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Research Paper

Inoculation Protocols Influence the Thermal Resistance of *Salmonella* Enteritidis PT 30 in Fabricated Almond, Wheat, and Date Products

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ABSTRACT

Inoculation methods in pathogen inactivation studies ideally represent conditions that might occur in real-world scenarios. Surface contamination in or on low-moisture foods affects *Salmonella* thermal resistance, which is critically important for process validation applications. The objective of this study was to quantify the effect of inoculation protocol on the thermal resistance of *Salmonella* Enteritidis PT 30 in fabricated low-moisture foods. Almond meal, almond butter, wheat meal, wheat flour, and date paste were inoculated via prefabrication and postfabrication protocols. In the prefabrication protocol, kernels and fruits were surface inoculated and equilibrated to a target water activity (a_w) (0.40 for almond and wheat products, 0.45 for date products) before fabricating meal, butter, flour, or paste and then reequilibrating the samples to the target a_w. In the postfabrication protocol, meal, butter, flour, and paste were fabricated before inoculation and equilibration. All inoculated and equilibrated samples were subjected to isothermal treatment (80°C), pulled sequentially during processing, cooled, serially diluted, and plated to enumerate survivors. Log-linear and Weibull-type models were fit to the *Salmonella* survivor data and were compared via the corrected Akaike information criterion. Pre- and postfabrication protocols resulted in significant differences ($P < 0.05$) in *Salmonella* thermal resistance in all products. Overall, the thermal resistance of *Salmonella* Enteritidis PT 30 in almond products was greater ($P < 0.05$) than in wheat products, which was also greater ($P < 0.05$) than in date paste. Additionally, *Salmonella* was more thermally resistant in almond products and date paste when inoculated pre- rather than postfabrication; however, the opposite was true for wheat products. These results indicate that the means of inoculation can significantly affect thermal resistance of *Salmonella* in low-moisture foods.

Key words: Bacteria; Inoculation; Low moisture; Pathogen; Thermal inactivation

Salmonellosis outbreaks associated with low-moisture foods, such as almonds, nut butter, and cereal products *(3, 4, 11, 19),* are an emerging and important food safety challenge. In one outbreak from 2012, 42 individuals across 20 states were infected by consuming contaminated peanut butter *(3)*. Dried fruit products have also been recalled *(26, 27),* with *Salmonella* having been detected on commercially available dried fruits, such as raisins and prunes *(30)*.

Previous reports of bacterial survival or inactivation in low-moisture foods are based on a range of inoculation methods. Ideally, the inoculation methods should yield bacterial responses that reflect actual contamination and processing scenarios. For inoculum preparation, *Salmonella* strains have been grown in tryptic soy broth (TSB) *(7, 16, 23)* or brain heart infusion broth *(8, 9)*. Bacteria were harvested and resuspended in peptone water *(14),* binary water-glycerol solution *(23),* or peanut oil (for peanut butter) *(8, 9)*. The means of dispersing the inoculum in the food matrix was product dependent and included hand mixing *(24),* machine stomaching *(23),* misting or transfer from sand *(1),* a mortar for food powder *(14),* or a sterile wooden tongue depressor for nut butter products *(2, 16)*. Initial pathogen inoculations in samples have been highly variable, ranging from 4.5 to 9.0 log for peanut butter *(12, 13, 16, 20)*.

The impact of certain aspects of inoculation protocols on *Salmonella* thermal resistance in low-moisture foods has been reported in a few studies. Ma et al. *(16)* found that *Salmonella* thermal resistance in peanut butter increased with increasing incubation time during inoculum preparation. Similarly, Keller et al. *(13)* reported that *Salmonella* growth procedures, including temperature and growth media, also impacted *Salmonella* thermal resistance in peanut butter. More recently, when Hildebrandt et al. *(10)* used five different methods for inoculating *Salmonella* into wheat flour, significantly different survival kinetics were obtained at the same water activity (0.45 a_w) and temperature (80°C). Additionally, a mist-inoculation procedure was shown to result in lower *Salmonella* survival than did a sand-inoculation procedure for stored dried fruit *(1)*.

