Sources and Risk Factors for Contamination, Survival, Persistence, and Heat Resistance of *Salmonella* in Low-Moisture Foods

RICHARD PODOLAK,* ELENA ENACHE, WARREN STONE, Darryl G. BLACK, AND PHILIP H. ELLIOTT

Grocery Manufacturers Association, 1350 I Street N.W., Suite 300, Washington, D.C. 20005, USA

MS 09-513: Received 24 November 2009/Accepted 2 June 2010

ABSTRACT

Sources and risk factors for contamination, survival, persistence, and heat resistance of *Salmonella* in low-moisture foods are reviewed. Processed products such as peanut butter, infant formula, chocolate, cereal products, and dried milk are characteristically low-water-activity foods and do not support growth of vegetative pathogens such as *Salmonella*. Significant food safety risk might occur when contamination takes place after a lethal processing step. *Salmonella* cross-contamination in low-moisture foods has been traced to factors such as poor sanitation practices, poor equipment design, and poor ingredient control. It is well recognized that *Salmonella* can survive for long periods in low-moisture food products. Although some die-off occurs in low-moisture foods during storage, the degree of reduction depends on factors such as storage temperature and product formulation. The heat resistance of *Salmonella* is affected by many factors, mostly by strain and serotypes tested, previous growth and storage conditions, the physical and chemical food composition, test media, and the media used to recover heat-damaged cells. *Salmonella* heat resistance generally increases with reducing moisture. Care must be taken when applying published $D$- and $z$-values to a specific food process. The product composition and heating medium and conditions should not be significantly different from the product and process parameters used by the processors.

Low water activity ($a_w$) is a barrier to growth for many vegetative pathogens, including *Salmonella* spp. (11). Processed products such as powdered milk, chocolate, peanut butter, infant foods, cereal, and bakery products are characteristically low-water-activity foods (35, 80, 92, 109, 126). While these products do not support the growth of *Salmonella*, all have been implicated in outbreaks of salmonellosis (22–24, 31, 71, 105, 110). Epidemiological and environmental investigations of these outbreaks have suggested that cross-contamination plays a major role in the contamination by *Salmonella* of these products (22–24, 31, 105, 110).

Cross-contamination is the transfer of bacteria from one surface, object, or place to another (91). Significant food safety risk might occur when transfer of a pathogen takes place where the product is ready to eat, with no additional *Salmonella* inactivation step in the process (103). In a 2004 review article, Reij et al. (103) cited a survey conducted by the World Health Organization (132), which indicated a significant proportion of European foodborne outbreaks could be traced back to cross-contamination. The report indicated that factors contributing to the presence of pathogens in prepared foods included insufficient hygiene (1.6%), cross-contamination (3.6%), processing or storage in inadequate locations (4.2%), contaminated equipment (5.7%), and contamination by personnel (9.2%). In a compilation of outbreaks in the United Kingdom where the contributing factor was known, cross-contamination accounted for 57% of all occurrences (99). In order to minimize the risk of salmonellosis from the consumption of low-moisture foods, it is crucial for manufacturers to apply best efforts to control various risk factors that could lead to cross-contamination.

It is expected that *Salmonella* may be present in or on any raw food materials (10), in part because *Salmonella* is widespread in nature. *Salmonella* can survive for weeks in water and for years in soil if environmental conditions such as temperature, humidity, and pH are favorable (120). Because of its ubiquitous nature, *Salmonella* may cycle through a host into the environment and back into another host, e.g., through animals to soil and water, and back to animals through contaminated water and food (32, 45, 131).

Studies showed that *Salmonella* may survive in dry foods and feeds for a long time (60, 65, 67). Janning et al. (65) studied the survival of 18 bacterial strains (including *Salmonella*) under dry conditions ($a_w$ of 0.2) at 22°C. After an initial decrease in cell numbers, the *Salmonella* strains evaluated remained stable for a very long time and 248 to 1,351 days were needed to achieve a 1-log reduction. *Salmonella* was more resistant to desiccation under the experimental conditions than were *Enterobacter cloacae* and *Escherichia coli*. Hiramatsu et al. (60) investigated the ability of *Salmonella* spp. to survive under dried conditions on paper disks with an $a_w$ of 0.5 to 0.6 and in selected dry foods such as dried squid chips and plain dried squid. They
reported that the survival of dried *Salmonella* cells substantially increased (up to 79 times) when sucrose (36%) was present in a desiccation model system compared with one without sucrose. Survival of *Salmonella* spp. inoculated in dried squid chips containing sucrose was 23 to 89 times greater than that in the dried paper disks, whereas the populations of *Salmonella* in dried plain squid without sucrose were almost equal to those in the dried paper disks (60). The authors also reported that *Salmonella* in the presence of sucrose might survive for months in foods such as chocolate, peanut butter, and potato chips. The combination of high fat and low water activity might have a synergistic effect on *Salmonella* survival (60).

The heat resistance of *Salmonella* in low-moisture products is affected by many factors (37). These include factors prior to heating (e.g., growth medium composition, growth phase, growth temperature, and stress such as heat or acid), and factors during heating (e.g., acidity, fat content, and addition of solutes to the matrix, as well as the *Salmonella* strains used) (56). Heat resistance observed in an aqueous system is not applicable to a low-moisture product. For example, a study by Ng and colleagues (94) found that *Salmonella* Senftenberg 775W was the most heat resistant among 300 strains evaluated in an aqueous solution. *Salmonella* Senftenberg 775W was 30-fold more resistant than was any other strain evaluated, while this strain was found to be less heat resistant than *Salmonella* Typhimurium in chocolate (49). *Salmonella* Enteritidis PT30, the target organism for raw almonds, was implicated in an outbreak and was found to be more resistant to dry heat than were many of the strains evaluated on almonds (3, 127).

The aim of this review is to provide insights into the sources and risk factors for contamination by *Salmonella* in low-moisture products and to address the survival and heat resistance of the pathogen with specific references that may be used to help to develop the appropriate formulations and processes for these products.

**SOURCES AND RISK FACTORS FOR CONTAMINATION BY SALMONELLA IN LOW-MOISTURE PRODUCTS**

*Salmonella* contamination in low-moisture foods has been traced to poor sanitation practices, substandard facility and equipment design, improper maintenance, poor operational practices and good manufacturing practices (GMPs), inadequate ingredient control, and other factors. Many such instances are not documented in the literature. This section summarizes and analyzes those reports that have been published.

**Contamination associated with poor sanitation practices.** Poor cleaning and sanitation is often cited as a contributing factor in many outbreaks of foodborne illness. The risk of cross-contamination has been considered lower when food contact surfaces are dry, partly because bacterial growth and survival would be reduced. However, *Salmonella* may be able to survive dry conditions on surfaces for extensive time. Kusumaningrum et al. (75) demonstrated that in the case of *Salmonella*, the effects of cross-contamination due to poor sanitation practices are enhanced by the organism’s ability to survive on dry surfaces for extended time, and then transfer to foods on contact. The authors showed that *Salmonella* Enteritidis remains viable on dry stainless steel surfaces and presents a potential for contamination for considerable time. *Salmonella* Enteritidis was readily transferred from these test surfaces to foods, with transfer rates of 20 to 100%. *Salmonella* Enteritidis was recovered from dry, highly contaminated (10^9 CFU/cm^2) stainless steel surfaces for at least 4 days and for 24 h from moderately contaminated surfaces (10^3 CFU/cm^2). This is significant because after undertaking cleanup and sanitation activities, manufacturers typically initiate new production much sooner than the times in these timetables studied by Kusumaningrum et al. (75). Residual concentrations of organisms used in the above study should not be found in a well-run establishment, but if they are, cross-contamination potential would be high due to the transfer rate cited here.

During investigations into outbreaks of salmonellosis, evidence of *Salmonella* has been found in plant processing environments (3, 15, 40, 123–125) where sanitation appeared substandard. An outbreak of *Salmonella* Agona associated with toasted oat cereal prompted examination of potential cross-contamination in the processing areas, air-handling systems, ingredients, and traffic flow of the manufacturing factory. Investigators found widespread low levels of the organism in the plant environment, including samples taken from the floor, production equipment, and the exhaust system in the plant (15). The investigators concluded that the unsanitary condition of the equipment (especially the air-handling systems), poor employee practices, and poor control of the vitamin spray mixing and holding process (e.g., multiple dead legs, direct connection of the vitamin supply line to the potable water supply without maintaining proper backflow protection) were ongoing factors, with the potential to produce contamination in the cereal product (123). An investigation of *Salmonella* Senftenberg contamination in infant cereal revealed that bulk cereal was contaminated with “cleaning remains” from milling machinery (105). Investigations into two consecutive *Salmonella* Enteritidis outbreaks in bakery products (43) showed that the second outbreak was most likely due to poor equipment sanitation. Piping nozzles used daily for making fresh cream cakes were inadequately cleaned, potentially allowing cross-contamination.

