Short communication: Decimal log reductions of *Salmonella* Senftenberg 775 W and other *Salmonella* serovars in nonfat milk and powder

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ABSTRACT

This study aimed to compare the thermal resistance of *Salmonella* Senftenberg 775 W with other serovars of *Salmonella* in nonfat dry milk (NDM) and hydrated NDM. The scientific literature suggests that *Salmonella* Senftenberg 775 W is the most heat-resistant serovar in high-water-activity foods such as milk, but little is known about the heat resistance of *Salmonella* Senftenberg 775 W compared with other *Salmonella* serovars in low-water-activity foods such as NDM. The 5 serovars of *Salmonella* used in this study were Enteritidis, Montevideo, Newport, Senftenberg, and Typhimurium. The hydration of NDM was conducted at 13% (wt/vol) total solids. The NDM was inoculated with the 5 individual serovars of *Salmonella* and dried again to its original pre-inoculation water activity. Hydrated NDM was prepared from individually inoculated NDM. The surviving *Salmonella* population at predetermined time-temperature intervals were enumerated using injury-recovery medium, and the average log reductions for the individual serovars were calculated. As expected, *Salmonella* Senftenberg 775 W was the most heat-resistant serovar in hydrated NDM at 59°C and 65°C. However, the heat resistance of *Salmonella* Senftenberg 775 W was found to be lower than or comparable to that of other serovars in low-water-activity NDM at 80°C and 90°C.

Key words: *Salmonella* Senftenberg 775 W, thermal resistance, log reduction

Short Communication

Salmonella can persist in hostile and dry environments of foods with low water activity (a_w), such as NDM, and multiply when NDM is hydrated (Enache et al., 2017; Crucello et al., 2019). Globally, *Salmonella* is one of the primary causes of bacterial foodborne ill-

nesses, causing 550 million illnesses each year, including 220 million cases in children under the age of 5 (Podolak et al., 2017; WHO, 2018). In the United States, *Salmonella* is responsible for causing 1.35 million illnesses, 26,500 hospitalizations, and 420 deaths each year (CDC, 2020). According to the Foodborne Disease Outbreak Surveillance System, from 2009 to 2015, dairy foods were implicated in 136 (11%) foodborne illness outbreaks in the United States (Dewey-Mattia et al., 2018). Foodborne diseases result in massive losses to the US economy, due to medical costs and product recalls. The infectious dose for *Salmonella* infection varies from 10 to 1,000 cells but can be as low as 1 cell, depending upon the age and health of an individual (Pal et al., 2016). Therefore, the inactivation of foodborne pathogens such as *Salmonella* is a critical processing step in the preparation of safe foods.

Foods such as chocolate, powdered milk, peanut butter, dried infant foods, and cereals are low-moisture foods. Although low-moisture foods do not support the growth of most foodborne pathogens, due to their low a_w , they still may be implicated in various foodborne illness outbreaks (Podolak and Black, 2017). For example, the outbreak of *Salmonella* Typhimurium attributed to peanut butter in 2008–2009 in the United States resulted in 714 illnesses in 46 states (Cavallaro et al., 2011). In 1993, *Salmonella* Tennessee caused 48 cases of infection, resulting in the recall of spray-dried milk powder (CDC, 1993). In 2008, *Salmonella* Give was involved in an outbreak of salmonellosis in France, forcing the recall of dry infant milk powder (Jourdan et al., 2008). Nonfat dry milk is widely used as an ingredient in dairy, confectionery, bakery, meat, and chocolate industries (Pal et al., 2016). Pasteurization of milk inactivates *Salmonella*, but *Salmonella* can survive spray-drying and other processing steps if the contamination of milk occurs post-pasteurization (Pal et al., 2016).

Heat resistance of *Salmonella* depends on the type of serovar, physical and chemical composition of the food matrix, and storage conditions (Podolak et al., 2017). Fat has a protective effect, due to its low heat conductivity and low a_w , which increases the thermal resistance of *Salmonella* (Murphy et al., 2003). Although *Salmo-*

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nella Senftenberg 775 W is not the most significant foodborne pathogen of concern, it is often used as a test organism in the thermal inactivation studies (Doyle and Mazzotta, 2000). It is assumed that if a particular thermal process destroys *Salmonella* Senftenberg 775 W in a specific high- a_w food, that process will also be sufficient for controlling most of the salmonellae in that food (Doyle and Mazzotta, 2000). However, little has been studied about the thermal resistance of *Salmonella* Senftenberg 775 W in low- a_w foods; therefore, the aim of this study was to compare the decimal reductions of *Salmonella* Senftenberg 775 W with other *Salmonella* serovars in NDM in dry or hydrated states separately at specific temperatures.

