



**ANNEX TO CONTROL OF *SALMONELLA* IN LOW-MOISTURE FOODS**

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## **AUTHORS AND ACKNOWLEDGEMENTS**

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

Low water activity ( $a_w$ ) is a barrier to growth for many vegetative pathogens, including *Salmonella* spp. Processed products such as powdered milk products, chocolate, peanut butter, infant formula, toasted cereal, and bakery products are characteristically low  $a_w$  foods. While these products do not support the growth of *Salmonella*, all have been implicated in outbreaks of salmonellosis (CDC, 1993, 1998 and 2007; Koch et al., 2005; Rushdy et al., 1998; Smith et al., 2004). Epidemiological and environmental investigations of these outbreaks have suggested that cross contamination plays a major role in the contamination by *Salmonella* of these products.

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 may lead to cross contamination. Cross contamination is the transfer of bacteria from one surface, object or place to another. Significant food safety risk may occur when this transfer takes place where the product is ready-to-eat with no additional *Salmonella* inactivation step in the process (Reij et al., 2004). In a 2004 review article, Reij et al. cited a survey conducted by the World Health Organization (WHO, 1995) that 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 rooms (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 (Powell and Atwell, 1998).

This section summarizes available literature on *Salmonella* contamination in low-moisture foods that has been traced to poor sanitation practices, substandard equipment design, improper maintenance, poor operational practices and GMPs, inadequate ingredient control, and other factors.

### **Contamination Associated with Poor Sanitation Practices**

*Salmonella* cross contamination due to poor sanitation practices is enhanced by the organism's ability to survive on otherwise clean surfaces for extended periods of time, then being transferred to foods upon contact. Kusumaningrum et al. (2003) showed that *Salmonella* can remain viable on dry stainless steel surfaces and present a potential for contamination for considerable periods of time. *S. Enteritidis* was readily transferred from these surfaces to foods with transfer rates of 20% – 100%. *S. Enteritidis* was recovered from dry, highly contaminated ( $10^5$  cfu/cm<sup>2</sup>) stainless steel surfaces for at least four days and it was recovered for 24 hrs from moderately contaminated surfaces ( $10^3$  cfu/cm<sup>2</sup>).

During investigations into outbreaks of salmonellosis, evidence of *Salmonella* has been found in plant processing environments. An outbreak of *Salmonella* Agona associated with toasted oats cereal prompted examination of potential cross contamination in the processing areas, air handling systems, ingredients, and traffic flow of the manufacturing factory. Investigators found

wide-spread low levels of the organism in the plant environment, including samples taken from the floor, production equipment and the exhaust system in the plant (Breuer, 1999; FDA, 1999). The investigators also found that a majority of the equipment was open to the atmosphere and that cleaning and sanitation was very difficult because insufficient space was allocated between pieces of equipment. 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 (FDA, 1999).

An investigation of *S. Senftenberg* contamination in infant cereal revealed that bulk cereal was contaminated with “cleaning remains” from milling machinery (Rushdy et al., 1998). Investigations into two consecutive *S. Enteritidis* outbreaks in bakery products (Evans et al., 1996) 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.

The mechanisms of *Salmonella* contamination in a Japanese oil meal (rapeseed or canola meal) factory were studied (Morita et al., 2006). The authors found *Salmonella* in 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 the study involving contaminated chocolate by Craven et al. (1975), investigators found inadequate separation between clean and unclean zones and recommended reducing opportunities for *S. Eastbourne* cross contamination by controlling airborne spread of dust. Butcher and Miles (1995) also indicated that dust was a major source of *Salmonella* contamination of poultry feed in processing mills.

### **Contamination Associated with Poor Facility and Equipment Design/Inadequate Maintenance**

Cross contamination as a result 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 maintained. Poor facility design and maintenance can also contribute to the problem of *Salmonella* contamination.

An international outbreak of *S. Eastbourne*, where 200 people were affected by contaminated chocolates, was traced to the factory. In a report on investigations following this outbreak, Craven et al. (1975) indicated that raw cocoa beans were the probable source of *Salmonella*, which survived heating during production. It was also suggested that valves in conches were arranged such that not fully-heated chocolate could accidentally be pumped directly to a storage tank.

The source of *S. 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 (Rowe et al., 1987). 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 (Booth, 2006). In a recent outbreak of *Salmonella* Tennessee infections associated with peanut butter in the US, the CDC report on the outbreak indicated that the source of the peanut butter contamination was unknown, and that the outbreak demonstrated that a low-moisture product can become contaminated even when the production process included a heat-treatment step (CDC, 2007). A company spokesperson indicated that the outbreak was traced to problems with a leaky roof and two instances of faulty sprinklers going off (Funk, 2007). FDA investigations of the outbreak included collecting samples from raw materials, finished products and the plant environment, examination of processing data, and inspection of the facility for sources of contamination (Zink, 2008). A total of 122 environmental samples were collected by FDA and two tested positive (a floor squeegee sample and a drain sample from the roaster room). According to Zink (2008), "water event(s)" in the facility might have increased the numbers of *Salmonella* and led to product contamination.

