

Assessment of *Pediococcus acidilactici* ATCC 8042 as potential *Salmonella* surrogate for thermal treatments of toasted oats cereal and peanut butter

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ABSTRACT

The control of *Salmonella* in low water activity foods poses a challenge for the food industry because of its thermal resistance. The use of surrogate bacteria in a food plant is considered a critical component to validate processing steps. The objective of this study was to evaluate the use of *Pediococcus acidilactici* ATCC 8042, a generally recognized as safe bacterium (GRAS), as potential surrogate for *Salmonella* in commercial toasted oats cereal (TOC) and peanut butter. *P. acidilactici* was compared to a five-serovar cocktail of *Salmonella* and *Enterococcus faecium* NRRL-B2354, separately. Cultures were inoculated into TOC and thermal kinetic parameters (δ , β) were determined at 80, 85, 90, and 95 °C using the Weibull model. In peanut butter, δ and β parameters were obtained at 63, 68, 73, and 77 °C. In TOC, the δ values (initial decimal reduction time) of *P. acidilactici* were 63 and 7 min at 80 and 95 °C, respectively, and at all four temperatures they were not significantly different from δ values of *E. faecium*. The δ value of *Salmonella* at 80 °C (139 min) was two-fold greater than the other two bacteria's values ($p < 0.05$). In peanut butter, δ values of *P. acidilactici* ranged from 31 min at 63 °C to 2.6 min at 77 °C, and at all temperatures they were not significantly different from *E. faecium*'s δ values. In peanut butter, all *Salmonella* cocktail's δ values were significantly smaller than *P. acidilactici*'s with values of 2 min at 63 °C and 0.4 min at 77 °C. These results indicated that *P. acidilactici* was as heat tolerant as *E. faecium* in these food matrices. However, the thermal inactivation kinetic parameters suggested that *P. acidilactici* can only be considered a *Salmonella* surrogate in TOC at temperatures above 85 °C. Because of its greater thermal tolerance in peanut butter, *P. acidilactici* may be used as *Salmonella* surrogate if an additional safety factor is recommended.

1. Introduction

Salmonella contamination of low water activity foods ($a_w \leq 0.6$) is a major concern for the food industry. While low water activity inhibits all microbial growth in food, it does not prevent contamination, which can cause illness. *Salmonella* contamination of almonds, peanut butter, breakfast cereal, and other products has caused large-scale outbreaks (Beuchat and Mann, 2015; CDC, 2009, 2007; 2004; Russo et al., 2013). Extruded toasted oat cereals were associated with two outbreaks due to *Salmonella* Agona contamination (CDC, 2008, 1998) These outbreaks have shown *Salmonella*'s potential to cause illness in foods that were previously thought to be low risk. Since low water activity foods can be ready-to-eat, the need for *Salmonella* control in those foods is essential.

Salmonella survives multiple harsh environmental conditions, including desiccation. Desiccated *Salmonella* is more resistant to subsequent stressors such as UV, sanitizers, bile salts, and heat (Gruzdev et al., 2011). *Salmonella* inoculated into a low water activity food, such

as wheat flour, is much more resistant to heat inactivation than it is in ground beef. As a comparison, *Salmonella* has a D-value of 20.7 min at 70 °C in wheat flour and a D-value of under 1 min in ground beef at the same temperature (Fudge et al., 2016; Juneja et al., 2014). This large change in heat resistance illustrates the effect of low water activity on enhancing thermal tolerance.

With the recent implementation of the Food Safety Modernization Act's Preventive Controls Rule, thermal inactivation controls must be validated through performing studies in the facility or relating the control to published scientific evidence (Buchanan and Williams, 2013). Validating a control in a facility is a viable method; however, pathogenic testing strains cannot be used in a food processing facility. In lieu of using pathogenic strains, a surrogate bacterium can be a viable alternative. Bacterial surrogates are non-pathogenic strains that act similarly to a target pathogen in the same food matrix and environmental conditions (Sinclair et al., 2012). An ideal surrogate has similar survival and growth characteristics to the target, is easy to use, easy to

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store, and genetically stable (Sinclair et al., 2012). For *Salmonella*, *Enterococcus faecium* NRRL-B2354, has been approved by the Almond Board of California for use in validating almond thermal processing processes (Stevenson et al., 2014). While *E. faecium* is nonpathogenic, it is not a GRAS microorganism. Using a GRAS microorganism as a surrogate may have the potential to be widely used in a food facility.

While not officially adopted by another commodity group, *E. faecium* has been investigated as a surrogate for *Salmonella* in other food matrices. These matrices have included high water activity foods, such as milk and ground beef, and low water activity foods, such as peanut paste and oat flour (Annous and Kozempel, 1998; Enache et al., 2015; Ma et al., 2007). The ability of *E. faecium* to model the inactivation of *Salmonella* species across varied food matrices shows potential for utilization in other foods. It was reported that *Pediococcus acidilactici* ATCC 8042 had similar survival and thermal inactivation characteristics to *Salmonella* in beef jerky and dry pet food (Borowski et al., 2009; Ceylan and Bautista, 2015). Unlike *E. faecium*, *P. acidilactici* is a GRAS status microorganism, which may allow for simpler adoption by industry (FDA, 2013).