The serovars of *Salmonella enterica* used in this study were Enteritidis (BAA-708), Montevideo (BAA-710), Newport (6962), Senftenberg 775W (43845), and Typhimurium (14028). All cultures were obtained from the American Type Culture Collection (ATCC, Manassas, VA). These serovars were selected on the basis of their involvement in outbreak and illness cases in the United States in last 3 years. The cultures were propagated according to the manufacturer's instructions (ATCC, 2015) and then individually transferred onto glycerol protectant cryogenic beads (Microbank, Richmond Hill, ON, Canada) and stored at -80°C . At the start of the research, a frozen bead of each culture was grown individually at 37°C for 24 h in 10 mL of brain heart infusion (BHI) broth (Difco/Becton, Dickinson and Co., Sparks, MD). For each replication, the individual cultures were streaked onto BHI agar (Difco/Becton, Dickinson and Co.) plates using cotton swabs (Puritan, Guilford, ME) to grow bacterial lawns (5 plates per culture) and incubated at 37°C for 24 h. Then, 2 mL of 0.1% peptone (Difco/Becton, Dickinson and Co.) was added to each plate, and the bacterial cells were loosened using sterile L-shaped plastic spreaders. Approximately 10 mL of cell solution for each serovar was collected in 15-mL sterile plastic tubes and used as master inocula for inoculating low-heat NDM (Darigold Inc., Seattle, WA). To determine the *Salmonella* populations in various master inocula, individual inocula were serially diluted using 9 mL of 0.1% peptone and then spread plated onto BHI agar. Plates were then incubated at 37°C for 24 h.

For each *Salmonella* serovar, NDM (~ 200 g) was weighed in a sanitized sealable plastic tub ($\sim 38 \times 26 \times 7$ cm; Rubbermaid Inc., Huntersville, NC), and evenly dispersed to form a uniform layer. The tubes containing master inocula were attached with spray nozzles, which were calibrated to spray ~ 1 mL of master inoculum per squirt. The NDM tubs were inoculated by mist spraying ~ 4 mL of individual master inocula of each *Sal-*

monella serovar. Subsequently, treated NDM was dried back to its original a_w weight by incubating with open lids at 37°C for ~ 4 h. The a_w of samples was measured at 25°C using a calibrated a_w meter (Neu-tec Group Inc., Farmingdale, NY). All dried, inoculated NDM was thoroughly mixed using a sanitized spatula. To stabilize *Salmonella* population, the inoculated NDM for each serovar was sealed with airtight lids and stored at room temperature ($\sim 25^{\circ}\text{C}$) for 24 h.

The hydration of inoculated NDM was performed at 13% (wt/vol) total solids to represent the regular skim milk composition. The milk was prepared just before the thermal treatment to have a stable bacterial population. Inoculated NDM (~ 5 g) or hydrated NDM (~ 5 mL) for individual *Salmonella* serovars were transferred into 5 custom-designed aluminum thermal-death-time (TDT) disks (Engineering Shop, Washington State University, Pullman, WA; Figure 1), sealed, and placed in a wire mesh plastic crate ($15.6 \times 20 \times 22.9$ cm; Sterilite, Townsend, MA) to ensure uniform heating and complete submersion in preheated water baths. The TDT disks had an internal height and diameter of 5 and 50 mm, respectively. One of these 5 TDT disks was connected to a thermocouple and a data logger. Digital thermometers (Fluke 51-2 Thermometer, Everett, WA), connected to K- and T-type thermocouples were used to monitor the temperature of the water and product inside the TDT disk, respectively. Thermal treatments were conducted at 59°C for 15 min and 65°C for 2 min for hydrated NDM, and 80°C for 60 min and 90°C for 14 min for NDM. These temperature-time combinations were selected based on preliminary work to ensure at least 3-log reductions for each *Salmonella* serovar population. The treatment times were started

Figure 1. Thermal-death-time (TDT) disks containing inoculated NDM (left) and inoculated hydrated NDM (right).

when sample temperatures inside the disks attained the target temperatures. At the end of each treatment, samples were quickly transferred into an ice-water bath to arrest any further *Salmonella* kill due to high temperatures.