Morita et al. (93) studied the mechanisms of *Salmonella* contamination in a Japanese oil meal (rapeseed or canola meal) factory. The authors found *Salmonella* on many environmental vectors including operators, processing area floors, dust in the air, and rodents. In particular, high concentrations of *Salmonella* were found in samples with high oil content from the floor of the manufacturing area. The authors concluded that high *Salmonella* contamination rates for the processing area represented the greatest risk for cross-contamination of the oil meal. They also stated that restricting the movement of operators remarkably reduced *Salmonella* contamination. In a study involving contaminated chocolate by Craven et al. (31), investigators...
Salmonella and Low-Moisture Foods

In a 2007 study, Tennessee infections associated with Salmonella. Cross-contamination by controlling airborne spread of dust. Butcher and Miles (20) also indicated that dust was a major source of Salmonella contamination of poultry feed in processing mills.

In Morita’s study (93), which sought to identify potential vectors of contamination in a Japanese oil meal factory, the authors also developed quantitative data and determined from this data that restricting the movement of operators could have a very positive effect in reducing the spread of Salmonella. They documented that 100% of manufacturing operators were contaminated with Salmonella after being effectively disinfected within 1 day. The authors also determined that 65% of floor swabs were positive. These findings clearly supported the author’s conclusion that restricting the movement of operators between factory areas (e.g., receiving versus manufacturing versus storage) and disinfecting the bottoms of work shoes when moving between zones is needed to prevent the spread of the organism.

Contamination associated with poor facility and equipment design and inadequate maintenance. Cross-contamination because of sanitary practice failures is not always attributed to procedural and human errors. In some cases, the manufacturing equipment is of poor sanitary design and/or has not been properly installed or maintained. Poor facility and equipment design and machinery maintenance can also contribute to the problem of Salmonella contamination.

Improper facility and equipment design, as well as maintenance issues at a company’s processing plant, were observed by government investigators in response to a 2008 to 2009 nationwide outbreak of Salmonella Typhimurium associated with various peanut products in the United States (121). In one case, investigators observed open gaps, as large as 0.5 in. (1.27 cm) by 2.5 ft (76.2 cm), at the air conditioner intakes located in the roof of the facility. There were several indications that rainwater had been leaking into the factory. The gaps were located in the production–packaging room and totes of finished, roasted product and a packaging line were located directly underneath the gaps.

In the same inspection, investigators noted other equipment and facility design issues that could have contributed to cross-contamination at the plant (123). A felt material was present on a final machine roller at the peanut roaster’s discharge. Felt is a material that cannot be adequately cleaned and sanitized. In addition, it was noted that the facility was not equipped with a ventilation system that would provide airflow from the factory’s finished goods section to the raw receipt and staging area, from its more sanitary sections towards the less hygienic areas. Such a design, often termed “positive pressure,” creates higher air pressure in parts of a factory after a lethality step has been applied, versus those areas where raw, nonprocessed goods are stored or staged. A positive pressure system prevents contaminated air originating in raw product areas from escaping to other parts of the facility.

Improper equipment and facility design was also a probable culprit in other salmonellosis outbreaks. In a report on investigations following an international outbreak of Salmonella Eastbourne, where 200 people were affected by contaminated chocolates produced at a Canadian factory, Craven et al. (31) indicated that raw cocoa beans were the probable source of Salmonella, which survived the heating step during production. It was also suggested that valves in conches were arranged such that chocolate that had not been heated could accidentally be pumped directly to a finished product storage tank. In addition, investigators cited inadequate separation between clean and unclean areas as a causative factor for potential product adulteration. During Breuer’s (15) investigation into a Salmonella Agona outbreak associated with toasted oat cereal, the investigators found poor facility design in the implicated factory, where a majority of the equipment was open to the atmosphere. The authors characterized cleaning and sanitation as “very difficult,” because insufficient space was allocated between pieces of equipment. Morita et al. (93) research in the Japanese oil meal factory demonstrated that choice of flooring material could be critical in factory design. The researchers tested different disinfection methods on diverse types of flooring material. They found that regardless of the disinfectant used with three different application methods, concrete could not be effectively rid of Salmonella.

In response to outbreaks of the past 10 years, the Grocery Manufacturers Association (53) recently published a sanitary equipment design checklist for low-moisture foods. The utilization of these principles may help minimize equipment design flaws, improve sanitation effectiveness, and minimize the risk of product contamination and outbreaks (15, 31, 123).

In the Craven report (31), designing processing hardware such that unprocessed material can bypass the lethality (cooking) step is a devastating oversight. It is hoped that this case can serve as a caveat to future engineers and designers. Any suspicion that a process bypass could occur must be investigated comprehensively and thoroughly. The outbreak reported by Craven et al. (31) occurred 35 years ago. The industry now has much more sophisticated control mechanisms available, which may be employed to prevent process bypasses. This can be a prudent design expense that can deliver repeated payback by preventing product bypass over the related public health consequences.

Even the most impeccably designed equipment and facilities can become sources of Salmonella contamination if they are not properly maintained. The source of Salmonella Ealing in an outbreak associated with infant dried milk was traced to poor equipment maintenance. A factory spray dryer had a hole in its inner lining, which allowed the escape and return of powder from the dryer’s contaminated insulation material (104). In another outbreak in England, 37 cases of foodborne illness during the spring and summer of 2006 caused by Salmonella Montevideo were linked to internationally distributed chocolate products. The manufacturer attributed the contamination to a leaking pipe at one of its main factories (14). In a 2007 outbreak of Salmonella Tennessee infections associated with
with peanut butter in the United States, a company spokesperson indicated that the outbreak was traced to problems with a leaky roof and two instances of faulty sprinklers being activated (46). U.S. Food and Drug Administration (FDA) investigations of the outbreak included collecting samples from the plant environment (134). One hundred twenty-two environmental samples were collected by the FDA and two tested positive (a floor squeegee sample and a drain sample from the roaster room). According to Zink (134), “water event(s)” in the facility might have increased the numbers of Salmonella and led to product contamination.

In addition to the potential microbial contaminants introduced from poor equipment and facility design, and maintenance issues such as leaky roofs, leaking pipes, and faulty sprinklers, these events introduced moisture into a normally dry environment. Moisture control is critically important in preventing Salmonella contamination in low-moisture products (63). 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, where normally the lack of moisture would prevent this. The subsequent growth caused by the introduction of moisture significantly increases the risk for product contamination. Moisture most likely contributed to the cross-contamination, in the United Kingdom chocolate (14) and U.S. peanut butter (46) outbreaks, by facilitating the growth of otherwise dormant Salmonella that might have come from the aforementioned sources.

Contamination associated with lack of GMPs. Processors, especially those supplying products that will receive little or no further lethality treatment from consumers, need to be aware of potential contamination hazards and employ GMPs to fully protect the health of their customers. Food manufacturers must thoroughly evaluate their operations and provide appropriate mitigations based on those hazards presented by their unique situations. Without these mitigation protocols, cross-contamination could occur and resulted in adulterated goods even when a lethality step is used in the process. The following case demonstrates such a situation.

In 2001, halvah, a candy made from sesame seeds and sugar, was implicated in an international outbreak of Salmonella Typhimurium DT 104 (16). As a follow up to this outbreak, Brockman et al. (16) examined several sesame products for the occurrence of the pathogen. In addition to finding Salmonella Typhimurium in the halvah involved in the outbreak, researchers also found different Salmonella Typhimurium strains in halvah from other manufacturers and other countries. As part of this same study, the authors also uncovered Salmonella Offa, Salmonella Tennessee, and Salmonella Poona in tahini (sesame paste) and sesame seeds. While sesame seeds can be contaminated with Salmonella during growth of the seeds, storage, or processing, the researchers stated that the organism should not survive during the production of halvah because of the high temperatures (120 to 140°C) that are used in the preparation, milling, and hot mixing processes of the candy manufacture. Consequently, they concluded that the likely cause of the outbreaks was cross-contamination of the halvah after the heat treatment step (55). This certainly is a plausible scenario, and a prudent manufacturer should have operational and GMP controls in place to prevent such an occurrence. Even though the temperatures involved in halvah processing appear similar to other reported literature values for obtaining multilog reductions of Salmonella (4, 84, 109), the authors did not specify if the halvah process was validated to achieve a particular log reduction of Salmonella. Quantifying such information would have lent more credence to the conclusion that the presence of the organism in the halvah was due to cross-contamination.

Contamination associated with poor ingredient control and handling. Even a well-designed equipment systems operating with detailed preventive maintenance programs and comprehensive operational practices cannot combat cross-contamination from poor choice, sourcing, and control of raw materials and ingredients. Contaminated ingredients used in products without a further kill step could carry the pathogen directly into finished products. For example, paprika powder contaminated with multiple serovars of Salmonella used in the manufacture of paprika-powdered potato chips was implicated in an estimated 1,000 cases of salmonellosis (78). Poor choice of formulation ingredients can have similar detrimental results. Marshmallows made with raw egg whites resulted in 36 cases of Salmonella Enteritidis PT4 infections (80).

