's food supply. One provision of this act is the identification of preventive controls to mitigate hazards identified during a facility's hazard analysis. The FSMA has listed environmental monitoring as a control measure that "may" be employed, though most industry practitioners regard EMPs as verification procedures (FSMA).

The decision to incorporate an EMP into a company's food safety plan for the manufacture of RTE products is often done following a product and process risk evaluation (Stone and Meyer 2011). The ability of pathogens to persist in the finished food following packaging and throughout the supply chain is a primary determinant for the incorporation and rigor of an EMP.

In the USA, the meat industry was among the first to implement an industry-wide program to address the presence of *Listeria* spp. in the processing environment and on product-contact surfaces as a verification tool to ensure that control programs were effective in preventing potential cross-contamination of finished products. Through collaborative efforts between food companies, industry associations, and

regulatory agencies, the industry was able to aggressively pursue a "seek and destroy" approach to identify possible harborage(s) of pathogenic microbes (Butts 2003). Recent data published by the Centers for Disease Control and Prevention (CDC) reveal a reduction in the number of outbreaks involving *L. monocytogenes* contamination of RTE meat since this approach has been implemented (CDC 2011).

In the author's experience, 5–10 years ago, very little environmental testing and monitoring was conducted in food plants, if at all. Food companies now recognize that the control of the in-plant environment is critical to the production of safe food. As a result, there has been a marked increase of in-house environmental control programs for *Listeria* species, *Salmonella* species, and aerobic plate counts. Thus, while not a regulatory requirement for most of the industry, EMPs are useful programs, which have gained considerable popularity over the past several years that certainly could provide another level of assurance to low-moisture food processors, and may serve to satisfy verification requirements, which may stem from the FSMA.

A survey was conducted in May 2007 to obtain information from members of the Grocery Manufacturers Association (GMA) on current practices and measures the industry employs to control *Salmonella* in manufacturing low-moisture products. The survey included producers of items such as cereal, chocolate, spray-dried milk, infant formula, and peanut butter. A total of 17 companies/plants responded to the survey. All respondents (100 %) had standard operating procedures to eliminate or minimize cross-contamination from raw ingredients or from the environment. Sixteen of 20 respondents had an EMP addressing non-product-contact surfaces for the presence of *Salmonella*; two of the 16 respondents (12.5 %) monitored *Salmonella* on product-contact surfaces on a routine basis. Fifteen of 17 respondents (88 %) had an environmental monitoring program for non-product-contact surfaces (GMA 2009).

E. coli *O157 H:7 and Non-O157 Shiga Toxin-Producing* E. coli *(STEC)*

In March of 2012, FSIS declared six non-O157 STEC serovars (O26, O45, O103, O111, O121, and O145) to be adulterants in raw non-intact beef products and product components. On June 4, 2012, FSIS initiated a testing program for these six non-O157 STECs in beef manufacturing trimmings, derived from cattle slaughtered on-site. Details of the program are described in FSIS Notice 63-12 (USDA 2014).

In this notice, FSIS indicated that they will treat positive test results for relevant non-O157 STEC the same as *E. coli* O157:H7-positive test results. With the issuance of this notice, FSIS began scheduling for-cause Food Safety Assessments (FSAs) in response to FSIS non-O157 STEC-positive results (confirmed presence of non-O157 STEC). If it is determined, during either a routine or a for-cause FSA, that an establishment cannot support its decisions regarding controlling adulterant STEC, regulatory or enforcement actions may result.

Additionally, FSIS indicated they would verify that establishments reassess their HACCP plans, when required as part of 9 CFR 417.3(b) corrective actions, in response to FSIS- or establishment-positive non-O157 STEC results. Alternatively, establishments can provide scientific support that their existing controls for *E. coli* O157:H7 effectively control the non-O157 STEC and demonstrate that the establishment is effectively implementing those controls.

If FSIS or the establishment found a product positive for either *E. coli* O157:H7 or non-O157 STEC and the establishment held the product or maintained control of the product, pending its own test results, FSIS would normally not take regulatory action, but all situations are evaluated on a case-by-case basis. For these reasons, a prudent establishment producing non-intact beef products or components may want to consider testing product(s) for pathogenic STEC in certain situations as a verification measure that its actions designed to control these microbes are effective. While there is no regulatory mandate for establishments to microbially test their non-intact beef items and their components, failure to have a robust, thorough, and comprehensive verification program could result in FSIS regulatory action against the firm.

Verification and Validation

The FDA defines validation as: "… documented evidence which provides a high degree of assurance that a specific process will consistently produce a product meeting its determined specifications and quality characteristics" (http://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm070336.pdf). Validation is an essential element in the establishment and implementation of a process procedure, as well as in determining what process controls are required in order to assure conformance to specifications. Successfully validating a process may reduce the dependence on more intensive in-process and finished product testing. "Processes" to be validated can run the gamut from a sanitation standard operating procedure (SSOP) designed to provide clean utensils to a low-acid canned food thermal process designed to destroy spores of *Clostridium botulinum*. Testing, while not the framework of a robust food safety program, is indeed an important tool to help processors validate a variety of food safety processes. While not specifically mandated in regulatory language, many such testing schemes have their base in such guidance.

Inoculation and challenge testing are common components of process validation throughout the food industry, be it for restaurants or food processors. Restaurants wanting to handle product differently than the procedures recommended in the FDA Food Code (e.g., refrigerated to unrefrigerated holding, extending shelf life, etc.) must submit the data to a state or local regulatory agency or directly to the FDA in the form of a variance application. State and local regulators who evaluate a variance application, based on laboratory evidence, need criteria to help them determine whether the study was adequately designed and whether the conclusions

are valid (NACMCF 2010). These regulatory agencies routinely use inoculation and challenge testing to determine the validity of such changes with respect to protecting the public health. NACMCF (2010) has recommended approaches to performance of such studies.

FDA HACCP regulations for both juice and seafood (21 CFR Part 120 and 123) have requirements for ongoing verification activities. Both regulations list “end-product or in-process” testing as a means of satisfying these requirements. In both regulations, such testing is allowed to be “at the option of the processor” (120.11(a)(1)(iii) and 123.8(2)(iii)). This type of independent confirmation of a pathogen lethality treatment can serve as a periodic, ongoing form of verification in various food safety/HACCP plans.

Validation of Cleaning and Sanitation Procedures

Validated cleaning and sanitization procedures are an integral part of an effective food safety program. Within a food safety program, a cleaning procedure would be considered “validated” when the procedure is shown to adequately remove organic residues, and in some cases food allergens, from food-contact surfaces whereas a validated sanitization procedure is one shown to remove targeted microbes. A validated process needs to be documented to assure consistent implementation. Once a visually clean status can be achieved on a reliable basis, additional validation should be provided to demonstrate that targeted microbes are removed when SSOPs are followed (Stone et al. 2009). This is especially prudent in the production of low-moisture foods, which typically avoid the introduction of water and employ “dry” cleaning techniques. Dry-cleaning methods can be especially difficult to validate.

In addition to using approved microbial surface analysis techniques such as swabbing or sponging for SSOP validation, one protocol that can be employed is to validate SSOP effectiveness by analyzing the first product produced after a changeover for the presence of the targeted microbe(s). These are typically microbiological hygienic indicator assays rather than pathogens. While often not applicable to low-moisture food producers, when using clean-in-place (CIP) systems, microbial examination of the final rinse water can also serve to validate an SSOP. Unfortunately, when using microbial surface swabs, it is impossible to predict how much contamination might exist in the finished product, based on a positive swab test of a food-contact surface. When using this technique, it is advisable to swab areas that are especially difficult to clean. “Dead spots” in the processing systems (e.g., valves, joints, and corners) present cleaning challenges and should be included in the sampling plan. A positive swab test would indicate that more cleaning is needed until a negative result is obtained. For low-moisture food producers, care must be taken that the swabbing/sponging technique does not introduce liquid into the processing environment (GMA 2009, 2010).

Validation is particularly important in the case of “push-through” or “purge” situations, a technique routinely employed by some low-moisture food processors.

In some cases, involving push-through of thick, highly viscous liquids, allergen residues have been found hours after changeover (Taylor and Hefle 2005), and thus a similar situation could exist for microbial contamination. In other cases, however, push-through has been effective.

After initial microbial validation, the sampling and testing protocol should be repeated occasionally to verify continued successful performance. Any changes in the SSOP or the detection of the microbe of concern may require reassessment and possibly revalidation. Changes in product formulation, preparation procedures, or processing operations may also trigger a need for reassessment of the SSOP through microbial analysis (Stone et al. 2009).

Adherence to Performance Standards

Other than the complete absence of a pathogen in a certain size sample, almost all food safety regulations fall short of an actual solid, quantitative value for microbial thresholds, such as "APC 500/g maximum." In place of this, many government regulations specify a performance standard that processors must achieve. A performance standard is an expression of the performance requirement(s) or expectation(s) that must be met to satisfy a food safety objective. Sometimes, regulatory agencies will offer process recommendations designed to achieve performance standards. For example, USDA FSIS' *Appendix A: Compliance Guidelines For Meeting Lethality Performance Standards For Certain Meat And Poultry Products* offers time-temperature combinations that meet the lethality performance standards for the reduction of *Salmonella* contained in Title 9 CFR Part 318.17(a)(1) and 381.150(a)(1) of the meat and poultry inspection regulations, which require a 6.5-log or 7-log reduction of *Salmonella* in cooked beef products (FSIS 1999).

Other performance standards are less specific. FDA's Juice HACCP regulations (21 CFR Part 120) contain a requirement for a 5-log reduction in the pertinent microorganism. FDA stopped short of telling processors how to achieve this standard; it simply mandated the objective that must be achieved.

Some performance standards are mandated by regulations other than common food safety rules. As a result of two *Salmonella* incidents in 2001 and 2004, the California almond industry approved a mandatory pasteurization plan for almonds. A voluntary industry initiative called for a change in the outgoing quality standards under the federal marketing order for almonds. On February 3, 2006, the Almond Board of California's (ABC) Board of Directors unanimously approved submitting the regulatory change to the USDA. On August 22, 2006, the Board of Directors amended several provisions of the previous regulatory language, and the amendments were submitted to the USDA in September 2006. The final rule was published in the Federal Register, on March 30, 2007, with an implementation date of September 1, 2007 (ABC).

The ABC determined, based on a risk assessment conducted for the ABC and reviewed by FDA, that a 4-log reduction in *Salmonella* was an appropriate level of

control for the processing of raw almonds. In other words, the ABC, in conjunction with FDA, established a performance standard. This standard was adopted by the USDA Agriculture and Market Services (AMS 2007) as an amendment to Marketing Order No. 981 (7 CFR part 981) regulating the handling of almonds grown in California (Federal Register). In the USA, a federal marketing order is a regulation of an executive agency, which sets prices and other conditions for the sale of certain goods. Accordingly, the 4-log reduction in *Salmonella* became a requirement under Title 7 CFR 981.442, quality control requirements for almonds grown in California.

Pasteurized Milk Ordinance

The United States Public Health Service (USPHS) activities in the area of milk sanitation began at the turn of the twentieth century with studies on the role of milk in the spread of disease. These studies led to the conclusion that effective control of milkborne disease required the application of sanitation measures throughout the production, handling, pasteurization, and distribution of milk and milk products. To assist states and municipalities in initiating and maintaining effective programs for the prevention of milkborne disease, the USPHS, in 1924, developed a model regulation known as the *Standard Milk Ordinance* for voluntary adoption by state and local milk control agencies. This model milk regulation, now titled the Grade "A" Pasteurized Milk Ordinance (Grade "A" PMO), incorporates the provisions governing processing, packaging, and sale of Grade "A" milk and milk products, including buttermilk products, whey and whey products, and condensed and dry milk products.

The PMO is a set of controls for the dairy industry (akin to the FDA Food Code) and is developed by the USPHS/FDA for use as the basic standard used in the voluntary Cooperative State-USPHS/FDA Program for the Certification of Interstate Milk Shippers, a program participated in by all 50 states, the District of Columbia, and US Trust Territories. While a federal program, the PMO is usually monitored and enforced by state authorities (Hight 2012).

The Grade "A" PMO is incorporated by reference in federal specifications for the procurement of milk and milk products, is used as the sanitary regulation for milk and milk products, and is recognized by public health agencies, the milk industry, and many others as the national standard for the sanitary production of milk products. Once the PMO was adopted by the states, counties, and municipalities in the USA, it became a mandatory condition, with the strength of a legal requirement, for all Grade "A" dairy products (Hight 2012).

Microbiological criteria for pasteurized Grade "A" milk and other milk products can be found in the PMO. In the 2011 online version, the requirements can be seen on page 29, Table 1*, Chemical, Physical, Bacteriological, and Temperature Standards* (Hight 2012), http://www.fda.gov/downloads/Food/GuidanceRegulation/UCM291757.pdf.

With respect to the microorganisms listed in Table 1, the PMO does not establish a regulatory mandate for testing. Microbial analysis to detect those organisms in milk products is voluntary. However, many dairy processors test for these organisms on a routine basis. Such microbial testing is very widespread throughout the dairy industry.

Conclusions

Low-moisture foods encompass of a broad market basket of wares. Spices, snack foods, confectionary, dairy, cereals, nuts, coffee, and bakery items are a small representation of foods that depend on low-water activity as a barrier against microbial contamination. Unfortunately, many of these product types have been involved in foodborne illness outbreaks in the past few decades.

The production of low-moisture foods often involves techniques that, while historically successful, may lack some of the common validations used in other facets of the overall food industry, such as canning. Roasting and baking procedures may have been used for years but never scientifically validated as a microbicidal step, for example. Validation of thermal processes in low-moisture foods is complicated by the fact that as water activity decreases, heat resistance of some microbes, such *Salmonella*, increases (Podolak et al. 2009, 2010). Factors such as this, coupled with the use of dry sanitation standard operating procedures, illustrate that, perhaps more so than some products, low-moisture foods benefit from a comprehensive, all-inclusive, thorough, and robust food safety program.

A sound sampling and testing plan is an integral part of a comprehensive safety system that minimizes safety risks due to microbial contamination of low-moisture foods. Routine microbiological testing is often used, as part of an overall food safety program, to determine the acceptance of purchased ingredients, raw materials, and finished products and may be performed by a vendor, customer, company, and/or regulatory agencies anywhere in the supply chain. However, in instances where the contamination rate is low, the reliance on microbiological testing as the lone measure of food safety may be misleading, as negative results do not always ensure safety (ASTA 2009; Scott et al. 2006).

For these reasons, it is rare that food safety regulations worldwide specify explicit testing for particular organisms. Out of the hundreds of thousands of pages of US food safety regulations, the six regulations requiring testing for an exact organism were listed above. In cases such as Juice HACCP (21 CFR 120.25), testing for *E. coli* is only required in unique situations, not across the entire scope of foods covered by the regulation.

In FDA's proposed "Current Good Manufacturing Practice and Hazard Analysis and Risk-Based Preventive Controls for Human Food Proposed Rule" stemming from the FSMA legislation, the agency appears intent on including some sort of mandated microbial testing regimen, most likely as a verification activity, in final regulations. However, because the proposed rule only asks for comments, it is difficult to project what those mandates might be at this time.

Nevertheless, it would be extremely rare to find a comprehensive food safety system, for low-moisture products or otherwise, that could exist without microbial evaluation. Even where specific government regulations for testing are absent, microbial evaluation can play an integral role through validation and verification systems, product conformance, and environmental monitoring. Successful results in such programs should not only help producers sleep better at night but may serve to illustrate compliance with future food safety regulations.

References

ABC (Almond Board of California) (2007) Almond action plan: pasteurization treatments. http://www.almondboard.com/Handlers/Documents/Pasteurization-Treatments.pdf. Accessed 15 Nov 2012

American Spice Trade Association (2009) Clean, safe, spices; guidance from the American Spice Trade Association (ASTA), Washington, DC

Butts J (2003) Seek & destroy: identifying and controlling *Listeria monocytogenes* growth niches. Food Safety Magazine, April/May Issue. http://www.foodsafetymagazine.com/magazine-archive1/aprilmay-2003/seek-destroy-identifying-and-controlling-listeria-monocytogenes-growth-niches. Accessed 14 Jan 2014

CDC (Centers for Disease Control and Prevention) (2011) Vital signs: incidence and trends of infection with pathogens transmitted commonly through food – foodborne diseases active surveillance network, 10 U.S. Sites, 1996–2010. Morbid Mortal Weekly Rep 60:1–7. www.cdc.gov/mmwr/pdf/wk/mm60e0607.pdf

CSPI (Center for Science in the Public Interest) (2000) Petition for regulatory action to require microbial testing by industry for *Listeria monocytogenes* in ready-to-eat meat and poultry products. (http://www.cspinet.org/foodsafety/listeria.html). Accessed 19 Nov 2012

Codex Alimentarius Commission (2003) General principles of food hygiene, Hazard Analysis and Critical Control Points (HACCP) and guidelines for its application, 3rd edn. http://www.fao.org/docrep/006/y5307e/y5307e00.htm#Contents. Accessed Oct 2012

EPA (Environmental Protection Agency) (2012) National primary drinking water regulations www.epa.gov/safewater/mcl.html. Accessed 15 Oct 2012

FDA (Food and Drug Administration) (2002) 21 CFR Part 120, Hazard Analysis and Critical Control Point (HACCP) Systems (Juice HACCP). http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm

FDA (Food and Drug Administration) (2009) Guidance for industry: measures to address the risk for contamination by *Salmonella* species in food containing a peanut-derived product as an ingredient. http://www.fda.gov/Food/GuidanceComplianceRegulatoryInformation/GuidanceDocuments/ProduceandPlanProducts/ucm115386.htm. Accessed 14 Nov 2012

FDA (Food and Drug Administration) (2011) 21 CFR Part 118; Production, storage, and transportation of shell eggs. http://www.fda.gov/Food/GuidanceComplianceRegulatoryInformation/GuidanceDocuments/FoodSafety/ucm285101.htm#B

Federal Register (2007) Rules and regulations 72(61). 30 Mar 2007 http://www.gpo.gov/fdsys/pkg/FR-2007-03-30/pdf/07-1557.pdf. Accessed 12 Nov 2012

Food Safety Modernization Act (2011) http://www.fda.gov/Food/FoodSafety/FSMA/ucm247548.htm. Accessed 22 Oct 2012

Gillibrand K (2010) Improving food safety. http://www.gillibrand.senate.gov/agenda/improving-food-safety. Accessed July 2012

GMA (Grocery Manufacturers Association) (2009) Control of *Salmonella* in low-moisture foods. http://www.gmaonline.org/science/SalmonellaControlGuidance.pdf. Accessed 12 Aug 2012

GMA (Grocery Manufacturers Association) (2010) Industry handbook for safe processing of nuts. http://www.gmaonline.org/downloads/technical-guidance-and-tools/Industry_Handbook_for_Safe_Processing_of_Nuts_1st_Edition_22Feb10.pdf. Accessed 12 July 2012

United States Department of Health and Human Services, Public Health Service, Food and Drug Administration (2011) Grade "A" Pasteurized Milk Ordinance. http://www.fda.gov/downloads/Food/FoodSafety/Product-SpecificInformation/MilkSafety/NationalConferenceonInterstateMilkShipmentsNCIMSModelDocuments/UCM291757.pdf. Accessed 13 Nov 2012

United States Department of Health and Human Services, Public Health Service, Food and Drug Administration (2013) Food Code. http://www.fda.gov/downloads/Food/GuidanceRegulation/RetailFoodProtection/FoodCode/UCM374510.pdf

Hight (2012) Timothy, personal correspondence

ICMSF (International Commission on Microbiological Specifications for Foods) (2002) Sampling plans and selection of cases and attributes plans. In: Microorganisms in foods 7: Microbiological testing in food safety management. Kluwer Academic/Plenum, New York, pp 123–172

Moorman M (2011) Laboratory testing: the price of bad data, Food Safety Magazine, October/November 2011. http://www.foodsafetymagazine.com/magazine-archive1/augustseptember-2011/laboratory-testing-the-price-of-bad-data/. Accessed 12 Jan 2013

National Advisory Committee on Microbiological Criteria for Foods (1997) Hazard Analysis and Critical Control Point (HACCP) and application guidelines. J Food Prot 61:762–775

National Advisory Committee on Microbiological Criteria for Foods (2010) Parameters for determining inoculated pack/challenge study protocols. Food Prot 73:140–202

Podolak R, Enache E, Stone W (2009) Sources and risk factors for contamination by *Salmonella* in low-moisture foods, Grocery Manufacturers Association, www.gmaonline.org/science/Salmonellaguidanceannex.pdf

Podolak R, Enache E, Stone W, Chen Y, Scott VN, Black DG (2010) Sources and risk factors for contamination, survival and persistence, and heat resistance of Salmonella in low-moisture foods. J Food Prot 73:1919–1936

Scott VN, Stevenson KE (ed) (2006) HACCP: a systematic approach to food safety, 4th edn. Food Products Association (Currently the Grocery Manufacturers Association), Washington, DC

Scott VN, Stevenson KE, Gombas DE (2006) Verification procedures. In: Scott VN, Stevenson KE (eds) HACCP: a systematic approach to food safety, 4th edn. Food Products Association, (Currently the Grocery Manufacturers Association), Washington, DC, pp 91–98

Scott VN (2012) Preventive controls – What's next, presentation at the Association of Food and Drug Officials 116th Annual Educational Conference, 5 June 2012, Providence RI

Scott VN, Kraemer D (2013) Webinar on the FSMA proposed Preventive Controls Rule. Grocery Manufacturers Association, Jan 2013

Stone WE, Jantschke M, Stevenson KE (2009) Key Components of a food allergen management program. In: Stone W, Stevenson K (eds) Managing allergens in food processing establishments. Grocery Manufacturers Association, Washington, DC, pp 25–40

Stone WE, Meyer J (2011) Grocery Manufacturing Association webinar, What's in Your Plant: Measuring Microbial Effectiveness. GMA Webinar, November 2011, www.gmaonline.org.

Stone WE (2012) Implications of the Food Safety Modernization Act, presentation at the Association of Food and Drug Officials 116th Annual Educational Conference, 5 June 2012, Providence, RI

Taylor SL, Hefle SL (2005) Allergen control. Food Technol 59(2):40–43, 75

USDA Agricultural Marketing Service (2007) Almonds grown in California; outgoing quality control requirements. Final Rule, Federal Register. 72(61):15021–15036 30 Mar 2007. http://edocket.access.gpo.gov/2007/pdf/07-1557.pdf. Accessed 8 July 2012

USDA Food Safety and Inspection Service (1996) 9 CFR Parts 310.25 and 381.94, Contamination with microorganisms; process control verification criteria and testing; pathogen reduction standards

USDA Food Safety and Inspection Service (1999) Appendix A: compliance guidelines for meeting lethality performance standards for certain meat and poultry products. http://www.fsis.usda.

gov/Frame/FrameRedirect.asp?main=http://www.fsis.usda.gov/OPPDE/rdad/FRPubs/95-033F/95-033F_Appendix_A.htm. Accessed 15 July 2012
USDA Food Safety and Inspection Service (2006) Title 9 CFR Part 430 – FSIS Requirements for specific classes of product, Control of *Listeria monocytogenes* in Ready-to-Eat meat and poultry products; Final Rule. http://www.fsis.usda.gov/oppde/rdad/frpubs/97-013f.htm. Accessed 31 Oct 2012
USDA Food Safety and Inspection Service (2014) FSIS notice 01-14 Verification Activities for non-O157 Shiga Toxin-Producing Escherichia Coli (Non-0157STEC) under MT60, MT52 and MT53 Sampling Programs, http://www.fsis.usda.gov/wps/wcm/connect/f1b04e09-0b76-44f6-a5db-a779b6eef9d3/01-14.pdf?MOD=AJPERES. Accessed 9 Feb 2013
USGPO (United States Government Printing Office), Federal Food Drug and Cosmetic Act (1938) (USC Subchapter 9 § 301 et seq.) Chapter 4: http://www.fda.gov/RegulatoryInformation/Legislation/FederalFoodDrugandCosmeticActFDCAct/default.htm. Accessed 13 Jan 2014
USGPO (United States Government Printing Office) (2012) Federal Register Notice. 77(105): 38975–38981. Accessed 11 Feb 2013
USGPO (United States Government Printing Office) (2013) Federal Register Notice. 78(11):3646–3824. Accessed 11 Feb 2013

Methodological and Sampling Challenges to Testing Spices and Low-Water Activity Food for the Presence of Foodborne Pathogens

Jean-Louis Cordier

Abstract Sampling and testing low-moisture foods for detecting foodborne pathogens are a challenge as there are several factors that influence detection that are unique to these types of products. These low-a_w products are characterized by a water activity <0.85, and they do not support the growth of foodborne pathogens. Case studies have revealed that several outbreaks of salmonellosis have been caused by products contaminated with very few cells. Furthermore, results obtained during investigations of several of these outbreaks as well as from laboratory studies revealed that low levels of pathogens such as *Salmonella* are able to survive for prolonged periods of time in the contaminated foods.

This chapter addresses factors contributing to pathogen contamination of low-moisture foods. These factors have an influence on the distribution of microorganisms within and in between manufactured lots of products and will ultimately affect the performance of sampling plans in their ability to detect pathogens.

Due to their characteristics and composition, several low-moisture foods are an analytical challenge. Factors such as the osmotic shock during rehydration as well as the presence of antimicrobial agents in several types of low-moisture foods influence the recovery of injured bacterial cells. This will be discussed as well as modifications of enrichment procedures that can improve the recovery and detection of *Salmonella*.

Keywords Low-moisture foods • *Salmonella* • Detection • Sampling plans • Testing

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Introduction

Low-moisture foods and low-moisture food ingredients encompass a large variety of products that have a water activity less than 0.85. Examples include dried dairy products such as milk powders, infant formulae or ingredients such as casein, whey protein concentrates, or lactose; cereal-based products such as flours, pasta, infant cereals, or breakfast cereals; cocoa powder, chocolate, and confectionery; egg powders; dried fruits and vegetables; different types of nuts including peanuts; seeds and grains; herbs, spices, and condiments; dry pet foods; and dried meat and fish products.

Many of these, as in the case of nuts, flours, certain spices, or chocolate, are naturally low in moisture. Additional drying, if applied to such foods and ingredients, is performed for technological reasons to obtain certain physicochemical or organoleptic characteristics or to further reduce the water activity in order to improve their shelf stability and to prevent mold spoilage and even the formation of mycotoxins associated with their growth.