The surviving populations of individual *Salmonella* serovars at particular time-temperature combinations were calculated as the log of colony-forming units per gram (log cfu/g). The log reduction for the individual serovars at a specific temperature was determined by subtracting the log of surviving bacteria population from the log initial population. The *Salmonella* population was enumerated following the method described by Michael et al. (2020) using an injury-recovery medium (i.e., BHI agar overlaid with xylose lysine deoxycholate, **XLD**; Difco/Becton, Dickinson and Co.). At each sampling point, the samples were added to 10 mL of 0.1% peptone in stomacher bags ($\sim 6.5 \times 10.5$ cm, Nasco Whirl-Pak, Fort Atkinson, WI). All samples were stomached for 1 min (using Seward Stomacher 80 Lab System, London, UK), thereupon serially diluted using the 0.1% peptone water solution and spread plated in duplicates on BHI agar plates. All plates were incubated at 37°C for 4 h and subsequently overlaid with the selective XLD agar. The overlaid plates were further incubated for 20 h at 37°C (Michael et al., 2020).

This study was designed as completely randomized with 3 replications. The average log reductions of indi-

vidual serovars at specific temperatures within dry or hydrated NDM were compared using one-way ANOVA and Tukey's test (SAS University Edition, SAS Institute Inc., Cary, NC) to determine differences at $\alpha = 0.05$.

The *Salmonella* populations in inoculated NDM for serovars Enteritidis, Montevideo, Newport, Senftenberg and Typhimurium were 7.6 ± 0.12 , 7.9 ± 0.20 , 7.3 ± 0.21 , 7.8 ± 0.18 , and 7.3 ± 0.23 log cfu/g, respectively; whereas, in hydrated NDM, the *Salmonella* populations were 8.2 ± 0.13 , 8.3 ± 0.23 , 8.4 ± 0.23 , 8.8 ± 0.18 , and 8.4 ± 0.18 log cfu/mL, respectively. The a_w of hydrated NDM was 0.991 ± 0.001 , whereas a_w of NDM was 0.199 ± 0.002 . As expected, *Salmonella* Senftenberg 775 W was statistically ($P < 0.001$ at 59 and 65°C) the most heat-resistant serovar in hydrated NDM, with the lowest log reductions of 3.7 ± 0.32 and 4.3 ± 0.12 log cfu/mL at 59°C and 65°C, respectively (Figure 2). At 59°C, in hydrated NDM, the other 4 *Salmonella* serovars had similar log reductions (varying from 6.8 to 7.2 log cfu/mL), which were significantly greater than that of *Salmonella* Senftenberg 775 W (Figure 2). Log reductions in hydrated NDM at 65°C heat treatment for *Salmonella* Montevideo, Newport, Typhimurium and Enteritidis were 7.6 ± 0.20 , 7.0 ± 0.21 , 6.3 ± 0.65 , and 5.9 ± 0.25 log cfu/mL, respectively (Figure 2). Ng et al. (1969) observed *Salmonella* Senftenberg 775W to be 30 times more heat resistant than *Salmonella* Ty-

Figure 2. Log reductions in 5 *Salmonella* serovar populations in hydrated NDM when heat-treated at 59°C and 65°C for 15 and 2 min, respectively. Within specific temperature, 59°C (a, b) or 65°C (A–C), bars (mean $\pm$ SE) with different letters are different ($P \leq 0.05$).

phimurium Tm-1 when cultured in trypticase soy broth under heat shock and limited carbohydrate levels.

However, the results were quite different in low- a_w NDM. The log reductions of *Salmonella* Senftenberg 775 W (4.4 ± 0.06 log cfu/g) in NDM at 80°C heat treatment were statistically similar to that of the other 4 *Salmonella* serovars (Figure 3). *Salmonella* Montevideo, Typhimurium, and Enteritidis (3.6 ± 0.32 , 3.7 ± 0.35 , and 4.6 ± 0.10 log cfu/g, respectively) were statistically the most heat-resistant serovars in NDM at 90°C heat treatment. Log reductions of *Salmonella* Senftenberg 775 W (4.8 ± 0.26 log cfu/g) in NDM at 90°C were similar to those of *Salmonella* Enteritidis and Typhimurium. *Salmonella* Newport was the least heat-resistant serovar in NDM at 90°C with 6.5 ± 0.26 log cfu/g log reduction (Figure 3). These results demonstrate that even though *Salmonella* Senftenberg 775 W was the most heat-resistant serovar in high- a_w hydrated NDM at 2 temperatures studied, the heat resistance of *Salmonella* Senftenberg 775 W in low- a_w NDM at 2 temperatures was either comparable to or lower than that of the other 4 *Salmonella* serovars. Therefore, although *Salmonella* Senftenberg 775 W could be included in thermal inactivation studies for milk powders, it should not be relied on as the most heat-resistant *Salmonella* serovar. Using a cocktail of various *Salmonella* serovars in thermal processing validation studies for milk powder would be a good option. Moreover,