The UK chocolate outbreak and the US peanut butter outbreak illustrate settings where poor facility maintenance can result in cross contamination by salmonellae. In addition to the potential contaminants introduced from leaky roofs and faulty sprinklers in the peanut butter processing facility, these events introduced moisture into a normally dry environment. Moisture most likely contributed to the cross contamination by facilitating the growth of otherwise dormant *Salmonella* that might have come from peanut dust.

### **Contamination Associated with Poor Ingredient Control**

Even the best designed equipment systems operating with exemplary preventive maintenance programs cannot combat cross contamination from poor control of raw materials and ingredients. Contaminated ingredients, used in products without a further kill step, carry the pathogen directly into finished products. For example, paprika powder contaminated with multiple serovars of *Salmonella* was added to paprika-powdered potato chips, which resulted in an estimated 1,000 cases of salmonellosis (Lehmacher et al., 1995). Poor choice of ingredients can have similar detrimental results. Marshmallows made with raw egg whites resulted in 36 cases of *S. Enteritidis* PT4 infections (Lewis et al., 1996).

Koch et al. (2005) investigated an *S. Agona* outbreak in Germany and reported that the organism was found among products from twelve 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. (1992) 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 *S. Javiana*.

## Other Factors for *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 insects can be vectors for *Salmonella* transmission. In a study involving seven species of common grain insects, Crumrine et al. (1971) demonstrated that *S. Montevideo* was transmitted by insects from inoculated wheat to clean wheat. The authors concluded that insects contaminated with *S. Montevideo* could contaminate large masses of grain. In another study, Kopanic et al. (1994) found that *S. Typhimurium* could be acquired and transmitted by cockroaches, which were implicated as potential vectors of the pathogen. Furthermore, infected cockroaches were capable of infecting other cockroaches, which could then serve as a potential source of *Salmonella* contamination.

In the Rushdy et al. (1998) investigation of eight reported cases of *S. Senftenberg* infections in infants, which occurred in 1995 in England, the illness was associated with the consumption of one brand of baby cereal. The supplier used common machinery to process heat-treated bulk cereal as well as other products that were not heat treated. They failed to identify the potential source for *Salmonella* to be introduced into the processing equipment system, and did not have control measures in place to mitigate the risk associated with it. The receiving company, in spite of receiving a previous shipment of bulk cereal contaminated with *S. Senftenberg*, did not thoroughly investigate the supplier and did not identify the ingredient as a possible source of *Salmonella* in their product.

An outbreak of *S. Enteritidis* associated with raw almonds occurred in Canada and the United States in 2000-2001. Environmental investigations conducted in response to the outbreak found that contamination and cross contamination risks exist within the tree nut processing facilities and on the farms (Isaacs et al., 2005; Elliott, 2005). *Salmonella* was found in 16 of 32 orchards samples. All of the growers involved indicated that manure or biosolids were not used on the land within the previous five 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 environmental 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.

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

*Salmonella* outbreaks have been linked to a number of food products with low  $a_w$ , including peanut butter, chocolate, dried milk, cereal products, fermented meat products, and food ingredients such as black pepper, paprika, and desiccated coconut. Because of a global and complicated food supply chain, there is a potential for more “new” foods to be linked to salmonellosis.

It is expected that *Salmonella* may be present in or on any raw food materials (Bell and Kyriakides, 2002), in part because *Salmonella* is wide-spread in nature. The organism can colonize a wide variety of hosts (e.g., mammals, birds, reptiles, amphibians, insects) and is widely disseminated in the environment (e.g., water, soil, plants). *Salmonella* can survive for weeks in water and for years in soil if environmental conditions such as temperature, humidity, and pH are favorable (Todar, 2006). 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 (Crumrine et al., 1971; Winfield and Groisman, 2003; Foster and Spector, 1995).

Studies show that *Salmonella* may survive in dry foods and feeds for a long period of time (Juven et al., 1984; Janning et al., 1994; Hiramatsu et al., 2005). Janning et al. (1994) 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 248-1,351 days were needed to achieve 1-log reduction. *Salmonella* was more resistant to desiccation under the experimental conditions than *Enterobacter cloacae* and *E. coli*. Hiramatsu et al. (2005) reported that the survival of dried *Salmonella* cells substantially increased (up to 79 times) when sucrose 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 dried plain squid without sucrose (Hiramatsu et al., 2005). It was reported that *Salmonella* may survive for months in foods such as chocolate, peanut butter and pepper. The combination of high fat and low  $a_w$  might have a synergistic effect on *Salmonella* survival (Shachar and Yaron, 2006).