Other products or ingredients, such as milk powders, infant formulae, dry dairy derivatives, and cereal-based products such as pasta, infant or breakfast cereals, are manufactured as high-moisture products and then subsequently dried in ovens, spray dryers, or roller dryers to obtain finished goods with a desired residual humidity.

The low water activity of these products will not support the growth of pathogens such as *Salmonella*, *Cronobacter* spp., *Listeria monocytogenes*, Shiga-toxin-producing *Escherichia coli*, or *Staphylococcus aureus*. However, occasionally, inappropriate drying conditions which do not allow for a sufficiently rapid decrease of the water activity to levels <0.85, i.e., the boundary for the growth of *Staphylococcus aureus*, have led to outbreaks of foodborne illness. A typical example is the outbreak caused by pasta contaminated with staphylococcal enterotoxins, which resulted from slow drying that provided the appropriate time and water activity conditions for growth and formation of toxins by the pathogen (Woolaway et al. 1986). However, such episodes are not frequently reported.

Several outbreaks of foodborne illness from consumption of contaminated low-*a*w foods have been attributed to a variety of pathogens. *Cronobacter* spp. have been associated with outbreaks related to infant formulae (FAO/WHO 2004, 2006); *Escherichia coli* O157:H7 was associated with low-moisture cookie dough (Neil et al. 2011), hazelnuts (Miller et al. 2011), and dried and fermented meats (Keene et al. 1997; Moore 2004); and *E. coli* O104:H4 was associated with an outbreak from contaminated sprouts grown from fenugreek seeds (Buchholz et al. 2011). However, outbreaks caused by low-moisture foods have resulted from contamination with *Salmonella*, and an extensive list of outbreaks is provided in recently published reviews by GMA (2009), Podolak et al. (2010), Zweifel and Stephan (2012), and Beuchat et al. (2013). This chapter will mainly focus on information and data published for *Salmonella* in low-moisture foods. In fact, several relevant aspects such as their behavior in low-moisture foods, factors influencing contamination, or

issues related to analytical procedures have been investigated in much more detail than for other pathogens. Relevant information available for other pathogens will be included as a complement to the *Salmonella* findings.

Although pathogens such as *Salmonella* are unable to grow in low-moisture products with a water activity <0.85, they are able to survive during prolonged periods of time, frequently over the entire shelf life of the product. Survival under such conditions is attributed to a greatly reduced metabolism of the bacteria and has been reported for numerous low-moisture foods as summarized by Beuchat et al. (2013).

Behavior of Pathogens in Processing Environments and in Low-Moisture Foods

Findings of investigations of foodborne outbreaks caused by contaminated low-moisture foods provide valuable information regarding the sources and routes of contamination of the implicated pathogens. The link between the influential factors discussed in this section and methodological and sampling challenges discussed in the subsequent sections is not immediately apparent. However, they have an important influence on the distribution of pathogens in food matrices, which in turn have an impact on the performance of sampling plans or on the ability of analytical methods to detect pathogens.

Mechanisms allowing the survival of *Salmonella* in foods and food-processing environments are not yet fully understood. This is particularly the case for processing environments remaining dry for prolonged periods of time, as can occur in the manufacture of dry milk products or infant formula (Cordier 2007 and CAC 2008). Considering these extreme conditions, phenomena such as the formation and establishment of biofilms, which are normally associated with moist environments, are difficult to investigate. Recent studies have highlighted the role of certain cell structures (e.g., fimbriae or pili), which may play a role in the attachment and adherence of pathogens to materials, such as stainless steel, Teflon®, or rubber, commonly present in food-processing environments and which may enable *Salmonella* to survive over prolonged periods of time (Waldner et al. 2012). *Salmonella* cells known to form specific morphological types, described as red, dry, and rough (rdar) morphotype, are more resistant to external stresses such as desiccation. This characteristic confers to cells the ability to be easily transferred from one surface to another and hence to contribute to the contamination of intermediate or finished products in contact with these surfaces.

The impact of environmental stresses such as nutrient limitations or starvation, exposure to low or high pH, exposure to low or high temperatures, and desiccation or exposure to chemicals such as oxidizing agents has been summarized by Spector and Kenyon (2012). Exposure to such stress factors such as desiccation can trigger either specific or more general cross-resistance, allowing the organisms to survive and persist under adverse conditions (Gruzdev et al. 2011).

Several *Salmonella* strains belonging to different serovars survived for at least 3 years in fish feed-processing environments (Nesse et al. 2003; Vestby et al. 2009). Another example demonstrating the survival and persistence of a specific strain over a prolonged period of time is provided in reports on two outbreaks caused by *Salmonella* Agona. This particular strain, indicative of the same source of contamination, was associated with breakfast cereals produced in the same manufacturing facility 10 years apart (Russo et al. 2013). The persistence of *Cronobacter* spp. in processing environments has been investigated as well by several authors. They have determined that different genetically distinct strains and clones can persist in processing environments and subsequently sporadically contaminate finished product (Mullane et al. 2007; Craven et al. 2010; Reich et al. 2010).

Another important factor to consider is the survival of pathogens in contaminated products. Many studies have revealed pathogen persistence in naturally contaminated, low-moisture products, as well as in artificially contaminated products. Survival of *Salmonella* or *Cronobacter* spp. for several weeks or months in products or ingredients (i.e., well beyond the end of their shelf life) has been reported by several authors and reviewed by Podolak et al. (2010), Walsh et al. (2012), and Beuchat et al. (2013).

Levels of pathogens in many low-moisture product-associated outbreaks have been determined to be extremely low. In the case of chocolate, for example, *Salmonella* populations of 1–3 CFU/g of a product have been reported by several authors (e.g., Craven et al. 1975; Gill et al. 1983; Werber et al. 2005). In fact, in one chocolate-associated outbreak, *Salmonella* populations were only 0.04–0.24 CFU/g (i.e., a few cells per 100 g or 100 times lower levels than was observed in other outbreaks) (Hockin et al. 1989). Similarly, low contamination levels (0.04–0.45 CFU/g) were reported in an outbreak caused by paprika-flavored potato chips contaminated with multiple serovars (Lehmacher et al. 1995). The occurrence of very low levels of contaminants represents a major challenge for performance of sampling plans as well as the analytical methods applied to analyze such products for the presence of *Salmonella*.

The number of cases involved in the outbreaks mentioned above ranged from a few individuals to, in the one caused by the contaminated potato chips, more than 1,000 patients. These outbreaks occurred despite the low total number of cells ingested when consuming the contaminated foods. The occurrence of strains having a resistance to gastric acids, the protective effect of certain components of the food such as fat or proteins, as well as a more rapid transit through the stomach due to a consumption after a main meal are likely contributing factors (Berk et al. 2005; Birk et al. 2012).

In the case of *Cronobacter* spp. in infant formulae, the reasons for the outbreaks have been attributed to the ingestion of large numbers of cells resulting from bacterial multiplication due to inappropriate handling and storage conditions between the time the formula was reconstituted and the time of consumption. However, in these outbreaks the initial levels of contamination of the dry infant formula were very low, i.e., ca. 1 cell per 100 g of product (FAO/WHO 2004, 2006).

Distribution of Microorganisms in Low-Moisture Foods

The distribution of microorganisms, including pathogens and hygiene indicator microbes, is an important factor influencing the performance of sampling and testing programs. The distribution of microorganisms in a particular food is a function of several factors and is related to the manufacturing conditions. Processing steps, such as heat treatments or other microbial inactivation steps, addition of ingredients, mixing, transportation, intermediate storage, or filling, can all play a role in contributing to the presence and distribution of pathogens such as *Salmonella*. A completely homogeneous distribution of microorganisms in a given food is rarely achieved, but good distribution can be expected in certain situations such as in well-mixed fluid products such as water or milk. In the case of low-moisture foods such as chocolate, which is maintained fluid during most of the process, several processing steps (such as grinding, milling, mixing, conching, and tempering) should ensure a rather homogeneous dispersion of microorganisms. This has been validated by findings in outbreak investigations.

The location of these processing steps, relative to others, will also play a role. For example, addition of ingredients at an early stage of the process may lead, if contaminated, to a wider distribution of the pathogen as opposed to adding the ingredients immediately before filling. Similarly, this would occur for a contamination from the processing environment or from the processing line such as product-contact surfaces in pieces of equipment. In the case of low-moisture foods, this distribution may vary greatly from one product to another, due to the very different types of processes applied in their manufacture.

Under normal operating conditions, microbial populations in a lot or a batch would rarely be homogeneously distributed. This is due to several factors, including the structural or natural heterogeneity of different food matrices that may contain particles, pieces, and flakes of different sizes and may be of different origins or mixtures thereof. Examples of low-moisture foods are breakfast cereals, nut-based products, dried fruits and vegetables, dried spices and herbs, and dry pet foods.

Results of several studies have revealed that distribution of a pathogen such as *Salmonella* can be affected by different processing steps, from primary production through manufacturing, storage, and distribution of finished goods up to the final preparation by the consumer. Therefore, knowledge of the origin and source of contamination may provide insights regarding the type of distribution. Product contamination with a given pathogen will have a certain pathogen mean (average) concentration and distribution throughout a manufactured lot. The standard deviation will determine the shape of the distribution curve—flat in the case of a large standard deviation (wide distribution) and narrow in the case of a small standard deviation. It can be assumed that very well-controlled processes would have much lower mean concentrations of a given pathogen, as well as a smaller standard deviation compared to poorly controlled processes which are prone to more frequent recontamination events with varying levels of pathogens. This is mainly due to a better control over parameters related to the increase of hazards which are described

below. The effect of much stricter control measures in facilities manufacturing infant formulae in reducing the level of contamination is discussed and illustrated in a previously published chapter by Cordier (2007). Subsequent publications have emphasized the importance of controlling the ingress and multiplication of *Salmonella* in processing facilities to minimize the risk of cross-contamination (e.g., Scott et al. 2009; Chen et al. 2009a, b). The implementation of effective control measures will significantly reduce or completely eliminate sporadic contamination events and, hence, minimize the heterogeneous occurrence of pathogens in products and ingredients.

The fate of microorganisms such as *Salmonella* during processing can be described by the following conceptual equation (ICMSF 2002), with the different elements being expressed in $\log_{10}$ units:

$$H_0 - \Sigma R + \Sigma I_{\mathrm{G+C}} \leq \mathrm{FSO\ or\ PO}$$

where FSO is the food safety objective, PO is the performance objective, H_0 is the initial load of the hazard, ΣR is the total (cumulative) reduction of the hazard, and ΣI is the total (cumulative) increase of the hazard. This increase can be further subdivided into growth (G) and/or contamination (C).

In the case of unprocessed products, i.e., those not submitted to any kill step, the pathogen prevalence and levels will be determined by the flora introduced during primary production and changes occurring during subsequent handling. Increases will be caused by growth in the agricultural raw material prior to the application of drying steps used to reduce the water content and ensure the shelf stability of the product. Increases may occur due to external contamination, such as from the field, during harvest, and further steps such as handling, drying, cleaning, sorting, grading, intermediate storage, etc. Almonds and nuts are examples of such products of which several investigations have been carried to obtain a better understanding on the fate of *Salmonella* (Isaacs et al. 2005; Danyluk et al. 2007). Similar studies have been conducted for other low-moisture foods such as dry herbs and spices (Sagoo et al. 2009), revealing the presence of *Salmonella* in 1.5 % of the production samples and 1.1 % of the retail samples. The authors concluded that reduction of contamination requires the application of good agricultural and good hygiene practices during in-field production, harvesting, and further processing.

Heterogeneous distribution due to recontamination during further processing can also occur for dry, unprocessed spices. During the investigation of the *Salmonella* Rissen outbreak associated with white pepper, the outbreak strain was not only detected in the raw materials used but as well in numerous samples of residues in processing equipment, thus further contributing to contamination during processing. In this case, the ground white pepper in wholesale packaging in sizes of 5–30 lbs was repackaged in smaller retail 5 oz or 5–10 lb plastic bags. During this procedure, the white pepper seeds were added to a hopper and then ground in a grinder. The ground pepper was then filled into cardboard drums before being filled in smaller packaging units. The investigation revealed that *Salmonella* was present in 63 out of 400 samples (raw materials, environmental, in process, and finished products), and

the presence of *Salmonella* Rissen, the outbreak strain, was confirmed in 25 samples (Myers et al. 2010).

In the case of processed ingredients or products, kill steps will reduce the initial pathogen load, while in the case of processes that do not include a kill step during further processing (as in the case of exclusive blending processes as applied for several types of low-moisture foods), no overall reduction will occur. The addition of contaminated raw materials will contribute to an increase in the pathogen populations, as will contamination from the processing environment or the processing lines. The pathogen distribution in the finished product will vary in size and population depending on the location of the addition of ingredients or of environmental contamination, as well as on the effect of subsequent processing steps contributing to their dispersion within the lot manufactured. The occurrence of pockets of pathogens due to contaminated individual particles or pieces or due to localized growth, for example, in clumps of higher water activity, will further contribute to the heterogeneous distribution of contamination.

Depending on the type of kill step applied (e.g., a mild heat treatment or technologies such as microwaves), survival may occur in a portion of the product or in colder spots, contributing further to the heterogeneity of the pathogen distribution (Vadivambal and Jayas 2010). In the case of heat treatments of particulate foods and in particular those at low water activity, the risk of a limited kill effect or even survival of injured cells is increased. Heat treatments, such as dry heating, applied on low-moisture matrices may also contribute to survival of fractions of the pathogen population. Since low water activity generally confers increased heat resistance to cells, validation of such processes is particularly important. The influence of low water activity or the composition of the food matrix on pathogen inactivation by heat treatments for low-moisture foods has been reviewed by several authors (Mattick et al. 2001; Komitopoulou and Peñaloza 2009; Podolak et al. 2010; Silva and Gibbs 2012). The risk of variation in the effect of kill steps is greatly reduced when kill steps are applied to well-mixed liquid products such as milk, which are then subsequently dried.

Antimicrobial Activity of Certain Low-Moisture Foods

The antimicrobial properties of dry herbs and spices or of their extracts are well established and have been discussed in numerous publications. Phenols and essential oils have the greatest inhibitory effect, followed by aldehydes, ketones, and alcohols. Sulfur-containing molecules present in seeds, such as mustard, play a role as well. Recent publications by Shan et al. (2007), Bakkali et al. (2008), Ito (2009), and Tajkarimi et al. (2010) provide details regarding the antimicrobial activity of different molecules as well as their variable occurrence among different herbs and spices. In the case of condiments using different dry herbs and spices, synergistic antimicrobial effects have been observed, which is further enhanced by the presence of high salt concentrations.

The antimicrobial molecules in tea leaves include polyphenols, tannins, and catechins, which represent up to 30–40 % of the dry weight of fresh green tea leaves. Further processing of tea leaves to transform green or white tea into partially or completely fermented teas (red or oolong and black tea, respectively) leads to the formation of larger polyphenolic molecules which are responsible for the darker color (Almajano et al. 2008; Bancirova 2010). The extent of the antimicrobial activity will depend on the type and the concentration of active molecules as well as on the susceptibility of bacterial strains (Mo and Zhu 2008; Kim et al. 2011).

Cocoa and chocolate, and in particular dark chocolate containing a large proportion of cocoa powder, contain polyphenols which have antimicrobial activity (Busta and Peck 1968; Wollgast and Anklam 2000; Lee et al. 2003; Daglia 2013).

The practical application of such antimicrobial components in, for example, high-moisture foods to inhibit pathogens has been limited. In fact, in most cases, addition of these food components at concentrations required to effectively inhibit pathogens leads to unacceptable organoleptic characteristics of the finished product. However, in the case of analyses for the presence of pathogens, these antimicrobial agents have a major impact and will negatively influence the ability of the assay to recover the target pathogen. During the enrichment phase for isolating *Salmonella* from foods, the growth of *Salmonella* can be inhibited or completely suppressed by such antimicrobial components. The type of effect (i.e., bacteriostatic or bactericidal) and its magnitude will depend on the type and concentration of antimicrobial chemicals present, the food matrix, as well as on the strain of *Salmonella* present in the food, the life cycle phase of the cells, and, especially, the degree of cell injury. This must be addressed in order to take the necessary measures to overcome inhibitory effects of the enrichment culture medium, thereby enabling the effective detection of pathogens, if present.

Detection-Pathogen Recovery During Analysis

Salmonella testing usually includes a pre-enrichment culture using a nonselective broth, frequently buffered peptone water (BPW), but others are used as well. This step is essential to enable the recovery of injured cells that may be present in low-moisture food. During pre-enrichment, the antimicrobial effect of components of the food matrix or of the broth as well as the way the analytical sample is prepared and handled may affect recovery and subsequent detection of cells. Injured cells require appropriate conditions and time to recover from sublethal injury before growth occurs. This period of injured cell recovery, during which repair takes place, results in an increased lag phase. The nonselective pre-enrichment broth is incubated for a period of 18 to 24h, during which cell recovery and growth occur. This pre-enrichment is defined in conventional cultural methods and represents the optimum conditions to achieve an effective recovery (ISO 2002; BAM 2011).

Omission of such a step during the analytical procedure can have a detrimental effect and lead to a substantial reduction in cell recovery, leading to a high probability

of obtaining false-negative results. Reducing the period of incubation of the pre-enrichment step may be detrimental and also lead to false-negative results for qualitative analyses. For this reason, many studies have been conducted to identify conditions that would minimize an adverse impact of enrichment conditions on cells present in different low-moisture foods, e.g., to minimize the impact of a dramatic and rapid increase of the osmotic stress occurring during reconstitution of the low-moisture food in enrichment broth or to overcome, e.g., by dilution, the inhibitory effect of antimicrobial agents.

Ray et al. (1971) determined that increasing the osmolarity of the recovery broth through the addition of varying amounts of solutes improved recovery of freeze-dried cells of *Salmonella anatum*. In order to avoid cumbersome additions to enrichment media, van Schothorst et al. (1979) determined that equilibration and slow rehydration of contaminated nonfat dry milk powder (NFDM) resulted in an increased recovery rate of *Salmonella* from contaminated samples. Sprinkling the powder onto the surface of the enrichment broth and then leaving the two components undisturbed for a period of time of up to 60 min allowed for slow moisture absorption. This resulted in a slow increase in product water activity in the enrichment medium and minimized the adverse effect of osmotic shock that occurs during a rapid rehydration. Other investigators have reported similar improved recoveries from NFDM as well as from dehydrated soy proteins when using this protocol, whereas results were not conclusive in the case of dried yeast powder (Andrews et al. 1983; Wilson et al. 1985). A collaborative study confirmed that this soak method improved recovery of *Salmonella*, and these results were used as the basis for including this approach in official methods, such as the BAM or ISO methods, in lieu of the rapid-soak method (Poelma et al. 1984).

Similar studies were conducted by D'Aoust and Sewell (1986) on naturally contaminated feeds and feed ingredients. While an increased number of *Salmonella*-positive samples was observed, this was attributed to the heterogeneous distribution of contaminating *Salmonella* rather than to the effect of the slow rehydration. The authors concluded that the slow rehydration method was of limited value and an extended application would require an individual assessment for each food matrix before being adopted as a standard method.

While the nonselective pre-enrichment procedure enhances the recovery and resuscitation of injured cells, followed by the growth of *Salmonella*, this step also allows recovery and growth of competitive microorganisms that are present in the analyzed samples. The resulting cell populations of this competitive flora will depend upon the type of low-moisture food being tested and will be higher and more diverse in the case of unprocessed products, such as herbs and spices. This may also be the case for environmental samples from factories manufacturing low-moisture foods. Hence, Van Schothorst and Renaud (1985) proposed that malachite green be added to the pre-enrichment broth to inhibit gram-positive bacteria and to enhance the detection of *Salmonella*. In other cases, the selective enrichment step applied after the pre-enrichment culture is designed to inhibit the competitive flora while allowing for further growth of *Salmonella*.

The overall analytical time is a reflection of the sensitivity of the detection system and, in particular, its ability to recover low numbers (ideally 1 cell) of *Salmonella* in the quantity of low-moisture product analyzed. A number of rapid methods have been developed trying to reduce the response time by reducing the period of pre-enrichment and subsequent selective enrichment or by eliminating one of the two steps. Considering the critical need to enable recovery of injured cells, a significant reduction in the time of one or both steps, or deletion of one of them, is often detrimental to the recovery of *Salmonella*. Such shortened pre-enrichment and selective enrichment steps need, therefore, to be carefully evaluated and validated to demonstrate that they do not adversely affect the recovery of *Salmonella*. Validation should be done according to internationally recognized protocols such as ISO (2003).

An additional consideration when testing low-moisture foods for *Salmonella* is the possibility of pooling analytical sample units during the enrichment culture in order to minimize the number of analyses carried out. Considering the constraints caused by the presence of injured cells that requires particular attention during rehydration and nonselective enrichment (as well as the presence of antimicrobial molecules requiring higher dilutions or modification of the enrichment broth), it is important that pooling of samples be carefully validated to confirm that such practices will not compromise the detection of pathogens when present (Jarvis 2007).

Several alternative methods have been developed to overcome the inhibitory effects of different food components on *Salmonella*. An example is the application of Dynabeads® for the immuno-magnetic separation of *Salmonella* to avoid the use of high dilutions by extracting the small cell populations of *Salmonella* present in a pre-enrichment culture as a consequence of growth inhibition. This method can also specifically bind *Salmonella* in the presence of large populations of closely related competitive *Enterobacteriaceae*. Results from a collaborative study revealed that the immuno-magnetic separation was an effective alternative obtaining *Salmonella* from enrichment cultures of herb and spice samples (Mansfield and Forsythe 1996). This technique has also been successfully applied in combination with either cultural methods or molecular methods for other types of low-moisture foods such as skimmed milk powder (Dziedkowiec et al. 1995) and pet chews (Wong et al. 2007). This method may also help overcome the specific or nonspecific inhibitory effects that some food components have on PCR-based detection methods (Mozola 2006).

In order to overcome the effect of antimicrobial molecules present in herbs and spices, *Salmonella* detection methods often include a generic requirement for a dilution of 1:100 instead of the traditional 1:10 as is applied for most food samples. Graubaum (2003) determined that there were differences in the inhibitory effect of herbs and spices depending on the type of herbs and spices analyzed. For herbs such as basil, galangal, ginger, garlic, peppermint, oregano, clover, and mustard seeds, a 1:100 dilution was effective; however, for oregano and cinnamon, a 1:1,000 dilution was necessary to overcome the inhibitory effect. For a group of 26 spices, including pepper and paprika, a 1:20 dilution was sufficient for recovery of sublethally injured cells of *Salmonella*.

In the case of the FDA BAM (2011), 1:10 dilutions in Trypticase soy broth are recommended for most of the spices, whereas a 1:100 dilution is recommended for

allspice, cinnamon, cloves, and oregano. For onion powder and flakes and garlic powder, the addition of potassium sulfite (K_2SO_3) is recommended to neutralize inhibitory substances present in these matrices. Different dilution rates, according to the type of spice and condiment, are also recommended in the official Canadian method MFHPB-20 (Anonymous 2009), with the most frequently recommended dilution rates being 1:10 and 1:20. Higher dilutions of 1:50 or more were only necessary in a few cases, such as for cloves or cinnamon.

For cocoa, confectionery, and chocolate products, modifications of the enrichment broth are necessary to minimize the effect of inhibitory substances such as tannins or polyphenols present in cocoa. The addition of casein neutralizes the effect of these chemicals and improves the recovery rate of *Salmonella* in contaminated products (Zapatka et al. 1977; Park et al. 1979). The addition of casein, nonfat dry milk, or UHT milk is described in official methods published by ISO (2002) or FDA BAM (2011) and is routinely used in the analysis of cocoa, chocolate, and confectionery products.

Hydrocolloids, which can be classified as low-moisture ingredients, are also a potential source of *Salmonella*, representing a particular analytical challenge. During pre-enrichment culture, they will absorb part of the liquid and lead to a thickening of the pre-enrichment broth as well as to a concomitant change in the pH of the solution. It is, therefore, important to prepare appropriate dilutions, to adjust the pH, and to add enzymes to reduce the viscosity of the solution to improve the recovery of *Salmonella* (Amaguaña et al. 1996, 1998). For gelatin, thickening of the pre-enrichment broth is prevented by using either a higher dilution (1:20 instead of the usual 1:10) or by adding papain to reduce the viscosity (Park et al. 1977; Amaguaña et al. 1998).

The ISO as well as the FDA BAM standard methods for the detection of *Salmonella* have included several modifications to the standard enrichment procedure in order to address specific issues related to low-moisture foods. Their application is important to ensure optimal detection in contaminated low-moisture products, and it is also important whether recommended modifications are applicable in all cases or need to be further adjusted. In the case of herbs and spices, for example, several ones, combinations thereof, or mixtures including other condiments such as salt may need a higher dilution rate to all for an effective detection of *Salmonella*, as highlighted, for example, by Graubaum et al. (2005). However, a review of publications addressing surveys of the microflora of low-moisture foods such as herbs and spices, dried dairy products, and others revealed that often no details are provided regarding the precise analytical protocols applied (e.g., Banerjee and Sarkar 2003; Willis et al. 2009 or Sospedra et al. 2010). In several cases, an unmodified method was used, which is likely to have had an adverse effect on *Salmonella* recovery. When data on the prevalence of pathogens in low-moisture ingredients or foods are evaluated, one needs to consider the limitations associated with published reports. On one side, results are frequently obtained from individual samples, and on the other side, detailed information on the analytical methods applied are not always available or incomplete; hence it is not possible to conclude which assay has the most optimal recovery of pathogens.

Performance of Sampling Plans

Considering the discussions in previous Sects. 4 and 5, one of the underlying assumptions of the performance of sampling plans should be that the analytical methods applied to test product samples are optimal and will allow effective detection of the target microorganisms.

If contamination of a sample is homogeneously distributed, the probability to detect the contamination is the same for each sample. In this case, the sampling plan does not influence the sampling performance based on the sample weight. The distribution of microorganisms is usually described as a random distribution, referring to an irregular distribution within the food matrix. An assumption which is often made is lognormality, since the distribution appears to best represent actual observations in foods or according to the Poisson distribution (ICMSF 2002; Jarvis 2008). Irregular clustering of microorganisms caused by sporadic contamination will lead to a heterogeneous distribution and impact the frequency distribution of the microbial population. Under these circumstances the sampling strategy applied to test a lot becomes extremely important.

