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Brigham Young University

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Isothermal Inactivation of *Salmonella, Listeria monocytogenes, and Enterococcus faecium* 
NRRL B-2354 in Peanut Butter, Powder Infant Formula, and Wheat Flour

Adam Robert Quinn

A thesis submitted to the faculty of 
Brigham Young University 
in partial fulfillment of the requirements for the degree of 
Master of Science

Bradley J. Taylor, Chair  
Laura K. Jefferies  
Frost M. Steele

Department of Nutrition, Dietetics, and Food Science  
Brigham Young University

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ABSTRACT

Isothermal Inactivation of *Salmonella*, *Listeria monocytogenes*, and *Enterococcus faecium* NRRL B-2354 in Peanut Butter, Powder Infant Formula, and Wheat Flour

Adam Robert Quinn
Department of Nutrition, Dietetics, and Food Science, BYU
Master of Science

Pathogens in low-moisture foods are an emerging food safety concern due to increased survival and thermotolerance in matrices with low water activity. However, limited data is publicly available for the thermotolerance of *Listeria monocytogenes*, *Salmonella* spp., and *Enterococcus faecium* NRRL B-2354 (a *Salmonella* surrogate). The aims of this study were to identify differences in thermal inactivation rates between these organisms in three different low-moisture foods. Three model low-moisture foods (peanut butter, powder infant formula, and wheat flour) were inoculated with either *E. faecium*, a *Salmonella* spp. cocktail, or a *L. monocytogenes* cocktail using a dry inoculation method for a total of 9 treatments. Samples were heat treated in a hot water bath at predetermined temperatures, and bacterial survival was detected via direct plating on tryptic soy agar with 0.6% yeast extract. In peanut butter and most of the powder infant formula treatments, *Salmonella* spp. had significantly higher D-values than *L. monocytogenes* using comparable temperatures (p < 0.05). However, D-values between *Salmonella* spp. and *L. monocytogenes* were comparable in wheat flour and one of the treatment temperatures in powder infant formula (p > 0.05). For all but one of the treatments at the same temperature, *E. faecium* had significantly higher D-values than *L. monocytogenes* and *Salmonella* spp. in each food matrix (p < 0.05). The observed matrix effect on thermotolerance for each of the bacteria was reported in descending order as powder infant formula > peanut butter > wheat flour in the majority of the comparable D-values. While *Salmonella* continues to be the pathogen of concern in low-moisture foods due to survival and outbreaks, these results indicate *L. monocytogenes* can exhibit similar thermotolerances in relevant model low-moisture foods matrices.

Keywords: food safety, low-moisture foods, water activity, thermal resistance, *Listeria monocytogenes*, *Salmonella*, *Enterococcus faecium* NRRL B-2354
ACKNOWLEDGEMENTS

My graduate chair, Dr. Brad Taylor, deserves the first acknowledgement in this work. He has been a great mentor and close friend throughout my program; I couldn’t ask for anyone better. Additional thanks to the other members of my committee, Dr. Laura Jefferies and Dr. Frost Steele, for their support of the project and more impactfully their belief in my capabilities and potential. Thank you to Kate Hartmann Gentry, my fellow graduate student, for her support, training, and encouragement in the lab. Additional thanks to Ruo Fen Liao and Thomas Smith for their many hours of work helping collect data. Finally, thank you to my wonderful wife, Kat, who has always believed in me and encouraged me to do difficult things.
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1. Introduction

*Listeria monocytogenes* is a significant, foodborne pathogen causing invasive listeriosis (septicemia, meningitis, and spontaneous abortion), especially among immuno-compromised individuals, pregnant women and their fetuses, newborn infants, and the elderly (> 65 years old) (Doyle, Meske, & Marth, 1985; ILSI Research Foundation, 2005). *L. monocytogenes* is ubiquitous in the environment and has unique characteristics including the ability to grow at refrigeration temperatures. While no outbreaks of *L. monocytogenes* in low-moisture foods have happened yet, recalls continue to occur throughout the United States (Maberry, 2017). Additionally, thermotolerance research has not been published or validated for a variety of low-moisture foods, including those responsible for recent recalls in the USA (Taylor, Quinn, & Kataoka, 2019).

The Food Safety Modernization Act (FSMA) requires the US food industry to rely more heavily on preventive controls to guarantee safe food; previous patterns of reacting to food safety hazards in finished goods or ingredients already out on the market are no longer an acceptable option. The risks of commercial foods and processes must be anticipated and managed via a comprehensive and specific food safety plan. High-moisture foods have water and nutrients readily available for the growth of microorganisms and are considered high risk. Low-moisture foods, on the other hand, cannot support most microbial growth and have therefore been traditionally seen as low-risk products. Though definitions of low-moisture foods vary, the US Food and Drug Administration (FDA) and Codex Committee on Food Hygiene define low-moisture foods as foods with water activity (*a*<sub>W</sub>) less than 0.85 (FDA, 2015; CCF, 2015).

Low-moisture foods are sources of pathogens in the food system, albeit typically in low numbers (CDC, 2007; FDA, 2018a; FDA, 2018b; Koch et al., 2005; Maberry, 2016; Maberry,
2017; Maberry, 2018; Rachon, Peñaloza, & Gibbs, 2016). In general, bacteria, including some foodborne pathogens, are more thermotolerant in low-moisture foods than in high moisture foods (Jin et al., 2018). These low $a_w$ food environments also allow for the persistence of bacteria in a dormant state for a longer period of time (Koseki, Nakamura, & Shiina, 2015; Taylor, Tsai, Rasco, Tang, & Zhu, 2018). From a quality perspective, low-moisture foods such as peanut butter are generally shelf-stable and therefore remain in the global supply chain and consumer pantry much longer than temperature sensitive foods. This means that a post-production contamination of a low-moisture food may be present throughout the product’s long shelf-life. This problem was illustrated with respect to salmonellosis when confirmed cases of *Salmonella* infections were reported from low-moisture food sources stored at ambient temperature with a shelf-life longer than 12 months (Kimber, Kaur, Wang, Danyluk, & Harris, 2012; Podolak, Enache, Stone, Black, & Elliott, 2010).

The ability of microorganisms of concern to survive in matrices that do not support growth is important to all partners (e.g., producer, distributor, regulator, and consumer). Data on pathogens and validated surrogates in low-moisture foods is needed for the enhancement of food safety practices globally and the fulfillment of newly enforced regulatory demands. This study reports on thermal inactivation rates of pathogens and a surrogate in three low-moisture foods.

2. Materials and methods

2.1 Experimental design

In this study, three model low-moisture food matrices were inoculated with pathogen cocktails (containing exclusively *L. monocytogenes* or *Salmonella spp.*) or with a single strain inoculum of *Enterococcus faecium* strain (NRRL B-2354) to test thermotolerance within a total
of 9 different experimental conditions. Three isothermal treatment temperatures were selected for each of the 9 bacteria/matrix treatments based on preliminary data and laboratory practicalities. A tight range of temperatures (60 to 90°C) was used to maximize the number of well-controlled $D$-value comparisons. For each of the 9 treatments, $D$-values collected at 3 temperatures were used to estimate $z$-values. Dry inoculums were prepared adopting the methods described by Enache et al. (2015), Liu, Xu, Xie, Zhu, & Tang (2019) and those applied in the infant formula industry (described in section 2.4). In addition, the $D$-values of organisms in dry inoculum matrix alone was determined for comparison.

2.2 Test microorganisms

Six *L. monocytogenes* strains and six *Salmonella spp.* strains were used as the test pathogens in this study. Stain descriptions are organized in Table 1. A single *Enterococcus faecium* strain (NRRL B-2354) was also included in this study as a possible surrogate. The stock cultures were stored at -80°C in tryptic soy broth supplemented with 20% (vol/vol) glycerol.

Table 1. Strain information

<table>
<thead>
<tr>
<th>Bacterium</th>
<th>Serotype/strain</th>
<th>Source</th>
<th>Isolate no.</th>
<th>Description</th>
<th>Lab ID</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Listeria monocytogenes</em></td>
<td>1/2b</td>
<td>Utah State University</td>
<td>FSL J1-177</td>
<td>Human isolate</td>
<td>LM 1</td>
</tr>
<tr>
<td></td>
<td>1/2a</td>
<td>Utah State University</td>
<td>FSL C1-056</td>
<td>Human isolate</td>
<td>LM 2</td>
</tr>
<tr>
<td></td>
<td>4b</td>
<td>Utah State University</td>
<td>FSL N3-013</td>
<td>Food isolate</td>
<td>LM 3</td>
</tr>
<tr>
<td></td>
<td>1/2a</td>
<td>Utah State University</td>
<td>FSL R2-499</td>
<td>Human isolate</td>
<td>LM 4</td>
</tr>
<tr>
<td></td>
<td>4b</td>
<td>Utah State University</td>
<td>FLS N1-227</td>
<td>Food isolate</td>
<td>LM 5</td>
</tr>
<tr>
<td></td>
<td>4b / Scott A</td>
<td></td>
<td>ATCC® 49594™</td>
<td></td>
<td>LM 6</td>
</tr>
<tr>
<td><em>Salmonella spp.</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Montevideo</td>
<td></td>
<td>University of Georgia</td>
<td></td>
<td>Food isolate</td>
<td>Sal M</td>
</tr>
<tr>
<td>Agona</td>
<td></td>
<td>University of Georgia</td>
<td></td>
<td>Food isolate</td>
<td>Sal A</td>
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<tr>
<td>Tennessee</td>
<td></td>
<td>University of Georgia</td>
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<td>Food isolate</td>
<td>Sal Tn</td>
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<tr>
<td>Weltevreden</td>
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<td>ATCC® BAA-2568™</td>
<td></td>
<td></td>
<td>Sal W</td>
</tr>
<tr>
<td>Senftenberg</td>
<td></td>
<td>ATCC® H385™</td>
<td></td>
<td></td>
<td>Sal S</td>
</tr>
<tr>
<td>Typhimurium</td>
<td></td>
<td>New Zealand (ESR)</td>
<td></td>
<td>Food isolate</td>
<td>Sal Ty</td>
</tr>
<tr>
<td>PT 42</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Enterococcus faecium</em></td>
<td>NRRL B-2354</td>
<td>Agricultural Research Service (USDA-ARS)</td>
<td></td>
<td></td>
<td>EF</td>
</tr>
</tbody>
</table>
2.3 Preliminary heat stress comparison

Prior to the development of final cocktails for inoculum preparation, initial screening of strains for fitness was performed using dry air to ascertain relative thermotolerances. All pathogenic strains were individually inoculated on filter paper and placed in a forced air oven held at 100°C for 10 min. An isolated colony from each strain was picked and transferred to 10 mL of tryptic soy broth supplemented with 0.6% yeast extract (TSBYE). After aerobic incubation at 37 ± 2°C for 24 h, an aliquot of 100 µL was spread onto tryptic soy agar supplemented with 0.6% yeast extract (TSAYE) and incubated at 37 ± 2°C for 48 h to form a lawn. The lawns were harvested after adding 3 mL of 0.1% sterile peptone water (PW) to each plate and loosening the lawn with a sterile spreader. Each plate produced 2 mL of inoculum of which 100 µL aliquots were added to filter papers and left to dry for 24 h. Inoculated filter papers were placed in a forced air oven at 100°C, and samples were withdrawn at 5 and 10 min. Surviving cells were resuspended in TSBYE and enumerated on TSAYE.