The National Advisory Committee on Microbiological Criteria for Foods also recommends using a cocktail for conducting heat inactivation studies (NACMCF, 2010).

Salmonella Senftenberg 775 W is known to be exceptionally heat resistant in high-moisture foods, and this may lead to a wrong perception that it is the most heat-resistant *Salmonella* serovar in all food types (Podolak et al., 2017). For instance, a study uncovered that the D-value (decimal reduction time) of *Salmonella* Typhimurium (75 min) at 90°C in chocolate milk was higher than that of *Salmonella* Senftenberg 775 W, which had a D-value of 36 min (Goepfert and Biggie, 1968). A study that compared the heat resistance of 8 *Salmonella* serovars (Alachua, Anatum, Anatum GF, Infantis, Montevideo, Senftenberg 775 W, Tennessee, and Typhimurium) in sucrose solutions at 57.2°C found *Salmonella* Senftenberg 775 W was not the most heat-resistant serovar as the a_w was reduced, and sucrose concentration was increased (Goepfert et al., 1970). Another study conducted by Sherry et al. (2004) compared inherent resistance of 40 *Salmonella enterica* serovars (including *Salmonella* Senftenberg 775 W) in a stationary phase (24 h) culture on the basis of injury by heat, pressure, and irradiation stress. In that study, *Salmonella* Senftenberg was found to be the most sensitive serovar, with D-value of 2.83 min as compared with 3.5 min for *Salmonella* Alachua at 450 MPa. Riemann (1968) compared the heat resistance (in terms of 10-

Figure 3. Log reductions in 5 *Salmonella* serovar populations in NDM when heat-treated at 80°C and 90°C for 60 and 14 min, respectively. Within specific temperature, 80°C (a, b) or 90°C (A–C), bars (mean $\pm$ SE) with different letters are different ($P \leq 0.05$).

fold reductions) of *Salmonella* Senftenberg 775 W and *Salmonella* Typhimurium in meat and bone meal with adjusted a_w in the range of 0.6 to 1. Riemann (1968) reported that *Salmonella* Typhimurium with 10-fold reduction at 75°C/40 min heating (a_w 0.88) was more heat tolerant than *Salmonella* Senftenberg 775 W.

In high- a_w foods, *Salmonella* Senftenberg 775 W has clearly shown the highest heat resistance. Humphrey et al. (1990) noted that *Salmonella* Senftenberg 775 W was the most heat-resistant serovar in homogenized whole egg and egg yolk at 55 and 60°C. Humphrey et al. (1990) also revealed that *Salmonella* Senftenberg 775 W was 5 times more heat resistant than *Salmonella* Enteritidis in the homogenized whole egg at 55°C. Read et al. (1968) demonstrated that *Salmonella* Senftenberg 775 W was the most heat-resistant serovar in milk compared with other salmonellae (Anatum, Binza, Cubana, Meleagridis, Newbrunswick and Tennessee).

Therefore, these results confirm that the overall heat resistance of the serovars are not solely dependent on the serovars themselves but also on the surrounding environments. *Salmonella* Senftenberg has better survivability in high-moisture foods; however, because of its weaker adaptability to the low- a_w environment, *Salmonella* Senftenberg had greater heat resistance in high- a_w hydrated NDM; however, due to its weaker adaptability in low- a_w environment, the heat resistance of *Salmonella* Senftenberg was either lower than or comparable to that of the other 4 serovars studied. To avoid the chance of selecting an incorrect target *Salmonella* serovar for thermal inactivation studies of low- a_w milk powders, *Salmonella* Senftenberg 775 W should be used within a cocktail, not as a single serovar. Moreover, recommendations from the National Advisory Committee on Microbiological Criteria for Foods must be reviewed before planning thermal validation studies. However, further research should be conducted to confirm the heat resistance of *Salmonella* serovars in other low-moisture dairy foods.

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