Given the need for validation processes for low water activity foods and *Salmonella*'s increased thermal resistance in a desiccated state, the use of surrogates in low water activity foods could be useful for validation. *E. faecium* NRRL-B2354 and *P. acidilactici* ATCC 8042 have been shown to be surrogates for *Salmonella* in some low water activity foods. The objective of this study was to evaluate the use of *P. acidilactici* ATCC 8042 as surrogate for *Salmonella* in two low water activity foods, toasted oats cereal and peanut butter comparing it to a cocktail of *Salmonella* serovars and to *E. faecium* NRRL-B2354.

2. Materials and methods

2.1. Bacterial culture preparation

The strains used in this research and their sources can be found in Table 1. Stock cultures were either prepared from frozen glycerol stocks from the Center for Food Safety's (CFS) culture collection or from lyophilized cultures from ATCC. Working cultures were cultivated at 37 °C in a shaker culture overnight. *Salmonella* cultures and *E. faecium* were grown in tryptic soy broth (TSB, BD, Franklin Lakes, NJ). *Pediococcus acidilactici* was grown in De Man, Rogosa, and Sharpe (MRS) broth (Research Products International, Mount Prospect, IL). All strains were frozen at –80 °C in a 20% glycerol stock. To test the *Salmonella* working stocks, they were streaked onto xylose lysine deoxycholate agar (BD) monthly.

2.2. Inoculation and thermal inactivation of toasted oats cereal

Inoculation methods were adapted from Chick (2011) (Chick, 2011). *Salmonella* serovars (Enteritidis, Agona, Typhimurium,

Table 1
Bacterial strains used in this research.

Strain	Source	Food source
<i>Salmonella</i> Enteritidis 2415	CFS ^a	Almonds
<i>Salmonella</i> Agona F5567	CFS	Cereal
<i>Salmonella</i> Typhimurium ATCC 14028	ATCC ^b	NA ^c
<i>Salmonella</i> Tennessee K4643	CFS	Peanut Butter
<i>Salmonella</i> Newport MH57137	CFS	NA
<i>Salmonella</i> Heidelberg MH27651	CFS	NA
<i>Enterococcus faecium</i> NRRL-B2354 (ATCC 8459)	ATCC	NA
<i>Pediococcus acidilactici</i> ATCC 8042	ATCC	NA

^a Culture Collection, Center for Food Safety, University of Georgia, Griffin, GA.

^b American Type Culture Collection.

^c Not applicable.

Tennessee, and Newport), *E. faecium*, and *P. acidilactici*, were grown overnight individually in 40 mL of TSB or MRS broth media at 37 °C with shaking. For *Salmonella*, 8 mL of each strain culture were mixed to a final volume of 40 mL. Cocktail or individual cultures (40 mL) were mixed with 20 g of commercially available extruded toasted oats cereal (TOC; ingredients according to the manufacturer's label: whole grain oats, modified corn starch, sugar, salt, tripotassium phosphate, wheat starch, vitamin E) and 360 mL sterile water in stomacher bags for 1 min. TOC samples had an initial (immediately after opening the package) a_w value ranging from 0.18 to 0.30. The inoculated TOC were strained with sterile metal strainer and spread across sterile metal trays. Trays were placed in a 37 °C incubator and dried for 18–24 h. The dried TOC were ground with a sterile mortar and pestle, and then placed in petri dishes for water activity equilibration. The ground TOC were stored in a desiccator with a saturated magnesium chloride solution (Sigma Aldrich, St. Louis, MO) to equilibrate the samples to a water activity of 0.33 for 7–14 d. Water activities were read using an AquaLab (Decagon, Pullman, WA) before thermal kinetics parameters were determined.

Samples of ground, inoculated TOC (approx. 0.05 g) were transferred to 0.2 mL sterile PCR tubes. The samples were placed in a heating block (Digital Dry Bath, Thermo Scientific, Pittsburgh, PA) for thermal inactivation. The times that the tubes took to reach the desired temperature (come up time) were consistently 46 ± 3 s. At pre-determined time points, nine samples were removed, placed in an ice bath and held in a 10% bleach solution for 10 s. The tubes were blotted dry with paper towels. The contents of three tubes were fully emptied with sterile small spatulas into 10 mL tubes of buffered peptone water (BPW). This created three biological replicates per time point. Subsequent dilutions were performed in 9 mL BPW, and 0.1 mL was plated in duplicate onto the appropriate solid media. Plates were incubated at 37 °C for 18–24 h. Thermal inactivation kinetics were determined at 80, 85, 90, and 95 °C. Each experiment was performed independently at least twice.

2.3. Inoculation and thermal inactivation of peanut butter

Peanut butter inoculation methods were adapted from Ma et al., 2009 (Ma et al., 2009). A *Salmonella* cocktail (Enteritidis, Agona, Typhimurium, Tennessee, and Heidelberg) and the two potential surrogates were grown overnight in a total of 100 mL broth cultures at 35 °C in a shaker culture. The inocula were prepared by washing the bacteria with phosphate buffered saline (PBS, pH 7.4), followed by centrifugation (900 × g, 10 min) twice. The washed cells were resuspended in 600 µL of PBS. These cell suspensions were used as inocula. Sixty grams of commercial creamy peanut butter (ingredients: roasted peanuts, sugar, molasses, hydrogenated rapeseed and soybean oils, mono and diglycerides, salt) were weighed into sterile stomacher bags. Bags were suspended in a water bath (Precision Instruments, Sarasota, FL) at 50 °C and held until the peanut butter reached 50 °C. Temperature was monitored by a thermocouple in a bag of uninoculated peanut butter. Before inoculation, bags were removed from the water bath and cooled to 37 °C. The inocula were added and mixed with a sterile spatula for 4 min. The inoculated peanut butter samples were then mixed in a stomacher (Seward, Worthing, UK) for 2 min. Peanut butter samples were stored at room temperature in sealed bags for 3–7 d. Water activity was determined before use in thermal kinetics trials. Samples with a water activity above 0.5 were discarded to reduce variability among measurements due to different water activity.