In still further examples, Koch et al. (72) investigated a Salmonella Agona outbreak in Germany and reported that the organism was found among products from 12 producers of herbal teas that contained aniseed. The contaminated aniseed was traced to a single importer, who indicated that the source of the contamination was a single batch of aniseed (cultivated in Turkey) that had been fertilized with manure. Hedberg et al. (57) reports a case where Salmonella-contaminated cheese was supplied to four separate shredding operations. While better sanitation practices at the shredding plants might have minimized the scope of the problem, these plants essentially were dealing with contaminated ingredients supplied by another company, which resulted in finished products contaminated with Salmonella Javiana. In June of 2007, the FDA warned consumers not to consume low-moisture children’s snack food, due to possible contamination with Salmonella Wandsworth (124). By mid-July, the Centers for Disease Control and Prevention had identified 60 people, mostly toddlers, from 19 states who had become ill. Five were hospitalized, but no deaths were reported. An FDA consumer update (124) indicated that the seasoning mix used in the snack food might have been the source of the contamination. In the aforementioned Rushdy et al. (105) investigation of eight reported cases of Salmonella Senftenberg infections in infants, which occurred in 1995 in England, the illness was associated with the consumption of one brand of baby cereal. One of the company’s suppliers
used common machinery to process heat-treated bulk cereal ingredients and other products that were not heat treated. The receiving company, in spite of receiving a previous shipment of bulk cereal contaminated with Salmonella Senftenberg, did not thoroughly investigate the supplier, and did not identify the ingredient as a possible source of Salmonella in their finished product. During the supplier’s risk assessment, the supplier failed to identify the use of common machinery as a potential source for introduction of Salmonella into the processing equipment system and therefore did not have control measures in place to mitigate this risk.

Ingredient control is not limited to programs designed to ensure the procurement and delivery of clean and wholesome raw materials. Once these materials are received, they must be stored and handled in a manner that does not enhance opportunities for cross-contamination. Poor handling, in fact, has lead to cross-contamination even in products subject to a lethality step. Poor control of ingredients that potentially contain Salmonella can allow it to colonize a facility resulting in the organism finding its way into fully processed finished products. In the U.S. nationwide outbreak of Salmonella contamination associated with peanut products (125) mentioned above, FDA investigators noted raw peanut storage and staging areas that were housed in the same open room, with no segregation, as finished product handling equipment. Finished goods packaging operations were also located near raw peanut handling, with no segregation. The FDA observed that totes of raw peanuts were stored directly next to totes of finished roasted peanuts. In another situation, environmental investigations conducted in response to an outbreak found contamination risks existed within tree nut processing facilities and on farms (41, 63). An outbreak of Salmonella Enteritidis associated with raw almonds occurred in Canada and the United States in 2000 and 2001 (41, 64). Salmonella was found in 16 of 32 orchard samples. All of the growers involved indicated that manure or biosolids were not used on the land within the previous 5 years. No livestock or poultry farms were nearby. However, Salmonella of the same phage type found in the orchards was isolated from environmental samples collected from the processing equipment, where 25% of equipment swabs cultured positive. It was postulated that Salmonella from field contamination colonized the plant environment and the processing equipment, which in turn could have contaminated almonds during processing.

In the Rushdy study (105), the authors cite the baby cereal supplier’s hazard analysis and critical control point (HACCP) system for failure to identify a potential hazard in their hazard analysis. However, in 1995, HACCP was in its developmental stages (114). Today, many HACCP practitioners include an in-depth and thorough evaluation of potential contamination sources from their suppliers in addition to those that might occur internally. Tools employed in this analysis could include on-site inspections, review of HACCP plans, requirements for certificates of analysis indicating the supplier’s goods have test negative for Salmonella, and ingredients (93). Implementation of such an approach today may help minimize the potential hazards identified by Rushdy et al. (105) 15 years ago and prevent such hazards from entering the supply chain, thus minimizing the risk of product contamination and outbreaks.

**Pest control and Salmonella contamination.** Pest control is an important food safety program in all manufacturing facilities. While the literature reviewed does not contain any documented cases where pest activity was directly implicated in Salmonella cross-contamination, there are studies that show that common rodents and insects can be vectors for Salmonella transmission.

In the previously cited study by Morita et al. (93), the researchers captured, autopsied, and analyzed the stomach contents of 48 rodents caught over the period of 1 year in a Japanese factory. Of rodents captured from the manufacturing area, 46% tested positive for Salmonella, while rodents captured from the receiving and storage areas all tested negative. Seven different serovars were found in those rodents testing positive along with several untypeable strains.

In a study involving seven species of common grain insects, Crumrine et al. (32) demonstrated that Salmonella Montevideo was transmitted by insects from inoculated wheat to clean wheat. The authors concluded that insects contaminated with Salmonella Montevideo could contaminate large masses of grain. In yet another study, Kopanic et al. (73) found that cockroaches are capable of acquiring and transmitting Salmonella Typhimurium and therefore are potential vectors of the pathogen. Furthermore, infected cockroaches were capable of infecting other cockroaches.

The identification of three different pest-oriented potential vectors (rodents, cockroaches, and grain insects) clearly indicates that pest control is not a program that can be ignored in a well-designed Salmonella-prevention strategy. The mobility of these insects and rodents could easily aid in widely transferring Salmonella throughout a facility from what was formerly an isolated niche.

**Sources and risk factors: summary.** This review demonstrates that cross-contamination by Salmonella can occur in a variety of low-moisture foods from an assortment of sources and vectors. In many of these cases, the causative factor was determined to be a single cause and in some situations, multiple factors were responsible. Manufacturers would be well served to identify potential sources of contamination and implement control measures against these.

The publication by Rushdy et al. (105) demonstrates the potential for the breakdown of several pathogens mitigation strategies when potential problems are not addressed by the manufacturer. First, the company had a breakdown in their HACCP system by not recognizing the potential problems that could be introduced by their suppliers. Then, in spite of receiving a batch of bulk cereal that contained Salmonella Senftenberg from their vendor, the company still did not take steps to address the supplier’s food safety and pathogen mitigation strategies and continued to use ingredients from this supplier. Had they investigated the situation with more
intensity, they would have discovered an incomplete supplier HACCP program, poor equipment and facility design (common equipment for cooked and noncooked goods), and poor sanitation practices (bulk cereal contaminated with cleaning remains). Finally, the company approached their food safety programs in this manner while manufacturing a product, baby cereal, which targets an immuno-sensitive population.

**SURVIVAL OF SALMONELLA IN LOW-MOISTURE FOODS**

*Salmonella* can easily adapt to extreme environmental conditions such as lower or higher than optimal temperatures, pH values, or desiccation. Although, the optimal growth temperature is 35 to 37°C, *Salmonella* can grow at temperatures as low as 2°C and as high as 54°C (12). While the optimal pH for growth of *Salmonella* is in the range of 6.5 to 7.5, growth has been observed at pH levels between 3.8 and 9.5 (12, 83, 128). In general, it is considered that no growth of pathogenic bacteria occur below approximately an aw value of 0.85 (83), but an aw as low as 0.93 is sufficient to support growth of *Salmonella* (12). When these conditions are below outside growth conditions, *Salmonella* may survive for months or even years in certain low-moisture foods. It was reported that survival and heat resistance of microorganisms increases as aw decreases (18, 62, 69, 87). Although water activity plays a major role, Goepfert et al. (50) stated that survival of the organism during heating is a function of a medium composition rather than water activity of the surrounding environment. In the same dry conditions, survival of *Salmonella* spp. may vary, depending on food matrix and medium composition (35, 49, 59, 60, 92). Air-dried *Salmonella* cells, in which water activity is lowered without the use of solutes, become more heat tolerant. Cells dried to an aw < 0.57 for 48 h showed increased resistance, but no significant change in shape of the survival curves occurred with longer periods of dehydration. Although a loss of viability was observed, it was attributed to the lethal damages occurring during the process of dehydration (70). It was demonstrated that, while an aw of 0.65 protected *Salmonella* at temperatures as high as 70°C or greater, it promoted more rapid cell destruction at lower temperatures (86). Hills et al. (59) hypothesized that the microbial stability of a food may be improved by manipulating the food microstructure of air-water distribution, making the water and nutrients unavailable to microbial cells. To prevent growth of *Salmonella*, it is important to keep the available water below the growth threshold so that cells that survive the initial osmotic shock phase will be unable to multiply and eventually die off due to starvation.

Several authors reported that reduced water activity has a protective effect against the inactivation of *Salmonella* in different food products, such as cake mix, peanut butter, chocolate, chocolate syrup, skim milk, onion soup, flummery, flour, dried squid chips, dry milk, and cocoa powder (6, 29, 60, 67, 87, 109, 129). While the water activity is an important controlling factor of microbial growth and survival, other factors such as medium composition (i.e., solutes used to decrease the water activity) (50, 60), or the microscopic air-water distribution in foods (59), might be as or more important as the water activity itself.