Sampling plans to test food products have the following characteristics:

n = number of analytical sample units that must conform to the criterion.
c = the maximum allowable number of defective sample units in a 2-class plan.
m = a microbiological limit which, in a 2-class plan, separates good quality from defective quality or, in a 3-class plan, good from marginally acceptable quality.
M = a microbiological limit, which in a 3-class plan separates marginally acceptable quality from defective quality.

The microbiological quality of a lot of manufactured products is normally assessed by drawing a certain number of samples, followed by subsequent testing of aliquots from these sample units. The performance of a sampling plan is defined as an ability to allow detection of microorganisms with a certain probability, usually a 95 % probability. However, in reality, the number of samples taken may vary widely, depending on the purpose of the sampling and testing as well as on the objectives of the entity carrying out this activity. The number of sample units may, therefore, range from a single sample (as frequently seen in surveys or surveillance programs) to a full set of sample units as defined in microbiological criteria for the food in question. As a consequence, the probability of detection of contaminated samples will vary dramatically.

Sampling and testing of individual samples of low-moisture foods is usually performed as research projects by academic institutions. Other stakeholders such as consumer organizations, public health authorities, or food business operators may apply the same approach in the form of surveys or during the establishment of baselines for a specific product or raw material. Sampling and testing of individual samples may also be performed in the framework of the evaluation and validation of analytical methods.

Sampling of a larger number of samples (n), up to the number defined in microbiological criteria, is usually done by official control laboratories to assess the

compliance of imported or commercialized products to established limits. In the case of outbreaks, public health authorities may need to increase the number of samples (i.e., beyond established microbiological criteria) in order to detect and identify the origin of pathogens. Tightened sampling plans will increase the probability of detection in suspected lots of products. Negative results may, however, be due to much lower levels of contamination or due to other vehicle(s) or source(s) of contamination than the one suspected. Food business operators normally sample finished products or raw materials as a verification of the effectiveness of hygiene control measures applied in their own facility, as well as by the supplier supplying ingredients used in the manufacture of finished goods. This is done to confirm compliance to established internal or regulatory microbiological limits or the ones defined in commercial agreements. The number of samples of finished products tested by food producers on a routine basis will, however, depend on several factors such as the sensitivity of consumers and the level and extent of control measures applied. The frequency of sampling and the number of samples drawn and tested may also depend on the outcome of the analyses of samples obtained in processing lines and processing environments. Information obtained from environmental monitoring will provide useful insight to define meaningful sampling and testing regimes for the finished product (ICMSF 2011; GMA 2009).

Random sampling throughout a manufactured lot is important to obtain representative sample units, which are then submitted for testing. Sample units represent small portions of the lot and the distribution of both numbers and types of microorganisms should be representative of the entire lot. For sampling that is conducted on lots for which there is no information regarding the application and effectiveness of hygienic control measures, there will be a reduction of the probability of detection if the contamination is clustered compared with lots having a Poisson-distributed contamination (ILSI 2010; Jongenburger et al. 2012a). The number of samples analyzed and their weight will affect the probability of detecting the relevant pathogen. The application of inadequate analytical methods, i.e., the use of standard general procedures irrespective of the sample matrix without applying specific modifications of pre-enrichment cultures such as increased dilutions, the addition of neutralizing agents, or rehydration procedures will further reduce the probability of detection. The 2010 ILSI report *Impact of Microbial Distribution on Food Safety* (ILSI 2010; Jongenburger et al. 2012a, b) addressed the role and influence of the distribution of pathogens across food batches and within food samples on human exposure. Other publications have described other models for the statistical distribution of microorganisms in foods, e.g., (Gonzales-Barron and Butler 2011; Commeau et al. 2012; Jongenburger et al. 2011).

Attribute sampling plans used for pathogens such as *Salmonella* or *Cronobacter* spp. are aimed at determining the pathogen's presence or absence. The basic aspects of sampling plans and their associated statistical aspects are discussed in reference books by the International Commission on Microbiological Specifications for Foods (ICMSF) (2002, 2011). The relationship between the number of samples (n) and the acceptable number of samples (c), usually 0 in the case of pathogens, can be plotted as an operating characteristic curve. The performance of a given sampling plan is,

therefore, determined by the probability of acceptance (Pa) (i.e., corresponding to the absence of *Salmonella*), when testing a lot with a defined true defect rate.

As described previously in Sect. 4, a homogeneous distribution of microorganisms can, in principle, be assumed when the food and the processing conditions are conducive to such a distribution of microorganisms. This can be the case in well-agitated fluid products such as water or milk or in the case of low-moisture foods such as chocolate, in which the processing conditions are likely to ensure a thorough distribution of microorganisms throughout the final product. When cells are evenly distributed throughout a liquid or solid food, the distribution approximates a Poisson distribution with a squared variance (σ^2) equal to the mean (μ). When cells occur more frequently in clumps and aggregates, then their spatial distribution becomes erratic with a squared variance (σ^2) larger than the mean (μ) representing, for example, a negative binomial distribution (Jarvis 2008). In general, however, the distribution of microorganisms present in the food is much more heterogeneous. This is due to the nature and structure of many products as well as to the fact that contamination, which can occur during processing (such as contamination events result from added raw materials or the processing environment), can occur at different steps of the process and may as well vary in their magnitude.

Jongenburger et al. (2012a) compared different statistical distributions commonly used for modeling and evaluated their strength and weaknesses using different criteria. The Poisson distribution was considered appropriate for processes that have thorough mixing, with the lognormal distribution considered appropriate in the case of high numbers and the Poisson-lognormal or negative binomial distributions for other situations.

Van Doren et al. (2013) studied the prevalence of *Salmonella* in sesame seeds and capsicum imported into the United States. They investigated the between- and within-lot distributions of *Salmonella* contamination in different shipments by testing samples for a total weight of 1,500 g per lot. They determined that 3.3 and 9.9 %, respectively, were contaminated considering a 95 % probability of detection, but concluded that based on the use of probabilistic models, these levels of contamination were probably underestimated. They also determined that there was an important between-lot variability, ranging between 10^{-2} and 10^{-4} MPN/g for these two types of low-moisture products, but that the within-lot contamination was not inconsistent with a Poisson distribution.

Considering the low level of contamination, the authors concluded that only sampling plans requiring the testing of 750–1,500 g, as applied by the FDA, would allow detection of a significant percentage of contaminated deliveries (25–50 %) for the observed prevalence as described in the previous paragraph. Testing of a single 25 g sample would only allow for the detection of 10–20 % of lots contaminated at the higher end of the levels of contamination. Such limitations were also identified in a 6-month survey of imported feed ingredients by the New Zealand Food Safety Authority. They determined a prevalence of 2.2 % *Salmonella* contamination; however, due to the limitation in the design of the sampling plan and the inability to collect samples systematically, they were not able to establish confidence limits of this prevalence (Anonymous 2010). These conclusions are in line with the discussions

on limitations of sampling and testing to reliably detect pathogens (as well as hygiene indicators), for very low prevalence of contamination (ICMSF 2011).

Sampling plans reflect a certain stringency of accepting a lot for different concentrations of microorganisms. This stringency has an associated level of performance, i.e., for a given standard deviation of the microbial distribution, a mean concentration can be calculated at which a lot will be rejected with a probability of at least 95 %. Geometric mean concentrations for different ICMSF cases, as well as for frequently encountered microbiological criteria, have been published in the Appendix of ICMSF book, volume 8 (ICMSF 2011). In the case of low-moisture infant formulae, for example, the following microbiological criteria have been recommended for two relevant pathogens (CAC 2008):

Salmonella	$n=60$, $c=0$, $m=0$ (in 25 g)
Cronobacter spp.	$n=30$, $c=0$, $m=0$ (in 10 g)

For *Salmonella*, the mean concentration detected is 1 CFU in 526 g (for an assumed standard deviation of 0.8 and a probability of detection of 95 %), and for *Cronobacter* spp., the mean concentration detected is 1 CFU in 340 g (for the same assumed standard deviation and probability of detection). Such values are also provided in recently published microbiological criteria for ready-to-eat foods and water and infant formulae by Codex Alimentarius (CAC 2007, 2008), and, depending on their purpose, an indication of the statistical performance will be required in microbiological criteria established in the future (CAC 1997).

The ability of sampling plans to detect low levels of contamination are frequently discussed and challenged. In a recent publication, Jongenburger et al. (2011) have investigated the performance of different sampling strategies on two lots of infant formulae naturally contaminated with *Cronobacter* spp. One lot was noncompliant with the requirements of the EC regulations (identical to those of Codex Alimentarius CAC 2008), and the other lot was used as reference. Contamination of the noncompliant, recalled lot resulted from incidents that occurred during processing whereby contamination was in clusters. The estimated average concentration in this batch was determined as being 2.78 log CFU/g, with a standard deviation of 1.10 log CFU/g. Various sampling strategies were evaluated, and it was determined that taking more and smaller samples (for a constant total analytical weight) improved the performance of the sampling plan. Stratified random sampling, i.e., samples obtained at regular intervals across the production run, improved the probability of detecting heterogeneous contamination when compared to random sampling. The sampling plan for *Cronobacter* spp. ($n=30$, $c=0$, $m=0$ (in 10 g)) proposed by Codex Alimentarius (CAC 2008) had the best performance of the different sampling plans applied, if carried out as a stratified sampling plan. Results were slightly less for a random sampling plan, i.e., 96.8 % versus 99.4 % probability of detecting at least one positive sample out of 30 drawn from this particular lot. This confirms the suitability of the Codex Alimentarius sampling plan in detecting contaminated lots which may have caused disease as determined in the risk assessment for *Cronobacter* spp. (FAO/WHO 2006).

Summary

Low-moisture foods have been the cause of numerous foodborne outbreaks during the last decades. Published reports and case studies on outbreaks reveal many common features which are discussed in this chapter. Important aspects related to the fate and behavior of pathogens in processing environments are discussed to gain a better understanding of the importance of contamination during processing.

This chapter also provides insight on sampling and testing of finished products and discusses factors contributing to the distribution of low levels of pathogens in low-moisture foods and the limitations of sampling plans to ensure the safety of products. Factors such as the presence of inhibitory substances or the effect of rehydration are discussed, and available information on their effect on the recovery of pathogens in low-moisture foods is presented as well as ways to mitigate and overcome them to improve detection. The chapter is completed by a section discussing the performance of sampling plan and associated statistical aspects are described, using available data related to low-moisture foods.

References

Almajano MP, Carbo R, Lopez Jimenez JA, Gordon MH (2008) Antioxidant and antimicrobial activities of tea infusions. Food Chem 108:55–63

Amaguaña RM, Sherrod PS, Hammack TS (1996) Usefulness of cellulose in recovery of *Salmonella* spp. from guar gum. J Assoc Off Anal Chem Int 81:853–857

Amaguaña RM, Hammack TS, Andrews WH (1998) Methods for the recovery of *Salmonella* spp. from carboxy-methylcellulose, gum, gum ghatti and gelatin. J Assoc Off Anal Chem Int 81: 721–726

Andrews WH, Wilson CR, Poelma PL (1983) Improved *Salmonella* species recovery in non-fat dry milk pre-enriched under reduced rehydration. J Food Sci 48:1162–1165

Anonymous (2009) Méthodes de la DGPS pour l'analyse microbiologique des aliments: MFHPB-20 isolement et l'identification des *Salmonelles* dans les aliments. Direction Générale de la Protection de la Santé, Ottawa (Canada)

Anonymous (2010) Survey of Salmonella in imported feed ingredients. http://www.foodsafety.govt.nz/elibrary/industry/salmonella-in-imported-animal-feeds-summary.htm. Accessed 6 Jan 2014

Bakkali F, Averbeck S, Averbeck D, Idaomar I (2008) Biological effects of essential oils – a review. Food Chem Toxicol 46:446–476

BAM (Bacteriological Analytical Manual) (2011) Chapter 5 *Salmonella*. http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm070149.htm. Accessed 6 Jan 2014

Bancirova M (2010) Comparison of the antioxidant capacity and the antimicrobial activity of black and green tea. Food Res Int 43:1379–1382

Banerjee M, Sarkar PK (2003) Microbiological quality of some retail spices in India. Food Res Int 36:469–474

Berk PA, de Jonge R, Zwietering MH, Abee T, Kieboom J (2005) Acid resistance variability among isolates of *Salmonella enterica* serovar Typhimurium DT104. J Appl Microbiol 99: 859–866

Beuchat L, Komitopoulou E, Betts R, Beckers H, Bourdichon F, Joosten H, Fanning S, ter Kuile B (2013) Low-water activity foods: increased concern as vehicles of foodborne pathogens. J Food Prot 76:150–172

Birk T, Kristensen K, Harboe A, Hansen TB, Ingmer H, De Jonge R, Takumi K, Aabo S (2012) Dietary proteins extend the survival of *Salmonella* Dublin in a gastric acid environment. J Food Prot 75:353–358

Buchholz U, Bernard H, Werber D, Böhmer MM, Remschmidt C, Wilking H, Deleré Y, an der Heiden M, Adlhoch C, Dreesman J, Ehlers J, Ethelberg S, Faber M, Frank C, Fricke G, Greiner M, Höhle M, Ivarsson S, Jark U, Kirchner M, Koch J, Krause G, Luber P, Rosner B, Stark K, Kühne M (2011) German outbreak of *Escherichia coli* O104:H4 associated with sprouts. N Eng J Med 365:1763–1770

Busta FF, Speck ML (1968) Antimicrobial effect of cocoa on *Salmonellae*. Appl Microbiol 16: 424–425

CAC (Codex Alimentarius Commission) (1997) Principles and guidelines for the establishment and application of microbiological criteria to foods. CAC/GL21-1997, Revised and renamed 2013. WHO/FAO, Geneva, Rome

CAC (Codex Alimentarius Commission) (2007) Guidelines on the application of general principles of food hygiene to the control of *Listeria monocytogenes* in foods. CAC/GL61 – 2007. WHO/FAO, Geneva, Rome

CAC (Codex Alimentarius Commission) (2008) Code of hygienic practice for powdered formulae for infants and young children. CAC/RCP66 – 2008. WHO/FAO, Geneva, Rome

Chen YU, Scott VN, Freier TA, Kuehm J, Moorman M, Meyer J, Morille-Hinds T, Post L, Smoot L, Hood S, Shebuski J, Banks J (2009a) Control of *Salmonella* in low-moisture foods. II: hygiene practices to minimize Salmonella contamination and growth. Food Prot Trends 29:435–445

Chen YU, Scott VN, Freier TA, Kuehm J, Moorman M, Meyer J, Morille-Hinds T, Post L, Smoot L, Hood S, Shebuski J, Banks J (2009b) Control of *Salmonella* in low-moisture foods. III: process validation and environmental monitoring – part three of a three-part series. Food Prot Trends 29:493–508

Commeau, N, Cornu, M., Albert, I., Denis, J.-B. and Parent, E. (2012) Hierarchical bayesian models to assess betwee-and within-batch variability of pathogen contamination in food. Risk Analysis, 32, 395–415

Cordier JL (2007) Production of powdered infant formulae and microbiological control measures. In: Forsythe DJ, Farber JM (eds) *Enterobacter sakazakii*. ASM Press, Washington, DC

Craven PC, Baine WB, Mackel DC, Barker WH, Gangarosa EJ, Goldfield M, Rosenfeld H, Altman R, Lachapelle G, Davies JW, Swanson RC (1975) International outbreak of *Salmonella eastbourne* infection traced to contaminated chocolate. Lancet 305:788–792

Craven HM, McAuley CM, Duffy LL, Fegan N (2010) Distribution, prevalence and persistence of *Cronobacter* (*Enterobacter sakazakii*) in the non-processing and processing environments of five milk powder factories. J Appl Microbiol 109:1044–1052

Daglia M (2013) Polyphenols as antimicrobial agents. Curr Opin Biotechnol 23:174–181

Danyluk MD, Jones TM, Abd SJ, Schlitt-Dietrich F, Jacobs M, Harris AJ (2007) Prevalence and amounts of *Salmonella* found on raw California almonds. J Food Prot 70:820–827

D'Aoust JY, Sewell AM (1986) Slow rehydration for detection of Salmonella spp. in feeds and feed ingredients. Appl Env Microbiol 51:1220–1223

Dziedkowiec D, Mansfield LP, Forsythe SJ (1995) The detection of *Salmonella* in skimmed milk powder enrichments using conventional methods and immunomagnetic separation. Letters Appl Microbiol 20:361–364

FAO/WHO (Food and Agriculture Organization/World Health Organization) (2006) *Enterobacter sakazakii* and *Salmonella* in powdered infant formula: Meeting report. Microbiological Risk Assessment Series 10. FA/WHO, Rome, Geneva

FAO/WHO (Food and Agriculture Organization/World Health Organization) (2004) *Enterobacter sakazakii* and other microorganisms in powdered infant formula. Microbiological Risk Assessment Series 6. FA/WHO, Rome, Geneva

Gill ON, Sockett PN, Bartlett CL, Vaile MS, Rowe B, Gilbert RJ, Dulake C, Murrell HC, Salmaso S (1983) Outbreak of *Salmonella napoli* infection caused by contaminated chocolate bars. Lancet 1:574–577

GMA (Grocery Manufacturers Association) (2009) Control of *Salmonella* in low moisture foods. http://www.gmaonline.org/downloads/technical-guidance-and-tools/SalmonellaControlGuidance.pdf. Accessed 6 Jan 2014

Graubaum D (2003) Zur Notwendigkeit einer Voranreicherung im Verhältnis 1:100 beim Nachweis von Salmonellen in Gewürzen. Inaugural Dissertation zur Erlangung des Grades eines Doktors der Veterinärmedizin. Freie Universität, Berlin

Graubaum D, Kleer J, Hildebrandt G (2005) Nachweis von Salmonellen in Gewürzen: Zur Notwendigkeit einer Voranreicherung im Verhältnis 1:100. Fleischwirtschaft 85:105–109

Gruzdev N, Pinto R, Sela S (2011) Effect of desiccation on tolerance of *Salmonella enterica* to multiple stresses. Appl Environ Microbiol 77:1667–1673

Hockin JC, D'Aoust JY, Bowering D, Jessop H, Khanna B, Lior H, Milling ME (1989) An international outbreak of *Salmonella nima* from imported chocolate. J Food Prot 52:51–54

ICMSF (International Commission on Microbiological Specifications for Foods) (2002) Microorganisms in foods – volume 7: microbiological testing in food safety management. Kluwer Academic/Plenum Press, New York

ICMSF (International Commission on Microbiological Specifications for Foods) (2011) Microorganisms in foods – volume 8: use of data for assessing process control and product acceptance. Springer, New York

ILSI (International Life Sciences Institute) (2010) Impact of microbial distributions in food safety. Commissioned by the ILSI Europe Risk Analysis in Food Microbiology Task Force, Brussels

Isaacs S, Aramin J, Ciebin B, Farrar JA, Ahmed R, Middleton D, Chandran AU, Harris LJ, Howes M, Chan E, Pichette AS, Campbell K, Gupta A, Lior LY, Pearce M, Clark C, Rodgers F, Jamieson F, Brophy I, Ellis A, Salmonella Enteritidis PT30 Outbreak Investigation Working Group (2005) An international outbreak of salmonellosis associated with raw almonds contaminated with a rare phage type of *Salmonella* Enteritidis. J Food Prot 68:191–198

ISO (International Standardization Organization) (2003) Microbiology of food and animal feeding stuffs – protocol for the validation of alternative methods. Geneva

ISO (International Standardization Organization) (2002) ISO6579:2002 – microbiology of food and animal feeding stuffs – Horizontal method for the detection of *Salmonella* spp, Geneva

Ito KA (2009) Food safety issues and the microbiology of spices and herbs. In: Heredia N, Wesley J, Garcia S (eds) Microbiologically safe food (chapter 16). Wiley, Hoboken, NJ, pp 337–342

Jarvis B (2007) On the compositing of samples for qualitative microbiological testing. Lett Appl Microbiol 45:592–598

Jarvis B (2008) Statistical aspects of sampling for microbiological. In: Statistical aspects of sampling for microbiological analysis (chapter 5). Elsevier, Amsterdam, pp 71–101

Jongenburger I, Reij MW, Boer EP, Gorris LG, Zwietering MH (2011) Actual distribution of *Cronobacter* spp. in industrial batches of powdered infant formula and consequences for performance of sampling strategies. Int J Food Microbiol 151:62–69

Jongenburger I, Bassett J, Jackson T, Zwietering MH, Jewell K (2012a) Impact of microbial distributions on food safety I. Factors influencing microbial distributions and modeling aspects. Food Control 26:601–609

Jongenburger I, Bassett J, Jackson T, Gorris LGM, Jewell K, Zwietering MH (2012b) Impact of microbial distributions on food safety II quantifying impacts on public health and sampling. Food Control 26:546–554

Keene WE, Sazie E, Rice DH, Hancock DD, Balan VK, Zhao T, Doyle MP (1997) An outbreak of *Escherichia coli* O157:H7 infections traced to jerky made from deer meat. J Am Med Assoc 277:1229–1231

Kim Y, Goodner KL, Park JD, Choi J, Talcott ST (2011) Changes in antioxidant phytochemicals and volatile composition of *Camellia sinensis* by oxidation during tea fermentation. Food Chem 129:1331–1342

Komitopoulou E, Peñaloza W (2009) Fate of *Salmonella* in dry confectionery raw materials. J Appl Microbiol 106:1892–1900

Lee KW, Kim YJ, Lee HJ, Lee CY (2003) Cocoa has more phenolic phytochemicals and a higher antioxidant capacity than teas and red wine. J Agr Food Chem 51:7292–7295

Lehmacher A, Bockemuhl J, Aleksic S (1995) Nationwide outbreak of human salmonellosis in Germany due to contaminated paprika and paprika-powdered potato chips. Epidemiol Infect 115:501–511

Mansfield L, Forsythe S (1996) Collaborative ring-trial of Dynabeads® anti-*Salmonella* for immunomagnetic separation of stressed *Salmonella* cells from herbs and spices. Int J Food Microbiol 29:41–47

Mattick KL, Jørgensen F, Wang P, Pound J, Vandeven MH, Ward LR, Legan JD, Lappin-Scott HM, Humphrey TJ (2001) Effect of challenge temperature and solute type on heat tolerance of *Salmonella* serovars at low water activity. Appl Env Microbiol 67:4128–4136

Miller BD, Rigdon CE, Ball J, Rounds JM, Klos RF, Brennan BM, Arends KD, Kennelly P, Hedberg C, Smit KE (2011) Use of traceback methods to confirm the source of a multistate *Escherichia coli* O157:H7 outbreak due to in-shell hazelnuts. J Food Prot 75:320–327

Mo H, Zhu Y (2008) Microbial fermented tea – a potential source of natural food preservatives. Trends Food Sci Technol 19:124–130

Moore JE (2004) Gastrointestinal outbreaks associated with fermented meats. Meat Sci 67: 565–568

Mozola MA (2006) Genetics-based methods for detection of *Salmonella* spp. in foods. J Assoc Off Anal Chem Int 89:517–529

Mullane NR, Whyte P, Wall PG, Quinn T, Fanning S (2007) Application of pulsed-field gel electrophoresis and trace the prevalence of *Enterobacter sakazakii* in an infant formula processing facility. Int J Food Microbiol 116:73–81

Myers C, Hernandez MF, Kennelly P (2010) Investigation of Union International Food Company *Salmonella* Rissen outbreak associated with white pepper. Report Prepared by: The Californian Department of Public Health Food and Drug Branch Emergency Response Team. California Department of Public Health; Sacramento, CA

Neil KP, Biggerstaff JK, MacDonald JK, Trees E, Medus C, Musser KA, Stoika G, Zink D, Sotir MJ (2011) A novel vehicle for transmission of *Escherichia coli* O157:H7 infections to humans: multistate outbreaks of *E. coli* O157:H7 infections associated with consumption of ready-to-bake commercial cookie dough – United States 2009. Clin Infect Dis 54:511–514

Nesse LL, Nordby K, Heir E, Bergsjoe B, Vardund T, Nygaard H, Holstad G (2003) Molecular analyses of *Salmonella enterica* isolates from fish feed factories and fish feed ingredients. Appl Environ Microbiol 69:1075–1081

Park CE, Rayman MK, Stankiewicz ZK, Hauschild AH (1977) Preenrichment procedure for detection of *Salmonella* in gelatin. Can J Micrbiol 23:559–562

Park CE, Stankiewicz ZK, Rayman MK, Hauschild AH (1979) Inhibitory effect of cocoa powder on the growth of a variety of bacteria in different media. Can J Microbiol 25:233–235

Podolak RE, Enache W, Stone W, Black DG, Elliott PH (2010) Sources and risk factors of contamination, survival, persistence, and heat resistance of *Salmonella* in low-moisture foods. J Food Prot 73:1919–1936

Poelma PL, Andrews WH, Wilson CR, Bennett B, Elliot PH, Estela LA, Flowers R, Jogan MJ, Lancette GA, Liddle K (1984) Recovery of *Salmonella* species from nonfat dry milk rehydrated and reduced pre-enrichment conditions: collaborative study. J Assoc Off Anal Chem 67:807–810

Ray B, Jezeski JJ, Busta FF (1971) Effect of rehydration on recovery, repair and growth of injured freeze-dried *Salmonella anatum*. Appl Microbiol 22:184–189

Reich F, König R, von Wiese W, Klein G (2010) Prevalence of *Cronobacter* spp. in a powdered infant formula processing environment. Int J Food Microbiol 140:214–217

Russo ET, Biggerstaff G, Hoekstra RM, Meyer S, Patel N, Miller B, Quick R, Salmonella Agona Outbreak Investigation Team (2013) A recurrent, multistate outbreak of salmonella serotype agona infections associated with dry, unsweetened cereal consumption, United States, 2008. J Food Prot 76:227–230

Sagoo SK, Little CL, Greenwood M, Mithani V, Grant KA, de McLauchlin J, Pinna E, Threlfall EJ (2009) Assessment of the microbiological safety of dried spices and herbs from production and retail premises in the United Kingdom. Food Microbiol 26:39–43