For thermal inactivation, three 1-g samples were weighed into 200 mL sterile stomacher bags and flattened into a rectangle, approximately 3 × 6 cm. The bags were suspended into a hot water bath, taken out at pre-determined time points, and held in an ice bath for 1 min. Samples were stomached for 2 min at 260 rpm in 9 mL of BPW with 0.1% Tween 80, then serially diluted in 9 mL BPW and plated in duplicate with volumes of 0.1 mL onto the appropriate media. Plates were incubated at 37 °C for 18–24 h. Thermal inactivation curves were performed at 63, 68, 73, and 77 °C. Temperature was monitored by placing

a bag of uninoculated peanut butter in the water bath and reading the temperature with a thermocouple. Each full set of heating experiments were conducted at least two times.

2.4. Data analysis

All data were generated from at least two independent experiments. Thermal inactivation parameters were analyzed using the Weibull model of inactivation (Mafart et al., 2002). Microsoft Excel (Microsoft, Redmond, WA) the add-in GInaFit was used to find the thermal kinetics parameters (Geeraerd et al., 2005). The Weibull model produces two values, the δ value and the β value. The δ value represents the time for the initial one log reduction in the population (van Boekel, 2002). The β value represents the shape parameter, a mathematical description of the shape of the curve (van Boekel, 2002). The R^2 value and root mean square error values (RMSE) were used to analyze the fit of the model. To compare between the *Salmonella* cocktail and the two surrogates, a one-way ANOVA, followed by Tukey Kramer HSD was performed on the δ and β values using JMP (SAS, Cary, NC). Statistical significance was determined at a 95% confidence level ($p < 0.05$). Once δ values were determined, z-values were calculated, and the regression coefficient determined. The z-value is the number of degrees (C) needed to change the δ value by one log. This calculation was done through mathematical equation or estimation using a graph. The equation used was $z = (T1-T2)/(\text{Log}(\delta1)-\text{Log}(\delta2))$.

3. Results

3.1. Thermal inactivation in TOC

At 80 °C, the populations of *Pediococcus* and *Enterococcus* declined more rapidly than the viable count of *Salmonella*, but the final reduction

after 3 h was approx. 1.6 Log CFU/g for all three bacteria (Fig. 1). At higher temperatures, the initial reduction for the three organisms was very similar within the first 30 min, but after longer incubations, the count of *P. acidilactici* had slightly lower counts than the other two bacteria (Fig. 1). When the thermal inactivation kinetic parameters were calculated, the δ value of *Salmonella* was at least two-fold larger ($p < 0.05$) than those of *E. faecium* and *P. acidilactici* at 80 °C (Table 2). The β value of *Salmonella* at 80 °C was also different as well. In the Weibull model, the shape of the curve can be concave ($\beta < 1$) or convex ($\beta > 1$) (van Boekel, 2002). At 80 °C, the β value of *Salmonella* was 1.36, indicating a convex curve. The β values of *E. faecium* and *P. acidilactici* were 0.71 and 0.56, respectively, that corresponded to a concave curve.

At 85 °C, the δ value of *Salmonella* inactivation was larger than the other two bacteria, but none of these differences was statistically significant (Table 2). At 95 °C, there was a marked reduction in δ values compared to the δ values at 90 °C. However, the inactivation of all three bacteria had similar δ values, with a one-way ANOVA ($p \geq 0.05$) resulting in no significant difference among them. Similarly, there were no significant differences among the β values at 85, 90 and 95 °C. Z-values were calculated based on δ values for each bacterium and ranged from 9.3 to 15.6 for *E. faecium* and *P. acidilactici*, respectively (Table 3). *E. faecium*'s regression coefficient (R^2) of z-value calculation were the only R^2 values smaller than 0.9.

3.2. Thermal inactivation in peanut butter

The range of temperatures used with peanut butter was markedly lower than with TOC to obtain measurable values of kinetic parameters (63–77 °C). The mean water activity of peanut butter samples measured right before thermal treatments was 0.39 ± 0.03 . At 63 °C, the *P. acidilactici* and *E. faecium* counts were reduced by 1.5 and 1.1 Log CFU/