**Chocolate and confectionary products.** Finished chocolate is probably the most consumed confectionary product in the world and has a very low moisture content (<8%) and an aw of 0.4 to 0.5 (10). In the last few decades, chocolate products have been implicated in a number of salmonellosis outbreaks (10, 31, 48, 51, 61, 68, 107, 129). In some cases, very low levels of contamination (1 to 3 cells per g) were detected in the finished product (31, 34, 51). Kapperud et al. (68) did not exclude the possibility that contaminated particles containing many viable *Salmonella* cells could be unevenly distributed in the product and that the infections were caused by large doses of *Salmonella* instead of small doses. The latter scenario was considered less likely because of the thorough mixing of the chocolate at the factory. It has been suggested that the high fat content of chocolate may protect *Salmonella* cells against the action of gastric acid in the stomach, which allows the cells to colonize the lower gastrointestinal tract and produce clinical symptoms, even when a very small number of the cells is present in the product (31, 34, 50).

Although *Salmonella* cannot grow in finished chocolate, it can survive for a long time and it represents significant risk, even at low levels of contamination (34). Barrile and Cone (8) found that lyophilized cells of *Salmonella* Anatum inoculated into milk chocolate at levels of 50 cells per 100 g was detected at a level of 14 most probable number (MPN)/100 g after 15 months of storage at room temperature. Tamminga et al. (117) demonstrated that *Salmonella* might survive for months in different types of chocolate (Table 1). The chocolate industry faces a difficult task in controlling *Salmonella* for a variety of reasons, which include (i) raw materials and ingredients such as raw cocoa beans or powdered milk may carry *Salmonella*; (ii) low water activity and high fat content, increases thermal resistance so that even considerable heating is required to eliminate *Salmonella*; and (iii) a small number of *Salmonella* can cause illness (11, 129).

In honey, which may be consumed as is or used as an ingredient in confectionary products, *Salmonella* may survive for over 29 weeks at 22°C (12). Halva is another confectionary product with very low aw of 0.18. The product consists of tahini (a paste of milled, roasted sesame seeds), sugar, citric acid, and soapwort root extract. Sometimes cocoa powder and pistachios or walnuts are mixed in with the halva to enhance flavor. Some of the ingredients (e.g., sesame seeds, cocoa powder, nuts, and flour) have the potential to be contaminated with *Salmonella*. Although *Salmonella* cells do not multiply because of the low water activity, the organism may survive for relatively long periods in the product. *Salmonella* Enteritidis survived in vacuum-packed halva stored for 8 months under refrigeration, longer than its survival in air-sealed halva stored at room temperature (74). The greatest decline in viable
Salmonella Enteritidis counts, from an initial inoculum of log 3.87 to log 2.15 CFU/g, was observed in air-sealed packed product stored after 8 months at room temperature. The author concluded that reduction of salmonellae during storage cannot be predicted solely on the basis of water activity. Interactions between low water activity and environmental factors such as temperature and storage in air or under vacuum appear to play an important role in Salmonella survival. Some examples of the survival of Salmonella in foods of low water activity are presented in Table 2.

**Peanut butter and nuts.** Salmonella inoculated into peanut butter and nut spreads may aggregate or clump within or near the water phase of the colloidal suspension of lipid and water in the peanut meal phase. If nutrient availability is affected by cell density within water droplets, then the viability of Salmonella would be expected to differ, depending on the size of the water droplets, which may vary with the product (18, 28, 42, 109). Viability of Salmonella in food products may also be influenced by storage temperature, level of contamination, and product formulation (13, 18, 121). For example, in peanut butter and peanut butter spread inoculated with 5.7 log CFU/g reductions of Salmonella in products stored for 24 weeks at 21 and 5°C were 4.1- to 4.5-log and 2.9- to 4.3-log reduced, respectively, depending on the product formulation. At a lower inoculum (1.5 log CFU/g), six of the seven products evaluated were positive for the pathogen at 5°C, while at 21°C, only one product was positive for Salmonella after storage for 24 weeks (18). If postprocess contamination of peanut butter and spreads occurs, it may result in survival of salmonellae in these products during their shelf life at 5°C and possibly at 21°C, depending on the formulation (18, 28).

**Thermal inactivation models** showed that Salmonella survived in peanut butter for a much longer time than predicted (86), highlighting the danger associated with the extrapolation of the predictive models beyond their intended application.

### TABLE 1. Survival of Salmonella in milk chocolate and bitter chocolate at 20°C

<table>
<thead>
<tr>
<th>Storage time</th>
<th>Typhimurium</th>
<th>Eastbourne</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Milk chocolate (a_w of 0.37)</td>
<td>Bitter chocolate (a_w of 0.42)</td>
</tr>
<tr>
<td>0</td>
<td>5.04</td>
<td>4.86</td>
</tr>
<tr>
<td>1 day</td>
<td>2.34–2.63</td>
<td>1.69–1.88</td>
</tr>
<tr>
<td>13 days</td>
<td>1.18–1.36</td>
<td>0.30–0.56</td>
</tr>
<tr>
<td>20 days</td>
<td>0.89–1.11</td>
<td>Neg–0.30</td>
</tr>
<tr>
<td>34 days</td>
<td>Neg–0.89b</td>
<td>Neg</td>
</tr>
<tr>
<td>41 days</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>48 days</td>
<td>Neg–0.89</td>
<td>ND</td>
</tr>
<tr>
<td>76 days</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>83 days</td>
<td>Neg–0.30</td>
<td>Neg</td>
</tr>
<tr>
<td>6 mo</td>
<td>Neg</td>
<td>Neg</td>
</tr>
<tr>
<td>9 mo</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

* Adapted from Tamminga et al. (117).

**Peanut butter and nuts.** Salmonella inoculated into peanut butter and nut spreads may aggregate or clump within or near the water phase of the colloidal suspension of lipid and water in the peanut meal phase. If nutrient availability is affected by cell density within water droplets, then the viability of Salmonella would be expected to differ, depending on the size of the water droplets, which may vary with the product (18, 28, 42, 109). Viability of Salmonella in food products may also be influenced by storage temperature, level of contamination, and product formulation (13, 18, 121). For example, in peanut butter and peanut butter spread inoculated with 5.7 log CFU/g reductions of Salmonella in products stored for 24 weeks at 21 and 5°C were 4.1- to 4.5-log and 2.9- to 4.3-log reduced, respectively, depending on the product formulation. At a lower inoculum (1.5 log CFU/g), six of the seven products evaluated were positive for the pathogen at 5°C, while at 21°C, only one product was positive for Salmonella after storage for 24 weeks (18). If postprocess contamination of peanut butter and spreads occurs, it may result in survival of salmonellae in these products during their shelf life at 5°C and possibly at 21°C, depending on the formulation (18, 28).

### TABLE 2. Examples of Salmonella survival in foods with low water activity

<table>
<thead>
<tr>
<th>Food</th>
<th>Salmonella serotype(s)</th>
<th>Inoculum (log CFU/g)</th>
<th>a_w</th>
<th>Length of survival</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dried milk products</td>
<td>Contaminated naturally with three serotypes</td>
<td></td>
<td>≤10 mo</td>
<td></td>
<td>100</td>
</tr>
<tr>
<td>Pasta</td>
<td>Infantis, Typhimurium</td>
<td>12% moisture</td>
<td>≤12 mo</td>
<td></td>
<td>102</td>
</tr>
<tr>
<td>Milk chocolate</td>
<td>Eastbourne</td>
<td>8.0</td>
<td>0.41</td>
<td>&gt;9 mo at 20°C</td>
<td>117</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5.0</td>
<td>0.38</td>
<td>≤9 mo at 20°C</td>
<td></td>
</tr>
<tr>
<td>Bitter chocolate</td>
<td>Eastbourne</td>
<td>7.0</td>
<td>0.51</td>
<td>≤9 mo at 20°C</td>
<td>117</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5.0</td>
<td>0.44</td>
<td>≤76 days at 20°C</td>
<td></td>
</tr>
<tr>
<td>Halva</td>
<td>Enteritidis</td>
<td>7.0</td>
<td>0.18</td>
<td>&gt;8 mo at refrigeration temp</td>
<td>74</td>
</tr>
<tr>
<td>Peanut butter</td>
<td>A composite of Agona, Enteritidis, Michigan, Montevideo, Typhimurium</td>
<td>5.7</td>
<td>0.20–0.33</td>
<td>≤24 wk held at 5 or 21°C</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.5</td>
<td>0.20–0.33</td>
<td>≤24 wk at 5°C</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>≤6 wk at 21°C</td>
<td></td>
</tr>
<tr>
<td>Paprika powder</td>
<td>Multiple serotypes</td>
<td></td>
<td>&gt;8 mo</td>
<td></td>
<td>78</td>
</tr>
</tbody>
</table>
range. Factors including, pH, and fat content could account for the differences seen between the predicted and the experimental results. In other words, it is important to have laboratory-based models with real foods, since the individual properties of foods may have a great impact on the survival of microorganisms within foods (86). Nut and seed products may be naturally contaminated with Salmonella; due to the nature of cultivation, harvesting, and epidemiologic history, Salmonella was identified as a biological hazard in this type of products. A large number of nut and seed products, including peanuts, pistachios, pecans, hazelnuts, and sesame and sunflower seeds, have been recalled due to Salmonella contamination. A study by Uesugi et al. (121) demonstrated the potential for long-term environmental presence or persistence of Salmonella in almond orchards. Salmonella was isolated from an almond farm over a period of 5 years, and all 53 isolates obtained were Salmonella Enteritidis PT 30, belonging to two pulsed-field gel electrophoresis patterns. This rare Salmonella strain was isolated in an outbreak in 2000 and 2001 that was linked to the consumption of raw almonds (121, 122). If almond hulls and shells are wet, Salmonella can grow by using nutrients available in the almond hull and/or shells, and penetrate the almond hulls into the kernels during wet conditions such as rainfall or from other water sources (121). Survival of Salmonella on pecans stored at different temperatures up to 32 weeks was inversely correlated to the storage temperature (13). Although storage for nuts and nut products (which have a relatively high fat level) at lower temperatures may be beneficial in preventing oxidative rancidity, lower temperatures may enhance the survival of foodborne pathogens such as Salmonella (121).