2.4 Dry inoculation preparation

Bacterial slurries for each strain were prepared using the same protocol used in the preliminary heat stress work. For *L. monocytogenes* and *Salmonella spp.*, every inoculum created from each strain was individually enumerated on TSAYE to confirm similar growth. Slurries from each bacterial species were combined to form the final cocktail. Cell populations in the three slurries of *L. monocytogenes*, *Salmonella spp.*, and *E. faecium* were enumerated on TSAYE to be 10.5, 10.5, and 9.8 log CFU/mL respectively.

Each of the final inoculums were created by adding the bacterial slurry to sterile hydrous magnesium silicate (talc) to achieve a target inoculation level of ~8.5 log CFU/g. Using good laboratory practices to limit inhalation, talc was autoclaved for 15 min in a disposable container.
The absence of contamination was verified through plating via serial dilution in 0.1% sterile peptone water (PW) onto TSAYE. Next, 18 mL of the bacterial slurry was added to sterile bags containing 25 g of talc. Bags were then hand massaged for approximately 5 min until a homogenous paste was formed. Open bags were dried for 48 h at 21°C in a biosafety cabinet, at which time the inoculated talc had returned to its original $a_w$ (~0.15). The dried inoculated talc was then aseptically placed back into a new sterile bag and pulverized into a powder. The final powder was placed in a sterile container and kept at 3°C for the duration of the study.

2.5 Sample inoculation

Three foods used in this study were all-purpose, white wheat flour (Deseret Mill, Kaysville UT, USA), peanut butter (Houston Cannery, Houston, TX, USA), and powder infant formula (Abbott Laboratories, Abbott Park, IL, USA). The lipid composition estimates and $a_w$ for each low-moisture food matrix are shown in Table 2. Analysis for $a_w$ was performed using the AquaLab TDL Water Activity Meter (METER Group, Inc. Pullman, WA, USA). To prepare low-moisture food samples per the 9 experimental conditions, inoculated talc was added at a ratio of 1% (wt./wt.) and either mixed in using a sterile spatula (for paste) or bag blending techniques (for powders). The samples were then left to incubate overnight (~20 h) at 21°C. Samples were placed into sterile bags at 500 mg per bag, sealed, and flattened to a thickness of ~1 mm.

<table>
<thead>
<tr>
<th>Matrix</th>
<th>Water Activity</th>
<th>% Lipids (wt/wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat Flour</td>
<td>0.45</td>
<td>&lt; 2%</td>
</tr>
<tr>
<td>Powder Infant Formula</td>
<td>0.20</td>
<td>27%</td>
</tr>
<tr>
<td>Peanut Butter</td>
<td>0.11</td>
<td>45%</td>
</tr>
</tbody>
</table>

Table 2. Characteristics of selected low-moisture foods
2.6 Isothermal treatment and enumeration

For each replication, two sample bags were placed in between two magnetic copper plates with dimensions of 20 cm by 5 cm (Copper Weld Backer; Eastwood Co., Pottstown, PA, USA). Six sets of copper plates with duplicate samples were placed in a water bath (VWR International, Radnor, PA, USA) vertically in a rack. The copper plates were used because of their high thermal conductivity and because the strength of the magnets was sufficient to hold the samples in place. Copper plate sets containing duplicate samples were removed at regular intervals and immediately cooled in cold water (~5 sec). The sample bags were aseptically opened, and 4.5 mL of PW was added to create a 1:10 dilution. Two unheated samples were included to calculate the initial level of inoculum. Samples were hand shaken and massaged until they were completely suspended. Serial dilution in PW on TSAYE was used, and colonies were counted after incubation at 37°C for 24 to 48 hours.

2.7 Statistical analysis

All experiments were done in triplicate. Linear regression lines of log survivors versus time were created, and the negative inverse of the slopes became the \( D \)-values at each temperature. In a similar way, \( z \)-values were determined using the linear regression lines of log \( D \)-values against temperature for each treatment (Microsoft Excel Office 365 software; Microsoft Corp, Redmond, WA, USA). The \( D \)-values were statistically compared using the Student’s \( t \) test with a pseudo-Bonferroni adjustment to determine if there were any significant differences (\( p < 0.05 \)) between comparable treatments (JMP Pro 14; SAS Institute Inc., Cary, NC, USA).
3. Results

3.1 Preliminary heat stress comparison and inoculum levels

After independently comparing $D_{100\degree C}$-values between the 6 strains of *L. monocytogenes* (Fig. 1 A), no strains were identified as statistically different from the group (ANOVA, p > 0.05). This was also true for the comparison of the 6 strains of *Salmonella* spp. (Fig. 1 B). Subsequently, all strains assessed in the preliminary study were used to develop the pathogen-specific inoculums. After the sterile talc was inoculated with the cocktails and dried, final population levels for *L. monocytogenes*, *Salmonella* spp., and *E. faecium* NRRL B-2354 inoculums were 9.1, 7.7, and 9.6 log CFU/g, respectively.

![Fig. 1. Comparison of strain and serovar $D_{100\degree C}$-values and standard deviations for A) L. monocytogenes and B) Salmonella spp.](image)

3.2 Matrix effect and z-values

Calculated z-values for the nine treatments are reported in Table 3. Thermotolerance measurements, shown as $D$-values, from the primary study and the corresponding statistically derived groupings are found in Table 4. The low-moisture food matrix had a significant effect on the bacterial thermotolerance in nearly all tested permutations (p < 0.05). Of interest and the exception, *Salmonella* spp. $D_{85\degree C}$-values between peanut butter and powder infant formula were not significantly different (p > 0.05). The following matrix effect on thermotolerance was
observed for the organisms tested: powder infant formula > peanut butter > wheat flour
(temperature range 60-90°C).

Table 3. Z-values (°C)

<table>
<thead>
<tr>
<th>Matrix</th>
<th>L. monocytogenes</th>
<th>Salmonella</th>
<th>E. faecium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat Flour</td>
<td>11.8</td>
<td>17.2</td>
<td>13.1</td>
</tr>
<tr>
<td>Peanut Butter</td>
<td>24.2</td>
<td>22.1</td>
<td>17.6</td>
</tr>
<tr>
<td>Powder Infant Formula</td>
<td>20.7</td>
<td>19.4</td>
<td>29.9</td>
</tr>
</tbody>
</table>

Table 4. D-values (min) for each of the 9 treatments at 3 various temperatures

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Food</th>
<th>L. monocytogenes</th>
<th>Salmonella</th>
<th>E. faecium</th>
</tr>
</thead>
<tbody>
<tr>
<td>60</td>
<td>Wheat Flour</td>
<td>53.0 ± 2.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>65</td>
<td>Wheat Flour</td>
<td>A 17.8 ± 0.70</td>
<td>A 18.1 ± 1.08</td>
<td></td>
</tr>
<tr>
<td>70</td>
<td>Wheat Flour</td>
<td>A 7.6 ± 1.66 a</td>
<td>A 8.3 ± 0.39</td>
<td>B 21.7 ± 1.12</td>
</tr>
<tr>
<td></td>
<td>Peanut Butter</td>
<td>15.3 ± 0.54 b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>75</td>
<td>Wheat Flour</td>
<td>A 9.0 ± 0.26 a</td>
<td>A 4.7 ± 0.36 a</td>
<td>B 10.3 ± 0.45 a</td>
</tr>
<tr>
<td></td>
<td>Peanut Butter</td>
<td>17.9 ± 3.00 b</td>
<td>B 14.6 ± 2.56 b</td>
<td>C 28.1 ± 1.70 b</td>
</tr>
<tr>
<td></td>
<td>Powder Infant Formula</td>
<td>10.0 ± 1.32 b</td>
<td>A 5.4 ± 0.70 a</td>
<td>B 12.9 ± 0.60 b</td>
</tr>
<tr>
<td>80</td>
<td>Wheat Flour</td>
<td>3.7 ± 0.13 a</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Peanut Butter</td>
<td>A 5.9 ± 0.70 a</td>
<td>B 8.9 ± 0.36 a</td>
<td>C 11.9 ± 0.29 b</td>
</tr>
<tr>
<td></td>
<td>Powder Infant Formula</td>
<td>10.0 ± 1.32 b</td>
<td>A 5.1 ± 0.07 a</td>
<td>A 7.4 ± 1.77 a</td>
</tr>
<tr>
<td>85</td>
<td>Peanut Butter</td>
<td>5.9 ± 0.63 a</td>
<td>A 6.2 ± 0.46 a</td>
<td>B 14.8 ± 0.35 b</td>
</tr>
<tr>
<td></td>
<td>Powder Infant Formula</td>
<td>11.6 ± 0.91</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Within each group (where available) of same temperature, same matrix, and two or three bacteria, means with different capital letters are significantly different (p < 0.05). At each temperature, means of the same bacteria between two or three matrices (where available) with different lowercase letters are significantly different (p < 0.05)

3.3 Thermotolerance of organisms

Thermotolerances for each organism grouped by low-moisture food matrix are shown in Fig. 2 A-C. The data supports E. faecium NRRL B-2354 as a surrogate for Salmonella spp. and L. monocytogenes since in nearly every permutation E. faecium had a statistically, significantly higher D-value (p < 0.05). In peanut butter, however, D$_{85°C}$-values of E. faecium and Salmonella spp. were not significantly different (p > 0.05). Salmonella spp. generally had higher D-values than L. monocytogenes, but there were several conditions where the analysis yielded no
statistical difference. The two comparable wheat flour $D$-values ($D_{65^\circ C}$ and $D_{70^\circ C}$) and powder infant formula $D_{85^\circ C}$-values were not significantly different for these organisms.