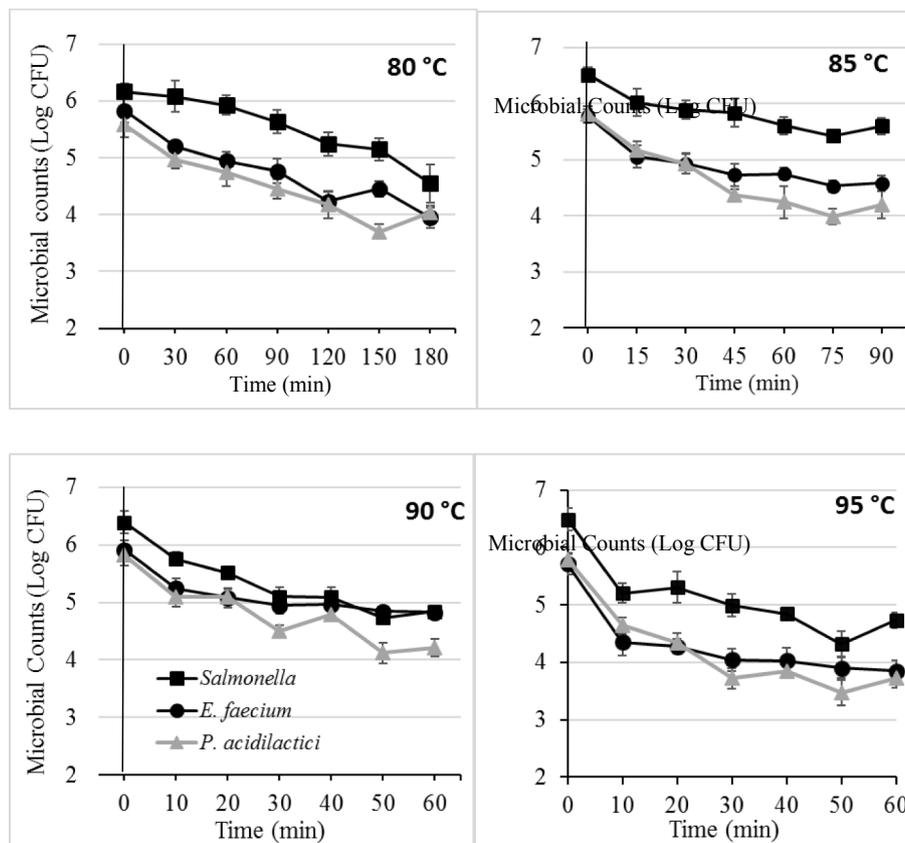


Fig. 1. Thermal death curves of *Salmonella*, *E. faecium* NRRL-B2354, and *P. acidilactici* ATCC 8042 at 80, 85, 90, and 95 °C in toasted oats cereal. *Salmonella* was a mixture that included five serovar strains (Agona, Typhimurium, Tennessee, Enteritidis and Newport).

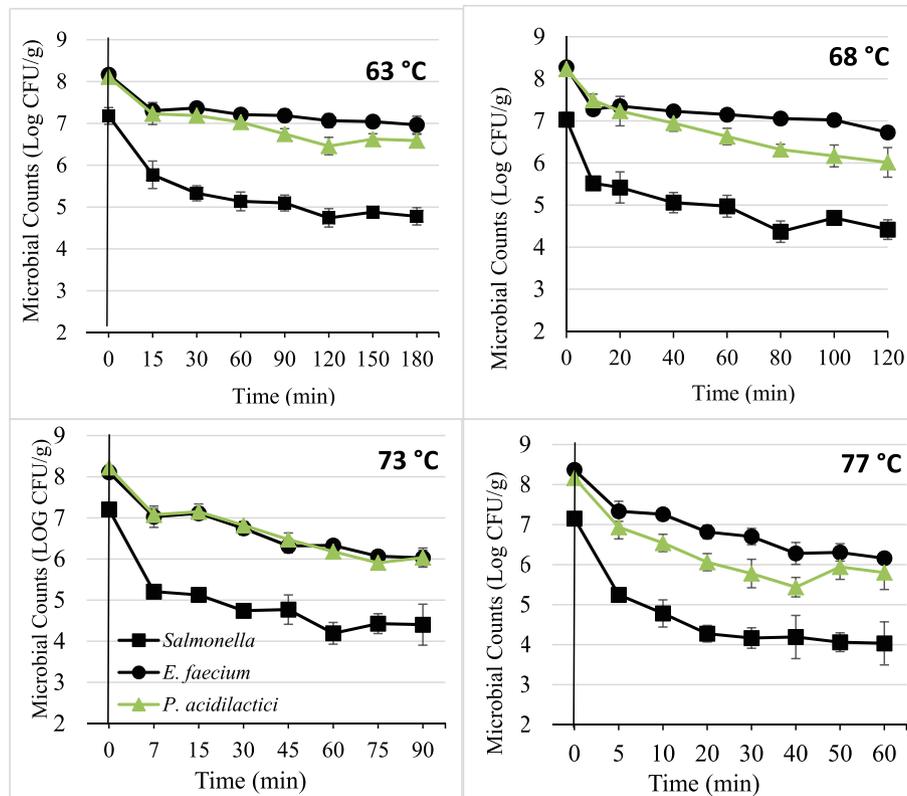


Fig. 2. Thermal death curves of *Salmonella*, *E. faecium* NRRL-B2354, and *P. acidilactici* ATCC 8042 at 63, 68, 73, and 77 °C in peanut butter. *Salmonella* was a mixture that included five serovar strains (Agona, Typhimurium, Tennessee, Enteritidis and Heidelberg).

Table 2

Thermal kinetics parameters of *Salmonella*, *E. faecium* NRRL-B2354, and *P. acidilactici* ATCC 8042 at 80, 85, 90, and 95 °C calculated with the Weibull model in toasted oats cereal. *Salmonella* was a mixture that included five serovar strains (Agona, Typhimurium, Tennessee, Enteritidis and Newport).