### **Survival of *Salmonella* in Chocolate and Cocoa and Confectionery Products**

Chocolate is considered an almost inert finished product with very low moisture content (<8%). It is probably the most consumed confectionery product in the world. In the last few decades chocolate products have been implicated in a number of salmonellosis outbreaks (Bell and Kyriakides, 2002; Craven et al., 1975; Gill et al., 1983; Greenwood and Hooper, 1983; Hockin et al., 1989; Kapperud et al., 1990; Scheil et al., 1998; Werber et al., 2005). In some cases very low levels of contamination were found, e.g., as low as 1–3 cell/g. Kapperud et al. (1990) 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 salmonellae instead of small doses. The latter scenario was, however, considered less likely because of the thorough mixing of the chocolate at the factory. It has been suggested that the high fat content of chocolates apparently protects *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 (Craven et al., 1975; D'Aoust, 1977; Greenwood and Hooper, 1983).

*Salmonella* cannot grow in chocolate but it can survive for long periods of time and it represents significant risk even at low levels of contamination (D'Aoust, 1977). Barille et al. (1970) found that lyophilized cells of *S. Anatum* inoculated into milk chocolate at levels of 50 cells/100g was detected at a level of 14 MPN/100g after 15 months of storage at room temperature. Tamminga et al., (1976) demonstrated that *Salmonella* may survive for months in different types of chocolate (Table A-1). The chocolate industry faces a difficult task in controlling *Salmonella* because of various reasons, which include: (i) raw materials and ingredients such as raw cocoa beans or powdered milk may carry *Salmonella*; (ii) low  $a_w$  and high fat content, which increases thermal resistance so that even considerable heating does not necessarily eliminate *Salmonella*; (iii) a small number of *Salmonella* can cause illness (Bell and Kyriakides, 2002; Werber et al., 2005).

Table A-1. Survival of *Salmonella* in milk chocolate and bitter chocolate at 20°C \*

Storage time	Level of <i>Salmonella</i> (log MPN/100g)			
	<i>S. Typhimurium</i>		<i>S. Eastbourne</i>	
	Milk chocolate ( $a_w$ 0.37)	Bitter chocolate ( $a_w$ 0.42)	Milk chocolate ( $a_w$ 0.38)	Bitter chocolate ( $a_w$ 0.44)
0 day	5.04	4.86	5.2	5.2
1 day	2.34-2.63	1.69-1.88	4.64	4.64
13 days	1.18-1.36	0.30-0.56	2.54-3.18	1.30-1.90
20 days	0.89-1.11	Neg-0.30	2.54-2.97	1.18-1.56
34 days	Neg-0.89	Neg	ND	ND
41 days	ND	ND	2.23-2.38	0.65-1.18
48 days	Neg-0.89	Neg	ND	ND
76 days	ND	ND	1.63-1.69	Neg-1.46
83 days	Neg-0.30	Neg	ND	ND
6 months	Neg	Neg	Neg-1.23	Neg
9 months	ND	ND	0.89-1.11	Neg

\* Adapted from Tamminga et al. (1976). Neg: *Salmonella* not detected. ND: not determined.

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 (Betts, 2007). Halva is another confectionary product with very low  $a_w$  (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) may have the potential to be contaminated with *Salmonella*. Although *Salmonella* cells do not multiply because of the low  $a_w$ , the organism may survive for relatively long periods of time in the product. *S. Enteritidis* survived in vacuum-packed halva stored for 8 months under refrigeration, longer than its survival in air-sealed halva stored at room temperature where a greater decline was observed (Kotzekidou, 1998). The

author concluded that reduction of salmonellae during storage cannot be predicted solely on the basis of  $a_w$ . Interactions between low  $a_w$  and environmental factors such as storage in air or under vacuum and temperature appear to play an important role in *Salmonella* survival.

### **Survival of *Salmonella* in Nuts and Peanut Butter**

Contaminated in-shell peanuts have been identified as the source of an international outbreak of *Salmonella* affecting countries on three continents. The product implicated in the outbreak originated from a fourth continent (Kirk et al., 2004). Although a range of *Salmonella* serotypes was identified in the implicated products, only the serotypes Stanley, Newport and Kottbus were associated with human infections (Kirk et al., 2004).

*Salmonella* inoculated into peanut butter and 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 upon the size of the water droplets, which may vary with the product (Burnett et al., 2000; Christian, 2000; European Commission, 2003; Shachar and Yaron, 2006). A study by Burnett et al. (2000) on the survival of *Salmonella* in peanut butter and peanut butter spread showed that, when products were inoculated with 5.7 log cfu/g of a five-serotype mixture, reductions of *Salmonella* in products stored for 24 weeks at 21 °C and 5 °C were 4.1-4.5 log and 2.9-4.3 log reduction, 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 after storage for 24 weeks at 5 °C. At 21 °C, all products (except one peanut butter spread) were negative for *Salmonella* after storage for 24 weeks. The investigators concluded that post-process contamination of peanut butter and spreads with *Salmonella* may result in survival in these products during their shelf life at 5 °C and possibly at 21 °C, depending on the formulation (Burnett et al., 2000).