Silva FVM, Gibbs PA (2012) Thermal pasteurization requirements for the inactivation of *Salmonella* in foods. Food Res Int 45:695–699
Scott VN, Chen YU, Freier TA, Kuehm J, Moorman M, Meyer J, Morille-Hinds T, Post L, Smoot L, Hood S, Shebuski J, Banks J (2009) Control of *Salmonella* in low-moisture foods. I: minimizing entry of Salmonella into processing facility. Food Prot Trends 29:341–353
Shan B, Cai Y-Z, Brooks JD, Corke H (2007) The in vitro antibacterial activity of dietary spice and medicinal herb extracts. Int J Food Microbiol 117:112–119
Sospedra I, Soriano JM, Mañes J (2010) Assessment of the microbiological safety of dried spices and herbs commercialized in Spain. Plant Food Hum Nutr 65:364–368
Spector MP, Kenyon WJ (2012) Resistance and survival strategies of *Salmonella* enterica to environmental stresses. Food Res Int 45:455–481
Tajkarimi MM, Ibrahim SA, Cliver DO (2010) Antimicrobial herb and spice compounds in food. Food Control 21:1199–1218
Van Doren JM, Blodgett RJ, Pouillot R, Westerman A, Kleinmeier D, Ziobro GC, Ma Y, Hammack TS, Vikas G, Muckenfuss MF, Fabbri L (2013) Prevalence, level and distribution of *Salmonella* in shipments of imported capsicum and sesame seed spice offered for entry to the United States: observations and modeling results. Food Microbiol 36:149–160
Vadivambal R, Jayas DS (2010) Non-uniform temperature distribution during microwave heating of food materials – a review. Food Bioprocess Technol 3:161–171
Van Schothorst M, Van Leusden FM, De Gier E, Rijnierse VFM, Veen AJD (1979) Influence of reconstitution on isolation of *Salmonella* from dried milk. J Food Prot 42:936–937
Van Schothorst M, Renaud AM (1985) Malachite green pre-enrichment medium for improved *Salmonella* isolation from heavily contaminated samples. J Appl Bacteriol 59:223–230
Vestby LK, Møretrø T, Langsrud S, Heir E, Nesse LL (2009) Biofilm forming abilities of *Salmonella* are correlated with persistence in fish meal- and feed factories. BMC Vet Res 5:20–26
Waldner LL, MacKenzie KD, Köster W, White AP (2012) From exit to entry: long term survival and transmission of *Salmonella*. Pathogens 1:128–155
Walsh D, Molloy C, Iversen C, Carroll J, Cagney C, Fanning S, Duffy G (2012) Survival characteristics of environmental and clinically derived strains of *Cronobacter sakazakii* in infant milk formula (IMF) and ingredients. J Appl Microbiol 110:697–703
Werber D, Dreesman J, Feil F, van Treeck U, Fell G, Ethelberg S, Hauri AM, Roggentin P, Prager R, Fisher IS, Behnke SC, Bartelt E, Weise E, Ellis A, Siitonen A, Andersson Y, Tschäpe H, Kramer MH, Ammon A (2005) International outbreak of *Salmonella* Oranienburg due to German chocolate. BMC Infect Dis 5:7–17
Willis C, Little C, Sagoo S, de Pinna E, Threlfall J (2009) Assessment of the microbiological safety of edible dried seeds from retail premises in the United Kingdom with a focus on *Salmonella* spp. Food Microbiol 26:847–852
Wilson CR, Andrews WH, Poelma PL, Wagner DE (1985) Recovery of *Salmonella* species from the dried foods rehydrated by the soak method. J Food Prot 48:505–508
Wollgast J, Anklam E (2000) Review of polyphenols in *Theobroma cacao*: changes in composition during the manufacture of chocolate and methodology for identification and quantification. Food Res Int 33:423–427
Woolaway MC, Bartlett CLR, Wieneke AA, Gilbert RJ, Murrell HC, Aureli P (1986) International outbreak of staphylococcal food poisoning caused by contaminated lasagne. J Hyg 96:67–73
Wong TL, Thom K, Nicol C, Heffernan H, MacDiarmid S (2007) *Salmonella* serotypes isolated from pet chews in New Zealand. J Appl Microbiol 103:803–810
Zapatka FA, Varney GW, Sinskey AJ (1977) Neutralization of the bactericidal effect of cocoa powder on salmonellae by casein. J Appl Bacteriol 42:21–25
Zweifel C, Stephan R (2012) Spices and herbs as source of *Salmonella*-related foodborne diseases. Food Res Int 45:765–769

Part V
Low a_w Food Decontamination

Irradiation, Microwave, and Alternative Energy-Based Treatments for Low-Water Activity Foods

Brendan A. Niemira

Abstract There is an increasing recognition of low-water activity foods as vectors for human pathogens. Partially or fully dried agricultural commodities, along with modern formulated dried food products, are complex and designed to meet a variety of nutritional, sensory, and market-oriented goals. This complexity means that advanced processing and treatment technologies are needed to achieve food standards. In this chapter, alternative energy-based antimicrobial processing interventions for low-water activity foods will be addressed, including advanced microwave processing, irradiation, and cold plasma. A discussion of existing commercial implementation for established technologies will provide a framework for examination of those recently emerging processes.

Keywords Irradiation • Microwave • Cold plasma • Nonthermal • Food processing • Spices • Nuts • Seeds

Introduction

Low-water activity (a_w) foods represent a unique challenge for decontamination. These foods, either naturally low in moisture or processed from high-moisture ingredients, present a microbiological environment which is already hostile to the growth of human pathogens, such as *Salmonella*, *Escherichia coli*, *Listeria monocytogenes*, and *Shigella* spp. Although the risk of the outgrowth of organisms after rehydration and reconstitution of the product is a known risk factor, growth within the dried food product itself is completely inhibited by a_w below ~0.60 (Beuchat et al. 2013). Therefore, where contamination exists, the organisms tend to be physiologically durable (as with *Clostridium* spp., *Bacillus* spp., and other sporeforming microbes) or in a metabolically quiescent state, reducing their susceptibility to conventional antimicrobial interventions.

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Many of the controls for pathogen contamination of low-a_w foods involve quality and cleanliness assurance in the production of raw ingredients, in the harvesting and transportation of those ingredients, and during subsequent processing. This can involve physical preparation such as peeling, shelling, cutting, chopping, or drying processes, or in the formulation of complex foods. These controls are intended to exclude pathogens from the pre- and postharvest production environments, thereby reducing the risks of contaminating the final low-a_w products. Postharvest interventions are intended to be an extra step incorporated into the food processing chain. A variety of nonthermal technologies have shown promise for inactivating pathogens on sensitive or difficult-to-treat commodities. New antimicrobial compounds and high pressure processing and other approaches have commercial applications. This chapter will focus on energy-based antimicrobial interventions.

Irradiation

Ionizing radiation can effectively eliminate microbes that cause foodborne illness, such as *Salmonella* and *Escherichia* coli (*E. coli*) (FDA 2013a). In addition to applications for food safety, irradiation increases shelf life, controls spoilage organisms and insects, delays sprouting and ripening, and, at higher doses, can sterilize foods for specialty applications. Irradiation operates primarily by the hydrolysis of water molecules into H^+ and OH^- ions (hence the term "ionizing" radiation) and generation of reactive oxygen species, as well as hydrogen (H·) and hydroxyl radicals (HO·). In most foods, water represents the greatest mass component and hence the greatest source of ions and radicals. Even in low-a_w foods, water remains the largest target for interaction with the ionizing particles. However, in low-a_w foods (e.g., dried foods), the mobility of these reactive species is limited, increasing the chance of simple recombination instead of interaction with target microorganisms (Diehl 1995). The practical result of this is that the D_{10} value (the irradiation dose required to achieve a 1-log reduction) for a given microorganism is higher in low-a_w foods than in fresh foods or liquids. In the United States, the allowable radiation dose limits for dry and frozen foods are higher than those set for fresh meats, poultry, seafood, and produce (CFR 2013). The facilities and equipment used to irradiate dry foods are the same as those used for other kinds of foods and food products.

Technologies

Electron Beam

Electron beams (e-beams) are produced by linear or cyclotron accelerators which impart a high velocity and kinetic energy to a stream of electrons. Commercial e-beam irradiators deliver a full processing dose in <5 s. The short exposure time

prevents any significant increase in temperature during processing. High doses are achieved by repeated exposure. The penetrability of the electron beam is much lower than that of gamma rays or X-rays; in commercial practice, many products are treated from multiple sides to enhance dose uniformity. Moreira et al. (2012) determined that the absorbed dose in a product of unit density (a tomato slice) can vary from 1.04 to 0.86 kGy over a distance of less than 4 cm. In general, food products to be treated with electron beam can typically be stacked no thicker than 6–8 cm before absorption results in unacceptable dose distributions.

X-ray

X-rays are high-energy photons with much higher penetrability and dose uniformity than e-beams. Pallet-sized loads can be irradiated without significant loss of penetration. The X-rays used in food irradiation are generated from the same types of accelerator systems used for e-beams. High-energy electrons are absorbed by a dense metal target, emitting X-rays. The conversion efficiency of this process is low, typically 5–10 %, with the rest of the energy lost as heat.

Gamma

Gamma rays are high-energy photons produced by the disintegration of radioactive isotopes, typically cobalt-60 and, less commonly, cesium-137. Like X-rays, gamma rays are photons and have comparably excellent penetrability, suitable for irradiating pallet- or crate-sized packages of product. The desired dose and the strength of the source will determine the time required for processing.

Applications

Herbs and Spices

In the United States, irradiation is allowed as an antimicrobial treatment for dry herbs and spices, with doses of up to 30.0 kGy (CFR 2013). Processing and drying of herbs and spices can lead to bacterial or fungal contamination, as well as infestation with insects or eggs. Irradiation at doses lower than 1 kGy will inactivate insect pests (phytosanitation); at doses up to 30 kGy, it is effective in treating fungal and bacterial contaminants. As a widely traded commodity, herbs and spices are typically subjected to control mechanisms to kill pathogens and for disinfestation. Historically, these have included steam treatments or fumigation with methyl bromide or, more recently, ethylene oxide (FDA 2013b). The technical difficulties of steaming low-a_w commodities, coupled with the regulatory phaseout of halogenic volatile chemicals

(e.g., methyl bromide, ethylene dibromide), have led to a significant commercial application for irradiation.

Dry herbs and spices are the most widely irradiated commodity in the United States, with 80,000 tons treated each year out of some 103,000 tons (i.e., 78 % of total herbs and spices) for all irradiated food commodities (Kume and Todoriki 2013). Prior to labeling regulatory changes in the European Union in 1999, irradiation was more commonly used to treat dry spices. Approximately 20,000 metric tons were irradiated in France in 1998, compared with 3,000 tons in 2005 (an 85 % reduction in 7 years) and 1,470 tons for the entire EU in 2010. Food irradiation overall in China increased from 145,000 tons in 2005 to more than 200,000 tons in 2010, with dried spices a leading irradiated commodity. India irradiated approximately 2,000 tons of spices in 2010, up from 1,400 tons in 2005 (Kume and Todoriki 2013).

Various markets allow different doses for different commodities. Regulators must be able to determine which foods have been irradiated (and to what dose). While this was a significant technical challenge for many years, advances in analytical chemistry since 1995, have led to the development of standardized chemical detection methods (Marchioni 2013). Dried fruits and other low-a_w commodities are amenable to analysis of electron spin resonance, a sensitive technique which measures the absorbed energy of the irradiation process. Also, dried products are typically treated with much higher doses than fresh, high-a_w commodities. This higher dose increases the differentiation in electron spin parameters for the irradiated food. Thus, a dried product may be identified as having been irradiated weeks or even months after the treatment. This is particularly useful for the accurate regulation and market control of dried herbs and spices, as their shelf life may be many months with proper packaging.

Dry Foods and Powders

Irradiation is effective against *Cronobacter* spp. (formerly *Enterobacter sakazakii*) in dry foods. The D_{10} is the dose required to reduce a population by 90 % (1 $\log_{10}$). A dose of 5 kGy reduced *Cronobacter* in dehydrated infant formula with an effective D_{10} value of 0.76 kGy (Lee et al. 2007). A similar value for *Cronobacter* in dehydrated infant formula was obtained by Osaili et al. (2007). In that study, the pathogen was much more susceptible in reconstituted formula, yielding an irradiation D_{10} of 0.24–0.37 kGy. Incorporation of irradiation as a terminal processing step, either immediately prepackaging or post-packaging, is regarded as a viable means to control *Cronobacter* in dry infant formulas (Osaili et al. 2008).

Application of irradiation to dried egg products was recently reviewed by Alvarez et al. (2013). Dried egg products such as dried whole egg, dried egg whites, or yolk powder are amenable to irradiation treatment to inactivate *Salmonella*. The D_{10} value obtained for these dry products was approximately 1.0 kGy, comparable to the *Salmonella* D_{10} obtained for other low-a_w products. Destruction of carotenoids leads to dose-dependent color changes in irradiated dried egg products. The threshold dose for color change in egg yolk powder was lower than that for dried whole egg, 1.5 versus 2.0 kGy, respectively. However, dried egg powder retained its aromatic

integrity at doses up to 4.0 kGy versus the development of off-odors in liquid whole egg after a much lower dose of 0.09 kGy (Katusin-Razem et al. 1989). Functionally, irradiated dried whole egg was comparable to nonirradiated product at doses up to 2.0 kGy. For example, sponge cakes and angel food cakes made with irradiated egg products were identical in volume, flavor, and texture to those made with conventional dried egg ingredients (Alvarez et al. 2013).

Nuts

Nuts, many of which are imported into the United States, are subject to phytosanitary measures such as chemical fumigation or heat treatment (Prakash 2013). Nuts may be irradiated with up to 1.0 kGy to inactivate insect pests and extend shelf life (CFR 2103). Irradiation has recently received new attention as a food safety intervention for nuts. Raw almonds contaminated with *Salmonella* were associated with foodborne illness outbreaks in 2001 (168 cases) and 2004 (47 cases) (Isaacs et al. 2005). These domestic almonds were contaminated with *Salmonella* during harvest, with contamination spreading during postharvest handling.

For nuts intended to be consumed raw or used as raw ingredients in complex foods, heat treatment (e.g., roasting or blanching) may be undesirable due to a negative impact on sensory characteristics. As a food safety intervention, irradiation can penetrate shells and seed coats in a way that chemical fumigation treatments cannot. However, as with many low-a_w, high-fat foods, contaminating pathogens are typically more resistant to irradiation than they are on fresh, moist foods. *Salmonella* on raw almonds had a D_{10} value of 1.25 kGy, indicating that a 5.0-kGy dose would be necessary to achieve a 4-log reduction, assuming irradiation was the sole intervention applied (Prakash et al. 2010).

The fat and oil content of many nuts makes lipid oxidation and induced rancidity a key limiting factor for the use of irradiation. While a phytosanitary dose of 0.6 kGy does not affect a significant sensory impact for irradiated walnuts, in-shell pistachios develop aroma changes after irradiation (Fuller 1986). The sensory response to irradiation is variety dependent and can be significantly influenced by treatment and storage conditions. Taipina et al. (2009) reported acceptable quality of pecans treated with 1.0 kGy, whereas Gölge and Ova (2008) reported no change in color, fatty acid composition, or sensory characteristics in pine nuts treated with up to 5.0 kGy. Nevertheless, Prakash (2013) concluded that, with proper processing controls and as part of an overall sanitation program, irradiation can be a viable treatment for bulk nuts.

Seeds for Sprouting

Fresh sprouts have been associated with many foodborne illness outbreaks in recent decades (Rajkowski and Bari 2013), one of the most severe of which was a 2011 event involving fenugreek seeds and an enterohemorrhagic strain of *E. coli*. In that instance, more than 4,000 cases were identified, which included some 900 hospitalizations and 52 deaths (King et al. 2012).

While a range of chemical treatments are used to inactivate pathogens on seeds used for sprouting, it is generally recognized that the intercalated nature of contamination on the seed coat makes chemical disinfection difficult. The National Advisory Committee on Microbiological Criteria for Foods (NACMCF 1999) advised treatments sufficient to achieve a 5-log reduction; however, even the recommended 20,000 ppm calcium hypochlorite treatment was insufficient to prevent a sprout-associated outbreak arising from seeds so washed (Proctor et al. 2001).

Irradiation treatment of seeds effectively inactivates pathogens. The D_{10} for *Salmonella* ranges from 0.60 to 1.24 kGy, depending on the strain and the suspending seed type (alfalfa, broccoli, or clover), whereas the D_{10} for *E. coli* O157:H7 varied more widely, ranging from 0.55 to 1.43 kGy (Rajkowski and Bari 2013). The United States allows irradiation of seeds as a food safety intervention, with a maximum allowable dose of 8.0 kGy (CFR 2013). However, it has been reported that doses above ~2 kGy result in decreased yield efficiency (the ratio of weight of sprouts produced per weight of seed used) (Rajkowski and Thayer 2001). Hence, the balance of antimicrobial inactivation with impact on seed germination and yield efficiency suggests that irradiation is best employed as part of a combination control strategy.

Microwave

Microwaves are electromagnetic waves with frequencies between 300 MHz (0.3 GHz) and 300 GHz in the electromagnetic spectrum. They are in a subset of the higher frequency end of radio waves, which have frequencies of 30 kHz to 300 GHz. In microwave ovens, relatively narrow bands of 2,450 MHz (or 915 MHz in some industrial applications) are used to excite molecules within foods, converting vibrational energy into heat (Schiffmann 2010). For foodstuffs with high-water content, the primary absorption mechanism is via water molecules. In low-a_w foods, several important factors are involved. Although less efficient at absorbing microwaves than water, constituent molecules such as sugars, fats, and oils absorb a greater proportion of the microwave energy under water-limited conditions. These molecules may undergo Maillard browning reactions which result from rapid, localized heating. This effect is compounded by the thermal conductivity of low-a_w foods, generally lower than in high-moisture food products. Nevertheless, with appropriate engineering controls, microwaves can be used for specific applications in the processing of low-a_w foods.

Applications

Herbs and Spices

Dried turmeric was treated with microwaves under vacuum at different power levels and drying times to determine the quality of the resulting product (Hirun et al. 2012). Models of the processing parameters indicated that the optimal processing

conditions were at high vacuum-microwave power (3,500–4,000 W) and long duration (27–30 min). This process is a significant improvement over conventional drying, primarily due to the suppression of browning reactions.

A microwave pretreatment of rosemary leaves enhanced the retention of carnosic acid and rosmarinic acid (RA) during subsequent storage (Sui et al. 2012), which increases the value of the leaves as a source of these extractable compounds. A 15-min treatment with microwaves at 2,450 MHz resulted in lower degradation rates, regardless of storage method, i.e., traditional sunlight, shade storage, heated storage (55 °C), or frozen storage (−20 °C). The investigators also determined that the microwave pretreatment was as effective as hydrodistillation in facilitating the extraction of essential oils. Microwave-assisted extraction of bioactive compounds was recently reviewed (Routray and Orsat 2012). This method is seen as particularly applicable to low-a_w foods such as seeds and dry rinds. Microwave energy absorbed in the cells will rupture tissues from within, releasing cellular subcomponents of interest into an extraction solvent, such as supercritical carbon dioxide.

Muesli

Muesli is a low-a_w breakfast meal product, consisting of dry grains (e.g., rolled oats, barley, etc.), dried fruits (e.g., raisins, dried currants, cranberries, etc.), and/or nuts. In a commercial processing facility, microwave batch processing of a low-a_w muesli mixture was successfully scaled up using larger 2,450 MHz sources (Schiffmann 2010). The product was run in continuous treatment, incorporating mixing and microwave heating for cooking and drying of the mixture. This commercial system is designed for a throughput of approximately 450 kg/h.

Cold Plasma

Cold plasma is a nonthermal food processing technology based on energetic, reactive gases. Using electricity and a carrier gas, such as air, oxygen, nitrogen, or helium, cold plasma inactivates pathogens on a variety of food products. This relatively new sanitizing method is a waterless process, in which antimicrobial chemical agents are not required (Niemira 2012a). The exact modes of action for cold plasma are dependent on the nature of the technology used and the processing conditions. Cold plasma systems can operate at atmospheric pressures or under low pressure in special treatment chambers. However, UV light and reactive chemical species are the primary mechanisms, with the relative antimicrobial significance of these being specific to the type of technology being tested (Niemira 2012a). UV is a key factor in low-pressure cold plasma systems (Lassen et al. 2005; Tran et al. 2008; Sureshkumar and Neogi 2009), whereas ambient pressure systems have little or no UV effect (Gweon et al. 2009; Machala et al. 2010). The true significance of the equipment design is a key area for future research to better elucidate mode(s) of action.

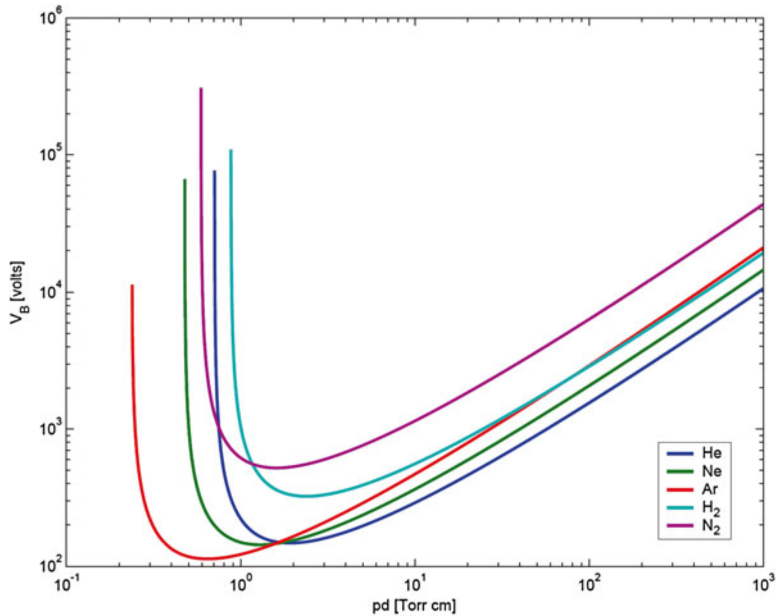

Fig. 1 Paschen ionization curves obtained for helium, neon, argon, hydrogen, and nitrogen. Breakdown potential (VB, in volts) as a function of pressure × distance (pd, in Torr cm^{-1}). Assumes parallel plate electrodes. Data taken from Lieberman & Lichtenberg 2005, Principles of Plasma Discharges, Wiley 2005 (Source: Wikimedia commons)

To date, food safety applications of cold plasma to low-a_w foods have been limited, but the waterless, chemical-free nature of cold plasma is an attractive attribute and is an area for additional research.

Technologies

Plasma is generated by ionizing the working gas of choice. The ionization potential is the level of energy which must be applied to strip the electrons from the gas molecules. The main factors which influence this ionization potential are (1) the type of gas, (2) the gas pressure, and (3) the shape and separation of the electrodes (Niemira 2012a). These interactions are illustrated in Fig. 1. Some gases, such as the noble gases (e.g., helium, neon, and argon), require lower potential energies to ionize, whereas other gases, such as nitrogen and oxygen, require higher energies. The gases used for cold plasma vary widely and are used either as a pure gas or as a defined mixture, depending on the application. Decreasing the gas pressure reduces the ionization potential for all gases. The pressure at which the minimum ionization potential is reached is gas specific. As a practical matter, cold plasma systems can be divided into two groups: (1) those which use reduced gas pressure to modulate the gas ionization potential and (2) those which operate at atmospheric pressure.

Because of the reduced ionization potential at low pressure, cold plasma systems operating under partial vacuum require less power to generate antimicrobially active plasmas. Although the reduced gas pressure means fewer gas molecules are produced per unit volume, the relative ease of ionization means there are more ionized species, free electrons, and active plasma for a given energy expenditure. This can result in a more efficacious plasma. However, the use of equipment that will establish and support partial vacuums can require a more robust chamber design than atmospheric pressure systems. Also, low-pressure plasma systems operate in batch processing mode only.

It requires more energy to produce atmospheric pressure plasmas because of the increased ionization potential of the working gas. This can be overcome, to some extent, with the design and spacing of the electrodes, but the physics of plasma formation means that atmospheric pressure plasmas will have a lower density of active chemical species for a given expenditure of energy. A compensatory advantage of this type of system is that it does not require a vacuum-tight processing chamber, which reduces equipment costs. Also, this type of cold plasma system is suitable for application as a continuous process.

Applications

Nuts and Grains

Nuts, grains, and other low-moisture foods have been studied by many researchers as potential commodities for use with cold plasma. In one study, almonds inoculated with *E. coli* K12 were treated with a dielectric barrier discharge, operating in air at frequencies of 1.0–2.5 kHz (Deng et al. 2007). The almonds were positioned between the dielectric material and the ground electrode, which exposed the almonds to a dense cold plasma field. A 30-s treatment at 25 kV, 2.0 kHz reduced *E. coli* populations by 4 log CFU/g. Treatments of the same duration at a higher frequency (2.5 kHz) resulted in a 5-log reduction.

Rather than placing almonds between the electrodes of a dielectric barrier discharge, the electrodes may be held above the product, with the cold plasma blown outward in a directionalized flow (Niemira 2012b). A “plasma jet” system of this type, operating at 15 kV, 47 kHz, and 524 W, was tested using either air or pure nitrogen as the feed gas. The cold plasma was applied to whole almonds inoculated with *Salmonella* or *E. coli* O157:H7. The greatest reduction observed in that study was 1.34 log of *E. coli* O157:H7 after a 20-s treatment with a 6-cm spacing between the almonds and the cold plasma emitter head. Nitrogen was generally less effective compared with dry air (Niemira 2012b). Greater microbial inactivation occurred with the dielectric barrier system than with the plasma jet system. However, increased flexibility of the former may be an advantage with respect to scale-up and commercialization. Similarly, a fluidized bed cold plasma (4.4 kV) system reduced *S. Enteritidis* PT 30 on almonds by 1.5–2.5 log CFU/g after 40 s of

treatment (Narayanan et al. 2012). In that study, varying the gas composition (helium or argon) and gas flow rate (4 or 14 L/min) did not influence the antimicrobial efficacy.

The different gases used in the studies discussed above ranged from air to nitrogen to the noble gases helium and argon. The design of equipment and the voltages used were directly related to the nature of the feed gas. A secondary impact was the cost and reactivity of the gas. In another study by Selcuk et al. (2008), air plasmas were directly compared with a more exotic gas, sulfur hexafluoride (SF_4). Seeds of tomato, wheat, bean, chick pea, soy bean, barley, oat, rye, lentil, and corn were placed in a partial vacuum chamber (500 mTorr) equipped with an RF plasma generator, operating at1 kHz, 20 kV, and with a power of 300 W. Seeds were treated from 30 s to 30 min. The researchers determined that fungal contamination was reduced in a time-dependent manner. Reductions of 99 + % CFU/g were obtained at the greatest time intervals, with germination reduced to 85 % under these conditions. Air and SF_4 treatments were similar in their antimicrobial efficacy, and plasma processing did not significantly influence quality or productivity factors such as root length, shoot height, etc. (Selcuk et al. 2008).