Temperature	Bacteria	δ value (min) \pm SD ^a	β value \pm SD ^a	R ² adjusted value	RMSE
80 °C	<i>Salmonella</i>	139.6 \pm 17.2 ^A	1.36 \pm 0.07 ^A	0.96	0.10
	<i>E. faecium</i>	69.5 \pm 7.0 ^B	0.71 \pm 0.2 ^{A, B}	0.95	0.16
	<i>P. acidilactici</i>	63.2 \pm 3.2 ^B	0.56 \pm 0.005 ^B	0.87	0.23
85 °C	<i>Salmonella</i>	86.8 \pm 47.2 ^A	0.50 \pm 0.18 ^A	0.92	0.11
	<i>E. faecium</i>	45.5 \pm 14.7 ^A	0.33 \pm 0.11 ^A	0.93	0.11
	<i>P. acidilactici</i>	27.0 \pm 4.0 ^A	0.52 \pm 0.1 ^A	0.91	0.28
90 °C	<i>Salmonella</i>	23.4 \pm 7.4 ^A	0.51 \pm 0.02 ^A	0.93	0.15
	<i>E. faecium</i>	41.1 \pm 8.1 ^A	0.26 \pm 0.03 ^A	0.97	0.06
	<i>P. acidilactici</i>	24.7 \pm 2.0 ^A	0.56 \pm 0.1 ^A	0.84	0.41
95 °C	<i>Salmonella</i>	7.8 \pm 2.9 ^A	0.31 \pm 0.06 ^A	0.87	0.28
	<i>E. faecium</i>	3.3 \pm 2.5 ^A	0.19 \pm 0.02 ^A	0.94	0.07
	<i>P. acidilactici</i>	7.0 \pm 2.8 ^A	0.36 \pm 0.01 ^A	0.92	0.22

^a Means with different capital letters are significantly different (p < 0.05) for each temperature.

Table 3

Z-values of *Salmonella*, *E. faecium* NRRL-B2354, and *P. acidilactici* ATCC 8042 in toasted oats cereal and peanut butter. *Salmonella* was a mixture that included five serovar strains (Agona, Typhimurium, Tennessee, Enteritidis and Newport).

Bacteria	Food matrix			
	Toasted oats cereal		Peanut butter	
	Z-value (°C)	r ²	Z-value (°C)	r ²
<i>Salmonella</i>	11.2	0.97	7.6	0.99
<i>E. faecium</i>	9.3	0.74	13.2	0.96
<i>P. acidilactici</i>	15.6	0.91	11.4	0.79

g by 120 min, but *Salmonella*'s count reduction was almost 2.5 Log CFU/g (Fig. 2). At 73 °C, the two Gram-positive bacteria had approx. the same 2.0 Log CFU/g reduction after 90 min, and the *Salmonella* count

was reduced by almost 2.9 Log CFU/g. At all four temperatures the reductions in *Salmonella* counts were always greater than *Enterococcus* and *Pediococcus*.

The thermal treatment of peanut butter samples inoculated with the *Salmonella* cocktail had smaller δ values than the two Gram-positive bacteria at all temperatures, but only at 73 °C the difference (1.5 vs. 15.5 min) with *P. acidilactici* was statistically significant (Table 4). At 63, 68 and 77 °C, the δ values of *P. acidilactici* inactivation were more than 15, 3 and 5-fold greater than those of *Salmonella*, but they were not significant. At 63 °C, *E. faecium* had a δ value almost 47-fold larger than the *Salmonella* cocktail (p < 0.05) and at 77 °C, this difference was more than 20 times greater. Only at 68 °C, there was a significant difference between the *E. faecium* and *P. acidilactici* β values. The z-values calculated from δ values ranged from 7.6 min for *Salmonella* to 13.2 min for *P. acidilactici* (Table 3). The 63 °C *Salmonella* δ value was not included in the z-value calculation, as it was 2.0 min at 63 °C and 6.0 min at 68 °C.

Table 4

Thermal kinetics parameters of *Salmonella*, *E. faecium* NRRL-B2354, and *P. acidilactici* ATCC 8042 at 63, 68, 73, and 77 °C calculated with the Weibull model in peanut butter. *Salmonella* was a mixture that included five serovar strains (Agona, Typhimurium, Tennessee, Enteritidis and Newport).

Temperature	Bacteria	δ value (min) \pm SD ^a	β value \pm SD ^a	R ² adjusted value	RMSE
63 °C	<i>Salmonella</i>	2.0 \pm 0.2 ^A	0.19 \pm 0.04 ^A	0.96	0.14
	<i>E. faecium</i>	94.2 \pm 26.0 ^B	0.16 \pm 0.1 ^A	0.93	0.09
	<i>P. acidilactici</i>	31.1 \pm 7.0 ^{A, B}	0.27 \pm 0.04 ^A	0.93	0.13
68 °C	<i>Salmonella</i>	6.0 \pm 2.0 ^A	0.27 ^{A, B}	0.90	0.27
	<i>E. faecium</i>	27.0 \pm 7.3 ^A	0.18 \pm 0.07 ^A	0.91	0.14
	<i>P. acidilactici</i>	21.1 \pm 0.9 ^A	0.46 \pm 0.02 ^B	0.99	0.05
73 °C	<i>Salmonella</i>	1.5 \pm 0.5 ^A	0.25 \pm 0.05 ^A	0.88	0.32
	<i>E. faecium</i>	13.0 \pm 3.4 ^{A, B}	0.37 \pm 0.07 ^A	0.94	0.17
	<i>P. acidilactici</i>	15.5 \pm 5.9 ^B	0.45 \pm 0.05 ^A	0.94	0.19
77 °C	<i>Salmonella</i>	0.4 \pm 0.1 ^A	0.22 \pm 0.05 ^A	0.89	0.28
	<i>E. faecium</i>	8.9 \pm 0.9 ^B	0.38 \pm 0.02 ^A	0.95	0.15
	<i>P. acidilactici</i>	2.6 \pm 1.9 ^{A, B}	0.27 \pm 0.1 ^A	0.87	0.33

^a Means with different capital letters are significantly different ($p < 0.05$) for each temperature.