Fig. 2. Thermotolerances of each organism in log $D$-value plotted against temperature in A) powder infant formula, B) peanut butter, and C) wheat flour.
4. Discussion

4.1 Effect of low-moisture food composition

The impact of $a_w$ on bacterial thermotolerance is well established. Simply stated, as $a_w$ decreases, thermotolerance and survival in low-moisture food matrices correspondingly increases (Syamaladevi et al., 2016). It has also been suggested that lipid content in food has a protective effect on bacteria during thermal treatments (Podolak & Black, 2017). This study supports those principles in confirming wheat flour, with the highest $a_w$ and lowest fat content, had the shortest $D$-value times. However, while peanut butter had the lowest $a_w$ and the highest fat content, it resulted in $D$-values that were significantly shorter than those from powder infant formula in every comparison except for Salmonella $D_{85^\circ C}$-values. These Salmonella $D_{85^\circ C}$-values in powder infant formula and peanut butter were not statistically different.

A possible explanation for these unexpected results may lie in the differences of form (i.e. powder versus paste). It is worth noting that the mean difference in $D$-values is smaller between peanut butter and powder infant formula than between peanut butter and wheat flour. At high isothermal treatments ($85^\circ$ and $90^\circ$C) there were physiochemical changes in the powder infant formula by the end of the trials. The powder yellowed and compacted into brittle sheets. Changes in material consistency may lead to differences in heat transfer as noted by Liu, Xu, Xie, Zhu, & Tang (2019). This study is indicative that $a_w$ and lipid content are not the only predictors of thermotolerance in low-moisture foods. More accurate predictive models across matrices may need to include additional variables such as glass transition temperature or high temperature $a_w$ (Syamaladevi et al., 2016).

Notably, the relationships between pathogen thermotolerance were not consistent in the low-moisture food matrices used in this study. Salmonella spp., though significantly more
thermotolerant than *L. monocytogenes* in peanut butter, was not statistically different than *L. monocytogenes* in wheat flour and in the 85°C treatment temperature in powder infant formula. A similar observation was also clearly reported by Rachon, Peñaloza, & Gibbs (2016) with the thermotolerance of *Salmonella* in model low-moisture food matrices of chicken meat powder (aw = 0.38) and confectionery powder (aw = 0.57) reported to be much higher than *L. monocytogenes* but nearly equivalent in other matrices including pet food (aw = 0.65) and culinary savory seasoning (aw = 0.67). Differentiation of thermotolerances between the two pathogens appears to be less noticeable in low-moisture food matrices at higher aw (Taylor, Tsai, Rasco, Tang, & Zhu, 2018). Due to the ubiquitous nature of *L. monocytogenes*, future studies are warranted to explore the effects of composition and physical chemical properties of low-moisture food impacting survival and thermotolerance.

4.2 *E. faecium* NRRL B-2354

This study confirmed *E. faecium* NRRL B-2354 had greater *D*-values across three low-moisture food matrices and temperatures than each of the pathogens in nearly all of the experimental conditions performed. Notably, while *E. faecium* did have estimated mean *D*$_{85°C}$-values higher than *Salmonella spp.* in peanut butter (7.4 and 5.1 min respectively), the values were not statistically significant. This is suggestive, but not definitive, that in some rare instances in low-moisture food matrices, *E. faecium* is not as conservative of a surrogate as desired. Rachon, Peñaloza, & Gibbs (2016) reported similar limitations in using *E. faecium* as a *Salmonella spp.* surrogate. They found that amongst the four model low-moisture foods tested, the high-sugar, confectionary formulations had shorter 5-log reduction times for the *E. faecium* than the *Salmonella*. These findings indicate the continued need for publicly available data to
evaluate *E. faecium*’s effectiveness as a surrogate in a diverse array of low-moisture foods and processing conditions.

4.3 Comparative studies

Publicly available thermotolerance data in low-moisture foods is insufficient, including a lack of robust values for *L. monocytogenes* (Taylor, Quinn, & Kataoka, 2019). However, several studies using *L. monocytogenes, Salmonella*, and/or *E. faecium*, commonly reported in one low-moisture food or category, can be compared to the results presented in this work across three matrices. Dealing with *L. monocytogenes* in wheat flour (a_\text{w} \sim 0.45), Taylor, Tsai, Rasco, Tang, & Zhu (2018) reported a mean $D_{70^\circ C}$-value of 17.4 min, which was higher than results from this study of 7.6 min. Enache et al. (2015) reported mean $D_{85^\circ C}$-values 1.1 and 2.5 min (*Salmonella Tennessee* and *E. faecium*, respectively) in a model peanut paste with a much higher a_\text{w} of ~ 0.60). Those values were notably lower than results from this study which found 5.1 and 7.4 min for *Salmonella* and *E. faecium*, respectively.

While comparing results between studies which used different methodologies introduces more variability, a recent publication displayed the consistency of results that can be obtained from laboratories using the same methodology (Hildebrandt, Marks, Anderson, & Grasso-Kelley, 2020). This study examined the thermotolerances of *Salmonella Agona 447967* in oat flour collected by six laboratories. It was concluded that cross-laboratory data was highly reproducible when aligned to predetermined protocols. However, the authors noted that small deviations in methodologies may yield differences approaching 50% of the mean $D$-value. This study contributes to the growing area and need for comparative published values.
4.4 Z-values

The relative $z$-values for the three organisms did not remain constant across the three low-moisture foods; no low-moisture food matrix consistently lead to highest or lowest values. One potential pattern is seen, however, in examining the matrix effect as wheat flour treatments consistently had the lowest $z$-value for all three of the bacteria. This may be due to wheat flour having the highest $a_w$ of the three low-moisture foods. A similar effect was reported by Taylor, Tsai, Rasco, Tang, & Zhu (2018) for $L. \text{monocytogenes}$ in wheat flour matrices with varying $a_w$ as well as $\text{Salmonella Typhimurium}$ in glucose enhanced nutrient broth reported by Aljarallah & Adams (2007).

4.5 Modeling

A log-linear model was used for all calculations of thermotolerance. While other models such as the Weibull model are able to better characterize data exhibiting shouldering, tailing, and other non-linear behavior in thermal death time curves, in consultation with experts, it was not justified for use with this data. The fit of the log-linear model was appropriately high with the mean and standard deviation of the $R^2$ values being $0.91 \pm 0.058$. Additionally, using the same, simple model for all treatments increased the ability to graphically represent and compare values. Visual inspection of survivor ratio ($\log N/N_0$) vs. time charts, found that the thermal death curves for $\text{Salmonella}$ in wheat flour showed evidence of minor tailing near the terminus. This is suggestive but not definitive that the effect of thermal treatment decreases over time. In contrast, $\text{Salmonella}$ showed an equivalent or greater log decrease than $L. \text{monocytogenes}$ for short treatment times. The contrary may be true for longer treatment times but best practices determining inactivation rates are commonly subject to limitations related to the maximum duration and limits of detection.
4.6 The use and influence of talc

Disparate inoculation methods have been developed to enable thermobacteriology research in foods. Among the published methods, few were feasible when applied to the three low-moisture food matrices evaluated in this study. We recognize that various methods of inoculation, including the selected method, have advantages and disadvantages. After consideration and prework, talc was selected as a dry inoculum in this study due to the following advantages: dried talc containing bacteria could be efficiently added to the three different food matrices with 1) minimal or no effect, even temporarily, on aw, 2) no second equilibrium in a conditioning chamber, and 3) complete avoidance of physical characteristic modification of the porous low-moisture food powders leading to lack of homogeneity and the introduction of undesired variables and complexity. Using dried talc inoculum containing defined cocktails allowed for the creation of a robust, uniform, and standardized approach applied to the predefined treatment conditions.

The dry inoculation method applied using talc as a carrier has a potential disadvantage – the carrier remains in the food matrix during isothermal treatments. To minimize the compositional impact, the level of talc in the low-moisture food was added at ≤ 1% on wt/wt basis. Some dry inoculum methods, such as the use of sand to inoculate walnuts (Blessington, Mitcham, & Harris, 2012), do not impact the final composition of the food or ingredient at all. While the use of sand or larger inert materials that could later be removed from the target matrix is feasible for inoculating powders and larger particles (e.g. shells), low-moisture food pastes like the peanut butter in our study cannot be handled in the same way.

A recent report by Ahmad, Öztabak, Marks, & Ryser (2019) reported on the impact of talc on bacterial thermotolerance indicating the need for a greater appreciation of the dry
inoculum’s impact when applied in low-moisture food thermobacteriology studies. We examined the effect of talc alone on the $D$-values for *E. faecium* by creating a sample of 1% inoculated talc in sterile talc and replicating the study methodology at two temperatures (75 and 80°C). The mean $D_{75\degree C}$- and $D_{80\degree C}$-values (3.4 and 1.6 min respectively) were lower than the lowest tested low-moisture food matrix treatment (Fig. 3); this is similar to the results reported by Ahmad, Öztabak, Marks, & Ryser (2019) in which the treatment of talc alone had the lowest thermotolerance amongst treatment variables. Our study confirmed this finding and showed that talc, when subjected to heat, alone does not provide much protection for the bacteria of interest during isothermal treatments. In any case, the possible effects of using talc would be applied to all treatment conditions equally and thus would not be a confounding variable to the internal conclusions of this work.