Spray-dried milk, eggs, and dry yeast. During the spray drying of foods, such as dairy products (whole milk, skim milk, and whey), egg products (whole egg, egg white, and egg yolk), and dry yeast, various factors may influence the survival of Salmonella in the final product (90). For example, 6.0-log reductions of Salmonella Typhimurium were observed in concentrated skim milk with 20% total solids in comparison with 3.3-log reductions in 40% total solids at moisture levels of 3.7 and 3.0%, respectively (90). Even at similar levels of moisture (6%), a greater destruction of Salmonella Typhimurium was observed in the process of drying of 20% solids concentrate, although the processing temperature was lower than that of 40% solids. The authors concluded that much less survival occurred in the less dense product. For the egg products, the greatest log reduction of 6.2 occurred in egg yolk (90). The authors pointed out that whole milk and whole eggs presented similar protection on artificially inoculated Salmonella cells when products were dried from 40% solid concentrates. Approximately the same degree of Salmonella death was achieved for both skim milk (20% solids) and concentrated yeast (25% solids) when dried under similar conditions (90). Several interrelated factors determine to what extent the enteric bacteria, such as Salmonella and E. coli, survive the spray-drying process. The most important factors that influence the survival of Salmonella in spray-dried products are product temperature during process, particle density, fat content, and strain variation (81, 90). Food processors should not depend on the drying process to replace adequate pasteurization prior to drying, and they should also be very cautious and avoid contamination during and after drying (90). Multiple factors, such as numbers of organisms present initially, serotype, type of product or processing, time, and temperature, may influence the survival of Salmonella in dried milk products during storage. Presence of salmonellae was detected in some samples after 1 year of storage (100). Jung and Beuchat (66) showed that Salmonella Typhimurium survival was enhanced as the water activity of egg white powder decreased. The investigators detected Salmonella in the powder at an aw of 0.13 but not at an aw of 0.34 after the product was stored at 54°C for 7 days.

Flours, pasta, and spices. Flour is typically used as an ingredient in more complex cooked or baked foods that receive effective killing steps for Salmonella and other vegetative pathogens before eating. Generally, the FDA does not consider flour a “sensitive ingredient” for Salmonella (113). However, there are circumstances where flour must be pretreated to eliminate the pathogen, e.g., when it is used as a carrier for nutraceuticals, pharmaceuticals, spices, and flavors or as a bulking-caloric agent in dried mixes, such as in ready-to-eat foods for elderly people or infants (113). Rayman et al. (102) were able to detect Salmonella Infantis and Salmonella Typhimurium from pasta after 360 days of storage, demonstrating that prolonged storage of pasta is not an effective means of decontamination for contaminated product. Spices and dried vegetable foods, such as mushrooms, parsley, asparagus, peppermint, and pepper, are occasionally contaminated with Salmonella. Reports on Salmonella outbreaks associated with the consumption of these types of foods have been published. For example, as noted previously, Lehmacher et al. (78) described a Salmonella outbreak associated with the consumption of paprika-powdered potato chips. Although low levels of Salmonella survived in the product (4 to 45 cells per 100 g), those levels were sufficient to cause illnesses, possibly because of the high fat content of the paprika-powdered potato chips, which may have protected Salmonella from gastric acidity.

Pet treats. In 1999, an outbreak of Salmonella Infantis in Canada was linked to contact with pet treats (30). In a survey, White et al. (130) reported that 41% of dog treat samples were positive for Salmonella. Raw hides used for preparation of dog chews are expected to be contaminated with salmonellae, and if Salmonella is not controlled adequately, pet treats could be potential sources of animal and human infections with Salmonella (27, 30, 97, 130).

Survival of Salmonella in other matrices. De Rezende et al. (36) suggested that an in vitro adaptation of Salmonella to dry environments might occur when the organisms are exposed to alternating levels of high and low water activity. The maximum survival of several vegetative
bacteria in dried milk was between $a_w$ values of 0.05 and 0.20. Maximum survival of *Salmonella* Newport in foods at neutral pH was at an $a_w$ of 0.11. According to Burnett et al. (18) and Christian (28), *Salmonella* Senftenberg and *Salmonella* Typhimurium survived in gelatin in a rubbery state (0.93 to 0.96 $a_w$) and a glassy state (0.45 to 0.28 $a_w$). *Salmonella* cells remained viable under low-water-activity conditions, and the lowest survival levels were observed at an intermediate $a_w$ between 0.55 and 0.74 (29).

Desiccated *Salmonella* cells can survive for a long time on work surfaces and in foods with low water activity, especially in those foods with a high fat content. Although some inactivation occurs in dehydrated foods during storage, the degree depends on relative humidity and storage atmosphere. Simulating conditions in dried foods, Hiramatsu et al. (60) showed that desiccated cells of different *Salmonella* strains inoculated on dried paper disks were inactivated after 35 to 70 days of storage at 25 and 35°C, but the cells survived 22 to 24 months when stored at 4°C. The investigators concluded that preserving dry foods contaminated with *Salmonella* and stored at refrigerated temperatures might present a higher food safety risk. Flowers (44) reported that the higher water activity, storage temperatures, and oxygen levels, the higher the death rates of *Salmonella*.

**Recovery of *Salmonella* stressed by low-moisture environments.** While there are several relatively straightforward methods for recovery of uninjured bacterial cells, the same cannot be said about sublethally injured cells surviving a processing treatment. More sensitive methods for recovery of the injured *Salmonella* cells, especially from low-moisture or desiccated foods, are needed. Factors, including the elimination of oxygen, gradual rehydration, enrichment broth, plating media, incubation time and temperature, and the addition of solutes (i.e. glycerol, glucose) may contribute to a better recovery of the cells injured by heat or desiccation (47, 50, 70, 86, 88, 101, 133). The recovery of the injured cells by heating at a water activity is improved by gradual rehydration, especially when using isotonic dilution media, prolonged incubation, and agents to protect against reactive oxygen. A rapid and large increase in water activity during the rehydration may result in cellular lysis, which will trigger an inaccurate estimation of the survivors. The use of solutions containing glycerol, lactose, sucrose, or milk solids to rehydrate the freeze-dried *Salmonella* resulted in higher recoveries than when the water was used for rehydration (86, 88, 101). Although slow rehydration of cells was found to be beneficial in some dried foods, it may not hold true for all food products. The dehydration procedure should be validated on each individual food basis (33). Mattick et al. (86) considered that gradual rehydration might have also an important contribution in accurate determination of the infectious dose for *Salmonella* associated with low-moisture food implicated in a food outbreak.

**Mechanisms for *Salmonella* survival.** *Salmonella* may enter a viable but nonculturable (VBNC) state, which represents a dormant state of the vegetative cells and a survival strategy for many nonsporulating species (21, 79). De Rezende et al. (36) also showed extensive filamentation of *Salmonella* Typhimurium DT104 cells after exposure to low water activity. Gupta et al. (54) succeeded in resuscitating the nonculturable organism by temperature increase and nutrient addition and confirmed the development of the VBNC state for *Salmonella* Typhimurium DT104. The investigators suggested that entering a VBNC state might enable the organism to maintain viability in inimical conditions and revert to the normal state under favorable conditions. It is not clear, however, whether *Salmonella* in a VBNC state maintains its pathogenic capacity and therefore is a concern for food safety (21, 79, 131). Several research groups have demonstrated the capacity of other bacteria (such as enteropathogenic *E. coli*, *Vibrio vulnificus*, and *Edwardsiella tarda*) to retain their pathogenicity in a VBNC state (38, 95, 98).

Biofilm formation is another way by which *Salmonella* survives the hostile conditions of the environment (112). However, based on available literature, it is not clear whether *Salmonella* cells form biofilms under low-moisture conditions.