4. Discussion

Low water activity foods, such as toasted oats cereal and peanut butter, were identified as *Salmonella* vehicles of concern. Due to the implementation of the Food Safety Modernization Act's Preventive Controls Rule, there is a need for validating thermal controls in low water activity foods. Bacterial surrogacy is a viable option for this, especially given *Salmonella*'s increased thermal resistance in low water activity foods. This study aimed to evaluate the use of a potential *Salmonella* surrogate, *P. acidilactici* ATCC 8042 comparing it also to *E. faecium* NRRL-B2354 in commercial toasted oat cereal and peanut butter.

For TOC, our findings suggested that *P. acidilactici* as well as *E. faecium* may be used as a *Salmonella* surrogate at temperatures between 85 and 95 °C. At 80 °C, *Salmonella*'s δ value was significantly higher than both surrogate bacteria's and the β value was significantly higher than *P. acidilactici*. *Salmonella*'s β value at 80 °C was above one, which was unique to that treatment. The β value in the Weibull model describes the shape of the curve. This instance is above one, which means the shape is convex, and the surviving cells have a greater probability of death than the initial population (van Boekel, 2002). When the curves were concave, it meant that the remaining microbial population had a decreased probability of death, compared to the initial population. Because of the differences in shape (β) and the time for the initial 1 Log/CFU reduction (δ) between *Salmonella* and both *E. faecium* and *P. acidilactici* at 80 °C they would not perform satisfactorily as surrogates.

For 85–95 °C, there were no significant differences among the δ and β values of *Salmonella* and the two Gram-positive bacteria. While the δ values for *Salmonella* were consistently larger than for both surrogates, they were not significantly different. The β values were not significantly different among the strains at 85–95 °C either. Interestingly, while *Salmonella* had consistently higher δ values, it did not have the highest z-value. Because of the sufficient similarities, we concluded that *P. acidilactici* ATCC 8042 could have potential as a surrogate for the thermal inactivation of *Salmonella* in toasted oats cereal at temperatures between 85 and 95 °C.

Comparing the inactivation rates obtained with the two matrices there were large differences of for all three bacteria. *Salmonella*'s inactivation in TOC at the lowest temperature (80 °C) was more than 200-fold slower ($\delta = 139.6$ min) than in peanut butter at 77 °C, the highest temperature tested ($\delta = 0.4$ min) (Tables 2 and 4). If the same comparison is made for *E. faecium* and *P. acidilactici*, the differences were not as high (7.8- and 24-fold, respectively), but still significantly smaller. Variations in thermal tolerance of *Salmonella* due to different food matrices has been previously recognized and the effect of fat content has been speculated as a factor that may contribute to reduce its ability to survive a heat shock (Santillana Farakos et al., 2014). In our study, the marked differences observed between TOC and peanut butter

may also be due to different matrix composition, but the influence of heat treatment methodology cannot be completely ruled out. The different rheological characteristics of these two foods led us to use different heat treatment studies. The inactivation in a powder such as ground TOC employed small plastic tubes in a dry heating block according to our methods previously published (Forghani et al., 2018; Maserati et al., 2017). In contrast, because peanut butter had a very different composition and texture, we decided to use the previously published protocol by Ma et al. (2009) that immersed the material in a water bath in a thin film inside of a plastic bag.

Studies investigating the use of *E. faecium* as a surrogate in almonds, oat flour, pet food, and peanut paste have all reported that *E. faecium* had greater heat resistance than *Salmonella* (Ceylan and Bautista, 2015; Enache et al., 2015; Sanghyup et al., 2011; Verma et al., 2018). Similar to previous studies, we also observed consistently higher δ values of *E. faecium* in peanut butter. As a potential surrogate, having a greater tolerance than the target organism can offer an additional safety factor that may address the potential variation in thermal tolerance of *Salmonella* strains and anticipate potential natural environmental factors that may enhance their thermal tolerance.

At all temperatures tested in peanut butter (63–77 °C), *P. acidilactici* had larger δ values than *Salmonella*. At 63 and 77 °C, *E. faecium* had a significantly greater δ value than *Salmonella*. At 73 °C, *P. acidilactici* also had a significantly greater δ value than *Salmonella*. There were no significant differences between the β values of either Gram-positive bacteria and *Salmonella*. While there were significant differences between the δ values of one surrogate and *Salmonella* at most temperatures studied, the potential surrogate was the more heat resistant bacteria tested. Since the potential surrogate was more resistant, it could serve as a conservative estimate for the thermal inactivation of *Salmonella* in peanut butter. Our findings indicated that *P. acidilactici* ATCC 8042 could be used as surrogate for *Salmonella* in peanut butter at 63–77 °C if a safety factor is desirable in validation testing.