The use of a dry inoculum method predicates that the data from this research will be most beneficial and applicable to food manufacturers of low-moisture foods seeking to understand and manage the risks of dry contamination of the processing and packaging lines. We acknowledge
that estimated $D$-values may be obtained by using other protocols, including wet inoculation methods. In recent reports, dry inoculation methods led to higher thermotolerances in pathogens and surrogates in contrast to liquid inoculations (Liu, Xu, Xie, Zhu, & Tang, 2019; Ahmad, Öztabak, Marks, & Ryser, 2019). The primary objectives of this study were to compare the matrix effects of three low-moisture foods and determine differences between bacterial thermotolerances in two pathogens and a well-established surrogate. Using the dry inoculation method and bacterial strain cocktails described, we met the objectives through a straightforward experimental design using a single method to obtain robust comparison data for these organisms in three model low-moisture foods.

5. Conclusion

This study investigated the matrix effects of three low-moisture foods on isothermal inactivation and determined differences between bacterial thermotolerances in $L.\ monocytogenes$ and $Salmonella\ spp.$ and a well-established surrogate, $E.\ faecium$ NRRL B-2354. Controlled heat treatments on organisms in inoculated powder infant formula exhibited the greatest thermotolerance while the same organisms in wheat flour had the lowest thermotolerance. $E.\ faecium$ was found to be an appropriate surrogate for both $L.\ monocytogenes$ and $Salmonella\ spp.$ in each of the model low-moisture foods. $Salmonella\ spp.$ generally was more thermotolerant than $L.\ monocytogenes$. However, no significant differences were determined between the thermotolerances of the two pathogens in the 85°C treatment temperature of powder infant formula and all wheat flour treatments. This represents the first report of comparable inactivation rates in these foods and highlights the importance of gathering thermotolerance data for $L.\ monocytogenes$ for two reasons: 1) isothermal inactivation in low-moisture foods is influenced
by more than $a_w$ and lipid content alone and 2) comparisons of isothermal inactivation rates in
these foods vary between *L. monocytogenes* and *Salmonella spp.*, warranting further
investigation.
References


Appendix A. Full Literature Review

*Low-moisture foods are defined by their water activity*

The Food Safety Modernization Act (FSMA) requires the food industry to rely more heavily on preventive controls to guarantee safe food; previous patterns of reacting to a food safety hazard in a finished good or ingredient that is already out on the market is no longer an acceptable option. The risks of commercial foods and processes must be anticipated and managed via a comprehensive and specific food safety plan. High-moisture foods (HMFs) have water and nutrients readily available for the growth of micro-organisms and are considered high risk. Low-moisture foods (LMFs), on the other hand, cannot support most microbial growth and have therefore been traditionally seen as low-risk products.

Though definitions of LMFs vary, the US Food and Drug Administration (FDA) and Codex Committee on Food Hygiene define LMFs as foods with water activity ($a_w$) less than 0.85 (FDA, 2015; CCF, 2015). The reason that this definition is centered on $a_w$ instead of moisture content is because the amount of water content is different than the amount of water that is available for physicochemical and biological reactions. This make $a_w$ a more useful parameter than water content in predicting product quality and stability (Bassal, Vasseur, & Lebert, 1993). The lack of available water is precisely why LMFs do not allow for the proliferation of bacteria such as potential pathogens (Fig. 4).

*Fig. 4. Water activity ($a_w$) is on a scale from 0 to 1.0. The right-hand side of the table lists typical foods at various $a_w$. Most spoilage bacteria cannot grow at $a_w < 0.9.*

<table>
<thead>
<tr>
<th>$a_w$</th>
<th>Foods</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>Fluid milk, cheese, bread, fresh fruit and vegetables</td>
</tr>
<tr>
<td>.9</td>
<td>Fermented sausage, fruit juice, sweetened condensed milk</td>
</tr>
<tr>
<td>.8</td>
<td>Jams, marshmallows, nuts dried fruit, honey</td>
</tr>
<tr>
<td>.6</td>
<td>Pasta, flour, peanut butter, dried milk, crackers, spices</td>
</tr>
<tr>
<td>&lt;.5</td>
<td>No microbial proliferation</td>
</tr>
</tbody>
</table>
Low water activity increases bacterial risk factors in several surprising ways

Unfortunately, there is an underestimation of the true risks that are associated with the production of LMFs. LMFs are sources of pathogens in the food system, albeit typically in low numbers (CDC, 2007; FDA, 2018a; FDA, 2018b; Koch et al., 2005; Maberry, 2016; Maberry, 2017; Maberry, 2018; Rachon, Peñaloza, & Gibbs, 2016). These problems have led to public health concerns in addition to “producer risk” (financial and reputational costs for food manufacturers). Three connected phenomena contribute to this miscalculation:

Lower $a_w$ results in greater thermotolerance. In general, bacteria, including some foodborne pathogens, are more heat resistant in LMFs than in (Jin et al., 2018). The thermotolerance and desiccation tolerance of bacteria is closely related, and both types of stresses are often experienced in LMFs. When faced by various stressors, proteins are produced by bacteria to help stabilize critical enzymes and structures in the cell against denaturation and aggregation (Sergelidis and Abrahim, 2009). These stress proteins can provide cross protection against future stresses (Laroche, Fine, & Gervais, 2005). This response to desiccation stress may explain the heightened thermotolerance in LMFs (Burgess et al., 2016). Fig. 5 summarizes this relationship. For example, in a manufacturing facility water from condensation can introduce bacteria into an LMF that will experience extreme desiccation stress only to become more resistant to future thermal processing. Alternatively, since ribosome unfolding is the main mechanism of cell failure in high-moisture environments (Mackey, Miles, Parsons, & Seymour, 1991), some

![Figure 5](image-url)
researchers have hypothesized that the low, molecular-mobility associated with low $a_w$ conditions helps to stabilize the ribosomal units against denaturation (Syamaladevi et al., 2016).

*Lower $a_w$ leads to longer survival.* Not only is it more difficult to thermally inactivate vegetative bacteria with decreasing $a_w$, but the bacteria are also able to persist in the food in a dormant state for a longer period of time (Taylor, Tsai, Rasco, Tang, & Zhu, 2018). For example, at ambient temperatures pathogens can remain in LMFs for a year with only about a 3-log decrease in cells. The decrease is reduced to only 1 log when the LMF is held at refrigeration temperatures (Koseki, Nakamura, & Shiina, 2015). Again, stress protein cross protection may be playing a role since many LMFs are thermally processed to remove water (see Figure 2). From a quality perspective, LMFs such as peanut butter are generally shelf-stable and therefore remain in the global supply chain and consumer pantry longer than other foods. This means that a post-production contamination of an LMF will be present throughout the product’s long shelf-life. This problem was illustrated with respect to salmonellosis when confirmed cases of *Salmonella* infections were reported from LMF sources stored at ambient temperature with a shelf-life longer than 12 months (Kimber, Kaur, Wang, Danyluk, & Harris, 2012). The ability of microorganisms of concern to survive in matrices that do not support growth is important to all parties (producer, distributor, regulator, and consumer).

*Low numbers of cells can cause disease.* Food producers are advised to study and apply controls to minimize the risk of foodborne illness from these organisms even in “no growth” LMFs because the risks associated with infection even at low numbers are severe. *Salmonella* is the primary foodborne pathogen of concern for producers of most LMFs, intermediates, and ingredients due to the survival and heat resistance of this organism in LMF matrices and a long history of outbreaks. (Podolak & Black, 2017; Sánchez-Maldonado, Lee, & Farber, 2018). A
number of salmonellosis outbreaks associated with the consumption of low-moisture products
(e.g., chocolate, powder infant formula, toasted oats breakfast cereal, infant cereals, peanut
butter, nuts, dry seasonings, and paprika-seasoned potato chips) have demonstrated that
Salmonella in dried foods can cause illness, even when present in low numbers (Greenwood &
Hooper, 1983; Lehmacher, Bockemühl, & Aleksic, 1995; Podolak, Enache, Stone, Black, &
Elliott, 2010; Werber et al., 2005).

In summary, in LMF matrices, fewer bacteria die as a result of thermal processing, they
are able to persist throughout the food’s shelf-life, and low numbers of cells can cause disease.
The combination of these factors increases the risks of LMFs. Though improved understanding
of all three of these dynamics are important to the food industry, our work will focus on the
differences in bacterial thermotolerances.

Aw alone is not the best predictor of the effect of LMFs on bacterial thermotolerances

As more studies document an increase of thermotolerance for bacteria in LMFs, the
variation in this increase also becomes apparent; the variation depends on the species of bacteria
and the food composition in addition to aw. Predicting the impact of LMFs on thermotolerances
will continue to be difficult until there is a fundamental understanding of these other two sources
of variation.

Macronutrient composition of an LMF influences bacterial thermotolerance. Bacteria in
two food environments with the same aw but different macronutrient compositions may exhibit
different levels of thermotolerance. Past reports have indicated that high-lipid foods have a
protective effect for bacteria undergoing thermal treatments (Podolak & Black, 2017). This effect
was demonstrated recently by comparing the thermo-tolerance of Salmonella in peanut butter
and wheat flour (Syamaladevi et al., 2016). Though the two foods had the exact same aw, the
*Salmonella* in the peanut butter showed much greater thermo-tolerance. Interestingly, the researchers suggested that the macronutrients affect thermotolerance of bacteria indirectly by influencing the high-temperature *a*<sub>w</sub> (Fig. 6). Since *a*<sub>w</sub> is affected by the temperature of the food, it is measured most commonly at room temperature. This research suggests that high-temperature *a*<sub>w</sub> may help predict the impact of LMF matrices.

The relative thermotolerances of different bacterial species fluctuates in various LMFs.

Many pathogens can cause foodborne illnesses and targeting or testing for all of them is not practical for food manufacturers and distributors. A common practice is to identify which pathogens may be present in the food, determine the species which has the highest thermotolerance, and then only test the food for that pathogen. The reasoning is that if you can sufficiently kill or exclude the strongest pathogen, then you can be confident that all other weaker pathogens are no longer of any concern. For this reason, the food industry is keenly interested in knowing which pathogen has the greatest thermotolerance in their specific food product.