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Heating is the most common method to inactivate *Salmonella,* and therefore, thermal resistance is of paramount importance in most pasteurization validations *(5).* Many factors affect thermal resistance of *Salmonella,* including the strains or serovars *(9)* and the a_w in studies that involve low-moisture foods. Thermal resistance generally increases when a_w decreases; Smith and Marks *(23)* reported that the $D_{80°C}$ for *Salmonella* Enteritidis PT 30 in wheat flour at 0.6 a_w was 1.3 min, but it increased to 7.3 min at 0.3 a_w. The $D_{90°C}$ for *Salmonella* in peanut butter at 0.2 a_w (7 min) was higher than at 0.6 a_w (3 min) *(9).* However, some studies have not reported initial postinoculation, or dynamic, a_w of the food matrix *(20, 21).*

Many studies have examined thermal resistance of bacteria; however, very few have assessed the thermal resistance of the same *Salmonella* strains in the same product at the same a_w and temperature. When similar treatments are used, large differences in the reported thermal resistances are sometimes seen. At the same a_w (~0.40 to 0.45) and temperature (90°C), the thermal resistance of *Salmonella* Tennessee in peanut butter, when inoculated by adding strains directly into the matrix *(9),* was five times less than in another study *(15)* in which the strains were suspended in peanut oil prior to introduction into the peanut butter. Consequently, increasing evidence suggests that the inoculum preparation and methodologies are likely key factors affecting thermal resistance and, therefore, any process validation relying on the resulting inactivation data or parameters.

Therefore, the objective of this study was to quantify the effect of inoculation protocol on the thermal resistance of *Salmonella* Enteritidis PT 30 in fabricated low-moisture foods (almond, wheat, and date products).

MATERIALS AND METHODS

Overall, the experimental design consisted of inoculating almond meal, almond butter, wheat meal, wheat flour, and date paste via two different inoculation protocols (prefabrication and postfabrication). Thereafter, the thermal resistances of *Salmonella* in these samples were compared by performing isothermal heat treatments in triplicate. In general terms, the prefabrication protocols entailed inoculation of intact natural products (i.e., whole almond kernels, wheat kernels, and date pieces), which would correspond to environmental, in-field, or preprocessing contamination, and then those products were processed to produce meal, flour, butter, or paste. In contrast, the postfabrication protocols entailed inoculation of the fabricated products after they were already produced, which would correspond to an in-plant or postprocessing contamination event. All known prior thermal inactivation studies with fabricated low-moisture products have been conducted using a postfabrication inoculation protocol.

Almond meal and almond butter. Almonds (Nonpareil, size 27/30, Select Harvest, Turlock, CA) were sourced from a retail supplier, vacuum packed (350 g per bag), and stored at ~2.5°C for up to a year. Almond meal and almond butter were fabricated using a food processor (model FP21, Hamilton Beach Brands, Inc., Glen Allen, VA). To produce almond meal, whole almonds (100 g) were ground at the lowest speed setting for 45 s and sieved through US standard sieves no. 20 and 80 (W.S. Tyler, Inc., Mentor, OH), capturing the material between the two sieves as the meal. Almond butter was produced by similarly grinding 200 g of almonds for 15 min total, while adding dry ice pellets (~30 mL) every 2 min to maintain product temperature below 40°C (confirmed via a handheld infrared thermometer, model 566, Fluke Corporation, Everett, WA).

Wheat meal and wheat flour. Organic soft white whole wheat kernels (*Triticum aestivum,* Eden Foods Inc., Clinton, MI) were stored in their original package at room temperature (~20°C) for up to 6 months. Wheat meal and wheat flour were fabricated by milling whole wheat kernels (50 g) for 45 s in a coffee mill (model 501, Jura-Capresso Inc., Montvale, NJ). Fabricated wheat samples were sieved through US standard sieves no. 20, 80, and 200. Ground product passing through a no. 20 sieve, but not through a no. 80 sieve, was called wheat meal, whereas ground product passing through a no. 80 sieve, but not through a no. 200 sieve, was termed wheat flour.

Date paste. Dates (medjool, jumbo) were purchased from a retail supplier (Nuts.com, Cranford, NJ) and stored in their original package at ~2.5°C for up to a year. Date paste was fabricated by feeding dates through a meat grinder plate with holes 1 cm in diameter (model K5-A, KitchenAid, Benton Harbor, MI). The resulting paste was then fed through the grinder two more times to ensure homogeneity, which was determined by sampling inoculated date paste and enumerating for *Salmonella* survivors in five subsamples per replication (~1 g each)

Inoculation and equilibration. The general inoculation preparation method was derived from the procedures of Danyluk et al. *(7). Salmonella enterica* serovar Enteritidis PT 30, previously obtained from Dr. Linda Harris (University of California, Davis), was kept frozen at −80°C in a concentrated culture containing 20% glycerol. The frozen culture was subjected to two successive 24-h (37°C) transfers in 17% TSB (m/m) (Difco, BD, Franklin Lakes, NJ) containing 0.6% yeast extract (Difco, BD). Thereafter, a plate (150 by 15 mm) of Trypticase soy agar (TSA; Difco, BD) containing 0.6% yeast extract (TSAYE) was spread for confluent growth and incubated for 24 h (37°C).