Several studies have investigated the thermal inactivation of *Salmonella* in peanut butter and there is at least one that explored the possibility of using *E. faecium* NRRL-B2354 as a surrogate organism in this food matrix (Kataoka et al., 2014; Ma et al., 2009; Shachar and Yaron, 2006). The δ values measured at 73 and 77 °C in this study (1.5 and 0.4 min, respectively) were very similar to those calculated in a study that used three serovars at 70 and 80 °C (0.25 and 1.1 min, respectively) (Shachar and Yaron, 2006). At 77 °C, Ma et al. (2009) reported 1-Log CFU/g inactivation times slightly greater (3.5–5.2 min) than our δ values they tested three groups of *Salmonella* serovars. A more recent study compared the inactivation of *E. faecium* NRRL-B2354 to individual strains of *S. Tennessee* and *S. Typhimurium* in peanut butter (Kataoka et al., 2014). In that report, kinetic parameters were not calculated, but it was observed that the count of *E. faecium* was reduced 1.4 Log CFU/g at 75 °C after 50 min. In our results, this strain

was reduced approx. 1.7 and 2.0 Log CFU/g at 73 and 77 °C, respectively.

While there were some differences between the *E. faecium* and *P. acidilactici* strains and *Salmonella*, there was only one difference between the two surrogates. The close relationship between the thermal kinetics parameters of *E. faecium* NRRL-B2354 and *P. acidilactici* ATCC 8042 suggested the potential for the use of *P. acidilactici* as a surrogate for *Salmonella* in other low water activity foods. Unlike *E. faecium* NRRL-B2354, *P. acidilactici* ATCC 8042 has GRAS status, which may allow for easier deployment for use as a surrogate.

This study investigated the potential for *P. acidilactici* ATCC 8042 to serve as a surrogate for *Salmonella* and generated additional data on *E. faecium* NRRL-B2354 for possible application in two low water activity foods, toasted oats cereal and peanut butter. The next phase of this study is to validate the surrogates' use through using processing equipment, such as extruders or heated mixers. A second potential study would be to expand the products used and validate the use of both surrogates in other low water activity foods.