A study by Mattick et al. (85) showed the presence of *Salmonella* filaments after 144 h of incubation in a broth medium supplemented with 8% NaCl (an approximate $a_w$ of 0.95); therefore, the authors hypothesized that filamentation may improve survival. Filaments occur as a consequence of exposure of *Salmonella* to marginal growth conditions, such as lower water activity, high or low temperatures (including refrigerated temperatures), and high or low pH values (69, 87). Kieboom et al. (69) showed that reduced water activity affected the morphology of *Salmonella* Enteritidis cells, which elongated and formed filaments when incubated at $a_w$ of 0.94 to 0.95 at 25°C for 6 days. Although cell filamentation increased the optical density of the broth culture, no increase in CFU was observed on plates, which suggests that filament cells form single colonies on the agar.

Research has also investigated other mechanisms that may enhance *Salmonella* survival. Abec and Wouters (2) showed that the adaptability of *Salmonella* Typhimurium to osmotic stress is most efficiently mediated by the accumulation of betaine (N,N,N-trimethyl glycine) via specific transporters. In response to increased osmotic pressure, *Salmonella* can modify the composition of its outer membrane (106). Optimal growth of *Salmonella* Typhimurium in media of high osmolarity and long-term survival during starvation in simple solutions of different osmolarity take place when both $\sigma^E$ and $\sigma^S$-regulated genes are functioning. The relative importance of $\sigma^E$ and $\sigma^S$ factors differed, depending on the environment. For example, at a concentration of 6% NaCl ($a_w$ of approximately 0.96), $\sigma^S$ was more important than was $\sigma^E$, whereas $\sigma^E$ was more important than was $\sigma^S$ for survival in a solution of 0.85% NaCl, especially at 37°C. The investigators concluded that these conditions are relevant to food preparation and storage, and $\sigma^E$ and $\sigma^S$ contribute toward survival of *Salmonella* Typhimurium in the food chain. The exposure of *Salmonella* Typhimurium to conditions that activate the
σE or σS pathways could trigger enhanced survival of the organism during food processing and storage (89). Hensel et al. (58) postulated that the water that is in close contact with the proteins inside a bacterial cell could be a factor determining the cell’s inactivation. As the cell is heated, water molecules begin to vibrate, and this vibration causes the disulfide bonds and hydrogen bonds in the surrounding proteins to weaken and break, altering the final three-dimensional configuration and possibly preventing the protein from functioning. As less water is present, these vibrations will be reduced, thus decreasing protein denaturation by this mechanism (39). It has also been suggested that with reduced water in the cells, the dipoles of the proteins within the cell interact and therefore stabilize both proteins and their subunits, i.e., peptides and amino acids, with formation of a stable complex (118). A larger amount of thermal energy would therefore be required to unfold the peptide chains, and the cell’s heat resistance would be increased in these low moisture ranges.

It is well recognized that Salmonella represents a real hazard for a wide range of low-moisture foods and food materials. Even though the organism does not grow, it may survive for a long time and cause illness. The ability of the organism to survive under adverse environmental conditions makes it difficult to control. Unlike other gram-negative bacterial strains (i.e., E. cloacae, E. coli), Salmonella seems to be supplied with a protection mechanism or structure that enables this organism to survive better under desiccated conditions (65). The mechanism by which Salmonella survives adverse conditions may include resistance to low water activity, biofilm formation, entry into a VBNC state, and activation of genes such as the σE or σS pathways (5, 36, 54, 89). However, these observations largely were made with studies conducted in a matrix with an aw above 0.85. The extent to which these mechanisms apply to a low-moisture product or the dry processing environment should be further investigated.

HEAT RESISTANCE OF SALMONELLA IN LOW-MOISTURE PRODUCTS

Thermal resistance of Salmonella is greatly enhanced in low-moisture foods and may be affected by other intrinsic and extrinsic properties of a food. For this reason, when evaluating published results of heat resistance of Salmonella in a particular food, one should be aware that it might be more meaningful to compare results within a study using similar food types and methods to determine heat resistance than to compare results from different studies. Due to variations in these parameters, it is important when using published D- and z-values or other inactivation models and applying them to certain food processes, that the conditions under which the values were obtained should not be significantly different from the product or process parameters used by the processor. Examples of published data on the heat resistance of Salmonella spp. in reduced-moisture food products such as chocolate, peanut butter, almonds, cereal grain flours, and spray dried milk are summarized here.

Chocolate and syrups. Chocolate and chocolate candies have such low-moisture content (aw of 0.4 to 0.5) that organisms heated in it are essentially subjected to dry heat. Increasing the amount of cocoa in the suspending medium, as well as agitation of the suspension before inoculation and heat treatment, enhanced the lethal effect on Salmonella (19). Several studies on the heat resistance of Salmonella in chocolate were conducted (Table 3) to assess the potential for the application of a heat process to eliminate the pathogen (9, 49, 76). A study conducted by Goepfert and Biggie (49) showed that in molten chocolate, Salmonella Typhimurium had a D-value of 396 min (6.6 h) and 816 min (13.6 h) at 71.1 and 65.6°C, respectively. Similar heat resistance was observed for milk chocolate (76), in which the D-values were 4.5, 4.6, and 6.6 h at 71°C for Salmonella Eastbourne, Salmonella Senftenberg, and Salmonella Typhimurium, respectively. Results from these two studies (49, 76) demonstrated that Salmonella Typhimurium was more heat resistant than was Salmonella Senftenberg 775W in milk chocolate. The curves obtained in the Goepfert and Biggie (49) study showed a rapid decline in numbers of survivors (3-log cycles) during the first few minutes of heating, followed by a slower rate of decrease thereafter. The rapid initial loss might be attributed to the death of cells injured during the lyophilization and inoculation methods used in this study. Salmonella cells were much more susceptible to destruction by heat when traces of water were added to the chocolate mass. Barrile and Cone (8) studied the effect of added moisture on the D-values of Salmonella Anatum in milk chocolate at 71°C. 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-values decreased as the level of added moisture increased. However, the change per increment of moisture was especially pronounced at or below 2.0% moisture level. D- and z-values for different Salmonella serotypes in chocolate are presented in Table 3.

Sumner et al. (116) determined the heat resistance of Salmonella Typhimurium in sucrose solutions with aw ranging from 0.98 to 0.83. The temperature data collected were analyzed with the general method (115) used to establish cumulative lethality for each heating time interval. The calculated lethality value was then used to determine the decimal reduction time (D). The D50,6°C was 0.29 at an aw of 0.98 and 40.2 min at an aw of 0.83. Authors also compared data collected in the sucrose solution to data generated using a food product; two thermal death time experiments were conducted with each of four chocolate syrups (A, B, C, and D) with aw values of 0.83, 0.84, 0.75 and 0.83, respectively. At an aw of 0.83 and temperature of 65.6°C, Salmonella Typhimurium was approximately three times more heat resistant in syrup D than in syrup A. The D-values for syrups A and D were 1.2 and 3.2 min, respectively. This observation was thought related to differences in compositions of the syrups, particularly sweeteners. D-values in chocolate syrups were more than 10-fold lower when compared with those in sucrose solutions at the same aw values. For examples, at an aw of 0.83 and temperature of 65.6°C, D-values were 3.2 and
TABLE 3. Heat resistance of Salmonella in chocolate"a

<table>
<thead>
<tr>
<th>Salmonella serotype</th>
<th>Heating medium</th>
<th>D-values (min) at temp indicated</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>65.6°C (150°F)</td>
<td>70°C (158°F)</td>
</tr>
<tr>
<td>Senftenberg</td>
<td>Molten chocolate</td>
<td>440b</td>
<td>116b</td>
</tr>
<tr>
<td></td>
<td>Molten chocolate</td>
<td>276c</td>
<td></td>
</tr>
<tr>
<td>Typhimurium</td>
<td>Molten chocolate</td>
<td>816b</td>
<td>222b</td>
</tr>
<tr>
<td></td>
<td>Molten chocolate</td>
<td>396c</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Chocolate syrup (A) (pH 5.10, aw = 0.83)</td>
<td>1.2d</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Chocolate syrup (B) (pH 5.10, aw = 0.84)</td>
<td>2.6d</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Chocolate syrup (C) (pH 5.65, aw = 0.75)</td>
<td>2.7d</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Chocolate syrup (D) (pH 5.35, aw = 0.83)</td>
<td>3.2d</td>
<td></td>
</tr>
<tr>
<td>Anatum</td>
<td>Molten chocolate (no moisture added)</td>
<td>1,200e</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Molten chocolate (1% moisture added)</td>
<td>510e</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Molten chocolate (4% moisture added)</td>
<td>210f</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Milk chocolate</td>
<td>11</td>
<td>24.2 (43.6)f</td>
</tr>
</tbody>
</table>

a Adapted in part from Doyle and Mazzotta (37).

b Goepfert and Biggie (49). Cells were grown to stationary phase and then inoculated into melted chocolate. Surviving cells were estimated by the most probable number after suspension in nutrient broth and incubation at 37°C for 48 h. The average D-values from three experiments are shown.

c Lee et al. (76).

d Sumner et al. (116). Cells were grown to stationary phase in brain heart infusion broth and then inoculated into chocolate syrup. Surviving cells were recovered in lactose broth, incubated at 30°C for 48 h, and plated on Hektoen enteric agar.

e Barrile et al. (9).

40.2 min for chocolate syrup (pH 5.35) and sucrose solutions, respectively (116).