Powders, Spices, and Formulated Foods

The low-moisture content, flowability, and low-lipid content of many powdered foods suggest that these may be amenable to cold plasma treatment (Misra et al. 2011; Niemira 2012a). Hati et al. (2012) reported that the modes of action for microbial inactivation were dependent on the design of the cold plasma system. With respect to powders and powdered foods, cold plasma processing equipment must be designed to account for the flow characteristics of each particular type of dehydrated material. A challenge for using cold plasma with spices is that the nature of the product relies on volatile flavor and aroma components. The oxidation/reduction potential of the active species within cold plasma discharges has led to their use in scrubbing volatile nitrous and nitric oxides from combustion facilities (Yan et al. 1999). For this technology to be used with spices and powdered foods, optimization of the plasma feed gas and ionization voltages is essential to avoid undue compromise of quality characteristics.

Conclusions

The technologies described in this chapter represent a range of options for use with low-water activity foods. Unlike conventional heating or chemical treatment, the interventions discussed herein are, for the most part, underutilized. For some technologies, such as irradiation, market forces unrelated to the efficacy of the process can be a limiting factor in adoption and commercial implementation. Conflicting regulations in international trade also have an influence with the respective

governing authorities for exporting and importing nations which are in disagreement as to allowable limits on various radiation-based treatments. For other technologies, such as microwave processing, improvements in equipment costs and engineering advances in control and implementation will drive the viability of the intervention as a reasonable alternative for select commodities. Relatively new technologies, such as cold plasma, await results of continued basic research into the modes of action for specific types of cold plasma devices, material advances in equipment design, and improvements in cost and throughput. As these advances are made, cold plasma technology will be an increasingly attractive alternative technology. Nevertheless, current regulations governing the use and application of cold plasma lag behind the scientific and engineering advances. It is anticipated that specific guidelines will be developed in the future. Each of the technology classes discussed in this chapter has specific advantages to producers and processers of low-water activity foods. The challenge, therefore, is to use them, singly and in combinations, to achieve the food safety and food quality goals consistent with consumer needs.

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References

Alvarez I, Niemira BA, Fan X, Sommers CH (2013) Ionizing radiation of eggs. In: Fan X, Sommers CH (eds) Food irradiation research and technology, 2nd edn. Blackwell, Ames, IA, pp 351–372

Beuchat LR, Komitopoulou E, Beckers H, Betts RP, Bourdichon F, Fanning S, Joosten HM, Ter Kuile BH (2013) Low-water activity food: increased concern as vehicles of foodborne pathogens. J Food Prot 76:150–172

Code of Federal Regulations (CFR) (2013) 21CFR, Section 179.26 Ionizing radiation for the treatment of food. Amended April 1, 2013. http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfCFR/CFRSearch.cfm?fr=179.26. Accessed 24 Oct 2013

Deng S, Ruan R, Mok CK, Huang G, Lin X, Chen P (2007) Inactivation of *Escherichia coli* on almonds using nonthermal plasma. J Food Sci 72:M62–M66

Diehl JF (1995) Safety of irradiated foods, 2nd edn. Marcel Dekker, Inc., New York, NY

U.S. Food and Drug Administration (FDA) (2013a) Food irradiation: what you need to know. http://www.fda.gov/Food/ResourcesForYou/Consumers/ucm261680.htm. Accessed 24 Oct 2013

U.S. Food and Drug Administration (FDA) (2013b) Risk profile: pathogen and filth in spices. http://www.fda.gov/Food/FoodScienceResearch/RiskSafetyAssessment/ucm367339.htm. Accessed 8 Nov 2013

Fuller G (1986) Quality evaluation of irradiation dried fruits and tree nuts. U.S. Dept. Energy, Energy Technology Division. Interagency Agreement No. DE-A104-83AL24327

Gweon B, Kim DB, Moon SY, Choe W (2009) *Escherichia coli* deactivation study controlling the atmospheric pressure plasma discharge conditions. Curr Appl Phys 9:625–628

Gölge E, Ova G (2008) The effects of food irradiation on quality of pine nut kernels. Rad Phys Chem 77:365–369

Hati S, Mandal S, Vij S, Minz PS, Basu S, Khetra Y, Yadav D, Dahiya M (2012) Nonthermal plasma technology and its potential applications against foodborne microorganisms. J Food Process Preserv 36:518–524

Hirun S, Utama-ang N, Roach PD (2012) Turmeric (Curcuma longa L.) drying: an optimization approach using microwave-vacuum drying. J Food Sci Tec. DOI: 10.1007/s13197-012-0709-9

Isaacs S, Aramini J, Ciebin B, Farrar J, Ahmed R, Middleton D, Chandran AU, Harris LJ, Howes M, Chan E, Pichette AS, Campbell K, Gupta A, Lior LY, Pearce M, Clark C, Rodgers F, Jamieson F, Brophy I, Ellis A (2005) An international outbreak of salmonellosis associated with raw almonds contaminated with a rare phage type of *Salmonella* Enteritidis. J Food Prot 68:191–198

King LA, Nogareda F, Weill FX, Mariani-Kurkdjian P, Loukiadis E, Gault G, Jourdan-DaSilva N, Bingen E, Macé M, Thevenot D, Ong N, Castor C, Noël H, Van Cauteren D, Charron M, Vaillant V, Aldabe B, Goulet V, Delmas G, Couturier E, Le Strat Y, Combe C, Delmas Y, Terrier F, Vendrely B, Rolland P, de Valk H (2012) Outbreak of Shiga toxin-producing *Escherichia coli* O104:H4 associated with organic fenugreek sprouts, France, June 2011. Clin Infect Dis 54:1588–1594

Kume T, Todoriki S (2013) Food irradiation in Asia, the European Union, and the United States: a status update. http://www.fipa.us/Setsuko.pdf. Accessed 24 Oct 2013

Katusin-Razam B, Razam D, Matie S, Mihokovic V, Kostromin-Soos N, Milanovic N. 1989. Chemical and Organoleptic Properties of Irradiated Dried Whole Egg Yolk. J Food Port 52(11): 781–786

Lassen KS, Bolette N, Reinar G (2005) The dependence of the sporicidal effects on the power and pressure of RF-generated plasma processes. J Biomed Mater Res B Appl Biomater 74B(1): 553–559

Lee JW, Oh SH, Byun EB, Kim JH, Kim JH, Woon JH, Byun MW (2007) Inactivation of *Enterobacter sakazakii* of dehydrated infant formula by gamma-irradiation. Rad Phys Chem 76:1858–1861

Machala Z, Chládeková L, Pelach M (2010) Plasma agents in bio-decontamination by DC discharges in atmospheric air. J Phys D Appl Phys 43:222001–222008

Marchioni E (2013) Detection of irradiated foods. In: Fan X, Sommers CH (eds) Food irradiation research and technology, 2nd edn. Blackwell, Ames, IA, pp 123–145

Misra NN, Tiwari BK, Raghavarao KSMS, Cullen PJ (2011) Nonthermal plasma inactivation of food-borne pathogens. Food Engin Rev 3(3–4):159–170

Moreira RG, Puerta-Gomez AF, Kim JS, Castell-Perez ME (2012) Factors affecting radiation D-values (D10) of an *Escherichia coli* cocktail and *Salmonella* Typhimurium inoculated in fresh produce. J Food Sci 77:E104–E111

Narayanan K, Anderson NM, Fleischman GJ, Keller S (2012) Inactivation of *Salmonella* Enteritidis PT 30 on almonds with a fluidized bed atmospheric pressure plasma (Doctoral dissertation). Illinois Institute of Technology, Bedford Park, IL

National Advisory Committee on Microbiological Criteria for Foods (NACMCF) (1999) Microbiological safety evaluations and recommendations on sprouted seed. http://www.fda.gov/food/foodborneillnesscontaminants/buystoreservesafefood/ucm078789.htm. Accessed 25 Oct 2013

Niemira BA (2012a) Cold plasma decontamination of foods. Annu Rev Food Sci Technol 2012(3):125–142

Niemira BA (2012b) Cold plasma reduction of *Salmonella* and *Escherichia coli* O157:H7 on almonds using ambient pressure gases. J Food Sci 77:M171–M175

Osaili TM, Shaker RR, Abu Al-Hasan AS, Ayyash MM, Martin EM (2007) Inactivation of *Enterobacter sakazakii* in infant milk formula by gamma irradiation: determination of D10-value. J Food Sci 72:M85–M88

Osaili T, Al-Nabulsi A, Shaker R, Ayyash M, Olaimat A, Abu Al-Hasan A, Kadora K, Holley R (2008) Effects of extended dry storage of powdered infant milk formula on susceptibility of *Enterobacter sakazakii* to hot water and ionizing radiation. J Food Prot 71:934–939

Prakash A, Lim F, Duong C, Caporaso F, Foley D (2010) The effects of ionizing irradiation on *Salmonella* inoculated on almonds and changes in sensory properties. Rad Phys Chem 79: 502–506

Prakash A (2013) Irradiation of nuts. In: Fan X, Sommers CH (eds) Food irradiation research and technology, 2nd edn. Blackwell, Ames, IA, pp 317–336

Proctor ME, Hamacher M, Tortorello ML, Archer JR, Davis JP (2001) Multistate outbreak of *Salmonella* serovar Muenchen infections associated with alfalfa sprouts grown from seeds pre-treated with calcium hypochlorite. J Clin Microbiol 39:3461–3465

Rajkowski KT, Thayer DW (2001) Alfalfa seed germination and yield ratio and alfalfa sprout microbial keeping quality following irradiation of seeds and sprouts. J Food Prot 64: 1988–1995

Rajkowski KT, Bari ML (2013) Irradiation of seeds and sprouts. In: Fan X, Sommers CH (eds) Food irradiation research and technology, 2nd edn. Blackwell, Ames, IA, pp 295–316

Routray W, Orsat V (2012) Microwave-assisted extraction of flavonoids: a review. Food Bioprocess Technol 5:409–424

Schiffmann RF (2010) Industrial microwave heating of food: principles and three case studies of its commercialization. In: Doona CJ, Kustin K, Feeherry FE (eds) Case studies in novel food processing technologies. Woodhead, Cambridge, UK, pp 407–426

Selcuk M, Oksuz L, Basaran P (2008) Decontamination of grains and legumes infected with *Aspergillus spp.* and *Penicillum spp.* by cold plasma treatment. Bioresource Technol 99(11): 5104–5109

Sureshkumar S, Neogi S (2009) Inactivation characteristics of bacteria in capacitively coupled argon plasma. IEEE Trans Plasma Sci 37(Suppl 2):2347–2352

Sui X, Liu T, Ma C, Yang L, Zu Y, Zhang L, Wang H (2012) Microwave irradiation to pretreat rosemary (*Rosmarinus officinalis* L.) for maintaining antioxidant content during storage and to extract essential oil simultaneously. Food Chem 131:1399–1405

Taipina MS, Lamardo LCA, Rodas MAB, del Mastro NL (2009) The effects of gamma irradiation on the vitamin E content and sensory qualities of pecan nuts (*Carya illinoensis*). Rad Phys Chem 78:611–613

Tran N, Amidi M, Sanguansri P (2008) Cool plasma for large scale chemical-free microbial inactivation of surfaces. Food Aust 60:344–347

Yan K, Kanazawa S, Ohkubo T, Nomoto Y (1999) Oxidation and reduction processes during NOx removal with corona-induced nonthermal plasma. Plasma Chem Plasma Process 19(3): 421–443

Heat and Steam Treatments

Elizabeth M. Grasso, Christina N. Stam, Nathan M. Anderson, and Kathiravan Krishnamurthy

Abstract Heat-based processes have been used for centuries for food preservation and still remain the most widely used preservation technique in food manufacturing. However, thermal processing treatments are not as efficacious in the destruction of pathogenic microorganisms at low water activities compared to moist environments. Several dry-heat processes have been applied to low-moisture foods and include drying, hot air and baking, air impingement, dry roasting, and oil roasting. These applications require higher temperatures and longer heat times to obtain equivalent lethality to moist-heat processes. Moist-heat processes, which include blanching, moist-air impingement, and controlled condensation steam, are very effective at inactivating microorganisms because the added moisture significantly reduces microbial thermal resistance. However, an increased moisture content of the product can reduce the shelf life, often resulting in the necessity for the (energy consuming) re-drying of product to remove the added moisture. Lastly, extrusion has been shown to be an effective process for microbial inactivation.

Keywords Baking • Blanching • Dry heat • Dry roasting • Drying • Extrusion • Frying • Hot air • Moist heat • Moist-air impingement • Oil roasting • Spray drying • Steam • Sun drying • Superheated steam drying

Introduction to Heat and Steam Treatments

Heat, as a method of food preservation, dates back over two centuries to Nicolas Appert, who in 1809, successfully preserved meats in glass bottles by heating them in boiling water (Jay et al. 2005). Since that time, there have been many developments

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in processing technology; however, heat treatments are among the most widely used in the manufacture of food. In this chapter, we briefly review the thermal resistance of microorganisms in low-moisture foods and explore the efficacy of dry heat, moist heat, and extrusion to reduce microorganisms.

Thermal Resistance of Microorganisms in Low-Water Activity Environments

Thermal treatments are not as efficacious in the destruction of pathogenic microorganisms at low water activities compared to moist environments. Barrile and Cone (1970) investigated the effect of added moisture on the heat resistance of *Salmonella* Anatum in milk chocolate. Lyophilized *S.* Anatum was inoculated into milk chocolate. The chocolate was heated to 71 °C. Moisture was added (0–10 %, via an aerosol mist) and *D*-values were calculated. The *D*-value was reduced from 20 to 4.0 h with the addition of 2.0 % moisture. As the amount of added moisture increased, the corresponding *D*-value decreased. The investigators did note that when the chocolate was inoculated at high initial levels (5-log CFU/g) and heated for periods of time greater than that calculated to assure a *Salmonella*-free product, viable cells were detected. This was most likely due to a decrease in moisture as the product was heated over time, resulting in a more protective environment. Archer et al. (1998) determined that thermal death curves were biphasic, with an initial rapid decline of *S.* Weltevreden in wheat flour. Regardless of the initial water activity prior to heating, the water activities decreased to <0.2 during the first 5 to 10 min. The *D*-value for *S.* Weltevreden in wheat flour was 29 min at water activity of 0.5. By decreasing the water activity from 0.4 to 0.1, the *D*-value increased by almost 3,000 % to 875 min. Goepfert et al. (1970) investigated the thermal resistance of a variety of *Salmonella* isolates of different serovars (*S.* Alachua, *S.* Anatum, *S.* Anatum GF, *S.* Montevideo, *S.* Senftenberg 775 W, and *S.* Tennessee) in sucrose and glycerol at water activities of 0.99, 0.90, and 0.75. They determined that *S.* Senftenberg 775 W, although traditionally regarded as an extremely heat-resistant serovar (in moist environments), was the least thermally stable serovar, of the serovars evaluated, as water activity decreased. While all serovars investigated exhibited a higher thermal resistance as water activity decreased, the resistance was dependent on the solute used to depress the water activity. The heat resistance of *Salmonella* is affected by many factors, including strain, growth conditions, storage conditions, and food composition. Because most of the published thermal inactivation data for pathogens has been obtained in high-water activity conditions, additional research is needed for pathogen survival during thermal processing in low-water activity foods. Podolak et al. (2010) reviewed the thermal resistance of *Salmonella* in a variety of food matrices, including chocolate and syrups, peanut butter, almonds, spray-dried milk, cereal grain flours, and dry animal feeds. Variations in processing and product

parameters may significantly affect actual thermal death kinetics; hence, care must be taken when applying published thermal death kinetics to a given food process (Podolak et al. 2010).

Dry Heat

Dry-heat processing of low-water activity food products is applied to achieve desired sensory attributes, extend shelf life, and provide microbial safety. The preservative effect of heat processing corresponds directly with the disruption of enzymatic activity, either through the dehydration or denaturation of proteins, which inhibits metabolism and growth of microorganisms. Dry-heat processes include drying, hot air and baking, air impingement, dry roasting, and oil roasting. As dry heat is less lethal than wet heat, higher temperatures and longer heating times are required to obtain equivalent lethality (Doyle and Beuchat 2007).

Drying

Drying is the application of heat to remove moisture from foods by evaporation. The main purpose of dehydrating foods is to extend the shelf life by reducing the water activity, which inhibits microbial growth and enzymatic activity. Examples of dried foods include raisins, powdered milk, pasta, grains, beans, nuts, and spices. Drying is accomplished using heated air, heated surfaces, sun drying, freeze-drying, and dielectrics. Potential applications of driers are provided in Table 1.

Table 1 Dryer types and applications

Drier type	Process	Batch or continous	Drying rate	Examples of products
Bin	Hot-air	Batch	Slow	Grains
Tray	Hot-air	Batch or continous	Moderate	Fruits, vegetables
Conveyor	Hot-air	Continous	Moderate	Cereals, snack foods, confectionary
Rotary	Hot-air	Batch or continous	Moderate	Cocoa beans, nuts
Fluidized bed	Hot-air	Continous	Moderate	Vegetables, powders, herbs
Spray	Hot-air	Continous	Fast	Instant coffee, powdered milk
Drum	Heated surface	Continous	Moderate	Potato flakes
Infrared	Radiant	Continous	Fast	Bakery products
Sun	Radiant	Batch	Slow	Fruits, vegetables, spices, herbs, tea
Microwave	Dielectric	Continous	Fast	Bakery products

Drying Using Hot Air

The capacity of air to remove moisture from a food is dependent on the temperature, relative humidity, volume, and velocity of the air passing over the food. Heat from drying air is absorbed by food and provides the latent heat necessary to evaporate water from the surface. The driving force for water removal from the food is the vapor pressure gradient established between the moist interior of the food and the surrounding air. An increase in air temperature or a reduction in relative humidity causes water to evaporate more rapidly from a wet surface. Water vapor diffuses through a boundary layer of air surrounding the food and is carried away by the hot air moving over the wet food. This boundary layer is a barrier to heat transfer and evaporation. The thickness of the boundary layer is determined primarily by air velocity. The more rapidly the air moves over the food, the thinner the boundary layer, which results in a higher vapor pressure gradient and a faster rate of drying.

After food is placed in a drier and the surface heats up, water moves from the interior of the food and initially evaporates from the surface at a nearly constant rate. The drying rate during this period is dependent on the rate of heat transfer. Water activity decreases rapidly during this stage of drying. As water activity decreases, a corresponding reduction in the rate of thermal destruction of *Salmonella* results (Archer et al. 1998; Doyle and Mazzotta 2000; Yoon et al. 2004; Phungamngoen et al. 2011, 2013; Nascimento et al. 2013). At the end of this constant-rate period, the moisture content decreases below a critical level and drying slowly decreases until equilibrium moisture content is reached. This is known as the falling-rate period (Fellows 2000). During this period, water moves from the interior of the food largely by diffusion and thus mass transfer is limited. When the initial moisture content of the food is below the critical moisture content, as is common for grain drying, the falling-rate period is the only rate observed. During the falling-rate period of drying, *Salmonella* survival will be high and, if temperatures are low enough, *Salmonella* growth may be observed during air drying (Nascimento et al. 2013).

In terms of microbial inactivation, it is well recognized that dry heat is less effective than moist heat (Archer et al. 1998; Doyle and Mazzotta 2000; GMA 2009). Hot-air drying has been reported to be ineffective for inactivating microbial populations (Archer et al. 1998; Beuchat and Mann 2011; Phungamngoen et al. 2013). In fact, preservation of microorganisms by drying has been the preferred method for the industrial production of probiotics, dairy starter cultures, baker's yeast, and biocontrol agents (Horaczek and Viernstein 2004; Morgan et al. 2006; Bansal and Garg 2008; Dimitrellou et al. 2008, 2009; Fu and Chen 2011). At air temperatures <38 °C, viable cells can survive at a rate greater than 90 % (Dimitrellou et al. 2008; Fu and Chen 2011). Archer et al. (1998) reported that 150 min was required to achieve a 3-log reduction of *Salmonella* in flour at 70 °C. Beuchat and Mann (2011) achieved reductions of 1- to 2-log CFU/g of *Salmonella* at 120 °C for 20 min in pecan nutmeats, but found that, regardless of the moisture content, hot-air treatment of pecan halves containing 0.77-log *Salmonella* CFU/g failed to eliminate *Salmonella*. The investigators concluded that hot-air treatment cannot be relied upon to reduce *Salmonella* by 5-log CFU/g (Beuchat and Mann 2011).

When starting at a very high moisture content and water activity (a_w), approximately 1- to 3-log reductions of *Salmonella* can be achieved with typical drying cycles for cabbage (60 °C for 270 min), carrots (60 °C for 6 h), and tomatoes (60 °C for 14 h) (Yoon et al. 2004; DiPersio et al. 2005; Phungamngoen et al. 2013). Samples subjected to pretreatments such as soaking in acid solutions, hot-water blanching, and steam blanching exhibited less resistance; however, none of these drying conditions was able to completely eliminate *Salmonella* (Yoon et al. 2004; DiPersio et al. 2005; Phungamngoen et al. 2013).

Spray Drying

Spray drying is accomplished by atomization of a fine mist of concentrated liquid sprayed into a large chamber whose atmosphere is heated to 150–300 °C (Fellows 2000). Drying is very rapid due to the large surface area of the droplets. Products such as milk, egg, cocoa, coffee, and cheese powder are typically spray dried. Literature on survival of microorganisms during spray drying is sparse. Though log reductions of 2–4.9 of *Salmonella* are achievable, *Salmonella* can survive spray drying in skim milk at commercial temperatures (LiCari and Potter 1970). Milk droplet size had no significant impact on *Salmonella* survival. Under all operating conditions, *S.* Tennessee was much more resistant to spray drying than other strains tested.

Superheated Steam Drying

Superheated steam drying uses superheated steam in place of hot air as the drying medium. Superheated steam (SS) is considered a dry steam produced when steam has been given additional energy to raise its temperature above the saturation point at the absolute pressure where the temperature measurement is taken. Superheated steam can cool somewhat, which will result in a drop in temperature, but will not result in condensation of the steam, as long as the temperature is greater than the saturation temperature at the processing pressure. Superheated steam has a higher rate of heat transfer and drying than hot air as a dry medium, and superheated steam drying is effective for microbial decontamination (Cenkowski et al. 2007; Nygaard and Hostmark 2008). Nygaard and Hostmark (2008) evaluated the microbial inactivation efficacy of superheated steam fluidized bed drying of fish meal from an initial moisture content of 37.6–6.3 %. The water activity of the fish meal was estimated to be between 0.4 and 0.5 following drying. They used linear kinetic models to describe microbial destruction that may not have accounted for dynamic water activity conditions observed during drying. *D*-values at 300 °C for *Escherichia coli* and spores of *Clostridium sporogenes* were 0.10 and 0.33 min, respectively. Comparatively, *D*-values of *E. coli* and spores of *C. sporogenes* were 1.12 and 54 min, respectively, for hot air. Phungamngoen et al. (2011) also determined that the destruction rates of *Salmonella* were significantly higher with superheated steam compared to hot air as a drying media in terms of the time to obtain a 3-log reduction of *Salmonella.* For superheated steam drying, 48 min was

required to reduce *Salmonella* by 3 log at 60 °C, whereas 3.3 h was required to obtain a similar destruction level using a hot-air drying at the same temperature (Phungamngoen et al. 2011).

Sun Drying

Sun or solar drying of vegetables, fruits, spices, herbs, and teas is a common international practice because it is very economical in many countries (Doymaz and Pala 2002; Toğrul and Pehlivan 2004; Chan et al. 2009; Sagoo et al. 2009; Sohail et al. 2011). Foods are dried in open air and exposed to sunlight. In many instances, foods are simply laid out on the bare ground in fields or on flat surfaces such as mats, concrete, roofs, or trays and turned regularly until dry. Turning is required for uniform drying and to prevent mold growth (Dhas and Korkanthimath 2003). Studies on survival of *Salmonella* during sun drying are lacking; however, UV rays and local environmental conditions may affect *Salmonella* survival during sun drying (Nascimento et al. 2013). Sun-dried foods are prone to contamination from dust, soil, insects, rodents, and birds flying overhead. To limit exposure of raw materials to pathogens and filth, drying should be accomplished on clean, elevated racks, concrete floors or mats, and not on bare ground. Covers should be in place to prevent contamination from birds flying overhead (ASTA 2011). Another drawback of sun drying is that it is weather dependent.

More sophisticated heat transfer methods collect solar energy and heat the air that is used for drying (Fellows 2000). These mechanical dryers improve hygiene and are in use in many countries to meet export standards (Axtell and Bush 2000).

Baking and Roasting

Baking and dry roasting are typically considered synonymous treatments. The product matrix commonly defines the terminology of these treatments. Baking is usually applied to flour-based foods, such as cakes, cookies, crackers, etc. Roasting commonly refers to nuts/seeds, beans, and meats.

Baking

Baking is a term that is defined as the operation of heating dough-based products in an oven (Potter and Hotchkiss 1995). The water activity of baked goods varies widely, ranging from 0.2 to 0.99. Higher-water activity foods are those such as cakes and pizza, and intermediate moisture foods are cream-filled pastries, donuts, and soft cookies. Low-water activity bakery products are considered those at a_w 0.6 and less, which would include such products as cookies, crackers, and pretzels (Smith et al. 2004). More than 300 foodborne illness outbreaks from 1999 to 2011 have

been associated with baked food products (CDC 2014). Most of these outbreaks were associated with the higher-water activity baked goods, such as cake and pizza. *Salmonella*, *Staphylococcus aureus*, and noroviruses were the most frequently implicated pathogens in these outbreaks. Noroviruses and *S. aureus* are frequent contaminants due to improper hygiene and human contamination during preparation or post-process handling. *Salmonella* contamination usually stems from a variety of sources, which may not have received a thermal kill step and can occur post-processing. These sources include flour, eggs, nut butters, chocolate, fruit, coconut, spices, flavorings, and fillings (Smith et al. 2004). Cross-contamination of processed dry foods from the post-processing environment with *Salmonella* may also occur.

Baking involves three different types of heating of the food product: (1) conduction, (2) convection, and (3) radiation (Therdthai et al. 2004). Briefly, conduction heating occurs from direct contact between the food and pans or conveyor belts (Feyissa et al. 2011). Convection heating results from the hot air inside the oven (Standing 1974). Radiation heating results from the emission of infrared radiation from hot metal surfaces that comprise the interior of the oven (Standing 1974). Farmiloe et al. (1954) studied the survival of *Bacillus* spores in bread baking and determined that, despite an oven temperature of 113 °C, the bread never reached a temperature above 38 °C, due to the cooling from evaporation, which is more rapid than the rate of heat transfer. The authors also incorporated *Salmonella* and *Staphylococcus aureus* into dough prior to baking and determined that the baking process was sufficient to destroy the *Salmonella* consistently, but did not completely inactivate *S. aureus*.