A study by Uesugi et al. (2007) 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 *S. Enteritidis* PT 30 belonging to two PFGE patterns. This rare *Salmonella* strain was isolated in an outbreak in 2000 that was linked to the consumption of raw almonds (Isaacs et al., 2005). Therefore, the *Salmonella* strain might have persisted in that environment for at least 7 years (Uesugi et al., 2007). Beuchat and Heaton (1975) reported that the survival of *Salmonella* on pecans stored at different temperatures up to 32 weeks was inversely correlated to the storage temperature. Although storage for nuts and nuts 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* (Uesugi and Harris, 2006).

### **Survival of *Salmonella* in Flours, Cereals, Spices and Pets Treats**

Flour is typically used as an ingredient in more complex cooked or baked foods, going through effective killing steps for *Salmonella* and other vegetative pathogens. Generally, FDA does not consider flour a “sensitive ingredient” for *Salmonella* (Sperber, 2007). 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 (Sperber, 2007). Six cases of *S. Senftenberg* infections were associated with the consumption of infant cereal in England in 1995 (Rushdy et al., 1998).

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 this type of foods have been published. For example, as noted previously, Lehmacher et al. (1995) described a *Salmonella* outbreak associated with the consumption of paprika-powdered potato chips. Low levels of *Salmonella* were found in the product (4 - 45 cells/100g); these levels were sufficient to cause illnesses because of the high fat content of the paprika-powdered potato chips, which may have protected *Salmonella* from gastric acidity.

In 1999 an outbreak of *Salmonella* Infantis in Canada was linked to contact with pet treats (Clark et al., 2001). In a survey, White et al. (2003) 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 adequately controlled, pet treats could be potential sources of animal and human infections with *Salmonella* (Clark et al., 2001; Pitout et al., 2003; White et al., 2003; Chiewchan et al., 2007). Some examples of the survival of *Salmonella* in foods of low water activity are presented in Table A-2.

Table A-2. Examples of *Salmonella* survival in foods with low water activity

Food	<i>Salmonella</i> serotype(s)	Inoculum (log cfu/g)	Water activity	Length of Survival	Reference
Dried milk products	Naturally contaminated with 3 serotypes			Up to 10 months	Ray et al., 1971, cited by Bell and Kyriakides, 2002
Pasta	Infantis, Typhimurium		0.12% moisture	Up to 12 months	Rayman et al., 1979, as cited by Bell and Kyriakides, 2002
Milk chocolate	Eastbourne	8.0 5.0	0.41 0.38	> 9 month at 20°C 9 months at 20°C	Tamminga et al., 1976
Bitter chocolate	Eastbourne	7.0 5.0	0.51 0.44	9 months at 20°C 76 days at 20°C	Tamminga et al., 1976
Peanut butter	A composite of Agona Enteritidis Michigan Montevideo Typhimurium	5.7 1.5	0.20-0.33 0.20-0.33	Up to 24 weeks held at 5°C or 21°C  Up to 24 weeks at 5°C  Up to 6 weeks at 21°C	Burnett et al., 2000
Paprika powder	Multiple serotypes			> 8 months	Lehmacher et al., 1995

## **Survival of *Salmonella* in Other Matrices**

De Rezende et al. (2001) suggested that an in vitro adaptation of salmonellae to dry environments may occur when the organisms are exposed to alternating levels of high and low  $a_w$ . The maximum survival of several vegetative bacteria in dried milk was at  $a_w$  0.05-0.20. Maximum survival of *Salmonella* Newport in foods at neutral pH was at  $a_w$  of 0.11. According to Burnett et al. (2000) and Christian (2000), *S. Senftenberg* and *S. Typhimurium* survived in gelatin at a rubbery state (0.93-0.96  $a_w$ ) and a glassy state (0.45-0.28  $a_w$ ). *Salmonella* cells remained viable under low  $a_w$  conditions and the least survival was observed at an intermediate  $a_w$  between 0.55 and 0.74 (Christian, 2000). Similar results were reported in a study by Jung and Beuchat (1999), which showed that *S. Typhimurium* survival was enhanced as the  $a_w$  of egg white powder decreased. The investigators detected *Salmonella* in the powder at  $a_w$  0.13 but not at  $a_w$  0.34 after the product was stored at 54 °C for 7 days.