In one of the first research publications dealing with the heat resistance of Salmonella in a low-water-activity environment, Goepfert et al. (50) examined the effect of various sugar and sugar-alcohol solutions on heat resistance by using several serotypes of Salmonella. They found that heat resistance was much greater when sucrose was used to lower the water activity than when fructose, glycerol, or sorbitol was used. Salmonella Senftenberg 775W showed less of an increase in heat resistance than did the other strains of Salmonella as the environment became drier. Growth in a reduced-water-activity environment prior to inoculation increased the heat resistance of Salmonella in glycerol solutions, but not in sucrose solutions. The novel conclusion of the early research was that although heat resistance did increase as the aw values, other factors such as the substance used to reduce the aw values had a significant effect. Therefore, it is not possible to take the heat resistance of an organism at a certain aw value in one food type and apply it to another.

**Peanut butter.** Shachar and Yaron (109) investigated the heat resistance of Salmonella serovars Agona, Enteritidis, and Typhimurium in peanut butter. The peanut butter was inoculated with the Salmonella serovars at 4 and 8 log CFU/g and incubated in water baths at 70, 80, or 90°C for 5 to 50 min at each of the temperatures. All Salmonella serovars tested, regardless of their initial cell concentration, showed no significant differences (P > 0.05) in heat resistance. All serovars were so heat resistant that even after 50 min at 90°C, only a 3.2-log reduction was observed. When peanut butter containing viable Salmonella cells of serotype Agona, Enteritidis, and Typhimurium at approximately 8 log CFU/g was exposed to heat for 5 min, a 1.4-log reduction was observed at 70°C, a 2.2-log reduction at 80°C, and a 2.5-log reduction at 90°C (109). It was observed that after an initial inactivation phase, cell death occurred at a slower rate. During the second inactivation phase, higher temperatures (80 and 90°C) were only slightly more effective in killing cells than was 70°C, but the differences were not statistically significant at heating intervals up to 50 min. The thermal inactivation curves were upwardly concave, indicating rapid death at the beginning (10 min), followed by lower destruction rates and an asymptotic tail. The authors applied the nonlinear Weibull model to describe the heat inactivation of Salmonella in peanut butter. This model predicted that more than 260 min (>4 h) would be needed to reduce Salmonella by 7 log units at 70°C, and more than 1 h would be needed at 90°C. Shachar and Yaron (109) concluded that some thermal treatments currently used in the industry to pasteurize peanut butter (e.g., 70°C for 20 min) are not sufficient to destroy vegetative cells of Salmonella. The authors concluded that a heat process of more than 4 h at 70°C or 1 h at 90°C would be adequate to deliver a 7-log reduction, but these processes may not have a practical application because they may adversely affect the sensory and quality properties of the product. Ma et al. (84) reported nonlinear inactivation of three outbreak strains of Salmonella Tennessee in peanut butter and used the Weibull model to fit the inactivation data. The resistance of Salmonella Tennessee strains was compared with the rates of inactivation of Salmonella strains of other serotypes (Enteritidis, Typhimurium, and Heidelberg). The authors found that 41 ± 3 min at 90°C achieved a 5-log reduction...
of a mixture of three outbreak-associated Salmonella Tennessee strains in peanut butter (26 ± 2 min were needed to inactivate a composite of other Salmonella isolates). Results of this study indicated that the outbreak associated with Salmonella strains were more thermostable than were the other Salmonella strains tested, and this greater thermal resistance was not serotype specific. Thermal treatments of 90°C for less than 30 min are not sufficient to kill large populations (5 log CFU/g) of Salmonella in highly contaminated peanut butter.

Shachar and Yaron (109) also studied the factors that affect the high heat resistance of Salmonella in peanut butter. They suggested that the combination of both high fat content (~55%) and low aw (0.2 to 0.33) in peanut butter had a protective effect on Salmonella. The authors also explained the higher heat resistance of Salmonella in peanut butter was based on the heterogeneous nature of the product. Since peanut butter is a highly concentrated colloidal suspension of lipid and water in a peanut-meal phase, the bacterial cells would be exposed to different local environments and could aggregate near the water phases. During the heat process, cells would die off at different rates, depending on the protective effect of the local environment.

Almonds. Traditional roasting of almonds involves using high temperature or a long roasting time. Commercial oil roasting of almonds is carried at temperatures higher than 260°F (126.7°C) and for longer than 2.0 min. This process is more than sufficient to yield a 5-log destruction of Salmonella in almonds (4). If there is a need for a shorter process, process parameters for a 4-log reduction are 1.6 min at a minimum oil temperature of 260°F (3, 4).

Abd et al. (1) evaluated the impact of prior storage temperature (4 and 23°C) on thermal inactivation at 121°C (250°F) of Salmonella Enteritidis PT30 on oil roasted almonds. Reductions of 4 or 5 log were consistently achieved after heating for 0.58 ± 0.08 or 1.18 ± 0.17 min, respectively, when almonds were stored at 4°C. In contrast, at 23°C, 4- or 5-log reductions were achieved after heating for 1.16 ± 0.36 and 2.06 ± 0.57 min.

New pasteurization techniques such as steam pasteurization and combined hot-air roasting and pasteurization process have been proposed for raw and roasted almonds (96). Both processes are designated to produce a boundary layer of humidity on the almond surface to maximal microbiological inactivation rates. The original product quality of the almonds are maintained and not impaired, since the temporary increase in moisture content is very little (96).

Lee et al. (77) studied the application of pasteurization treatment for the reduction of Salmonella Enteritidis on an almond surface. Two varieties of California raw shelled almonds (Nonpareil and Mission) were inoculated with Salmonella Enteritidis and treated with steam at 93°C for 5, 15, 25, 35, 45, 55 or 65 s. A higher D-value (16.13 s) was calculated for Salmonella Enteritidis Mission almonds than for the Nonpareil variety (12.22 s). The data suggested that steam treatments of 61- and 81-s durations would be required to achieve 5-log reductions in Nonpareil and Mission almonds, respectively (77).

Spray-dried milk. An increase in bacterial resistance as solute concentration of the heating medium increases (7, 35, 92) has been reported in several publications. It has been suggested that this increase in resistance is a consequence of reduced water activity. Dega et al. (35) conducted research on the influence of milk solids concentrate at 10, 30, 42, and 51% (wt/wt) on the thermal resistance of Salmonella Typhimurium and Salmonella Alachua grown in tryptic soy broth at 37°C. The study showed that increasing the solids level resulted in an increase in resistance to heat of both strains of Salmonella. In addition, Salmonella Alachua was more heat resistant in milk containing 10, 30, 42, and 51% solids than was Salmonella Typhimurium (Table 4). The researchers also observed that the z-value increased as the solids level in milk increased. For examples, Salmonella Alachua z-values were reported as 4.1, 6.2, and 6.9°C at 10, 42, and 51% solids, respectively. The authors also demonstrated that the growth of Salmonella Typhimurium in 42% milk solids for 24 h did not greatly enhance the thermal resistance of the organisms when milk solutions were heated at atmospheric pressure to obtain 42% solids concentrate (35).

McDonough and Hargrove (88) observed that a cocktail of Salmonella (Salmonella Senftenberg, Salmonella Typhimurium, and Salmonella New Brunswick) was extremely resistant to destruction by dry heat in non-fat dried milk powder (Table 5). Neither 60 nor 76.6°C destroyed Salmonella cells starting with an initial population of 10ªCFU/g after 10 h (10-g samples). The moisture level in milk powder significantly influenced the heat resistance of Salmonella. For example, 2 h was insufficient to kill Salmonella in 4 and 7% moisture powders at 85°C, although 30 min was sufficient at the 25% moisture level. The degree of heat required for destruction at a high temperature (115.5°C for 1 h) at 4% moisture was too intense and imparted a yellow, burned appearance to the milk powder. Salmonella was not detected in milk powders containing 15% moisture treated at 148.8°C for 6 min. It was concluded that if the moisture content of milk powder was greater than 15%, milk powder might form larger agglomerates, slowing the rate of heat conductance (88).