Dry Roasting

Dry air roasting is a direct heating method in which the heat, usually gas, comes into direct contact with the food product. Food products can be held stationary or moved continuously via a conveyor or rotary roaster (ABC 2007b). Continuous roasters may have single stages, where processing conditions are held constant, or multiple stages with a variety of temperature controls (ABC 2007a). In an oven, heating usually occurs via (a) conduction, as heat is absorbed into the food product, and (b) convection from hot air (Fellows 2000). Many commercial ovens are designed with fans to supplement the natural convection currents to provide more uniform heat distribution (Fellows 2000). As the heat contacts the surface of the food product, surface evaporation begins to occur, resulting in slower microbial death as the water activity decreases. To increase the efficacy of the thermal inactivation, product bed depth, air temperature and velocity, chamber humidity, and product moisture must all be optimized.

Nut products are commonly roasted between 130 and 155 °C for 40–55 min at lower temperatures or 10–15 min at higher temperatures to achieve a light to medium roasted product (ABC 2007a). While categorized together, research has been independently conducted on different varieties of nuts, such as almonds, pecans, peanuts, and sesame seeds. Harris (2009), in conjunction with the Almond

Board of California, completed extensive research, validating dry roasting "pasteurization" of almonds. They determined that dry roasting for 100 min at 121 °C or 9 min at 149 °C provided a 4-log *Salmonella* CFU/g reduction. Further research (Harris 2009) determined appropriate validation considerations for a surrogate microorganism, used in lieu of *Salmonella. Pantoea agglomerans,* an accepted surrogate for *Salmonella* Enteritidis PT 30, under dry-heat almond-processing conditions, was used to develop dry-roast guidelines for *S.* Enteritidis PT 30 (ABC 2007a). The dry-heat *D*-value at 250 °F is 21.1 min. To achieve a 4-log CFU/g reduction with an internal D250°F of 21.1 min, almonds must be processed for 100 min at 250 °F, 50 min at 265 °F, 23 min at 280 °F, 12 min at 295 °F, or 9 min at 300 °F, based on the most conservative data from two ABC-sponsored studies and yield a *Z*-value of approximately 47 °F (ABC 2007a). Beuchat and Mann (2011) investigated the use of mild and traditional thermal treatments on the survival of *Salmonella* on pecan nutmeats. Pecan nutmeats, pieces, and halves were inoculated via immersion with a mixture of 5 nut-associated *Salmonella* serovars. For mild treatments, pecan pieces and halves were heated at 60–120 °C for up to 20 min. Significant reductions were obtained by heating wet pieces and halves of nutmeats at 120 °C for 20 min. Regardless of the moisture content of the nutmeat (wet or dry), significant *Salmonella* reductions were observed for halves at 120 °C for 20 min, 2.04 (wet)-log, and 1.18 (dry)-log CFU/g, respectively. These time-treatment conditions, typically used by commercial pecan shellers to dry nutmeats, failed to eliminate the pathogen, regardless of the moisture, size, or initial microbial count. For more traditional roasting conditions, they investigated the effects of roasting for up to 20 min at 130–170 °C. A 2.89-log CFU/g reduction was achieved at 130 °C for 20 min. Dry roasting *Salmonella*-inoculated pecan pieces at 140 °C and below failed to produce a 5-log CFU/g reduction. *Salmonella* reductions were greater than 7-log CFU/g within 15 min at 160 °C and above, but these conditions failed to completely eliminate the *Salmonella* in 2 of 6 samples at 170 °C. Goodfellow investigated the thermal inactivation of *Salmonella* on unblanched Virginia peanuts by dry roasting (Doyle 2009). Results revealed that 129 °C for 45 min provided a 4.3-log CFU/g reduction. Increasing the oven temperature to 146 °C for 15 min provided a 4.9-log CFU/g reduction. Pasteurization of peanuts was achieved at 163 °C for 10 min with a 5.8-log CFU/g reduction (Doyle 2009). Kahyaoglu and Kaya (2006) determined the optimum processing conditions for hot-air roasting of hulled sesame seeds using a response surface model. The optimum roasting temperature and time range for the production of tahini (ground, hulled sesame seeds) to obtain the desired color and texture was 155–170 °C for 40–60 min. Torlak et al. (2013) investigated the rate of *Salmonella* destruction during the dry-heat roasting of sesame seeds. A three-serovar mixture of *Salmonella* (*S.* Typhimurium, *S.* Newport, and *S.* Montevideo) was inoculated onto raw sesame seeds and heated from 0 to 60 min at 110, 130, and 150 °C in a forced-heat oven. Complete inactivation occurred following 60 min at 110 °C, 50 min at 130 °C, or 30 min at 150 °C, with an initial inoculum level of 5.9-log CFU/g. Elevated temperatures may provide additional lethality.

Cocoa bean roasting is one of the most critical of control steps of chocolate manufacture and is the principal stage at which microbial reduction occurs. Prior to roasting, the cocoa bean undergoes fermentation and drying, which reduces moisture levels to about 40–50 % (Doyle and Beuchat 2007). As in many other low-water activity commodities, roasting cannot be conducted at extreme processing conditions, as it would adversely affect the organoleptic properties of the resulting product. Cocoa nibs are commercially dry roasted for 30 min to 2 h at 110–150 °C (Bell and Kyriakides 2002). By the end of the roasting, cocoa nibs have dried to <3 % moisture content, which greatly affects the thermal death of microorganisms (Bell and Kyriakides 2002). Barrile et al. (1971) analyzed the survival of general microflora on cocoa beans roasted at 135, 150, 165, and 180 °C for 30 min. Dry roasting provided a 1- and 2-log CFU/g reduction at 135 and 150 °C, respectively. Following roasting at 180 °C for 30 min, there were no microorganisms detected on the cocoa beans by direct plate count enumeration on plate count agar. Nascimento et al. (2012) investigated the inactivation of *Salmonella* during cocoa nib roasting. A 5-serovar mixture of *Salmonella* (*S.* Typhimurium, *S.* Oranienburg, *S.* Senftenberg, *S.* Eastbourne, and *S.* Enteritidis) was surface inoculated onto nibs and dried for 10 min at 35 °C, to minimize water activity changes of the chocolate nib. Inoculated nibs were dry roasted using a forced-air oven at 110, 120, 130, and 140 °C for up to 50 min. Processing the nibs at 110 or 120 °C for 50 min failed to reduce *Salmonella* populations below detectable limits. Processing cocoa nibs at 130 and 140 °C for 30 and 20 min, respectively, reduced *Salmonella* to below detectable levels. Greater than 5-log CFU/g reductions were achieved for each temperature tested, 140 °C for 10 min, 130 °C for 20 min, and 120 and 110 °C for 50 min.

Jerky is made from thinly sliced or ground and formed meat that is smoked, dehydrated, and seasoned. Strips of jerky are dried slowly over relatively mild thermal treatments for an extended period of time. A 1993 salmonellosis outbreak associated with beef jerky highlighted the importance of process controls (Jay et al. 2005). Processing conditions must be controlled to inhibit the proliferation of pathogenic microorganisms that may be on the raw meat. When drying to below 0.86 water activity within 3 h, pathogen growth is unlikely to occur (Holley 1985). Yoon et al. (2009) modeled the effect of temperature on *Salmonella* inactivation during drying of beef jerky. Raw beef slices, 0.6 cm thick, were inoculated to approximately 5.5-log CFU/g with a mixture of *Salmonella* serovars. The mixture consisted of 4 serovars of *S.* Typhimurium and 1 serovar of *S.* Agona. Following inoculation, the beef slices were heat treated in an American Harvest Gardenmaster Dehydrator (model FD-1000, Nesco, Chaska, MN) at 52, 57, and 63 °C for up to 10 h to produce jerky. The surface temperature of the beef slices took 5 h to reach the target temperature. Survival curves revealed a rapid decrease of salmonellae within the first 2–8 h of drying. After 10 h treatment, a 3-log CFU/g decrease was seen at 52, 57, and 63 °C. During this processing (10 h at 52, 57, or 63 °C) the water activity of the jerky decreased from 0.986 to 0.469–0.666. For all of the investigated temperatures studied, a 3-log CFU/g decrease was observed after 10 h treatment, with a corresponding decrease at water activity from 0.986 to 0.469–0.666.

Harper et al. (2009) evaluated the thermal processing effects of a small-scale and large-scale jerky production schedule at low relative humidity on the elimination of *Salmonella* in chopped and formed beef jerky. Mixtures of *E. coli* O157:H7 and *Salmonella* serovars were inoculated into fresh-chopped all beef jerky batter. The batter was extruded and processed via large-scale or small-scale processing parameters. For large-scale production, the product is heated at 55 °C for 44 min with a medium blower speed in a smokehouse. Temperature is then increased to 77.8 °C and held for 46 min with a medium blower speed (240.4 ± 16.1 m/min). Temperature is then held constant and the blower is increased to high speed (347.7 ± 34.1 m/min) for 434 min. Following large-scale processing for 44 min at 55.6 °C with <10 % RH, followed by 46 min at 77.8 °C, both pathogens were reduced by ≥5-log CFU/g. To provide a water activity of 0.67 and a moisture-to-protein ratio of 0.77, an additional 3.5 h at 77.8 °C was needed. Small-scale jerky production follows a more ramped heating profile, with temperatures of 52 °C (stage 1) and 57 °C (stage 2) for 45 and 60 min, respectively, with medium blower speed. The blower speed is then increased to high for 45 min at 60 °C (stage 3), 46 min at 63 °C (stage 4), 90 min at 68 °C (stage 5), and 120 min at 77 °C (stage 6). Following small-scale jerky production, *Salmonella* was reduced more rapidly with a >5-log reduction following stage 3. Similar reductions of *E. coli* were not observed until partway through stage 6.

Oil Roasting

Oil roasting or deep fat frying is commonly used to produce flavorful, stable, and microbially safe food products. Frying provides a reduced water activity and thermal destruction of microorganisms and enzymes. Commercially produced, oil-roasted foods use a preheated oil tank, which is usually equipped with a continuous conveyor (ABC 2007b). When raw foods are immersed in hot oil, the surface temperature increases rapidly and the water is vaporized as steam (Fellows 2000). The surface then begins to dry out as in dry air roasting. The surface temperature of the food then increases to that of the oil and the internal temperature increases toward 100 °C (Fellows 2000). The degree of roasting, reduction of water activity, and inhibition of microorganisms are affected by thermal processing conditions (time and temperature) as well as product characteristics (size, surface area, and initial water activity). In general, oil roasting is much more rapid than dry air roasting (ABC 2007b). Oil roasting is commonly conducted at 135–180 °C for as little as 3–15 min (Vorria et al. 2004; ABC 2007b). Nuts, chips (potato and tortilla), and baked goods can be heat treated via immersion in hot oil (ABC 2007b; Doyle and Beuchat 2007).

Following the 2007 *Salmonella* outbreak associated with almonds, a minimum pasteurization effect of 4-log *Salmonella* CFU/g reduction was recommended for almond producers (ABC 2007b). Harris (2009), along with the Almond Board of California (ABC 2007b), determined the appropriate critical limits for almond roasting in hot oil. Critical limits for almond exposure are at least 2 min at 127 °C to achieve pasteurization (5-log CFU/g reduction), with corresponding process-dependent operational limits of 138–149 °C for 3–10 min (Harris 2009). Critical limits were proposed

because processing at 126.7 °C for 1.6 and 2 min provided a 4- and 5-log CFU/g reduction of *Salmonella* on the surface of almonds, respectively (ABC 2007b). These operating conditions are required to obtain the desired level of almond roast (Doyle and Beuchat 2007). Abd et al. (2012) determined the impact of storage temperature and time on the thermal inactivation of *Salmonella* Enteritidis PT 30 on oil-roasted almonds. Whole almonds inoculated with *S.* Enteritidis PT 30 were stored at either 4 °C or 23 °C for up to 48 weeks. Almonds were subsequently heat treated at 121 °C for 0.5 and 2.5 min. Survivors were enumerated and results were analyzed using a Weibull model. In almonds previously stored at refrigeration temperatures, heat treatment times of 0.85 ± 0.16 min and 1.8 ± 0.43 min were required to achieve a 4- or 5-log CFU/g reduction, respectively. At ambient temperatures of 23 °C, heat treatment times increased to 1.6 ± 0.53 and 3.2 ± 1.0 min for a 4- or 5-log CFU/g reduction, respectively. Du et al. (2010) evaluated the reduction of *Salmonella* on inoculated almonds exposed to hot oil. Whole almonds were inoculated with *S.* Senftenberg 775 W or *S.* Enteritidis PT 30. Room temperature almonds were submerged in hot oil at 116, 121, and 127 °C for up to 4 min. Rapid reductions were observed during the first 30 s, of at least 3-log CFU/g. Inactivation occurred at a much slower rate during the balance of the processing time. Using Weibull prediction modeling, 0.74 and 1.3 min would be required to provide a 4- or 5-log CFU/g reduction, respectively, for *S.* Enteritidis PT 30 at 127 °C. Neither *Salmonella* serovar (*S.* Senftenberg 775 W or *S.* Enteritidis PT 30) could be recovered following treatment of inoculated almonds at 127 °C for 1.5 min (N_o = 5-log CFU/g). Beuchat and Mann (2011) determined the effect of oil roasting at temperatures of 110–138 °C for up to 4 min on the survival of *Salmonella* on pecan pieces. Industry-significant reductions (>5-log CFU/g) were obtained by heating pieces at 127 °C for 1.5 min or at 132 °C for 1 min. The rate of inactivation during the first 1–1.5 min was rapid, with slower inactivation rates observed for longer exposure times. Similar results were obtained for oil roasting of *Escherichia coli*-inoculated black walnut meats (Meyer and Vaughn 1969). *E. coli* (sample 33b) recovered from walnut meat was used to inoculate samples of uncontaminated walnut for subsequent thermal analysis. *E. coli* was recovered from black walnut meats heat treated by immersion in coconut oil at 100 °C in all cases, except after heat exposure of 3.5 min or more. At 125 and 150 °C, *E. coli* was not detected after as little as 75 and 60 s of exposure, respectively.

Vorria et al. (2004) completed a hazard analysis and critical control point assessment of fried foods. An analysis of safety hazards of the production of potato chips and French fries was conducted. Frying was determined to be a critical control point in the production of fried foods. The frying temperature was identified as the critical limit. The required temperature range is 165–185 °C, with an optimum temperature of 177 °C.

Infrared Heating

Infrared heating, a part of electromagnetic spectrum (0.78–1,000 μm), is known for its advantages over conventional heating such as having a reduced heating time, uniform heating, reduced quality losses, and significant energy savings (Krishnamurthy

et al. 2008). Infrared heating has been successfully used for baking, blanching, drying, pasteurization, roasting, and sterilization (Krishnamurthy et al. 2008). Infrared heating has a poor penetration capacity and, thus, foods (especially solid foods) absorb infrared radiation in a thin surface layer. The heat is further transferred by heat conduction.

Infrared radiation is absorbed by water and organic components of foods such as proteins, lipids, and sugars (Sandu 1986). Due to the energy absorption by proteins, lipids, and sugars, infrared heating can be effective in low-moisture environments as well. Several researchers have successfully demonstrated that infrared heating can inactivate microorganisms in low-moisture foods such as almonds. Brandl et al. (2008) treated almonds at temperatures up to 109 °C using a catalytic infrared heater for inactivation of *Salmonella*. They demonstrated that *Salmonella* Enteritidis PT 30 populations can be reduced by up to 5.3 log without changing the color or morphology of the almonds. Following the infrared heat treatment, almonds were cooled slowly at ambient conditions to allow further thermal lethality and enhance the pasteurization process. In an another study, researchers achieved up to an 8-log reduction of *Enterococcus faecium* (NRRL-B-2354; used as a surrogate for *Salmonella* Enteritidis PT 30) when almonds were infrared heat treated at 120 °C, followed by cooling under ambient conditions until the almond temperature reached 90 °C and then held for 15 min (Bingol et al. 2011). The authors also found that holding almonds at 90 °C for 15 min, following the infrared heat treatment, can contribute more than 5 logs of inactivation. There was no significant difference in appearance, flavor, texture, and overall quality of almonds treated by infrared heating (Bingol et al. 2011). Staack et al. (2008) investigated the effects of temperature, pH, and water activity on inactivation of *Bacillus cereus* spores in paprika powder. The authors used a very high heat flux to rapidly increase the temperature. They were able to obtain a 4.5-log CFU/g reduction in *Bacillus cereus* spores within a 6-min treatment at 95–100 °C, at a water activity of 0.88.

Other researchers used infrared heating in combination with conventional treatments such as hot-air drying, sanitizers, hot water, and superheated steam to further increase the effectiveness of the treatment. For example, Yang et al. (2010) used a sequential infrared and hot-air roasting (SIRHA) for pasteurization of almonds. They reported 4.10-, 5.82-, and 6.96-log reductions after SIRHA roasting at 130, 140, and 150 °C, respectively. The time saving of SIRHA compared to hot-air roasting alone was 38, 39, and 62 % at 130, 140, and 150 °C, respectively (Yang et al. 2010). Bari et al. (2010) used a combination of superheated steam and gas catalytic infrared heat treatment to inactivate *Salmonella* on raw almonds. The authors suggested that superheated steam treatment at 125 °C for 70 s, followed by catalytic infrared heat treatment for 70 s, will deliver a 5.73 ± 0.11-log CFU/g reduction in *Salmonella* population. Bari et al. (2009) reported that almonds treated by sanitizers alone did not reduce the population significantly. When the sanitizer treatment was followed with infrared drying, the population was reduced to 3-log CFU/g from an initial 5.73 ± 0.12-log CFU/g. Similarly, dry heating at 60 °C for 4 days followed by infrared drying for just 70 s resulted in an additional 1-log CFU/g reduction in the *Salmonella* population. When hot-water treatment (85 °C for 70 s) was followed by infrared drying (for 70 s),

Salmonella was reduced to an undetectable level by plating (detection limit was 1-log CFU/g). However, the sample tested positive after, indicating that the population was below the detection limit of the plating approach, and/or some of the sublethally injured cells were able to recover. In another study, Ha and Kang (2013) investigated the efficacy of simultaneous near-infrared heating and ultraviolet radiation for inactivating *Escherichia coli* O157:H7 and *Salmonella enterica* serovar Typhimurium in powdered red pepper (*Capsicum annuum* L.). Simultaneous infrared and UV combination for 5 min resulted in a 3.34-log reduction of *S.* Typhimurium, which included a synergistic effect of 1.86-log reduction. Similarly, the infrared and UV combination for 5 min resulted in 2.78-log reduction of *E. coli* O157:H7 and included a synergistic effect of 1.31-log reduction. This treatment did not have any significant impact on the color of the powdered red pepper.

Moist Heat and Steam

Blanching/Hot Water

Blanching is defined as a heat treatment designed to inactivate enzymes (Potter and Hotchkiss 1995). Blanching is achieved via saturated steam or hot-water immersion. Blanching is typically a pre-drying step used to inactivate enzymes on fruit and vegetable products, as well as to reduce contamination by dirt and microorganisms on the produce surfaces before further preservation by freezing or canning. Steam blanching conditions vary from product to product, with typical treatments ranging from 95 to 100 °C (for steam) for a few seconds to up to 11 min (Nieto et al. 2001; Puupponen-Pimiä et al. 2003; Andress 2006). In steam blanching, there is often poor uniformity of heating through the mass of food, if the food is stacked too high (Fellows 2000). Conventional hot-water blanchers operate at 70–100 °C and there is a risk of contamination by thermophilic bacteria (Fellows 2000). Blanching freshly harvested raw spices provides thermal inactivation of enzymes, inhibits mold growth, which prevents mycotoxin formation, and results in significant reductions in microbial populations (Schweiggert et al. 2007). Harris et al. (2012) investigated the efficacy of hot-water blanching for reducing *Salmonella* on the surface of almonds. The D_{88}°C-values for *S.* Enteritidis PT 30 and *S.* Senftenberg 775 W were 0.39 and 0.37 min, respectively. Neither serovar of *Salmonella* was detectable on samples inoculated with 5-log CFU/g and heated at 88 °C for 2 min.

Moist-Air Impingement

In moist-air impingement systems, an air-steam mixture is forced through an array of nozzles or slots at high velocity. This yields a high heat transfer rate by reducing boundary layer thickness at the surface of the product (Walker and Li 1993; Fellows 2000).

Steam is condensed on the product surface at the initial stage of the process, which results in a high rate of heat transfer as compared to other heating mediums such as hot air or water. Since moist heat is more effective than dry heat at inactivating microorganisms, the moist-air impingement technology can be an effective method for surface decontamination because the moisture condensing on the surface improves inactivation of bacteria. As the product surface temperature exceeds the dew-point temperature of the surrounding air-steam mixture, condensation stops and evaporation begins, which has a negative impact on the bacterial inactivation rate (Jeong et al. 2009). Because traditional thermal inactivation kinetics for microorganisms are insufficient to predict surface pasteurization results for the moist-air impingement process, Jeong et al. (2009) modified the traditional *D*- and *Z*-value models to account for the dynamic water status at the surface of the product under humid heating conditions. The modified model, which utilizes the dew-point temperature of the processing air and dynamic surface temperature history during heating, was more robust and accurate than the traditional *D*- and *Z*-value models.

Steam

Steam had been successfully used for pasteurization and sterilization of food products for decades. Steam is a preservation agent owing to its large amount of latent heat deposit on the product upon condensation and a large convective heat transfer coefficient. Wet steam is the most commonly used method to treat spices in the United States, as well as the most effective process (ASTA 2011). Wet steam is similar in principle to an autoclave and can be performed in closed batch or in a continuous process. Due to steam condensation, the moisture content of the low-moisture foods increases significantly during processing. An increased moisture content can reduce the shelf life of the product, allowing for growth of molds and other organisms. A drawback to the wet steam treatment, then is that the product has to be re-dried to remove the added moisture, which is very energy consuming (Ratti 2001). Yesair and Williams (1942) determined that heating black pepper by wet steam achieved a 5-log reduction of total vegetative cells and spores, and further drying after treatment resulted in a 6-log reduction. After sensory testing, the pepper had a 10 % reduction in flavor potency, based on a taste panels' assessment. One such method that has been successfully applied to spices is the Ventilex® pasteurization system (Anonymous 2012). The system uses saturated, high-pressure steam (130–220 kPa). Due to the high temperature (107–123 °C), the treatment time is very short (25–50 s) (Anonymous 2012). Then a cooler/dryer is used to remove the excess moisture buildup in the Ventilex® system (Anonymous 2012), resulting in a dried finished product.

Another strategy that can be employed in steam processing is controlling condensation on the product. These processes are broadly classified as controlled condensation steam (CCS) processes. In these systems, additional heat is added to the system to raise the steam temperature just above the saturation point. If steam

cools below the saturation point, condensation will result. Thus, CCS processes are often referred to as *dry* steam processes.

The basic principles of CCS processes are as follows:

1. Reduce the amount of steam needed through supplemental electric or resistance heating.
2. Use flash cooling or other methods (drying, etc.) to rapidly remove the added moisture (typically cold and dry air is used to enable efficient cooling and moisture removal).

The efficacy of the controlled condensation process to inactivate microorganisms while maintaining the quality depends upon the combination of pressure, time, and temperature. At higher pressures, bacterial spores can be effectively inactivated as well (Sangi 2011). Spices are an ongoing vehicle of concern for foodborne outbreaks in low-moisture foods. In particular, *Salmonella* can persist for years during storage of spices (Keller et al. 2013). However, spices and other low-moisture foods are very sensitive to high temperatures. One of the considerations for inactivating pathogens in spices is the negative impact steam treatment has on flavor, aroma, color, and the loss of volatile oils (Schweiggert et al. 2007). Therefore, some CCS processes operate under a vacuum and, thereby, reduce the pasteurization temperature. Lower processing temperatures result in preserving the nutritional qualities (Ivarsson 2011). Ensuring uniform exposure to steam is a challenge when treating low-moisture foods with CCS processes. Therefore, many commercial CCS processes use vibrators, screw conveyors, or fluidized beds to ensure uniform mixing, steam exposure, and temperature of the product. To our knowledge, several pieces of commercially available equipment (e.g., Log5®,Napasol®, Revtech®, Safesteril®, Steripure®, Steristep®, and Torbed®) utilize controlled condensation processes. A brief overview of these systems is presented as follows:

CCS processing is often performed in a closed chamber with temperatures ranging from 108 to 125 °C (Leistritz 1997). Hirasa and Takemasa (1998) determined that dry steam was effective in inactivating 8-log CFU/g of bacterial microorganisms and sporeformers in ground spices and also effective in inactivating mold counts of 5-log CFU/g (Hirasa and Takemasa 1998). In Log5® and Napasol®, the controlled condensation process takes place under negative pressure (vacuum) to maintain a saturated steam environment at a reduced pasteurization temperature and thereby preserve the product quality. The Log5® system has an extremely low moisture input and uses a mixing screw to agitate the material and provide uniform treatment (Sangi 2011). In contrast, product treated in Napasol® system is static, often in bags on racks. A 4-min treatment of pistachio kernels, artificially inoculated with *Enterococcus faecium,* in a Napasol® system at 88 °C provided at least 4-log CFU/g reduction (Ivarsson 2011). In the Steripure® steam sterilization system, a vacuum-steam-vacuum process is utilized. Firstly, a high vacuum is drawn in the treatment chamber to evacuate the air, then steam is injected into the chamber, and lastly, a high vacuum is drawn to evacuate the steam and remove any added moisture (Anonymous 2014e).