Unfortunately, identifying the pathogen with the greatest thermotolerance is not as useful as previously thought in making predictions for other products. This problem is clearly demonstrated in a seminal study that compared the thermotolerances of three different bacteria in four different model food products (Rachon, Penaloza, & Gibbs, 2016). *Enterococcus faecium* is
often used as a surrogate for *Salmonella* because it generally has a greater thermotolerance. In the study, the order of bacteria from most to least thermotolerant was not consistent in every food (Table 5). Data on thermotolerance of one pathogen cannot be confidently used to predict how other species will act in different LMFs. Since not every bacterial species has been studied in every LMF, important knowledge gaps exist in food production and distribution.

Table 5. Average of $D_{80^\circ C}$-values (min) of several bacterial species in four model food products. The foods varied in composition and aw. Higher $D_{80^\circ C}$-values indicate greater thermotolerance. No obvious patterns can be seen in the relative thermotolerances.

<table>
<thead>
<tr>
<th>Products</th>
<th>$a_w$</th>
<th>L. monocytogenes Cocktail</th>
<th>Salmonella Cocktail</th>
<th>Enterococcus faecium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chicken Meat Powder</td>
<td>0.383</td>
<td>2.0</td>
<td>8.3</td>
<td>23.5</td>
</tr>
<tr>
<td>Confectionery Powder</td>
<td>0.565</td>
<td>0.9</td>
<td>6.7</td>
<td>4.6</td>
</tr>
<tr>
<td>Pet Food</td>
<td>0.653</td>
<td>0.6</td>
<td>0.7</td>
<td>1.9</td>
</tr>
<tr>
<td>Culinary Savory Seasoning</td>
<td>0.655</td>
<td>1.8</td>
<td>1.8</td>
<td>8.1</td>
</tr>
</tbody>
</table>

Currently, no model can accurately predict the changes to bacterial thermotolerance in LMFs because of the combination of pathogens’ variation and macronutrient effects. We are left to experimentally determine the effect of LMF matrices for each pathogen in each food. As of yet, very little data is publicly available concerning the impact of LMFs on the thermotolerance of *L. monocytogenes*.

*L. monocytogenes* is a dangerous pathogen and causes recalls in LMFs.

*L. monocytogenes* is a significant, foodborne pathogen causing invasive listeriosis (septicemia, meningitis, and spontaneous abortion), especially among immuno-compromised individuals, pregnant women and their fetuses, newborn infants, and the elderly (> 65 years old) (Doyle, Meske, & Marth, 1985; ILSI Research Foundation, 2005). *L. monocytogenes* is ubiquitous in the environment and has unique characteristics including the ability to grow at refrigeration temperatures. Using heat, the organism can be readily inactivated in HMFs by reaching a cooking temperature greater than 70 °C (Todd, 2006).
*L. monocytogenes* contaminations in LMFs have already been causing problems in the food industry. Of the 196 U.S. recalls due to *L. monocytogenes* in 2016, more than 50 were due to sunflower seeds and products containing sunflower kernels causing a cascade of affected products including trail mixes; protein, energy and granola bars; nut butters; and salad toppings (Maberry, 2017). Two more recent examples of recall due to potential contamination with *L. monocytogenes* occurred during 2017 and 2018 in roasted cashew butter and a seed butter respectively (FDA, 2017; FDA, 2018c). *L. monocytogenes* and *Salmonella* caused another cascade of product recalls in October 2018 due to vegetable ingredients manufactured at a single plant (FSIS, 2018). It should be noted that no domestic outbreaks were associated with these voluntary recalls. Rather, they were conducted out of caution for the potential presence of *L. monocytogenes*. Additionally, thermotolerance research has not been published or validated for a variety of LMFs, including those responsible for recent cascades of voluntary recalls.
References


Appendix B. Published Review Manuscript

Listeria monocytogenes in Low-Moisture Foods and Ingredients

Bradley J. Taylor, Adam R. Quinn, and Ai Kataoka
Food Control 103 (2019) 153-160

Abstract

Food manufacturers and distributors are tasked with applying science-based approaches and preventative controls to minimize the risk of foodborne illness from Listeria monocytogenes. Concerns regarding “no growth” low-moisture foods (LMFs), defined as ingredients and finished goods with a water activity < 0.85, have escalated because LMFs may harbor foodborne pathogens, albeit in low numbers, and result in illness. Small outbreaks are occurring at a greater frequency presenting a severe and systemic form of listeriosis. Sources include foods not traditionally associated with the organism. Though fortuitously no Listeria outbreaks were directly attributed to LMFs in the U.S., recent cascades of voluntary recalls addressing producer risk related to the potential presence and survival of L. monocytogenes in LMFs are relatively new and complex. This review assembles and assesses the publicly available information on the prevalence, survival, and thermal resistance of L. monocytogenes in LMFs. Traditional dried meats and hard cheese were out of scope. L. monocytogenes is reported to survive in relevant “no growth” model products for months, if not years. Though data is limited, the D-values for L. monocytogenes in model matrices including peanut butter, indicate the organism exhibits thermal resistance and survival comparable to Salmonella and other pathogens of interest in LMFs. A compellation of L. monocytogenes survival and thermal resistance data in LMF matrices was undertaken to build on current preventative controls for effective food safety programs that protect consumers and brands. Recently issued technical guidance and additional applied research in representative LMF matrices and ingredients will continue to improve control of this invasive pathogen throughout food manufacturing systems.
1. Overview and introduction

The food industry is experiencing significant changes in applied approaches to product safety. In the United States, thanks in part to the evolution of Hazard Analysis Critical Control Point (HACCP) and the preventive controls approach advanced via the Food Safety Modernization Act (FSMA), previous patterns of reacting to a food safety hazard in a finished good or ingredient after a product is in commerce are not an acceptable option.

Low-moisture foods (LMFs) are generally considered “lower risk” by food safety program and risk managers supporting food manufacturers and product distributors as intrinsic factors including water activity ($a_w$) limit bacterial growth of foodborne pathogens. LMFs such as peanut butter are shelf-stable and therefore remain in the global supply chain and consumer pantry longer than other foods that do not maintain desired quality attributes at ambient temperatures. Definitions of LMFs vary in terms of water activity limits. However, the US Food and Drug Administration (FDA) and Codex Committee on Food Hygiene define low-moisture foods as foods with $a_w$ less than 0.85 (FDA, 2015; CCF, 2015).

Food safety authorities have established criteria on *L. monocytogenes* for refrigerated Ready-to-Eat (RTE) foods based on a risk assessment conducted by FAO/WHO (2004). Appendix II of Codex Guidelines on Control of *L. monocytogenes* in Foods includes microbiological criteria for three categories of RTE foods: 1) foods for which no criteria are needed, 2) RTE foods in which *L. monocytogenes* growth will not occur, and 3) RTE foods in which growth can occur (Codex Alimentarius Guidelines, 2007). Microbiological criteria for category 2) foods could have this organism <100 CFU/g in food based on a scientifically valid sampling scheme (ICMSF, 1974). Other countries and government bodies such as Canada and European Union adapted these microbial criteria for the verification and control of *L.*
monocytogenes in RTE foods (European Commission, 2005; Health Canada, 2010).

Comparatively, US FDA and USDA FSIS utilizes a ‘zero-tolerance’ policy with regard to L. monocytogenes in any RTE foods (Shank, Elliot, Wachsmuth, & Losikoff, 1996).

Food producers are advised to assess and apply controls to minimize the risk of foodborne illness from these organisms including “no growth” LMFs due to the severe consequences of an infection. The case-fatality rate for invasive listeriosis is 20-30% despite adequate antimicrobial treatment and nearly all cases of listeriosis result from eating food contaminated with L. monocytogenes (Swaminathan & Gerner-Smidt, 2007). Notably, newborn infants may contract listeriosis if their mothers consume contaminated food during pregnancy.

Compared to “high moisture” RTE foods, the opportunity and likelihood of LMF matrices serving as a vehicle of infection ranges from low to high depending on several factors including the intended use of the food or ingredient. Therefore, the robustness and integrity of food safety programs operating during production and, to a lesser degree, the prevention of post-processing contamination (e.g., sanitation programs, product segregation, and bulk packaging) play a critical preventative role to minimize consumer risk. The post-processing contamination risk is primarily controlled by strict avoidance of water using appropriate seals and visual inspection reports. In the evaluation of a specific ingredient or product, it is necessary to determine if Listeria challenge tests are needed based on availability of published data, government guidance documents, and specifications of the physio-chemical properties of the product or ingredient (Álvarez-Ordóñez, Leong, Hickey, Beaufort, & Jordan, 2015).

The ability of microorganisms of concern to survive in matrices that do not support growth, such as LMFs, is important to all parties (producers, distributors, regulators, and consumers). If conditions allow, surviving pathogenic organisms, including L. monocytogenes,
present in intermediates or ingredients may cause illness when consumed in a final product. This problem was illustrated specific to salmonellosis when confirmed cases of *Salmonella* infections were reported from LMFs sources stored at ambient temperature with a shelf-life >12 months (Kimber et al., 2012). In general, bacteria, including some foodborne pathogens, are more heat resistant in LMFs than in high-moisture matrices (Jin et al., 2018). For example, *Salmonella* serotypes implicated in outbreaks have been shown to be more heat resistant in chocolate than in a slurry or dilute solution with a$_w$ 0.86 and above (Podolak & Black, 2017). Microbial viability and survival during storage in dried foods is organism-specific and can vary both with product composition and time.

LMFs are sources of pathogens, albeit typically in low numbers, in the food system (CDC, 2007; FDA, 2018a; FDA, 2018b; Koch et al., 2005; Maberry, 2016; Maberry, 2017; Maberry, 2018; Rachon, Peñaloza, & Gibbs, 2016). *Salmonella* is currently the primary foodborne pathogen of concern for producers of most LMFs, intermediates and ingredients due to its survival and heat resistance in LMFs matrices and a long history of outbreaks in LMFs (Podolak & Black, 2017; Sánchez-Maldonado, Lee, & Farber, 2018). The case-fatality rates for *Salmonella* vary among serotypes but are generally $>10\times$ smaller than *L. monocytogenes*. A review by Jones et al. (2008) reported case-fatality rates of 0.3% for Newport, 0.6% for Typhimurium, and 3% for Dublin.