Cereal grain flours. Sperber et al. (113) reported that the incidence of Salmonella in wheat flour ranged from 0.14 to 1.32%. Flour is typically an ingredient in food that is to be cooked or further processed before consumption. If there is a possibility that the flour will be consumed without further processing (even if that is not the intended use of the food product), then use of flour that has been heat treated to eliminate Salmonella may be desirable. Archer et al. (6) reported that the D-values for Salmonella Weltevrede in flour ranged from a D-value of 875 min at 60 to 62°C and an initial aw of 0.4 to a D-value of 29 min at 63 to 65°C and at an initial aw of 0.5 (Table 6). The z-values obtained in flour ranged from 15.2 to 53.9°C for Salmonella Weltevrede in wheat flour, and they were considerably larger than
TABLE 4. Influence of milk solids concentration on the heat resistance of Salmonella Typhimurium and Salmonella Alachua grown in Trypticase soy broth at 37°C

<table>
<thead>
<tr>
<th>Salmonella serotype</th>
<th>10% solids</th>
<th>30% solids</th>
<th>42% solids</th>
<th>51% solids</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Temp (°C)</td>
<td>Mean D-value (min)</td>
<td>Temp (°C)</td>
<td>Mean D-value (min)</td>
</tr>
<tr>
<td>Typhimurium</td>
<td>57.1</td>
<td>1.4</td>
<td>58.0</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td>55.7</td>
<td>3.2</td>
<td>55.0</td>
<td>11.0</td>
</tr>
<tr>
<td></td>
<td>52.5</td>
<td>22.5</td>
<td>51.7</td>
<td>59.8</td>
</tr>
<tr>
<td></td>
<td>51.4</td>
<td>49.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alachua</td>
<td>59.2</td>
<td>0.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>57.8</td>
<td>1.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>57.0</td>
<td>1.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>55.0</td>
<td>6.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>54.1</td>
<td>9.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>53.0</td>
<td>20.4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a Adapted in part from Dega et al. (35).
b Temperature values, ±0.2°C.
c D-value, decimal reduction time; it represents an average of two to five trials at each temperature.

dry animal feeds. Animal feeds are frequently contaminated with Salmonella (20, 82, 111). Liu et al. (82) determined the thermal resistance of Salmonella Senftenberg 775W in dry animal feeds (artificially contaminated and simulated naturally contaminated) at various moisture levels. Increasing feed moisture levels decreased heat resistance, with a declining effect starting between 15 and 20% moisture. Data obtained from thermal death time tubes indicated that, except for initial fast killing rates, the heat resistance of Salmonella Senftenberg 775W in dry feeds was an exponential function of heating time. Heat resistance was higher with contamination by the simulated natural method than by broth cultures. The simulated naturally contaminated feedstuffs were prepared by inoculation of Salmonella Senftenberg 775W into a sterile suspension of meat and bone meal in distilled water, whereas artificially contaminated feedstuff was prepared by adding a tryptic soy broth culture to feed. The D-value at 140°F in simulated naturally contaminated feed was 2.8 min at a 5% moisture level, and was approximately 2.9, 27.5, 37.9, 103, and 258 times as much at respective moisture levels of 10, 15, 20, 25, and 30%. The z-values were in the range of 18 to 20°F (10.0 to 11.1°C) (82).

TABLE 5. Survival of Salmonella in non-fat dried milk subjected to dry heat

<table>
<thead>
<tr>
<th>Exposure time</th>
<th>60.0°C</th>
<th>76.6°C</th>
<th>85.0°C</th>
<th>115.5°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>6.9 x 10^5</td>
<td>7.3 x 10^5</td>
<td>9.4 x 10^4</td>
<td>9.4 x 10^4</td>
</tr>
<tr>
<td>15 min</td>
<td>5.4 x 10^5</td>
<td>ND</td>
<td>ND</td>
<td>4.2 x 10^4</td>
</tr>
<tr>
<td>30 min</td>
<td>4.5 x 10^4</td>
<td>1.35 x 10^4</td>
<td>7.1 x 10^3</td>
<td>8.0 x 10^2</td>
</tr>
<tr>
<td>45 min</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>2.0 x 10^3</td>
</tr>
<tr>
<td>1 h</td>
<td>4.7 x 10^5</td>
<td>4.5 x 10^4</td>
<td>8.7 x 10^2</td>
<td>&lt;1</td>
</tr>
<tr>
<td>2 h</td>
<td>3.0 x 10^5</td>
<td>5.0 x 10^3</td>
<td>3.5 x 10^3</td>
<td>&lt;1</td>
</tr>
<tr>
<td>3 h</td>
<td>3.8 x 10^5</td>
<td>3.0 x 10^3</td>
<td>8.0 x 10^2</td>
<td>&lt;1</td>
</tr>
<tr>
<td>4 h</td>
<td>ND</td>
<td>2.9 x 10^3</td>
<td>5.0 x 10^2</td>
<td>&lt;1</td>
</tr>
<tr>
<td>5 h</td>
<td>3.0 x 10^5</td>
<td>1.4 x 10^3</td>
<td>2</td>
<td>&lt;1</td>
</tr>
<tr>
<td>10 h</td>
<td>4.0 x 10^5</td>
<td>3.2 x 10^2</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
</tbody>
</table>

a Adapted from McDonough and Hargrove (88). A thin layer of conventional (4% moisture) powder was heated in an oven. Negative results from 10-g samples recorded as <1.
b ND, not determined.
Bucher et al. (17) studied the thermal resistance of Salmonella strains isolated from raw, frozen chicken nuggets/strips, nugget meat, and pelleted broiler feed to determine whether they exhibited enhanced thermal resistance. Salmonella Enteritidis and Salmonella Orion were isolated from pelleted broiler feed. For Salmonella Enteritidis, D-values ranged from 6.93 to 0.15 min at 55 and 62 °C, respectively, and the z-values from 4.10 to 5.17 °C. For Salmonella Orion, D-values ranged from 3.58 to 0.18 min at 55 and 62 °C respectively, with a z-value of 5.17 °C. Results of this study indicated that Salmonella Enteritidis and Salmonella Orion strains did not exhibit unusually high thermal resistance, and that normal heating (71 °C) prior to consumption, should eliminate these organisms from chicken nuggets/strips (17).

Application of published heat resistance data for establishing lethal processes in low-moisture foods. It is highly recommended that food processors determine the heat resistance of Salmonella in their specific low-moisture product(s) rather than directly apply published D- and z-values from the literature in establishing a lethal process. Published values obtained from the same or similar type food products, heating temperatures, and aw values can serve as guidance in making conservative assumptions about sampling times at various heating temperatures. As can be seen throughout this review of heat resistance data, product composition can have an equal or greater affect than just the water activity value on the destruction of Salmonella. The specific serotype of Salmonella used in a new study as well the method used to recover surviving cells can also have a significant impact on the heat resistance values. For these reasons, it important that processors understand the thermal death kinetics of Salmonella in their specific products rather than relying solely on published values from similar products when validating a thermal process.

### CONCLUSION

Salmonella is extensively populated throughout nature and can be associated with many foods, in part because the organism can inhabit a multiplicity of hosts (11). Accordingly, to prevent the ingress of Salmonella into the factory, prudent processors should identify both food and non-food sources of the organism and either reject or securely isolate these sources whenever possible. When the production of a given product involves the receipt and handling of known sources of Salmonella, such as for processors of raw agricultural commodities, establishments should have con-
trol measures to mitigate the risks associated with handling these potential sources. These include minimizing vectors for the transfer of Salmonella to other locations throughout the facility by utilizing programs such as proper storage practices, balance of air flow, preventive maintenance, control of employee traffic patterns, and other GMPs. Additional efforts should be made to prevent the organism from becoming embedded or otherwise well-established in the factory environment, such as sanitary design of the facility and processing equipment, effective sanitation practices, and preventing the introduction of moisture into normally dry environments.

Due to the ability of Salmonella to adapt to the stresses of extreme physical and chemical conditions such as desiccation, temperature, pH, lack of nutrients, etc., it may persist for a long time in dry environments. Even though the organism does not grow in low-moisture food products such as dry milk powder, chocolate, or peanut butter and almonds (11, 18, 121, 132), it can remain viable for extended periods, especially when stored at refrigeration temperature. Accordingly, Salmonella spp. represent a potential severe hazard for a wide range of low-moisture foods and food materials if not controlled.

Thermal resistance of Salmonella is greatly enhanced in low-moisture foods. Given the fact that the heat resistance of Salmonella is affected by many factors, comparing heat resistance among studies can be misleading. Comparing differences in heat resistance from experiments within the same study is more accurate than comparing data from different experiments or studies using different conditions. Due to variations in these parameters, it is important, when using published D- and z-values or other inactivation models and applying them to certain food processes, that the conditions under which the values were obtained should not be significantly different from the product or process parameters used by the processor. Survivor curves of heat inactivation of Salmonella can be nonlinear and may have a significantly asymptotic tailing effect, which can affect the efficacy of some processes. Often, nonlinear models, particularly the Weibull model, have been used to describe more accurately the thermal resistance of Salmonella in a variety of low-moisture foods when compared with traditional log-linear techniques. Therefore, expert microbiologists and thermal process authorities with low-water-activity food experience should be consulted for determining the appropriate thermal process for these food products.

The attributes mentioned in our review characterize a low-moisture food processor’s challenges: a widespread, highly adaptive organism with considerable heat resistance under low-moisture conditions. Low-moisture food processors would be well advised to enact a multitude of highly disciplined control measures to address the organism and combat it to the fullest. In 2008, in response to the Salmonella outbreaks in low-moisture foods issues, the Grocery Manufacturers Association formed a join Salmonella Control Task Force to develop industry guidance. Scientific data and information, summarized in this review, were used in part to develop those guidance documents (25, 26, 52, 108).

ACKNOWLEDGMENTS
The authors gratefully acknowledge the valuable assistance of Virginia N. Scott (GMA, currently with FDA), Yuhuan Chen (GMA), and Ai Kataoka (GMA).

REFERENCES