Desiccated *Salmonella* cells can survive for long periods of time on work surfaces and in foods with low  $a_w$ , especially in those foods with a high fat content. Although some die-off occurs in dehydrated foods during storage, the degree depends on relative humidity and storage atmosphere. Simulating conditions in dried foods, Hiramatsu et al. (2005) showed that desiccated cells of different *Salmonella* strains inoculated on dried paper disks died after 35 to 70 days of storage at 25 °C 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 (2004) reported that the higher the  $a_w$ , temperatures and oxygen levels, the higher the death rates of *Salmonella*.

## **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 (Caro et al., 1999; Lesne et al., 2000). De Rezende et al. (2001) also showed extensive filamentation of *S. Typhimurium* DT104 cells after exposure to low  $a_w$ . Gupte et al. (2003) succeeded in resuscitating the nonculturable organism by temperature increase and nutrient addition and confirmed the development of VBNC state for *S. Typhimurium* DT104. The investigators suggested that entering a VBNC state may 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 VBNC state maintains its pathogenic capacity and therefore is a concern for food safety (Caro et al., 1999; Lesne et al., 2000; Winfield and Groisman, 2003).

Biofilm formation is another way by which *Salmonella* survives the inimical conditions of the environment. However, based on available literature, it is not clear whether *Salmonella* cells form biofilms under low-moisture conditions. Under high-moisture conditions, cellulose production and biofilm formation may be an important factor for the survival of *S. Enteritidis* on surface environments (Solano et al., 2002). In biofilms on solid surfaces salmonellae display an increased resistance to chlorination. These biofilm cells, which are not inactivated during usual chlorination procedures, could be a source of contamination for water and foods (Scher et al., 2005). *S. Enteritidis* can adhere to both hydrophobic (e.g., Teflon) and hydrophilic (stainless

steel) surfaces common in the food industry. A *Salmonella* outbreak was linked to residual bacteria on blender blades, which were apparently unaffected by routine sanitizing procedures (Austin et al., 1998).

The presence of at least four fimbrial systems suggests that attachment to cellular or non-cellular surfaces may be a critical step in the survival of *S. Typhimurium* in the environment (Darwin and Miller, 1999). The fact that cells in biofilms are more resistant to heat, detergents, and sanitizers emphasizes the importance of cleaning of the equipment prior to sanitizing in order to control biofilms. Trisodium phosphate is effective against *S. Typhimurium* biofilm cells (Montville and Matthews, 2007).

A study by Mattick et al. (2000) showed the presence of live filaments after 144 hr of incubation in a broth medium supplemented with 8% NaCl (approximately  $a_w$  0.95), which suggests that filamentation may improve survival. Filaments occur as a consequence of exposure of *Salmonella* to marginal growth conditions, such as lower  $a_w$ , high or low temperatures (including refrigerated temperatures) and high or low pH values (Mattick et al., 2003; Kieboom et al., 2006). Kieboom et al. (2006) showed that reduced water activity affected the morphology of *Salmonella* Enteritidis cells, which elongated and formed filaments when incubated at  $a_w$  of 0.94-0.95 at 25 °C for 6 days. Although cell filamentation increased the optical density of the broth culture, no increase in colony forming units was observed on plates, which suggests that filament cells form single colonies on the agar. Based on available literature, it is not clear whether *Salmonella* cells form filaments under low-moisture conditions at  $a_w$  less than 0.85.

Research has also investigated other mechanisms that may enhance *Salmonella* survival. Abee et al. (1999) showed that the adaptability of *S. Typhimurium* to osmotic stress is most efficiently mediated by the accumulation of betaine (*N,N,N*-trimethylglycine) via specific transporters. In response to increased osmotic pressure, *Salmonella* can modify the composition of its outer membrane (Rychlik and Barrow, 2005). Optimal growth of *S. 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 (approximately  $a_w$  of 0.96),  $\sigma^S$  was more important than  $\sigma^E$ , whereas  $\sigma^E$  was more important than  $\sigma^S$  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 towards survival of *S. Typhimurium* in the food chain. The exposure of *S. Typhimurium* to conditions that activate the  $\sigma^E$  or  $\sigma^S$  pathways could trigger enhanced survival of the organism during food processing/storage (McMeechan et al., 2007).

In summary, the ability of the organism to survive under adverse environmental conditions makes it difficult to control. The mechanism by which *Salmonella* survives adverse conditions may include resistance to low water activity, biofilms formation, entry into a VBNC state, and activation of genes such as the  $\sigma^E$  or  $\sigma^S$  pathways (Anriany et al., 2001; de Rezende, 2001; Gupta et al., 2003; McMecchan et al., 2007). However, these observations largely were made with studies conducted in a matrix with  $a_w$  above 0.85. To what extent these mechanisms apply to a low-moisture product or the dry processing environment remains to be further investigated.

## HEAT RESISTANCE OF *SALMONELLA* IN LOW-MOISTURE PRODUCTS

### Chocolate

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 (Goepfert and Biggie, 1968; Barrile and Cone, 1970; Lee et al., 1989). Chocolate and chocolate candies have such low-moisture content ( $a_w$  0.3-0.5) that organisms heated in it are 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 salmonellae (Busta and Speck, 1968). The authors concluded that agitation and heating resulted in chemical alteration of the suspending medium or an enhanced extraction of lethal component(s) of the cocoa suspension.