A number of salmonellosis outbreaks associated with the consumption of low-moisture products (e.g., chocolate, powder infant formula, toasted oats breakfast cereal, infant cereals, peanut butter and nuts, dry seasonings, paprika-seasoned potato chips) have demonstrated that *Salmonella* in dried foods can cause illness, even when present in low numbers (Greenwood and Hooper, 1983; Lehmacher, Bockemühl, & Aleksic, 1995; Podolak, Enache, Stone, Black, &
Elliott, 2010; Werber et al., 2005). Powder infant formula and other medical foods are also routinely tested for the presence of Cronobacter sakazakii in addition to Salmonella. Pathogenic Escherichia coli strains are known to survive in low moisture conditions and were associated with an outbreak linked to seeds and flour (Breuer et al., 2001; CDC, 2016; Soon, Seaman, & Baines, 2013).

Recalls and market withdrawals of low-moisture food products due to potential contamination with Salmonella in the United States from 1998 to the present demonstrate a predominance of recalls of nuts, seeds, and products produced from them, followed by spices and herbs, cereals, soup mixes, peanut butter, powdered protein products, and a category consisting of a heterogeneous mix of food products for which the root cause of contamination could be any of the ingredients (Beuchat et al., 2011).

In the limited literature on the topic of survival and heat resistance values of L. monocytogenes in a variety of low-moisture matrices, the organism is reported to exhibit lower or equivalent levels of heat resistance and survivability when compared to other foodborne pathogens such as Salmonella. Therefore, processing parameters that target Salmonella have, in most cases, been assumed to be sufficient for L. monocytogenes. The understanding and validation of this assumption across model LMF matrices is of critical importance to advance the application of food safety programs which include effective raw material controls and specifications throughout the supply chain.

2. Objective and method

The objective of this review is to compile applied microbiological studies and methodically present information available to food safety program managers and regulators. We address the
following questions specific to *L. monocytogenes* in LMFs focused on preventing and controlling the organism in production and processing:

1. How long can *L. monocytogenes* survive in relevant “no growth” model products with aw of < 0.85?

2. Does *L. monocytogenes* exhibit heat resistance similar to other organisms of concern and potential non-pathogenic surrogates in low-moisture food matrices?

3. Are the food sources and organisms implicated in recalls and/or outbreaks consistent with previous observations?

By advancing the understanding of the survival and thermal resistance of *L. monocytogenes* in LMFs matrices, we aim to enhance current approaches to preventative controls for effective food safety programs in global supply chain of the food industry to address risks associated with this foodborne pathogen. Dried meats and hard cheeses, though considered ‘dry foods’ were not included.

2.1 Recalls

Of the 196 U.S. recalls due to *Listeria monocytogenes* in 2016, more than 50 were due to sunflower seeds and products containing sunflower kernels causing a cascade of affected products including trail mixes, protein, energy and granola bars, nut butters and salad toppings (Maberry, 2017). In May of 2017, products with roasted cashew butter were voluntarily recalled due to potential contamination with *L. monocytogenes* and a seed butter was recalled in December 2018 due to potential *L. monocytogenes* contamination (FDA, 2017; FDA, 2018c). *L. monocytogenes* and *Salmonella* caused another cascade of product recalls in October 2018 due to vegetable ingredients manufactured at a single plant (FSIS, 2018). Most recently, several nut
butters including sunflower, peanut, almond and hazelnut were voluntarily recalled due to potential contamination with *L. monocytogenes* (FDA, 2019).

It should be noted that no domestic U.S. outbreaks were associated with these voluntary recalls. Rather, they were conducted out of caution for the potential presence of *L. monocytogenes* and the desire to minimize producer risk. A review of *L. monocytogenes* outbreaks, virulence, dose-response, ecology, and risk assessments was published in 2017 (Buchanan, Gorris, Hayman, Jackson, & Whiting, 2017). Again, no LMFs were specified. However, the U.S. did experience listeriosis outbreaks attributed to foods considered to be “moderate risk” or “low risk” by the existing risk assessments including fruits, vegetables, and ice cream. Producer risk under the zero-tolerance policy, in contrast to consumer risk, drives additional efforts to understand the survival of *L. monocytogenes* in “no growth” matrices and opportunities to eradicate this organism in LMFs.

2.2 Survival of *L. monocytogenes* in LMFs

*L. monocytogenes* is widely distributed in the environment. It persists after colonizing harborage sites in a variety of food production facilities including non-meat food producers of powder dairy products, nut butters, flours, vegetables and spices. In general, LMFs are distributed and consumed over longer periods than other ingredients and products that have *a_w* ≥ 0.85.

Studies reporting the length of survival of *L. monocytogenes* in LMFs at various temperatures are found in Table 6. In general, “presence” of survivors in food matrices is assessed by determining the presence/absence of *L. monocytogenes* in 25-gram samples and is typically reported by microbiology laboratories as <1/25g or <0.04/g of ingredient or finished product (absence equals less than 1 cell in 25 g, or less than 0.04 cell in 1 g). The studies listed
evaluated the organisms’ length of survival in days, weeks or months after holding the samples at various temperatures in LMF matrices that do not support the growth of microorganisms under standard or common conditions used for food production and distribution.

Table 6. Survival of *Listeria monocytogenes* in low-moisture food matrices

<table>
<thead>
<tr>
<th>Food</th>
<th>Inoculum (log CFU/g)</th>
<th>Water Activity (aw)</th>
<th>Length of Survival Tested at Temperature (°C)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonfat dry milk</td>
<td>5.2</td>
<td>aw not reported; moisture level of 3.7%</td>
<td>12 weeks at 25°C</td>
<td>Doyle et al., 1985</td>
</tr>
<tr>
<td>Peanut butter</td>
<td>4.4</td>
<td>0.33 or 0.65</td>
<td>24 weeks at 20°C</td>
<td>Kenney and Beuchat, 2004</td>
</tr>
<tr>
<td>Chocolate and peanut butter spread</td>
<td>3.4</td>
<td>0.33 or 0.65</td>
<td>24 weeks at 20°C</td>
<td>Kenney and Beuchat, 2004</td>
</tr>
<tr>
<td>Raw walnuts</td>
<td>2.5</td>
<td>0.4</td>
<td>105 days at 23°C</td>
<td>Blessington et al., 2012</td>
</tr>
<tr>
<td>Almond kernels</td>
<td>~ 4.4</td>
<td>0.4</td>
<td>12 months at -19, 4, 24°C</td>
<td>Kimber et al., 2012</td>
</tr>
<tr>
<td>Raw in-shell pistachios</td>
<td>~ 4.4</td>
<td>0.4</td>
<td>12 months at -19, 4, 24°C</td>
<td>Kimber et al., 2012</td>
</tr>
<tr>
<td>Raw peanut kernels</td>
<td>4.1</td>
<td>aw not reported; moisture level of 3.8%</td>
<td>12 months at -24, 4, 22°C</td>
<td>Brar et al., 2015</td>
</tr>
<tr>
<td>Raw pecan kernels</td>
<td>5.3</td>
<td>aw not reported; moisture level of 2.6%</td>
<td>12 months at -24, 4, 22°C</td>
<td>Brar et al., 2015</td>
</tr>
<tr>
<td>Infant formula</td>
<td>5.0 at 35°C</td>
<td>0.28</td>
<td>4 months at 35°C</td>
<td>Koseki et al., 2015</td>
</tr>
<tr>
<td></td>
<td>3.3 at 22°C</td>
<td></td>
<td>12 months at 22°C</td>
<td>Taylor et al., 2018</td>
</tr>
<tr>
<td>Wheat flour</td>
<td>8.0</td>
<td>0.31 or 0.56</td>
<td>6 months at 22°C</td>
<td>Taylor et al., 2018</td>
</tr>
</tbody>
</table>

Abbreviations: CFU, colony forming unit; aw, water activity

Doyle, Meske, & Marth (1985) conducted pioneering research on the survival of *L. monocytogenes* throughout the production and storage of nonfat dry milk (NFDM). Skim milk was inoculated before being processed through a spray dryer. NFDM was then held at room temperature for 16 weeks and a cell count was performed nine times throughout that period. The rates of decline were not reported, but in two trials with an initial inoculation of > 5 CFU/g, *L. monocytogenes* survived and was detected at week 12, but not at week 16 (Doyle et al., 1985).
*L. monocytogenes* was studied in peanut butter and peanut chocolate spread to determine how long it could survive. The water activity was adjusted to test two conditions (0.33 and 0.65 \(a_w\)). *L. monocytogenes* was not eliminated in either food after storage for 24 weeks at 20°C. The initial inoculation levels were 4.4 log CFU/g, and greater survival was observed in samples with a lower water activity (Kenney & Beuchat, 2004).

Starting in 2010-2011, more applied work was conducted and published testing the survival rates of three major pathogens, including *L. monocytogenes*, in tree nuts. This work addressed outbreaks and national recalls of nuts and nut products; walnuts were recalled due to the isolation of *L. monocytogenes* (Blessington, Mitcham, & Harris, 2012). The initial study focused on the inoculation of walnuts. When compared with later experiments, the *L. monocytogenes* work executed on walnuts considered the fewest number of variables and ran for the shortest period of time. The purpose of the study was to compare the rates of decline for three different inoculation levels. Two single-strain experiments (*L. monocytogenes* serovar 4b) used high and moderate inoculation levels, while the lone five-strain cocktail experiment used a lower inoculation level. *L. monocytogenes* inoculated in a five-strain cocktail was detected in 55 of the 90 samples during the course of the study. At the conclusion of the work, all but three samples tested positive after enrichment on day 105 though counts were below the limit of detection for the plating method (Blessington et al., 2012).