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References

- Annous, B.A., Kozempel, M.F., 1998. Influence of growth medium on thermal resistance of *Pediococcus* sp NRRL B-2354 (formerly *Micrococcus freudenreichii*) in liquid foods. *J. Food Prot.* 61, 578–581.
- Beuchat, L.R., Mann, D.A., 2015. Survival of *Salmonella* in cookie and cracker sandwiches containing inoculated, low-water activity fillings. *J. Food Prot.* 78, 1828–1834.
- Borowski, A.G., Ingham, S.C., Ingham, B.H., 2009. Validation of ground-and-formed beef jerky processes using commercial lactic acid bacteria starter cultures as pathogen surrogates. *J. Food Prot.* 72, 1234–1247.
- Buchanan, R., Williams, E., 2013. Hazard analysis and critical control point system: use in managing microbiological food safety risks. In: Doyle, M.P., Buchanan, R. (Eds.), *Food Microbiology: Fundamentals and Frontiers*. ASM Press, Washington, DC, pp. 1039–1057.
- CDC, 2009. Multistate outbreak of *Salmonella* infections associated with peanut butter and peanut butter-containing products — United States, 2008–2009. *Morb. Mortal. Wkly. Rep.* 58, 85–90.
- CDC, 2008. Multistate outbreak of *Salmonella* Agona infections linked to rice and wheat puff cereal. <https://www.cdc.gov/salmonella/2008/rice-wheat-puff-cereal-5-13-2008.html>.
- CDC, 2007. Multistate outbreak of *Salmonella* serotype Tennessee infections associated with peanut butter — United States, 2006–2007. *Morb. Mortal. Wkly. Rep.* 56, 521–524.
- CDC, 2004. Outbreak of *Salmonella* serotype Enteritidis infections associated with raw almonds—United States and Canada, 2003–2004. *Morb. Mortal. Wkly. Rep.* 53, 484–487.
- CDC, 1998. Multistate outbreak of *Salmonella* serotype Agona infections linked to toasted oats cereal — United States, April–May, 1998. *Morb. Mortal. Wkly. Rep.* 47, 462–464.
- Ceylan, E., Bautista, D.A., 2015. Evaluating *Pediococcus acidilactici* and *Enterococcus faecium* NRRL B-2354 as thermal surrogate microorganisms for *Salmonella* for in-plant validation studies of low-moisture pet food products. *J. Food Prot.* 78, 934–939.
- Chick, M., 2011. Thermal Inactivation Kinetics of *Salmonella* Serovars on Dry Cereal. University of Minnesota, St. Paul, MN.
- Enache, E., Kataoka, A., Black, D.G., Napier, C.D., Podolak, R., Hayman, M.M., 2015. Development of a dry inoculation method for thermal challenge studies in low-moisture foods by using talc as a carrier for *Salmonella* and a surrogate (*Enterococcus faecium*). *J. Food Prot.* 78, 1106–1112.
- FDA, 2013. GRN No. 463. [WWW Document]. GRAS Not. https://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=463&sort=GRN_No&order=DESC&startrow=1&type=advanced&search=%C2%A4%C2%A4lactic%20acid%20bacteria%C2%A4.
- Forghani, F., den Bakker, M., Liao, J.-Y., Payton, A.S., Futral, A.N., Diez-Gonzalez, F., 2018. *Salmonella* and enterohemorrhagic *Escherichia coli* serogroups O45, O121, O145 in wheat flour: effects of long-term storage and thermal treatments. *Front. Microbiol.* <https://doi.org/10.3389/fmicb.2019.00323>.
- Fudge, J., Dunn, M., Pike, O., Robison, R., Steele, F., 2016. He isolation and identification of *Pantoea dispersa* strain JFS as a non-pathogenic surrogate for *Salmonella* Typhimurium phage type 42 in flour. *Int. J. Food Microbiol.* 219, 1–6.
- Geeraerd, A., Valdramidis, V., Van Impe, J.F., 2005. GlnaFIT, a freeware tool to assess non-log-linear microbial survivor curves. *Int. J. Food Microbiol.* 102, 95–105.
- Gruzdev, N., Pinto, R., Sela, S., 2011. Effect of desiccation on tolerance of *Salmonella enterica* to multiple stresses. *Appl. Environ. Microbiol.* 77, 1667–1673. <https://doi.org/10.1128/AEM.02156-10>.
- Juneja, V., Mukhopadhyay, S., Marks, H., Mohr, T.B., Warning, A., Datta, A., 2014. Predictive thermal inactivation model for effects and interactions of temperature, NaCl, Sodium pyrophosphate, and sodium lactate on *Listeria monocytogenes* in ground beef. *Food Bioprocess Technol.* 7, 437–446. <https://doi.org/10.1007/s11947-013-1102-z>.
- Kataoka, A., Enache, E., Black, D.G., Elliott, P.H., Napier, C.D., Podolak, R., Hayman, M.M., 2014. Survival of *Salmonella* Tennessee, *Salmonella* Typhimurium DT104, and *Enterococcus faecium* in peanut paste formulations at two different levels of water activity and fat. *J. Food Prot.* 77, 1252–1259. <https://doi.org/10.4315/0362-028X.JFP-13-553>.
- Ma, L., Kornacki, J.L., Zhang, G.D., Lin, C.M., Doyle, M.P., 2007. Development of thermal surrogate microorganisms in ground beef for in-plant critical control point validation studies. *J. Food Prot.* 70, 952–957.
- Ma, L., Zhang, G., Gerner-Smidt, P., Mantripragada, V., Ezeoke, I., Doyle, M.P., 2009. Thermal inactivation of *Salmonella* in peanut butter. *J. Food Prot.* 72, 1596–1601.
- Mafart, P., Couvert, O., Gaillard, S., Leguerinel, I., 2002. On calculating sterility in thermal preservation methods: application of the Weibull frequency distribution model. *Int. J. Food Microbiol.* 72, 107–113.
- Maserati, A., Fink, R.C., Lourenco, A., Julius, M.L., Diez-Gonzalez, F., 2017. General response of *Salmonella enterica* serovar Typhimurium to desiccation: a new role for the virulence factors *sopD* and *sseD* in survival. *PLoS One.* <https://doi.org/10.1371/journal.pone.0187692>.
- Russo, E.T., Biggerstaff, G., Hoekstra, R.M., Meyer, S., Patel, N., Miller, B., Quick, R., 2013. A recurrent, multistate outbreak of *Salmonella* serotype Agona infections associated with dry, unsweetened cereal consumption, United States, 2008. *J. Food Prot.* 76, 227–230. <https://doi.org/10.4315/0362-028X.JFP-12-209>.
- Sanghyup, J., Marks, B.P., Ryser, E.T., 2011. Quantifying the performance of *Pediococcus* sp. (NRRL B-2354: *Enterococcus faecium*) as a nonpathogenic surrogate for *Salmonella* Enteritidis PT30 during moist-air convection heating of almonds. *J. Food Prot.* 74, 603–609.
- Santillana Farakos, S.M., Schaffner, D.W., Frank, J.F., 2014. Predicting survival of *Salmonella* in low-water activity foods: an analysis of literature data. *J. Food Prot.* 77, 1448–1461. <https://doi.org/10.4315/0362-028X.JFP-14-013>.
- Shachar, D., Yaron, S., 2006. Heat tolerance of *Salmonella enterica* serovars Agona, Enteritidis, and Typhimurium in peanut butter. *J. Food Prot.* 69, 2687–2691.
- Sinclair, R.G., Rose, J.B., Hashsham, S.A., Gerba, C.P., Haase, C.N., 2012. Criteria for selection of surrogates used to study the fate and control of pathogens in the environment. *Appl. Environ. Microbiol.* 78, 1969–1977. <https://doi.org/10.1128/AEM.06582-11>.
- Stevenson, K., Ito, K., Beuchat, L., Marks, B.P., Ashton, D., 2014. Guidelines for using *Enterococcus faecium* NRRL B-2354 as a surrogate microorganism in almond process validation. [WWW Document]. https://www.almonds.com/sites/default/files/guidelines_for_using_enterococcus_faecium_nrri_b_2354_as_a_surrogate_microorganism_in_almond_process_validation.pdf.
- van Boekel, M.A.J.S., 2002. On the use of the Weibull model to describe thermal inactivation of microbial vegetative cells. *Int. J. Food Microbiol.* 74, 139–159.
- Verma, T., Wei, X., Lau, S.K., Bianchini, A., Eskridge, K.M., Subbiah, J., 2018. Evaluation of *Enterococcus faecium* NRRL B-2354 as a surrogate for *Salmonella* during extrusion of low-moisture food. *J. Food Sci.* 83, 1063–1073.