A study conducted by Goepfert and Biggie (1968) showed that in molten chocolate *S. Typhimurium* had a D-value of 396 minutes (6.6 hrs) and 816 minutes (13.6 hrs) at 71.1°C and 65.6 °C, respectively. Similar heat resistance was observed for milk chocolate (Lee et al., 1989) where the D-values were 4.5, 4.6 and 6.6 hrs at 71 °C for *S. Eastbourne*, *S. Senftenberg* and *S. Typhimurium*, respectively. Results from two studies (Goepfert and Biggie, 1968; Lee et al., 1989) demonstrated that *S. Typhimurium* was more heat resistant than *S. Senftenberg* 775W in milk chocolate.

*Salmonella* cells were much more susceptible to destruction by heat when traces of water had been added to the chocolate mass. Barrile and Cone (1970) studied the effect of added moisture on the D-values of *S. 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 hr to 4 hrs. D-values decreased as the level of added moisture increased. However, the change per increment of moisture was especially pronounced at or below 2% moisture level. D- and z-values for different *Salmonella* serotypes in chocolate are presented in Table A-3.

Table A-3. Thermal resistance of *Salmonella* in chocolate <sup>ξ</sup>

<i>Salmonella</i> serotype	Heating medium	D-values (min) at temperature indicated					z value in °C or (°F)
		65.6 °C (150°F)	70 °C (158°F)	71.1 °C (160°F)	80 °C (176°F)	90 °C (194°F)	
Senftenberg	Molten chocolate		440 <sup>a</sup>		116 <sup>a</sup>	36 <sup>a</sup>	18.0 (32.4) <sup>a</sup>
	Molten chocolate			276 <sup>b</sup>			
Typhimurium	Molten chocolate		816 <sup>a</sup>		222 <sup>a</sup>	75 <sup>a</sup>	19.0 (34.2) <sup>a</sup>
	Molten chocolate			396 <sup>b</sup>			
	Chocolate syrup (pH 5.10, a <sub>w</sub> =0.83)	1.2 <sup>c</sup>					6.2 (11.2) <sup>c</sup>
	Chocolate syrup (pH 5.65 a <sub>w</sub> =0.75)	2.7 <sup>c</sup>					8.3 (15.0) <sup>c</sup>
Anatum	Molten chocolate (no moisture added)			1,200 <sup>d</sup>			
	Molten chocolate (1% moisture added)			510 <sup>d</sup>			
	Molten chocolate (4 % moisture added)			210 <sup>d</sup>			

<sup>ξ</sup> Partially adopted from Doyle and Mazzotta, 1999.

<sup>a</sup> Goepfert and Biggie (1968). 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 hrs. The average D-values from three experiments are shown.

Lee et al. (1989).

<sup>c</sup> Sumner et al. (1991). 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 hrs and plated on Hektoen enteric agar.

<sup>d</sup> Barrile and Cone (1970).

### **Peanut Butter and Almonds**

Shachar and Yaron (2006) investigated the heat resistance of *Salmonella enterica* serovars Agona, Enteritidis, and Typhimurium in peanut butter. The peanut butter was inoculated with *Salmonella* serovars at 10<sup>4</sup> cfu/g and 10<sup>8</sup> cfu/g and incubated in water baths at 70, 80 or 90 °C for 5-50 minutes at each of the temperature. All tested *Salmonella* serovars and their initial cell concentration had no significant effects (P>0.05) on the heat resistance of these organisms in peanut butter. However, all the serovars were highly resistant to heat; even after 50 minutes at a temperature as high as 90 °C only 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 was observed at 80 °C, and a 2.5-log reduction was observed at 90 °C (Shachar and Yaron, 2006). The investigators did not observe significant differences between the serotypes. After the initial inactivation phase, cell death occurred at a slower rate. During the second inactivation phase, higher temperatures (80 and 90°C) were slightly more effective in killing cells than was 70 °C, but the differences were not statistically significant at different heating intervals up to 50 min. The thermal inactivation curves were upwardly concave, indicating rapid death at the beginning (10 min) followed by lower death rates and an asymptotic tail. Therefore, the authors used the nonlinear Weibull model to describe the heat inactivation of *Salmonella* in peanut butter. Based on their calculation, more than 260 min (>4 hrs) would be needed to reduce *Salmonella* by 7 logs units at 70 °C, and more than 60 min (1 hr) would be needed at 90 °C.