In a later study, almonds and pistachios were inoculated with *L. monocytogenes* and held at -19, 4, and 24°C for a period of twelve months. Although the initial rates of decline were calculated to be 0.71 and 0.86 CFU/g/month for almonds and pistachios respectively, culturable *L. monocytogenes* colonies were confirmed in the samples throughout the entire study via an enrichment process (Kimber et al., 2012). A study of peanuts and pecans conducted two years ago...
later used the same methods and found similar results. *L. monocytogenes* survived the year-long duration of the study at -24, 4, and 22°C (Brar, Proano, Friedrich, Harris, & Danyluk, 2015).

*L. monocytogenes* can survive for at least a year on tree nuts, and the survival rates are comparable to those of *Salmonella* and *Escherichia coli* O157:H7. In the three studies above investigating the survival of foodborne pathogens in various tree nuts with a water activity near 0.40, *L. monocytogenes* was observed to decline more rapidly than *Salmonella* and *E. coli* O157:H7. In Japan, a similar hypothesis was specifically tested by researchers who conducted a comparison study of *L. monocytogenes*, *S. enterica* serovars Typhimurium and Enteritidis, *C. sakazakii*, and *E. coli* O157:H7. Using the Weibull model to analyze the data in powder infant formula (0.28 aw), the relative desiccation tolerances of these bacteria were as follows: *C. sakazakii* > *S. enterica* > *L. monocytogenes* = *E. coli* (O157:H7) (Koseki, Nakamura, & Shiina, 2015). Again, *L. monocytogenes* demonstrated it can survive for 12 months at 22°C in a representative infant formula matrix.

In a recent study, the survival of *L. monocytogenes* in wheat flour at different water activities was determined by Taylor, Tsai, Rasco, Tang, & Zhu (2018). The flour was adjusted to 0.31 or 0.56 aw and inoculated with a high (~10^8 CFU/g) concentration of *L. monocytogenes*. Over a period of six months at 22°C, the lower aw samples were reduced by 2.5 log CFU/g while, in contrast, the higher aw samples were reported to have a 6.3 log CFU/g reduction. This study demonstrated a clear effect of aw on the survival of *L. monocytogenes* in a low-moisture food ingredient typically not consumed raw.

There are important limitations to the data available regarding *L. monocytogenes* survival. Only four example LMFs products or ingredients have been studied (tree nuts, nut spreads, powder milk, and wheat flour), and the holding times have often been shorter than is
needed to accurately report how long *L. monocytogenes* can survive. Results in the studies listed in Table 6 demonstrate that *L. monocytogenes* was recovered successfully from food samples throughout the time course and at the conclusion of several studies. Therefore, there is a reasonable likelihood that, when present, *L. monocytogenes* will survive the entire duration of typical shelf-lives of some LMFs products including shelled nuts. As an example, shelled nuts typically have a shelf-life of 4 months at room temperature and 6 months at refrigeration temperatures. Other common low-moisture food products, including peanut butter have a shelf-life 6 to 9 months at room temperature (Boyer & McKinney, 2013).

2.3 Thermal resistance of *L. monocytogenes* in LMFs

It is well established that the thermal resistance of *L. monocytogenes* is higher in LMFs than in foods with aw greater or equal to 0.85 (Lian, Zhao, Yang, Tang, & Katiyo, 2015; Podolak & Black, 2017). Additionally, each food matrix provides unique microenvironmental variables that affect pathogen survival during heat treatments. Various complexities have been described in determining the thermal inactivation kinetic values. For example, many LMFs are high-moisture products before processing; the organisms are in an aqueous environment in these cases (Podolak & Black, 2017). The study by Doyle et al. (1985) cited previously, featured the processing of liquid milk (a high-moisture food) to NFDM (a low-moisture food and ingredient). A 1 to 1.5 log reduction of *L. monocytogenes* was reported after the samples were spray dried. It should be noted that the log reduction of 1 to 1.5 log of *L. monocytogenes* is less than the reductions reported in similar, early research on *Salmonella* and *E. coli* (Miller, Goepfert, & Amundson, 1972).

High-fat, low-moisture matrices of peanut and chocolate products were used in *L. monocytogenes* thermal resistance and survival studies conducted by Kenny & Beuchat (2004).
The samples were inoculated with *L. monocytogenes* from a suspension of washed cells. The samples were subjected to a heat treatment through immersion in circulating water at 60°C for 0, 5, 10, 15, 20, 25, and 30 min. The samples were then enumerated on two different plates – brain heart infusion agar (BHIA) and modified Oxford agar (MOX). Differences in the calculated $D_{60^\circ C}$ values were not statistically significant when comparing the two products within the same agar types. The calculated $D_{60^\circ C}$ values are listed in Table 7 (Kenney & Beuchat, 2004). These findings are similar to those of published thermal resistance values for *Salmonella* in peanut butter; the $D_{70^\circ C}$ values were approximately 30 min with a $z$-value of approximately 40-50°C (Ma et al., 2009). Preliminary thermal resistance work in peanut butter ($a_w$ 0.60 and 47% fat) conducted in the GMA laboratory found that *Salmonella* and *L. monocytogenes* had $D_{75^\circ C}$ values of 12.5 ± 1.7 and 16.7 ± 0.46, respectively.

<table>
<thead>
<tr>
<th>Heating medium</th>
<th>BHIA</th>
<th>MOX</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peanut butter</td>
<td>25.99</td>
<td>39.04</td>
</tr>
<tr>
<td>Chocolate-peanut spread</td>
<td>37.47</td>
<td>29.18</td>
</tr>
</tbody>
</table>

Table 7. $D_{60^\circ C}$ values of *L. monocytogenes* in LMF pastes determined on BHIA and MOX

Abbreviations: BHIA, brain heart infusion agar; MOX, modified Oxford agar

Taylor et al. (2018) examined the effect of $a_w$ on the thermal resistance of *L. monocytogenes* in wheat flour. The $a_w$ of the flour was adjusted to either 0.3, 0.45, or 0.6, and the inoculated flour was placed in an ethylene glycol bath at either 70, 75, or 80°C. The calculated $D$- and $z$-values are shown in Table 8 (Taylor et al., 2018). When each temperature is considered separately, $D$-values increased as $a_w$ decreased. However, the change in the respective $z$-values corresponding with the increasing $a_w$ values did show the same inverse relationship. Rather, the $z$-value of the 0.45 $a_w$ samples was higher than that reported for the 0.3 and 0.6 $a_w$ samples.
Table 8. $D$-values and $z$-values of *L. monocytogenes* in wheat flour with varying water activities

<table>
<thead>
<tr>
<th>$a_w$</th>
<th>Temp (°C)</th>
<th>$D$-value (min)</th>
<th>$z$-value (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.30</td>
<td>70</td>
<td>37.1</td>
<td>12.9</td>
</tr>
<tr>
<td></td>
<td>75</td>
<td>19.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>80</td>
<td>7.1</td>
<td></td>
</tr>
<tr>
<td>0.45</td>
<td>70</td>
<td>17.4</td>
<td>14.2</td>
</tr>
<tr>
<td></td>
<td>75</td>
<td>7.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>80</td>
<td>3.1</td>
<td></td>
</tr>
<tr>
<td>0.60</td>
<td>70</td>
<td>16.9</td>
<td>9.9</td>
</tr>
<tr>
<td></td>
<td>75</td>
<td>4.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>80</td>
<td>1.6</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: $a_w$, water activity

Rachon et al. (2016) examined the heat resistance of *L. monocytogenes* in four unique food matrices: culinary savory seasoning, chicken meat powder, pet food, and confectionery powder. These LMFs matrices represented independent compositions across a spectrum of foods and received an irradiation treatment prior to inoculation to eliminate background flora. A cocktail of five *L. monocytogenes* strains was used to assess and compare $D_{80^\circ C}$ values between organisms of interest.

The inoculated samples were held at 16°C for 21 days; the $D_{80^\circ C}$ values were calculated using an oil bath on days 0, 3, 7, and 21. The microbial loads remained within the same log CFU/g value while they were being held at 16°C. The samples showed small changes in $a_w$ that were statistically significant yet were deemed inconsequential from a practical perspective. The average values for the organisms tested in the four matrices are presented in Table 9.

The heat resistance of *L. monocytogenes*, represented as an average of $D_{80^\circ C}$ values, was slightly lower or identical to those reported for *Salmonella* in culinary savory seasoning and the pet food matrices with $a_w$ 0.655 and 0.653, respectively. Overall, there was little variation in the $D_{80^\circ C}$ values of each food over the 21-day experiment. The $D_{80^\circ C}$ value of *L. monocytogenes* in the confectionery powder increased by 0.4 min while the $D_{80^\circ C}$ value decreased by 0.76 min in the culinary savory seasoning. Though the data is limited, evidence exists that the heat resistance
of *L. monocytogenes* is comparable to that of *Salmonella* LMF matrices including model peanut butter, pet food, and savory seasoning.