In the Revtech®, Safesteril®, and Steristep® systems, atmospheric steam is introduced into the product and condensation is controlled by the addition of sensible heat provided by supplemental electric or resistance heating. In the Revtech® technology systems, low-moisture food is fed into an electrically heated, vibrating spiral tube at a constant flow rate. This setup ensures uniform heating. Once the temperature of the product is increased to the desired level, a very small amount of steam (3–10 %) is directly injected. This system results in very minimal condensation on the food product. The product is cooled down by cold, dry air in a second spiral tube (Anonymous 2014d). The reduction of aerobic mesophilic bacteria counts was 5.34-, 3.72-, 3.44-, 4.97-, and 4.58-log CFU/g for black pepper, cumin, parsley, paprika, and chamomile, respectively, under optimum processing conditions. Under these conditions, enterobacteria, yeast, and mold counts were below the detection limit. In the Safesteril® system, the majority (~90 %) of the energy comes from the induction-heated screw conveyor. This reduces the total amount of moisture absorbed by the food product. The food product is then cooled down in a cold and dry atmosphere using dehumidified air to remove the excess moisture. Safesteril® processing reduces the aerobic mesophilic bacteria count, coliforms, yeasts, and molds by 1 to 5 log, depending upon the product, processing conditions, and microbial load (Anonymous 2013). Following treatment by the Safesteril® system, the uninoculated sample's pathogenic microbial load, *Escherichia coli, Salmonella spp., Campylobacter spp., and Shigella spp.,* was below detection limit (Anonymous 2014a). Safesteril® technology can reduce *Enterococcus faecium* by 7-log CFU/g on low-moisture dry sunflower seeds (Anonymous 2014b). The Steristep® (Natprocess, Lyon, France) conveys food material along set of covered, linear vibrating conveyors that are heated by electrical resistance (Anonymous 2014c). The vibrating conveyors agitate the product to provide uniform heat treatment.

The Torbed® system (Torbed® food systems, Cambs, England) treats spices in a shallow fluidized bed using high-velocity hot air (heated by electricity) under low pressures. The moisture released from the spices creates a humid, steam-air environment within the process chamber. Due to the moisture loss, products treated with Torbed® systems are moderately hygroscopic in nature (Anonymous 2001).

Extrusion

Extrusion is a process that combines several unit operations, including mixing, kneading, shearing, cooking, and forming. Extruded products are often processed further by drying, baking, and frying. Cold extrusion, at temperatures near ambient, is used to produce products such as pasta and meat products, typically with a single-screw extruder. Viable microorganisms are unaffected by cold extrusion processes. Walsh et al. (1974) determined that *S.* Typhimurium survived extrusion at temperatures from 35 to 55 °C at a screw speed of 12 rpm. Faster screw speeds (12–30 rpm) resulted in greater reductions of *S.* Typhimurium populations at 35 °C due to mechanical destruction. Walsh and Funke (1975) studied the effects of temperature

and screw speed on survival of *Staphylococcus aureus* during extrusion of spaghetti and determined that a 1-log reduction of *S. aureus* was obtained at 35–55 °C and 20–50 rpm. The abovementioned studies were done without consideration for the effect of moisture content on heat resistance. Hsieh et al. (1976) concluded, in their investigation of pathogens in pasta products, that to insure safety from pathogens, intermediate moisture food components may have to be pasteurized before mixing to the lower a_w.

Thermal extrusion occurs at temperatures typically above 100 °C with twin-screw extruders. Typical products include a variety of low-density, puffed cereals and snack foods. The high-temperature short-time (HTST) processes of thermal extrusion are effective at reducing microbial contamination. Quéguiner et al. (1989) used a twin-screw extruder to study the reduction of *Streptococcus thermophilus* in whey protein powder with low moisture content (4–5 %, w/w), a barrel temperature of 80–204 °C, and a screw speed of 50 rpm. Inactivation was 4.2 log of *Streptococcus thermophilus* in a 500-mm barrel length at a 143 °C barrel temperature and 4.9 log in a 1,000-mm barrel length at 133 °C barrel temperature. Likimani et al. (1990) evaluated inactivation of *Bacillus globigii* spores during extrusion of a corn/soybean mixture (70/30 %, w/w), at 18 % moisture content, and observed reductions of 1 to 7 log. The extrusion variables included screw speeds of 80–160 rpm and barrel wall temperatures of 80 °C in zone 1 and 110–130 °C in zone 2. Ukuku et al. (2012) determined that cornmeal product extruded at ≥75 °C and whey protein isolate (WPI) extruded at 95 °C enhanced reduction of *Escherichia coli* K-12 (ATCC 23716), resulting in populations below the detection limit (<20 CFU/g). Li et al. (1993) reported 2-log and 4- to 5-log reductions at 93.3 and 115.6 °C, respectively, for *C. sporogenes* spores in a mixture of mechanically deboned turkey and white corn flour processed in a twin-screw extruder. Crane et al. (1972) determined that thermal extrusion completely eliminated *Salmonella* when temperatures were above 93.3 °C. These findings are consistent with those of others (Okelo et al. 2006; Anderson et al. 2014; Bianchini et al. 2014). Okelo et al. (2006) reported that in preliminary experiments, *S.* Typhimurium was inactivated at 83 °C with 28.5 % moisture content and 7 s residence time. Anderson et al. (2014) studied destruction of *S.* Agona in oat flour extruded in a single-screw extruder at 65–100 °C and 14–28 % moisture content (a_w 0.72–0.96) and observed reductions of less than 1-log to greater than 7-log CFU/g. Destruction was greatest at higher temperatures with extruded mixtures having higher moisture levels with little to no destruction observed at low water activity and low temperature. At process conditions above 82 °C and a_w of 0.89, a 5-log reduction was achieved. Oat flour was selected as the model food due to its high (about 8 %) indigenous fat content, which has a protective effect on microbial inactivation. Bianchini et al. (2014) determined that *Enterococcus faecium* NRRL-B-2354 is an appropriate surrogate for *Salmonella* in a balanced carbohydrate-protein meal typical of an extruded pet food formula at 28 % over a temperature range of 55–100 °C. *Salmonella* was reduced by 5-log CFU/g at 60.6 °C; however, the inoculum used in these experiments was cultured in broth, which is less heat resistant than lawn-grown cells (Uesugi and Harris 2006; Izurieta and Komitopoulou 2012; Keller et al. 2012). A 5-log reduction of *E. faecium* was achieved at 73.7 °C and was below the limit of detection above 80.3 °C. A 5-log

reduction of *E. faecium* NRRL-B-2354 was also achieved at 73.5 °C and 28 % moisture content during extrusion processing of a balanced carbohydrate-protein mix pet food (Bianchini et al. 2012). The moisture content and temperature ranges used in the study were 24.9–31.1 % and 65–85 °C, respectively.

Conclusion

Heat-based processes are among the most widely used in food processing. Thermal treatments are not as efficacious in the destruction of pathogenic microorganisms at low water activities, compared to moist environments. Thus, dry-heat processes such as drying, hot air and baking, air impingement, dry roasting, and oil roasting require higher temperatures and longer treatment times to obtain equivalent lethality to moist-heat processes. Oil roasting and superheated steam drying are more effective at microbial destruction than hot-air processes. Moist-heat processes, including blanching, moist-air impingement, and controlled condensation steam, are very effective at inactivating microorganisms because the added moisture significantly reduces thermal resistance. Since an increased moisture content can reduce the shelf life of the product, often the product has to be re-dried to remove the added moisture, which is very energy consuming. Lastly, extrusion has been shown to be an effective process for microbial inactivation.

References

ABC (2007a) Guidelines for validation of dry roasting processes. http://www.almondboard.com/Handlers/FoodQualitySafety/Pasteurization/PasteurizationPRogram/ValidationGuidelines/. Accessed Nov 2013

ABC (2007b) Guidelines for validation of oil roasting processes. http://www.almondboard.com/Handlers/FoodQualitySafety/Pasteurization/PasteurizationPRogram/ValidationGuidelines/. Accessed Nov 2013

Abd SJ, McCarthy KL, Harris LJ (2012) Impact of storage time and temperature on thermal inactivation of *Salmonella enteritidis* PT 30 on oil-roasted almonds. J Food Sci 77(1):M42–M47

Anderson NM, Keller S, Mishra N, Gradl D, Pickens S, Conway C, Hartter T, Rokey G, Grasso E, Stam C, Chirtel S (2014) Survival of *Salmonella* related to temperature and water activity during extrusion of a simple oat flour model food. IFT Annual Meeting and Food Expo, New Orleans, LA

Andress EL (2006) How do i freeze. http://nchfp.uga.edu/how/freeze/blanching.html. Accessed Jan 2014

Anonymous (2001) Pasteurization of spices: fact sheet no. 17a. T. F. Systems. Cambs, England

Anonymous (2012) On the safe side: effective decontamination of herbs and spices with a ventilex continuous steam sterilizer for “ultra pasteurization.” I. Imtech Ventilex USA. Middletown, OH

Anonymous (2013) Safesteril® steam sterilization: frequently asked questions. http://www.safesteril.com/en/download.php. Accessed Dec 2013

Anonymous (2014a) Herb and botanical steam sterilization with safesteril®. http://www.safesteril.com/en/download.php. Accessed Dec 2013

Anonymous (2014b) Safesteril® pasteurization and sterilization systems for seeds, nuts and dried fruits. http://www.safesteril.com/en/download.php. Accessed Dec 2013

Anonymous (2014c) Steam sterilization process "steristep". http://www.natprocess.com/steam.php. Accessed Jan 2014

Anonymous (2014d) Sterilization/pasteurization of dried products. http://www.revtech-process-systems.com. Accessed Feb 2014

Anonymous (2014e) Steripure steam sterilization services. http://www.steripure.fr. Accessed Feb 2014

Archer J, Jervis ET, Bird J, Gaze JE (1998) Heat resistance of *Salmonella* Weltevreden in low-moisture environments. J Food Protect 61(8):969–973

ASTA (2011) Clean, safe spices: guidance from the American Spice Trade Association. American Spice Trade Association, Washington, DC

Axtell BLA, Bush A (2000) Small scale drying. IT Publications, London

Bansal T, Garg S (2008) Probiotics: from functional foods to pharmaceutical products. Curr Pharm Biotechnol 9(4):267–287

Bari L, Nei D, Sotome I, Nishina IY, Hayakawa F, Isobe S, Kawamoto S (2010) Effectiveness of superheated steam and gas catalytic infrared heat treatments to inactivate *Salmonella* on raw almonds. Foodborne Path Dis 7(7):845–850

Bari L, Nei D, Sotome I, Nishina IY, Isobe S, Kawamoto S (2009) Effectiveness of sanitizers, dry heat, hot water, and gas catalytic infrared heat treatments to inactivate S*almonella* on almonds. Foodborne Path Dis 6(8):953–958

Barrile JC, Cone JF (1970) Effect of added moisture on the heat resistance of *Salmonella anatum* in milk chocolate. Appl Microbiol 19(1):177–178

Barrile JC, Ostovar K, Keeney PG (1971) Microflora of cocoa beans before and after roasting at 150 °C. J Milk Food Technol 34(7):369–371

Bell C, Kyriakides A (2002) *Salmonella*: a practical approach to the organism and its control in foods. Iowa State University Press, Ames, IA

Beuchat LR, Mann DA (2011) Inactivation of *Salmonella* on pecan nutmeats by hot air treatment and oil roasting. J Food Protect 74(9):1441–1450

Bianchini A, Stratton J, Weier S, Hartter T, Plattner B, Rokey G, Hertzel G, Gompa L, Martinez B, Eskridge AM (2012) Validation of extrusion as a killing step for *Enterococcus faecium* in a balanced carbohydrate-protein meal by using a response surface design. J Food Protect 75(9): 1646–1653

Bianchini A, Stratton J, Weier S, Hartter T, Plattner B, Rokey G, Hertzel G, Gompa L, Martinez B, Eskridge KM (2014) Use of *Enterococcus faecium* as a surrogate for *Salmonella enterica* during extrusion of a balanced carbohydrate-protein meal. J Food Protect 77(1):75–82

Bingol G, Yang J, Brandl MT, Pan Z, Wang H, McHugh TH (2011) Infrared pasteurization of raw almonds. J Food Eng 104(3):387–393

Brandl MT, Pan Z, Huynh S, McHugh TH (2008) Reduction of *Salmonella* Enteritidis population sizes on almond kernels with infrared heat. J Food Protect 71:153–158

CDC (2014) Foodborne outbreak online database. http://wwwn.cdc.gov/foodborneoutbreaks/Default.aspx. Accessed Jan 2014

Cenkowski S, Pronyk C, Zmidzinska D, Muir WE (2007) Decontamination of food products with superheated steam. J Food Eng 83(1):68–75

Chan EWC, Lim YY, Wong SK, Lim KK, Tan SP, Lianto FS, Yong MY (2009) Effects of different drying methods on the antioxidant properties of leaves and tea of ginger species. Food Chem 113(1):166–172

Crane FM, Hansen M, Yoder R, Lepley K, Cox P (1972) Effect of processing feeds on molds, *Salmonella*, and other harmful substances in feeds. Proceedings of the symposium: effects of processing on the nutritional value of feeds, National Academy of Sciences, Gainesville, FL

Dhas PHA, Korkanthimath VS (2003) Processing and quality of black pepper – a review. J Spices Aromatic Crops 12(1):1–13

Dimitrellou D, Kandylis P, Kourkoutas Y, Koutinas AA, Kanellaki M (2009) Evaluation of thermally-dried *Kluyveromyces marxianus* as baker's yeast. Food Chem 115(2):691–696

Dimitrellou D, Tsaousi K, Kourkoutas Y, Panas P, Kanellaki M, Koutinas AA (2008) Fermentation efficiency of thermally dried immobilized kefir on casein as starter culture. Process Biochem 43(12):1323–1329

DiPersio PA, Yoon Y, Sofos JN, Kendall PA (2005) Inactivation of *Salmonella* during drying and storage of carrot slices prepared using commonly recommended methods. J Food Sci 70(4): M230–M235

Doyle M (2009) Preventing *Salmonella* contamination in peanut products. http://www.worldpeanutinfo.com/HotTopics/2009/documents/Doyle2009hottopic.pdf. Accessed Dec 2013

Doyle ME, Mazzotta AS (2000) Review of studies on the thermal resistance of *Salmonellae*. J Food Protect 63(6):779–795

Doyle MP, Beuchat LR (2007) Food microbiology: fundamentals and frontiers. ASM Press, Washington, DC

Doymaz İ, Pala M (2002) Hot-air drying characteristics of red pepper. J Food Eng 55(4):331–335

Du WX, Abd SJ, McCarthy KL, Harris LJ (2010) Reduction of *Salmonella* on inoculated almonds exposed to hot oil. J Food Protect 73(7):1238–1246

Farmiloe FJ, Cornford SJ, Coppock JBM, Ingram M (1954) The survival of *Bacillus subtilis* spores in the baking of bread. J Sci Food Agri 5(6):292–304

Fellows P (2000) Food processing technology principles and practice. CRC Press, Boca Raton, FL

Feyissa AH, Gernaey KV, Ashokkumar S, Adler-Nissen J (2011) Modelling of coupled heat and mass transfer during a contact baking process. J Food Eng 106(3):228–235

Fu N, Chen XD (2011) Towards a maximal cell survival in convective thermal drying processes. Food Res Int 44(5):1127–1149

GMA (2009) Control of *Salmonella* in low-moisture foods. Grocery Manufacturers Association, Washington, DC

Goepfert JM, Iskander IK, Amundson CH (1970) Relation of the heat resistance of *Salmonellae* to the water activity of the environment. Appl Microbiol 19(3):429–433

Ha JW, Kang DH (2013) Simultaneous near-infrared radiant heating and UV radiation for inactivating *Escherichia coli* O157:H7 and *Salmonella enterica* serovar Typhimurium in powdered red pepper (capsicum annuum l). Appl Environ Microbiol 79(21):6568–6575

Harper NM, Roberts MN, Getty KJ, Boyle EA, Fung DY, Higgins JJ (2009) Evaluation of two thermal processing schedules at low relative humidity for elimination of *Escherichia coli* O157:H7 and *Salmonella* serovars in chopped and formed beef jerky. J Food Protect 72(12): 2476–2482

Harris LJ (2009) Process validation for low-water activity foods. http://www.foodprotection.org/files/rr_presentations/RR_07.pdf. Accessed Nov 2013

Harris LJ, Uesugi AR, Abd SJ, McCarthy KL (2012) Survival of *Salmonella* Enteritidis PT 30 on inoculated almond kernels in hot water treatments. Food Res Intl 45(2):1093–1098

Hirasa K, Takemasa M (1998) Spice science and technology. Marcel Dekker, Inc., New York, NY

Holley RA (1985) Beef jerky: Viability of food-poisoning microorganisms on jerky during its manufacture and storage. J Food Protect 48(2):100–106

Horaczek A, Viernstein H (2004) *Beauveria brongniartii* subjected to spray-drying in a composite carrier matrix system. J Microencapsulation 21(3):317–330

Hsieh F-H, Acott K, Labuza TP (1976) Death kinetics of pathogens in a pasta product. J Food Sci 41(3):516–519

Ivarsson C (2011) Nut pasteurization: minimising impact on appearance, colour and flavor. AgroFood Industry Hi-Tech 22(3):22–24

Izurieta WP, Komitopoulou E (2012) Effect of moisture on S*almonella* spp. heat resistance in cocoa and hazelnut shells. Food Res Intl 45(2):1087–1092

Jay JM, Loessner MJ, Golden DA (2005) Modern food microbiology. Springer, New York, NY

Jeong S, Marks BP, Orta-Ramirez A (2009) Thermal inactivation kinetics for *Salmonella* Enteritidis PT30 on almonds subjected to moist-air convection heating. J Food Protect 72(8):1602–1609

Kahyaoglu T, Kaya S (2006) Determination of optimum processing conditions for hot-air roasting of hulled sesame seeds using response surface methodology. J Sci Food Agric 86:181–185

Keller SE, Grasso EM, Halik LA, Fleischman GJ, Chirtel SJ, Grove SF (2012) The effect of growth on the thermal resistance and survival of *Salmonella* Tennessee and Oranienburg in peanut butter using a new thin-layer thermal death time device. J Food Protect 75(6):1125–1130

Keller SE, VanDoren JM, Grasso EM, Halik LA (2013) Growth and survival of *Salmonella* in ground black pepper (piper nigrum). Food Microbiol 34(1):182–188

Krishnamurthy K, Khurana HK, Jun S, Irudayaraj J, Demirci A (2008) Infrared heating in food processing: an overview. Comp Rev Food Sci Food Safety 7:2–13

Leistritz W (1997) Methods of bacterial reduction in spices. Spices: flavor chemistry and antioxidant properties. American Chemical Society, Washington, DC, pp 7–10

Li Y, Hsieh F, Fields ML, Huff HE, Badding SL (1993) Thermal inactivation and injury of C*lostridium sporogenes* spores during extrusion of mechanically deboned turkey mixed with white corn flour. J Food Process Preserv 17(5):391–403

LiCari JJ, Potter NN (1970) *Salmonella* survival during spray drying and subsequent handling of skimmilk powder. Effects of drying conditions. J Dairy Sci 53(7):871–876

Likimani TA, Sofos JN, Maga JA, Harper JM (1990) Methodology to determine destruction of bacterial spores during extrusion cooking. J Food Sci 55(5):1388–1393

Meyer MT, Vaughn RH (1969) Incidence of *Escherichia coli* in black walnut meats. Appl Microbiol 18(5):925–931

Morgan CA, Herman N, White PA, Vesey G (2006) Preservation of micro-organisms by drying: a review. J Microbiol Methods 66(2):183–193

Nascimento MDSD, Pena PO, Brum DM, Imazaki FT, Tucci MLS, Efraim P (2013) Behavior of *Salmonella* during fermentation, drying and storage of cocoa beans. Intl J Food Microbiol 167(3):363–368

Nascimento MS, Brum DM, Pena PO, Berto MI, Efraim P (2012) Inactivation of *Salmonella* during cocoa roasting and chocolate conching. Intl J Food Microbiol 159(3):225–229

Nieto A, Castro MA, Alzamora SM (2001) Kinetics of moisture transfer during air drying of blanched and/or osmotically dehydrated mango. J Food Eng 50(3):175–185

Nygaard H, Hostmark O (2008) Microbial inactivation during superheated steam drying of fish meal. Drying Technol 26(2):222–230

Okelo PO, Wagner DD, Carr LE, Wheaton FW, Douglass LW, Joseph SW (2006) Optimization of extrusion conditions for elimination of mesophilic bacteria during thermal processing of animal feed mash. Anim Feed Sci Technol 129(1–2):116–137

Phungamngoen C, Chiewchan N, Devahastin S (2011) Thermal resistance of *Salmonella enterica* serovar Anatum on cabbage surfaces during drying: effects of drying methods and conditions. Intl J Food Microbiol 147(2):127–133

Phungamngoen C, Chiewchan N, Devahastin S (2013) Effects of various pretreatments and drying methods on *Salmonella* resistance and physical properties of cabbage. J Food Eng 115(2): 237–244

Podolak R, Enache E, Stone W, Black DG, Elliott PH (2010) Sources and risk factors for contamination, survival, persistence, and heat resistance of *Salmonella* in low-moisture foods. J Food Protect 73(10):1919–1936

Potter NN, Hotchkiss JH (1995) Food science. Chapman & Hall, New York, NY

Puupponen-Pimiä R, Häkkinen ST, Aarni M, Suortti T, Lampi A-M, Eurola M, Piironen V, Nuutila AM, Oksman-Caldentey K-M (2003) Blanching and long-term freezing affect various bioactive compounds of vegetables in different ways. J Sci Food Agric 83(14):1389–1402

Quéguiner C, Dumay E, Cavalier C, Cheftel JC (1989) Reduction of *Streptococcus thermophilus* in a whey protein isolate by low moisture extrusion cooking without loss of functional properties. Intl J Food Sci Technol 24(6):601–612

Ratti C (2001) Hot air and freeze-drying of high-value foods: a review. J Food Eng 49(4): 311–319

Sagoo SK, Little CL, Greenwood M, Mithani V, Grant KA, McLauchlin J, de Pinna E, Threlfall EJ (2009) Assessment of the microbiological safety of dried spices and herbs from production and retail premises in the United Kingdom. Food Microbiol 26(1):39–43

Sandu C (1986) Infrared radiative drying in food engineering: a process analysis. Biotechnol Prog 2(3):109–119

Sangi D (2011) Heading for the future. Diagram (official magazine) of Buleher (158)

Schweiggert U, Carle R, Schieber A (2007) Conventional and alternative processes for spice production – a review. Trends Food Sci Technol 18(5):260–268

Smith JP, Daifas DP, El-Khoury W, Koukoutsis J, El-Khoury A (2004) Shelf life and safety concerns of bakery products – a review. Crit Rev Food Sci Nutr 44(1):19–55

Sohail M, Ayub M, Ahmad I, Ali B, Dad F (2011) Physicochemical and microbiological evaluation of sun dried tomatoes in comparison with fresh tomatoes. Pakistan J Biochem Mol Biol 44(3):106–109

Staack N, Ahrne L, Borch E, Knorr D (2008) Effects of temperature, pH, and controlled water activity on inactivation of spores of *Bacillus cereus* in paprika powder by near IR radiation. J Food Eng 89:319–324

Standing CN (1974) Individual heat transfer modes in band oven biscuit baking. J Food Sci 39(2):267–271

Therdthai N, Zhou W, Adamczak T (2004) Three-dimensional cfd modelling and simulation of the temperature profiles and airflow patterns during a continuous industrial baking process. J Food Eng 65(4):599–608

Toğrul İT, Pehlivan D (2004) Modelling of thin layer drying kinetics of some fruits under open-air sun drying process. J Food Eng 65(3):413–425

Torlak E, Sert D, Serin P (2013) Fate of *Salmonella* during sesame seeds roasting and storage of tahini. Intl J Food Microbiol 163(2–3):214–217

Uesugi AR, Harris LJ (2006) Growth of *Salmonella* Enteritidis phage type 30 in almond hull and shell slurries and survival in drying almond hulls. J Food Protect 69(4):712–718

Ukuku DO, Onwulata C, Mukhopadhyay S (2012) Behavior of *Escherichia coli* bacteria in whey protein concentrate and corn meal during twin screw extrusion processing at different temperatures. J Food Process Technol 3(4):150

Vorria E, Giannou V, Tzia C (2004) Hazard analysis and critical control point of frying – safety assurance of fried foods. Eur J Lipid Sci Technol 106(11):759–765

Walker C, Li A (1993) Impingement oven technology. Part III: combining impingement with microwave. Am Inst Baking Technol Bull 15:1–6

Walsh DE, Funke BR (1975) The influence of spaghetti extruding, drying and storage on survival of *Staphylococcus aureus*. J Food Sci 40(4):714–716

Walsh DE, Funke BR, Graalum KR (1974) Influence of spaghetti extruding conditions, drying and storage on the survival of *Salmonella typhimurium*. J Food Sci 39(6):1105–1106

Yang J, Bingol G, Pan Z, Brandl MT, McHugh TH, Wang H (2010) Infrared heating for dry-roasting and pasteurization of almonds. J Food Eng 101(3):273–280

Yesair J, Williams OB (1942) Spice contamination and its control. J Food Sci 7:118–126

Yoon Y, Geornaras I, Kendall PA, Sofos JN (2009) Modeling the effect of marination and temperature on *Salmonella* inactivation during drying of beef jerky. J Food Sci 74(4):M165–M171

Yoon Y, Stopforth JD, Kendall PA, Sofos JN (2004) Inactivation of *Salmonella* during drying and storage of roma tomatoes exposed to predrying treatments including peeling, blanching, and dipping in organic acid solutions. J Food Protect 67(7):1344–1352

Part VI
Research Needs

Research Gaps and Needs Pertaining to Microbial Pathogens in Spices and Low-a_w Foods

Margaret Hardin

Abstract The following sections include discussions of research opportunities and needs associated with low-moisture/low-water activity foods. Preventing contamination of low-moisture foods begins with developing an understanding of the microbiological ecosystems associated with commodities and ingredients that comprise low-a_w foods beginning at growing and harvesting through their primary and final processing. This includes developing more rapid and sensitive microbiological methods for isolating foodborne pathogens and identifying newly emerging microorganisms of concern.

The effectiveness of any food process relies on the validation of its efficacy to kill or control foodborne pathogens; hence, many processes used in the production of low-moisture foods need to be validated to ensure their usefulness in mitigating food safety hazards. This also highlights the need for appropriate validated surrogate microorganisms and protocols to perform these studies for a seemingly unlimited number of low-moisture foods and food processes.

Keywords Prevalence • Risk assessment • Surrogate microorganism • Validation • Interventions • Sanitation

Introduction

Reducing the water available in foods to prevent the growth and metabolism of microorganisms has been used as a method of preservation for centuries. Until recently, low-moisture foods have had a relative good food safety history. This safety record is largely based on the theory that if the available water is controlled, either by removing water or by adding solutes, microbial growth (not necessarily microbial survival) and the production of associated toxins will not occur. However, in recent years there has been an increase in the number of recalls and outbreaks of foodborne illnesses associated with low-moisture foods. This has led to a need to

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identify the gaps, opportunities, and research needs associated with these types of foods. The task may seem straightforward; however, it can easily become overwhelming considering how many different food products and processes comprise the category of "low water activity" or "low moisture."