Shachar and Yaron (2006) concluded that some thermal treatments currently used in the industry to pasteurize peanut butter (e.g., 70 °C/20 min) are not sufficient to destroy vegetative cells of *Salmonella*. The authors concluded that a heat process of more than 4 hrs at 70°C or 1 hr 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. (2008) reported non-linear inactivation of *Salmonella* in peanut butter and used the Weibull model to fit the inactivation data. Recent research (Doyle and Ma, 2009) found that, based on the non-linear Weibull model, 42±8 min at 90 °C achieved a 5-log reduction of a mixture of three outbreak-associated *S. Tennessee* strains in peanut butter (49±12 min were needed to inactivate a composite of other *Salmonella* isolates). On oil-roasted almonds, 2.06 ±0.57 min at 121 °C was needed to achieve a 5-log reduction of *S. Enteritidis* PT 30, based on using the Weibull model to fit the heat inactivation curve (Abd et al., 2008). When heat resistance parameters were determined based on the linear portion of the inactivation curve for *Salmonella* on oil-roasted almonds, the D-value was 0.85 min at 121 °C and the z-value was 27 °C (Harris, 2008), which indicates that 4.25 min at 121 °C would be needed for 5-log reduction based solely on the D-value derived from the linear portion of the thermal inactivation curves.

Shachar and Yaron (2006) 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 water activity (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 over time and a tailing effect. 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 would aggregate into clumps near the water phases. During the heat process, cells would die off at different rates depending on the protective effect of the local environment.

### **Spray Dried Milk**

Several papers demonstrated an increase in bacterial resistance as the solute concentration of the heating medium increases (Baird-Parker et al, 1970; Dega et al., 1972; Moats et al. 1971). It has been suggested that this increase in resistance is a consequence of reduced water activity. Dega et al. (1972) conducted research on the influence of milk solids concentrate at 10, 30, 42, and

51% (w/w) on the thermal resistance of *S. Typhimurium* and *S. 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, *S. Alachua* was more heat resistant in concentrated milk than *S. Typhimurium* (Table A-4). The researchers also observed that the z-value increased as the solids level in milk increased. For examples, for *S. 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 *S. Typhimurium* in 42% milk solids for 24 hr did not greatly enhance the thermal resistance of the organism when heated in a fresh 42% solids concentrate.

Table A-4. Influence of milk solids concentration on the heat resistance of *S. Typhimurium* and *S. Alachua*<sup>a</sup>

<i>Salmonella</i> serotype	10 % solids		30% solids		42 % solids		51% solids	
	Temp (°C) <sup>b</sup>	Mean D-value <sup>c</sup> (min)	Temp (°C) <sup>b</sup>	Mean D-value <sup>c</sup> (min)	Temp (°C) <sup>b</sup>	Mean D-value <sup>c</sup> (min)	Temp (°C) <sup>b</sup>	Mean D-value <sup>c</sup> (min)
Typhimurium	57.1	1.4	58.0	2.5	60.8	2.9	65.0	1.7
	55.7	3.2	51.7	11.0	59.6	4.1	62.8	3.8
	52.5	22.5	53.8	59.8	58.8	5.4	62.3	4.5
	51.4	49.0	52.8		58.5	5.9	61.0	6.7
					57.0	9.9	57.0	26.6
					55.1	18.3		
Alachua	59.2	0.5			61.1	3.0	64.0	2.8
	57.8	1.1			59.7	4.3	63.0	4.8
	57.0	1.6			58.7	5.9	60.0	13.5
	55.0	6.2			56.9	12.5	58.0	21.0
	54.1	9.5			55.0	21.6	57.1	33
	53.0	20.4			53.3	41.7	56.7	38.0

<sup>a</sup> Adapted from Dega et al. (1972). Cells were grown in tryptic soy broth at 37°C.

<sup>b</sup> Values ± 0.2°C.

<sup>c</sup> Decimal reduction time; represents an average of 2 to 5 trials at each temperature.

McDonough and Hargrove (1968) observed that a cocktail of *Salmonella* (*S. Senftenberg*, *S. Typhimurium* and *S. New Brunswick*) was extreme resistant to destruction by dry heat in non-fat dried milk powder (Table A-5). Neither 60 °C or 76.6 °C destroyed *Salmonella* cells at the initial population of 10<sup>4</sup> CFU/g after 10 hrs. The moisture level in milk powder significantly influenced the heat resistance of *Salmonella*. For example, 2 hrs was insufficient to kill *Salmonella* in 4% and 7%-moisture powders at 85 °C, although 30 min. was enough at the 25%-moisture level. The degree of heat required for destruction at a high temperature (115.5 °C/1 hr) at 4% moisture was too intense and imparted a yellow burnt appearance to the milk powder. *Salmonella* was not detected in milk powders containing 15% moisture, treated at 148.8 °C for 6 minutes. It was concluded that if the moisture content of milk powder was greater than 15%, it might form larger agglomerates, slowing the rate of heat conductance. Table A-5 shows dry heat resistance of *Salmonella* (cfu/ml) in non-fat dried milk (McDonough and Hargrove, 1968).