Table 9. Average of $D_{80^\circ C}$-values (min) in low-moisture foods held at 16°C throughout a 21-day storage period

<table>
<thead>
<tr>
<th>Products</th>
<th><em>L. monocytogenes</em> Cocktail</th>
<th><em>Salmonella</em> Cocktail</th>
<th>Enterococcus faecium NRRL B 2354</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chicken Meat Powder</td>
<td>2.0</td>
<td>8.3</td>
<td>23.5</td>
</tr>
<tr>
<td>($a_w = 0.383$)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Confectionery Powder</td>
<td>0.9</td>
<td>6.7</td>
<td>4.6</td>
</tr>
<tr>
<td>($a_w = 0.565$)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pet Food</td>
<td>0.6</td>
<td>0.7</td>
<td>1.9</td>
</tr>
<tr>
<td>($a_w = 0.653$)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Culinary Savory Seasoning</td>
<td>1.8</td>
<td>1.8</td>
<td>8.1</td>
</tr>
<tr>
<td>($a_w = 0.655$)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: $a_w$, water activity

3. Discussion

3.1 Listeriosis and associated risk assessments

*L. monocytogenes* is a significant foodborne pathogen causing invasive listeriosis (septicemia, meningitis, and spontaneous abortion) especially among immuno-compromised individuals, pregnant women and their fetuses, newborn infants, and the elderly (>65 years old) (Doyle, 2001; ILSI Research Foundation, 2005). *L. monocytogenes* is ubiquitous in the environment and has unique characteristics including the ability to grow at refrigeration temperatures. Well documented intrinsic food conditions that do not support *L. monocytogenes* growth include a pH < 4.4, $a_w < 0.92$, or a combination of pH < 5.0 and $a_w < 0.94$, NaCl >16%; freezing (−18 °C) is also an effective extrinsic condition (Buchanan et al., 2017). In other words, growth is completely suppressed at or near these values though the organism survives for extended periods. Using heat, the organism can be readily inactivated in high-moisture foods by reaching a cooking temperature of > 70 °C, (Todd, 2006). These characteristics should factor into foodborne illness prevention strategies involving this organism. Past risk assessments indicated that high risk foods are those which support the growth of *L. monocytogenes*, have a long shelf-
life, and are consumed frequently (FAO/WHO, 2004; FDA/FSIS, 2003). Refrigerated Ready-to-Eat (RTE) foods that have a likelihood to be contaminated with this organism before packaging and allow growth include well-known high-risk foods such as deli meats. Also, the risk assessments concluded that foods with low levels of *L. monocytogenes* (e.g., <100 CFU/g) pose very little risk (Chen, Ross, Scott, & Gombas, 2003; FAO/WHO, 2004).

LMFs, frozen foods, acid and acidified food with pH less than 4.3 have not been considered as high-risk food for listeriosis because *L. monocytogenes* will not grow out in these food matrices even if present at <100 CFU/g. However, recent listeriosis outbreaks with ice cream (CDC, 2015) and caramel apples (Angelo et al., 2017) as well as the advent of whole genome sequencing (WGS) technology, may challenge previous classification of foods as high- or low-risk in the near future (Buchanan et al., 2017). Recent studies (Lee et al., 2018; Maury et al., 2016) utilized WGS and found that certain *L. monocytogenes* strains (e.g., serotype 4b, clonal complexes CCs such as CC1, CC2, CC4 and CC6) appear to be hypervirulent. These strains may be rarely reported in foods and environment; however, they are highly associated with clinical cases (Lee et al. 2018; Maury et al., 2016). These studies established that *L. monocytogenes* is a highly heterogeneous species with regards to pathogenicity, and is composed of hypervirulent and hypovirulent clones. This may influence our current understanding of high-risk food or “growth” / “no growth” properties of *L. monocytogenes* in foods and food processing facilities.

Controlling *L. monocytogenes* in food processing and mitigating the likelihood of contamination in all food types, especially RTE foods, through improved current Good Manufacturing Practices (cGMPs) and systematic preventative controls (PCs) are paramount for food manufacturers. For high-moisture RTE foods, this strategy to control *Listeria* has been well-elaborated, and guidance documents and references have been extensively published for
supplier control, segregation of raw and finished products, cleaning and sanitation, verification programs (environmental monitoring program: EMP) and formulation of product with growth inhibitors (FSIS, 2014; GMA, 2018; Malley, Butts, & Wiedmann, 2015; Tompkin, 2002).

For LMFs, wet cleaning is not recommended (GMA, 2009). If water is introduced to a dry facility and is not completely dried immediately afterwards, it can create opportunities for pathogenic organisms such as Salmonella (or Listeria) to establish themselves and spread in the processing environment. Therefore, effective dry cleaning and EMPs are central to the food safety programs of facilities producing LMFs exposed to the environment post-lethality (GMA, 2009).

3.2 Prevalence

A 2014 report suggests that L. monocytogenes is not commonly found in LMFs and no illness and outbreaks of listeriosis in LMFs were reported (FAO/WHO, 2014). The risk ranking of L. monocytogenes in LMFs was estimated very low based on available data of prevalence, outbreak, and illness reports (FAO/WHO, 2014; GMA, 2009). Recent recalls of LMFs due to L. monocytogenes, however, suggest this organism, independent of the presence or absence of Salmonella, should not be overlooked as a biological food safety hazard in foods with aw < 0.85 depending on usage of the food and/or target customers.

Since L. monocytogenes is pervasive in nature and in food manufacturing plants, it is difficult to completely eliminate in the food system. The prevalence of L. monocytogenes in consumer food products is determined by the presence in food ingredients, the survival during processing, and contamination of finished product. All three factors should be characterized and considered by food manufacturers.
Information on prevalence of *L. monocytogenes* in LMFs as finished products or ingredients is generally not publicly available. Addressing this knowledge gap is a logical starting point for further characterization of the key considerations addressing risks in LMFs. Presence, or more commonly counts of >100 CFU/g of the organism or another indicator organism, could suggest an issue with prerequisite programs or preventative controls in the firm’s food safety plan.

### 3.3 Thermal resistance

Processors should use appropriate techniques and heat resistance data to validate transformation and processing steps. This may be supported by applying data from peer-reviewed studies or conducting their own product-specific studies. It is also advisable to model the persistence of specific pathogens in the product, taking into consideration the realistic vectors and kinetics of the organism. This modeling approach, designed to be a conservative assessment, can be used throughout the process and gives the processor or researcher an advantage in determining the scale and scope of hazard analysis and validation procedures.

Thermal resistance research available for this type of work is limited for *L. monocytogenes* in terms of the amount of data and the LMFs matrices or ingredients; most studies have focused on *Salmonella* or other pathogens. Within the published reports on multiple organisms, fewer parameters and replications and shorter times were used for *L. monocytogenes*. We noted that thermal resistance research has not been published or validated for a variety of LMFs, including ingredients and products involved or associated with recent cascades of voluntary recalls.

Rachon et al., (2016) reported similar thermal resistance values for *Salmonella* and *L. monocytogenes* in two LMF models, pet food and culinary savory seasoning. Findings from our
preliminary work found a similar but slightly greater thermal resistance in \textit{L. monocytogenes} than \textit{Salmonella} in a model nut butter with 0.60 \textit{a}_w. These model LMF examples, in the context of increased virulence, warrant further research specific to the thermal resistance of \textit{L. monocytogenes} in additional LMFs and ingredients. Notably, the limited amount of data for \textit{L. monocytogenes} indicates that the organism exhibits an increase in thermal resistance with a decrease in \textit{a}_w as previously reported with \textit{Salmonella} and \textit{E. faecium} in model LMFs.

The thermal resistance of \textit{L. monocytogenes} varies from product to product. \textit{D}_{80^\circ C} \textnormal{ values} (min) of \textit{L. monocytogenes} and \textit{Salmonella} are similar \textit{D}_{80^\circ C} \textnormal{ values} in model LMFs with 0.65 \textit{a}_w. In contrast, \textit{D}_{80^\circ C} \textnormal{ values} (min) of \textit{L. monocytogenes} are smaller than corresponding \textit{D}_{80^\circ C} \textnormal{ values} for \textit{Salmonella} in model LMFs with 0.565 and 0.383 \textit{a}_w, respectively (Rachon et al., 2016). Variation in heat resistance could potentially result from changes in \textit{a}_w at elevated temperatures (Syamaladevi et al., 2016). Logarithmic thermal death curves are not linear, but clearly show increasing resistance during processing with heat at lower \textit{a}_w values.

Viability and thermal inactivation curves are less available for evaluating the safety of LMFs using predictive models. In “high moisture foods”, where more data is abundant, these models help food safety managers estimate the microbial non-thermal survival, growth, and thermal inactivation under a set of conditions including temperature, pH, and \textit{a}_w based on peer-reviewed published reports or trusted sources. For example, the ComBase Predictor (https://www.combase.cc/index.php/en/) managed by the ComBase Consortium, consisting of the Institute of Food Research in the United Kingdom, the USDA Agricultural Research Service (USDA-ARS) in the U.S. and the University of Tasmania Food Safety Center in Australia is a searchable database featuring core inputs for predictive growth and survival models of pathogens including \textit{L. monocytogenes}, \textit{Salmonella}, and \textit{E. coli} 0157:H7 in foods. Relevant LMF data
starting to be available and searchable in ComBase though the date added is not always available. For example, a recent search resulted in data sets of *L. monocytogenes* in non-dairy cream with 0.6-0.70 aw at various pHs and temperatures from Champden and Chorleywood Food Research Association in the UK. In addition, similar data for *Salmonella* in peanut butter with 0.45 aw, pH 5.12 and various temperatures originally from Ma et al., (2009) at the Center for Food Safety at the University of Georgia is searchable in ComBase.

4. Conclusion

Due to survival, heat resistance, and outbreak occurrences, *Salmonella* is the target organism in most LMF raw matrices such as almonds and peanut butter (0.2-0.4 aw). The risk ranking of *L. monocytogenes* in LMFs is very low based on prevalence, outbreak, and illness reports. Despite this ranking, *L. monocytogenes*, not *Salmonella*, is at the center of increasing numbers of cascading voluntary recalls. These recalls are not based on outbreaks of human illness but rather triggered as a precautionary measure. Some firms are likely recalling LMFs or low-moisture ingredients containing or potentially containing *L. monocytogenes* to actively manage producer risk if the organism was subsequently provided with conditions supporting growth prior to consumption.

The *D*-values and survival data for *L. monocytogenes* in model 0.5-0.6 aw matrices captured in this review of publicly available data indicate that the organism can, under relevant conditions, exhibit survival patterns and thermal resistance comparable to *Salmonella* and represents a potential knowledge gap. This gap is critical as food safety professionals working with LMFs and ingredients need additional science-based preventative controls to minimize the risk of foodborne illness from *L. monocytogenes* that specifically address survival, thermal resistance and prevention of post-processing contamination in foods including “no-growth”
foods. The findings of current and future studies will benefit technical validations and opportunities for inactivation technologies.


Rachon, G., Peñaloza, W., & Gibbs, P. A. (2016). Inactivation of *Salmonella, Listeria monocytogenes* and *Enterococcus faecium* NRRL B-2354 in a selection of low moisture


Appendix C. Photographs of Methodology