The products included in the category of low-a_w foods range from soup mixes to nuts, from spices and herbs to dried fruits and vegetables, dried meats, powdered milk and infant formula, snacks, pet foods, cocoa, honey, egg powders, hydrolyzed vegetable protein, grains, and seeds. The number of foods, food ingredients, and combinations of ingredients included in this category of foods seems limitless. The multitude of processes involved in growing, harvesting, processing, and preparation of low-a_w foods are extensive and wide ranging. In addition, growing, harvesting, and primary processing may occur in one or more of over one hundred different countries, including the most remote areas of the world. The secondary or final processing of these foods may include numerous processes and types of equipment and involve baking, drying, roasting (dry and oil), blanching, extruding, and infrared or microwave technology, just to mention a few. Most of these processes were designed to impart certain quality characteristics to a product, such as color and flavor for roasted nuts or setting starch in a baked product, and were not necessarily designed as lethality processes. Therefore many microorganisms, including pathogenic bacteria, may be capable of surviving these processes. Although the opportunities and research needs may not equal the number of products in this category, they almost all begin in the field and do not end until final preparation and consumption by the end user.

Prevalence Studies

Many low-moisture foods are derived from plant material and are, therefore, in contact with the soil and the environment. There are many potential contributing factors influencing the exposure of food products and ingredients to microbial contaminants in their natural environment, including soil, air, dust, water (irrigation and rain), wild animals (including birds and pests), people, and equipment. An effective and efficient approach to enhancing the safety for these types of food commodities is through farm-to-plant control of the supply chain, beginning at the source. Many commodities, such as spices and herbs, are globally sourced and cultivated in various areas of the world, often in developing countries and in areas with varying levels of good agricultural practices and sanitation. Historically, management of this supply chain has included strategic vendor alliances and control of source materials, which includes monitoring and control of the manufacturing process. Prevention-based management of microbial issues often includes material risk assessments, supplier risk assessments, and a combination of product specifications, auditing, and microbiological monitoring programs. A significant amount of data exists and is utilized within companies to manage the risks associated with the raw materials and suppliers specific to their own processes and products. For the industry as a

whole, however, updated and in-depth surveys and prevalence data are needed to identify the actual food safety risks and data gaps within each specific commodity. While this particular task may be cumbersome and require the cooperation and participation of entities on an international level, the data will be valuable for improving the understanding and control of microbial food safety hazards and enable scientifically informed decision-making. Pathogen prevalence and the concentration of a particular pathogen in raw ingredients influence the overall risk assessment associated with microbiological hazards for growing, harvesting, processing operations, and end-user preparation. Some risk profiles and microbiological guidelines exist for certain products; however, many are outdated and incomplete, focusing on one step in the process or on only one organism of concern (ESR 2010; Pafumi 1986). Oftentimes, these published literature reports or food industry estimates are used as guidance for predicting prevalence. However, these historical data and risk assessments, when based on estimates or outdated data, may become less reliable, particularly as recalls and outbreak data identify new risks. In addition, they often do not keep up with changing agricultural best practices, the implementation of new interventions, or changes in regulatory focus and international trade (Sperber et al. 2007).

Available technology and agricultural best practices do not currently allow for complete removal of pathogenic bacteria in the growing and primary processing stages of production, especially if those primary processing stages also occur in the field. The challenge, then, becomes managing the risk of contamination and putting procedures in place to limit the growth of pathogenic bacteria on product. This also includes understanding the risk of current practices and procedures as well as recognizing that prevalence may also be influenced by seasonal and geographical factors, weather, and other environmental factors, as well as by sampling and testing methodologies. Many questions may be answered by a thorough survey, prevalence study, or risk assessment. An example may be, "what is the food safety risk associated with contamination by insects, animals, and pathogens during the growing cycle in different parts of the world for different agricultural products?" Keep in mind that contamination from birds and pollinating insects is often a natural part of the growing cycle. It is sometimes a common practice for animal (and sometimes human) waste ("night soil") to be used to fertilize soil to grow agricultural products. By documenting the actual risks associated with current practices, solutions and best practices can be developed to address such issues as composting manure in a practical manner attainable to developing countries where sanitation and food handling practices may not be adequate. The same approach can be used to identify risks associated with different sources of irrigation water and may extend into primary processing and transportation.

Primary processing of many agricultural products on an international level, where automated technologies and equipment are not affordable or available, often includes human handling of the product, such as shelling, deseeding, and sorting. In these cases, opportunities abound for close attention to be paid to proper sanitation and human hygiene. In addition, developing countries with limited economic capital and income do not tend to waste resources and often use and reuse whatever

is at hand, including packaging materials. While the industry recommends only new, unused bags be used to pack product, in actual practice, bags that originally held animal feed, pesticides, fertilizers, or bird guano may also be used as final or intermediary packaging for products destined for human consumption. As previously mentioned, a well-designed study can help to measure the actual risks, if any, from these types of practices and aid in the development of best practices, depending on availability, for cleaning and sanitizing these bags before use.

Many agricultural products such as spices, herbs, and fruits include a drying step during processing. There is always the risk of airborne or other environmental contamination during drying, thus various practices may be employed to reduce microbial risks and contamination, such as sun drying product on mats, platforms, overhead, in greenhouses, or above ground. Valid microbiological data that would support these different processes would further assist growers, processors, and manufacturers in managing actual and practical risks and in obtaining needed resources, which may be required to implement them. Additional handling that could contribute to the risks associated with these products include mode of transportation, whether it is a bicycle, farm truck, or a pack animal. Secondary processing offers opportunities for contamination associated with personnel hygiene practices, particularly if these processes occur in a developing country where the expected level of sanitation and hygiene is oftentimes less than what occurs in the home.

All surveys and prevalence studies have challenges, including the volume of samples required, which can become costly and resource intensive due to the hundreds of samples that may be required to determine prevalence of a specific microbe with some degree of confidence. Such testing must take into account seasonal and geographical factors, often during multiple growing seasons, weather and other environmental factors, the expected (and unexpected) level and occurrence of the target microbe(s) in the sample, as well as the sampling and testing methodologies. These studies should also take into consideration how the microbiological concentration and prevalence within an ingredient may change throughout the production and supply chain to the end user.

A review of the current literature, outbreaks of foodborne illnesses, and product recalls will aid in determining the microbe(s) of concern for most low-moisture foods. Examples of target microbes of concern in a few types of low-moisture foods include: *Salmonella*, *Listeria monocytogenes*, *Bacillus cereus*, and Shiga toxin-producing *Escherichia coli* (STECs) in spices and spice processing; *Salmonella* and STECs in cereal and grain commodities and milling; *Salmonella*, STECs, and *Listeria monocytogenes* for certain baked goods; *Salmonella*, *Listeria monocytogenes*, and STECs in tree nuts, including almonds, pecans, pistachios, and hazelnuts; *Salmonella* in powdered milk plus *Cronobacter* in infant formula; and *Salmonella* in extruded and dry pet food and treats. A word of caution in identifying the pathogen(s) of concern: just because a target microbe, such as *Salmonella,* has an epidemiologic link to a product does not mean there are no other potential pathogens of concern present that should be considered.

Sampling and Testing Methodology

The food industry, in general, typically faces both low-frequency contamination and low pathogen survivability events with regard to ready-to-eat commodities; therefore, sampling methods should be developed to detect even sporadic, low levels of key pathogens. This can be a challenge in determining and obtaining a representative sample of a low-moisture or dry product, as they are often produced in batches or lots of hundreds or even thousands of pounds. When a contamination event does occur, it is often sporadic and tends to be isolated. Additional challenges associated with the research of low-moisture foods include the methodology used to detect a pathogen. For example, enumeration methods for *Salmonella* are not widely used and are often based on forms of the MPN (most probable number) method. Methods of isolation also frequently include at least one nonselective pre-enrichment and/or resuscitation step, particularly if the microorganism may have been stressed due to processing (drying, heating, freezing), prior to enumeration, which may also interfere with determining the actual cell numbers of the microbe present. This is followed often by a selective enrichment. Proper enumeration of the target microbe in the matrix of concern is important in determining the incoming loads, levels of the microbe needed to effectively validate lethality and intervention methods, and in determining an infective dose for the consuming population. Some herbs and spices, such as oregano, rosemary, cinnamon, cloves, and allspice, present an additional challenge to the microbial isolation and recovery process as they contain naturally occurring antimicrobials (i.e., essential oils; Tajkarmi et al. 2010) that may interfere with conventional and rapid microbiological methods. The method most often employed to circumvent the inhibitory effect of an antimicrobial in the product has been to dilute the sample to a level at which the antimicrobial is no longer effective. However, dilution also reduces the sensitivity of the assay and thus is not always the best solution. Research is needed on methods to separate or extract the antimicrobial fraction (or the target organism) in some products such as spices and herbs, without further diluting the sample or adversely affecting the microbial population in the sample. Realizing the limitations associated with low-a_w foods, there is a need for more rapid microbiological methods with improved sensitivities, which allow for the detection of low levels of foodborne pathogens (Beuchat et al. 2013). Additional research is needed to develop practical tools, such as molecular subtyping of isolates, to facilitate microbial source tracking from the patient and product to ingredients, and to differentiate resident from transient strains in the environment (Chen et al. 2009).

Validation and Surrogates

Perhaps the greatest single need for low-a_w food research is the need for validation studies to provide scientific data to support pathogen control measures during processing. Significant challenges exist in validating processes for low-moisture foods,

including, but not limited to, (a) a need to revisit *D*-values for certain products and processes, (b) applying suitable inoculation methods for challenge studies used in validating processes, (c) improvement in methods to isolate and enumerate target microbes, and (d) selection and use of the most appropriate surrogate microorganism(s) best suited for the target microbe(s) of concern as well as for the specific products and processes being validated.

Revisiting D-Values

The ability of a microbe to survive and grow in a low-a_w food can be affected by a variety of factors. In some instances, there is a need to revisit and update published data and assumptions which have been made for low-a_w foods and their associated target microbes throughout the years. What is known is that *D*-values of target microbes, such as *Salmonella,* increase in the presence of reduced moisture content or a_w. In addition, the heat resistance of *Salmonella* is affected by other factors such as the strain and serotype tested, previous growth and storage conditions, heating method (dry vs. moist heat), food composition (fat, protein, moisture content, ingredients, preservatives), and test media and recovery method (Podolak et al. 2010). *D*- and *z*-value data published in the literature for low-moisture foods are often inadequate if *D*-values were calculated using only two to three points on a graph and when the concept of “integrated lethality” was not applied (Scott and Weddig 1998). Caution must be taken when using published *D*-values as the *D*-value for *Salmonella* in milk chocolate may be vastly different than those in bitter chocolate or cereal of the same water activity owing to differences in the chemical composition of the food (D’Aoust 1977).

Surrogate Microorganisms

There is a pressing need for surrogate microorganisms and standardized protocols to assist companies (large and small) in the validation of critical process operations that are necessary for the control of foodborne pathogens (Borowski et al. 2009). Some initial validation testing can be accomplished in a laboratory setting using specific pathogens of concern. The results of laboratory research, however, must be interpreted with caution, as it is not possible to reproduce all industrial processes in a laboratory setting. It is also often difficult to extrapolate laboratory results to the processing facility if the initial research is performed in a controlled laboratory setting. In addition, when plant process line validation or in-plant confirmation is required, the use of a surrogate microbe is essential due to the risks associated with introduction of pathogens into a food processing environment. For example, some processes such as sterilization interventions used to treat spices, including propylene oxide (PPO), ethylene oxide (ETO), steam heat, dry heat, and irradiation, are

not easily reproduced in a laboratory. These antimicrobial interventions implemented for pathogen reduction in the spice industry must be validated in the processing environment to demonstrate their efficacy under actual, in-plant operation conditions. In such cases, laboratory research can be used as a reference point; however, it is not an equivalent substitute for actual, in-plant process validation. The availability of nonpathogenic bacteria, which have similar or correlated responses to specific food processes as the pathogenic bacteria, offers the food processor the ability to validate a process within the plant under actual processing conditions without the use of human pathogens. An effective surrogate is often described a microbe whose resistance to the kill step is well known, relative to the pathogen(s) of concern. Surrogates are nonpathogenic microorganisms that are stable and possess similar growth and inactivation characteristics and respond to a particular treatment or to processing parameters (i.e., pH, temperature, oxygen, heat, cold, antimicrobials, sterilization) in a manner that is ideally equivalent to or more resistant than that of a target pathogen (Hardin 2012). Prior to using a microbe as a surrogate for a pathogen, extensive testing must be done in the laboratory to ensure that the surrogate will perform in a way that allows the behavior of the pathogen to be predicted in industrial processes in the matrix of concern. Such testing provides confidence in the outcome of any tests that were done utilizing the surrogate. Surrogates are specifically used to evaluate the effects of and responses to selected processing treatments applied to specific food matrices. A surrogate is considered a suitable replacement for a target pathogen if the processing treatment results in a reduction in surrogate levels that is equal to or not significantly greater than observed for the pathogen. For some pasteurization technologies, the surrogate may be significantly more resistant to the process than the target pathogen(s). For thermal treatments, validation of a surrogate usually takes the form of establishing thermal death time curves (time to death of a microbial population at several temperatures) so that there is some flexibility in implementing the processing treatment. Several different surrogates may be required to include all possible processing treatment types, products, and/or conditions. This is particularly true for spices for which there are many different types of spices and where there are often many different forms of the same spice (whole, coarse ground, fine ground, or powder) that may affect pathogens differently by the same process. Research should include not only identification and validation of surrogates but also standardized and validated protocols for use of surrogates in process and/or hurdle technology (multiple antimicrobial approaches with an enhanced cumulative effect) including the intrinsic properties of the product and ingredients as well as any preservatives used in formulation. Some of these processes include the validation of the proofing and/or baking steps involved in baking processes, the roaster in a peanut roasting process, pet food extrusion processes, as well as the lethality treatments applied to other low-moisture foods, such as dry spices, seasonings, beans, and tree nuts. One size does not fit all regarding surrogates. For example, a surrogate identified for use in almond processing may not be the best surrogate to validate roasting of peanuts or walnuts, or for a lethality step used in treating spices or pet food, even if the target microbe is the same (i.e., *Salmonella*). This may be valid within a product category, but a

previously identified surrogate that is appropriate for validating the processing of black pepper may not be the most appropriate microbe for validating a process designed for oregano.

Process and Product Validation

Low-moisture food products require specific considerations in the development, validation, and implementation of an effective kill step based on the inherent ability of a target pathogen, such as *Salmonella*, to resist thermal or nonthermal processing. Continuing research in the validation of new practices and current processes used to reduce or control a target pathogen is needed to scientifically prove and ultimately validate that a process is effective. Collectively, validation includes:

- Reviewing scientific data for related products and processes.
- Determining a proper reduction of the target microbe of concern for a specific process based on the expected incoming pathogen levels and food safety objectives.
- Determining defined critical parameters to achieve the targeted reduction.
- Confirming that the equipment and process can consistently deliver the required parameters to meet these limits. This will often require that appropriate measures be applied to eliminate controllable causes of variation in order to ensure that the product will consistently meet specifications.
- Documenting the parameters and protocols necessary to maintain the process and ensure that the process, when operated within specified limits, will consistently produce product meeting predetermined specifications.

Validation studies should also consider the entire process being used to prevent, reduce, and control recontamination of the finished product. Further, use of raw materials in the validation should represent “worst case scenario” conditions to best assess process performance and, therefore, adequately validate extreme conditions should they occur. The effectiveness of a process should be determined using a strain or serotype of the target microbe that best represents the worst case scenario. An example would be validating a heat treatment to inactivate *Salmonella* using the *Salmonella* serotype that has the greatest level of heat resistance in that product and under the specific processing conditions the product or ingredients would be exposed to. Validation conditions must be carefully developed for low-moisture food products, including considering the effects of previous storage conditions, product matrix, integrated lethality, and nonlinear patterns for microbial inactivation in some matrices. It is helpful to identify process authorities with knowledge and experience in conducting process validation studies for similar matrices. Ideally, validation of targeted process control points could be simulated in a laboratory setting so that appropriate strains of the actual microbe of concern could be used for the evaluation. However, as previously mentioned, unique processes, or those not able to be simulated, may need to be validated using a surrogate microbe.

Intervention Methods

The demand for a highly effective "silver bullet" continues to be sought by the food industry. There is a continual need for cost-effective technologies and ingredients to control, reduce, or eliminate pathogens from food. These pathogen control measures may take a physical, chemical, or biological form. Some of these interventions are applied to food-contact surfaces, directly to the product itself such as in product formulations, to packaging, or to in-package products (depending on the regulatory approvals and labeling requirements), and the overall desired effect on product quality and safety. Their use is particularly important for food products that are exposed to the processing environment post-lethality and prior to final packaging. Additional studies addressing the synergistic inhibitory effects of various food safety interventions that combine more than one antimicrobial control parameter (hurdle technologies) would provide fundamental information to support their use.

Dry Sanitation Technology

The development and validation of additional dry cleaning methods and improved sanitary design of equipment used in dry-food manufacturing are needed to help minimize the risk of post-processing contamination. Most equipment used in dry-food manufacturing is designed to operate for many hours and even weeks at a time and is not designed to be readily disassembled accessible for cleaning and sanitation. Enabling the breakdown of equipment is an area of opportunity for equipment design improvement that will facilitate many factors that contribute to an effective sanitation program. Such factors include the cleaning process itself, pre- and post-operational inspections, and sanitation and environmental monitoring, sampling, and testing. Processors and producers of low-moisture foods have the additional challenge of cleaning equipment without the use of water. As soon as water is introduced into the environment, the risk of pathogen growth increases substantially. Water containment has been the traditional approach to controlling pathogens of concern in dry-food processing facilities. Water-based sanitation procedures, if employed, are usually conducted on an infrequent basis and must include a complete dry out before production resumes, as the presence of water may lead to the enhanced risk of microbial growth and the potential development of microbial growth niches.

Additional validation methods for dry cleaning different types of equipment containing different soil types are greatly needed. Many methods have been developed to clean dry processing environments, including flushing the system with food grade materials such as dry ice or coarse salt, or the use of high-efficiency vacuum systems, and wiping equipment with alcohol-based compounds (Beuchat et al. 2013; Jackson and Al-Taher 2010). While vacuum systems have garnered much interest and may be effective in removing visible particles, they may not be as effective for

cleaning equipment to a microbiologically safe level. Jackson and Al-Taher (2010) reported that alcohol-moistened wipes were more effective in removing allergen residues from multiple surface types than a high-efficiency vacuum cleaner, particularly for food cooked onto surfaces. From a practical perspective, alcohol wipes provide a mechanical action vitally important in the cleaning process and necessary to physically remove food particles, allergens, and any associated microorganisms of concern.

Technology Transfer

Not all research needs and opportunities for improvement of processing facilities are researchable. Research has oftentimes been completed and published, yet the last crucial, often-overlooked step is technology transfer, implementation, education, and training of food industry employees. Providing useful data, however, can go a long way to support these programs. The challenge is in determining the best and most effective vehicles for communication, education, and training programs for growers, suppliers, processors, and customers. A considerable amount of time, effort, and, often time, significant capital must be invested in learning about the country of origin for many low-a_w products, in order to effectively change a current practice or behavior. This includes learning about the current food production practices, methods for distribution, differences in culture and language, available technology, the food safety rules or lack thereof, and associated resources to adopt and enforce food safety policies in their country of origin. Companies must acknowledge that different countries have different levels of food safety expertise and experiences, as well as different levels of acceptable risk. Some countries may perceive a certain food safety risk as totally acceptable, whereas others may place a low or high priority on addressing the same risk. Understanding these differences will serve to improve communication and further facilitate behavioral changes, where needed.

Other Considerations

Multicomponent Foods

Many low-moisture foods are used as ingredients or as subparts of a multicomponent food. There is a need for better understanding of the protective effects afforded by food safety interventions such as thermal processing and of certain food components when used singly and in combination with multicomponent foods. Multicomponent foods, such as sandwiches and pies, are often comprised of a combination of dry and intermediate- or high-moisture food components, which may also vary in the amount of fat, salt, sugar, and water that are added. Care must be taken when analyzing multicomponent foods, as the effective measurements of a_w

may not reflect the actual a_w values in the microenvironment or at the interface between or among different food components. Interfaces between food components require validation as they can create new microenvironments, particularly over the shelf life of a multicomponent product (Taormina 2012). In these cases, controlling factors such as a_w should be measured at the interface areas of the food, as well as in potential microenvironments. These factors need to be measured throughout the product's expected shelf life and under varying environmental conditions of temperature and humidity to which the product may be exposed during storage.

Preparation, Handling, and Storage by the End User

In addition to carefully assessing how the product is produced and distributed, it is important to consider how the food will ultimately be prepared, handled, and/or stored by the end user, particularly for not-ready-to-eat (NRTE) products that for safety purposes require cooking by consumers before consumption. This includes accounting for the risks associated with any product misuse or mishandling, such as only partial cooking or failure to cook an item or rehydration of a dried food, followed by prolonged storage at ambient temperature (Beuchat et al. 2013). Two highly publicized outbreaks of foodborne illness, in recent years, have highlighted the need for evaluating the risk of certain consumer practices for products, such as low-moisture foods, which were once thought to be of low microbial risk. In one outbreak which was associated with frozen pot pies, flour was not ruled out as a potential source of the causative agent (i.e., *Salmonella*), leading the industry to now use heat-treated flour in many of these NRTE products. In another case, an outbreak of foodborne illness of *E. coli* O157:H7 was associated with the risky behavior of eating raw cookie dough. Although no "root cause" was determined, flour was determined to be the only ingredient not cleared at the supplier level, and the company is now using heat-treated flour in its refrigerated dough to enhance the safety of its products. Validating the safety of consumer cooking directions for NRTE products, under different methods of preparation (conventional oven, microwave oven), is important in ensuring the safe consumption of these products. Additional studies evaluating end-user (foodservice, institutional, and in-home) preparation practices (correct use and misuse), including those products that are NRTE but may have the potential to be handled and consumed as ready-to-eat, may provide valuable insight into additional safeguards needed to protect public health.

Concluding Comments

Recent outbreaks of foodborne illness associated with low-moisture foods have revealed gaps in our knowledge of the microbiological safety of such products and highlighted the need for additional research to develop more effective food interventions. The best defense against microorganisms entering food products and the

production environment is a good offense, including (a) up-to-date information regarding the prevalence and population of foodborne pathogens in raw materials, (b) application of the most sensitive and reliable methodology to isolate and identify target microbe(s), and (c) implementation of processes and interventions designed and validated to reduce or eliminate the pathogens of concern from both product and the environment. The American Feed Industry Association (AFIA) in their *Salmonella* Control Guidelines (2010) suggests that when common sense is applied to such activities as procurement of raw ingredients, processing and distribution of ingredients and pet food and good housekeeping will preclude most problems associated with pathogen contamination. However, the problem with depending on common sense is that it is not always that common. Therefore, these control measures must be delineated, reinforced, and disseminated in written form, as several industry associations representing low-moisture foods have done (AFIA 2010; APC 2009; ASTA 2011). Critical questions need to be asked and answered to adequately determine the risks associated with low-moisture foods and the potential misuse by the end user. Consumers expect safe food even if they do not prepare the product properly.

References

American Feed Industry Association (2010) *Salmonella* control guidelines. http://www.afia.org/AFIA/Files/GUIDANCE%20DOCUMENTS/SALMONELLA%20CONTROL%20GUIDELINES%202010%20FINAL.pdf

American Peanut Council (2009) Good manufacturing practices and industry best practices for peanut product manufacturers. http://www.peanutsusa.com/MainMenu/Food-Safety/Good-Management-Practices/Good-Management-Practices-2009-revision.pdf

American Spice Trade Association (2011) Clean, safe spices: guidance from the American Spice Trade Association. http://www.astaspice.org/i4a/pages/index.cfm?pageID=4200

Beuchat LR, Konitipoulou E, Beckers H, Betts RP, Bourdichon F, Fanning S, Joosten HM, Ter Kuile BH (2013) Low-water activity foods: increased concern as vehicles of foodborne pathogens. J Food Prot 76:150–172

Borowski AG, Ingham SC, Ingham BH (2009) Validation of ground-and-formed beef jerky processes using commercial lactic acid starter cultures as pathogen surrogates. J Food Prot 72:1234–1247

Chen Y, Scott VN, Freier TA, Kuehm J, Moorman M, Meyer J, Morrille-Hinds T, Post L, Smoot L, Hood S, Shebuski J, Banks J (2009) Control of *Salmonella* in low-moisture foods III: process validation and environmental monitoring. Food Protect Trends 29:493–504

D'Aoust JY (1977) *Salmonella* and the chocolate industry. A review. J Food Prot 40:718–727

Hardin MD (2012) Food process validations. In: Taormina PJ (ed) Microbiological research and development for the food industry. CRC, Boca Raton, FL

Institute of Environmental Science and Research Limited (ESR) (2010) Risk profile: *Salmonella* (non typhoidal) in cereal grains. http://www.foodsafety.govt.nz/elibrary/industry/salmonella-in-cereals.pdf

Jackson LS, Al-Taher F (2010) Efficacy of different dry cleaning methods for removing allergenic foods from food-contact surfaces. International Association for Food Protection Annual meeting, Anaheim, CA, pp 1–47

Pafumi J (1986) Assessment of the microbiological quality of spice and herbs. J Food Prot 48:958–963

Podolak R, Enache E, Stone W, Black DG, Elliot PH (2010) Sources and risk factors for contamination, survival, persistence, and heat resistance of *Salmonella* in low-moisture foods. J Food Prot 73:1919–1936

Scott J, Weddig L (1998) Principles of integrated time-temperature processing. Processed Meat Industry Research Conference, Philadelphia, PA

Sperber WH, The North American Millers Association Microbiology Working Group (2007) Role of microbiological guidelines in the production and commercial use of milled grains: a practical approach for the 21st century. J Food Prot 79:1041–1053

Tajkarmi MM, Ibrahim SA, Oliver DO (2010) Antimicrobial herb and spice compounds in food. Food Control 21:1199–1218

Taormina PJ (2012) Food product validations. In: Taormina PJ (ed) Microbiological research and development for the food industry. CRC, Boca Raton, FL

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