Table A-5. Dry heat resistance of *Salmonella* in non dried milk <sup>a</sup>

Exposure time	<i>Salmonella</i> count (cfu) at temperature indicated			
	60 °C	76.6 °C	85 °C	115.5 °C
0	6.9 x 10 <sup>5</sup>	7.3 x 10 <sup>5</sup>	9.4 x 10 <sup>4</sup>	9.4 x 10 <sup>4</sup>
15 min	5.4 x 10 <sup>5</sup>	ND	ND	1.6 x 10 <sup>4</sup>
30 min	4.5 x 10 <sup>5</sup>	1.35 x 10 <sup>5</sup>	7.1 x 10 <sup>3</sup>	8.0 x 10 <sup>2</sup>
45 min	ND	ND	ND	2.0 x 10 <sup>1</sup>
1 hr	4.7 x 10 <sup>5</sup>	4.5 x 10 <sup>4</sup>	8.7 x 10 <sup>2</sup>	<1
2 hr	3.0 x 10 <sup>5</sup>	5.0 x 10 <sup>4</sup>	3.5 x 10 <sup>2</sup>	<1
3 hr	3.8 x 10 <sup>5</sup>	3.0 x 10 <sup>3</sup>	8.0 x 10 <sup>1</sup>	<1
4 hr	ND	2.9 x 10 <sup>3</sup>	5.0 x 10 <sup>1</sup>	<1
5 hr	3.0 x 10 <sup>5</sup>	1.4 x 10 <sup>3</sup>	2	<1
10 hr	4.0 x 10 <sup>3</sup>	3.2 x 10 <sup>2</sup>	0	<1

<sup>a</sup> Adapted from McDonough and Hargrove (1968). A thin layer of conventional (4% moisture) powder was heated in an oven. Negative results from 10 g samples recorded as < 1.

ND: not determined

### Cereal, Flour and Infant Foods

There is little published information related to the heat resistant of *Salmonella* in cereals. VanCauwenberge et al. (1981) investigated the use of dry heat to inactivate a number of *Salmonella* serotypes, including Newington, Typhimurium, Anatum, Kentucky, Cubana, Senftenberg, Thompson, and Tennessee in corn flour at 10 and 15% moisture. The flour was spray inoculated at 10<sup>5</sup> cfu/g and then treated with dry heat at 49 °C (120 °F). After 24 hrs at either 10% or 15% moisture level, 99.9% of the *Salmonella* cells (serotypes Newington, Typhimurium, Anatum and Kentucky) were killed. A product moisture level of 15% was slightly more effective than a 10% moisture level in reducing the cell population from 10<sup>5</sup> to 10<sup>3</sup> cfu/g. *S. Thompson* and *S. Tennessee* were more resistant to heat inactivation than the other serotypes. The investigators suggested that *Salmonella* in contaminated flour could be significantly reduced with a treatment of low temperature for a long period of time (VanCauwenberge et al., 1981).

The initial a<sub>w</sub> before heating is a significant parameter affecting the heat resistance of an inoculated product (Archer et al., 1998). Archer et al. (1998) reported that the D-values for *S. Weltevreden* in flour ranged from D<sub>60-62°C</sub> of 875 min at an initial a<sub>w</sub> of 0.4 to a D<sub>63-65°C</sub> of 29 min at an initial a<sub>w</sub> of 0.5. The results demonstrated the lower the initial water activity of the sample prior to heating, the higher the heat resistance of the cells. Although flour is too dry to support *Salmonella* growth, cells can remain viable for several months. The z-values obtained in flour ranged from 15.2 to 53.9 °C for *S. Weltevreden* in wheat flour and they were considerably higher than those obtained in moist environments (where a typical z-value would be 5.7°C) for *Salmonella* serotypes (Thomas et al., 1966).

## Influencing Factors

The heat resistance of *Salmonella* in low-moisture products is affected by many factors. These include factors prior to heating, (e.g., growth medium composition, growth phase, growth temperature, 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) (Harris, 2008). Heat resistance observed in an aqueous system is not applicable to a low-moisture product. For example, a study by Ng and colleagues (1969) found that *S. Senftenberg 775W* was the most heat resistant among 300 strains evaluated in an aqueous solution (*S. Senftenberg 775W* was 30-fold more resistant than any other strain evaluated), while this strain was found to be less heat resistant than *S. Typhimurium* in chocolate (Goepfert and Biggie, 1968). *S. Enteritidis PT30*, the target organism for raw almonds, was implicated in an outbreak and was found to be more resistant to dry heat than many of the strains evaluated on almonds (ABC, 2007; Wang, 2008).

Given the fact that the heat resistance of *Salmonella* is affected by many factors, it would be much easier to compare differences in heat resistance from experiments within the same study than to compare 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.

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