For prefabrication inoculation of almond and wheat products, the lawn cultures were each harvested in 10 mL of 0.1% peptone water. Thereafter, 8 mL of the liquid suspension (~$10^{7.5}$ to 10^9 CFU/mL) was added directly to 100 g of either almond or wheat kernels and mixed in a sterile plastic bag for 1 min. These wet-inoculated samples were placed on filter paper (P8, Fisher Scientific, Pittsburgh, PA) in an open plastic container, dried (~3 h) in a biosafety cabinet, and then placed in an equilibration chamber (described in the "Equilibration" section) until they reached the target a_w (0.40 ± 0.02). After equilibration, the samples were processed into meal, flour, or butter and were reequilibrated as described below.

For postfabrication inoculation of almond and wheat samples, the *Salmonella* inocula (8 mL, grown and harvested as described above) were pelleted by centrifugation (model Sorvall RC 6 plus, SS-34 rotor, Thermo Fisher Scientific, Waltham, MA) at 2,988 × *g* for 15 min. To minimize a_w change of sample during inoculation (and to prevent physical changes caused by the addition of water to the meals and powder), the *Salmonella* pellet was introduced into 50 g of almond meal, almond butter, wheat meal, or wheat flour and hand mixed for 3 min in a sterile 24-oz (710-mL) plastic bag (Nasco, Fort Atkinson, WI). Inoculated samples then were equilibrated (as described below) until they reached the target a_w (0.40 ± 0.02).

In the pre- and postfabrication protocols, date samples were inoculated using cell pellets that were produced by the same method as the postfabrication protocol (described above) for almond and wheat samples. Based on preliminary tests, insufficient homogeneity of inoculation was achieved by directly introducing the pellet into the date paste, because the highly viscous or semisolid structure of the paste impeded uniform distribution of a solid pellet. Therefore, the pellets were resuspended in 2 mL of 0.1% peptone water and were homogenized using a vortex (model G-560, Scientific Industries Inc., Bohemia, NY). This highly concentrated suspension for inoculation contained $\sim 10^{11}$ CFU/mL.

For prefabrication inoculation, whole dates were each cut into 12 pieces (~1.8 g each) for faster equilibration. Each date piece was spot inoculated (200 μL of total inoculum across 12 pieces) on the date skin, dried for ≥20 min in a biosafety cabinet, and then conditioned to ~0.45 a_w in an equilibration chamber (described below) for up to 1 week. Date paste was fabricated by grinding the inoculated date pieces, as previously described. If the a_w after grinding was not 0.45 ± 0.02, the paste was returned to the chamber and reequilibrated to the target a_w (0.45). However, if the number of days the product spent reequilibrating as paste exceeded the number of days spent originally equilibrating as inoculated pieces, it was considered unusable and was discarded, in order to control the overall treatment for both the intact date and paste.

For postfabrication inoculation, dates were passed once through the grinder (previously described), and 600 μL of the concentrated *Salmonella* suspension then was added to 60 g of ground dates. The inoculated date paste was then passed through the grinder four more times to evenly distribute the inoculum prior to equilibration to 0.45 ± 0.02 a_w in the equilibration chamber.

Equilibration. Samples were placed in custom-designed equilibration chambers *(23)* to adjust and control the sample a_w. Controlled-humidity air (±0.2%) obtained by mixing air passed through a desiccant column (dry air) or a water column (wet air) was monitored and controlled by a humidity sensor (DHT 22, Adafruit Industries, New York, NY) and a microcomputer. Batches of samples (~300 g of almonds, 100 g of wheat, and 50 g of dates) were equilibrated to 0.40 ± 0.02 (almonds and wheat) or 0.45 ± 0.02 (dates) a_w. Total equilibration times were 6 to 9 days for the almond meal, wheat meal, and wheat flour, and 11 to 14 days for the almond butter and date paste.

a_w measurement. a_w of representative samples (pulled after mixing the bulk inside the equilibration chamber) was measured daily (a_w meter, AquaLab 3TE, Decagon Devices, Pullman, WA) to confirm that the target a_w was reached.

Thermal treatment. After equilibration to the target a_w, samples (~0.7 g of almond meal, 1.2 g of almond butter, 0.6 g of wheat meal, 0.5 g of wheat flour, and 1.2 g of date paste) were loaded into sealed aluminum test cells *(6)* in the equilibration chamber to prevent a_w changes. Sample thickness in the aluminum test cells was less than 1 mm. Samples were heated in an isothermal water bath set at 80.5°C (GP-400, Neslab, Newington, NH). Come-up time for the product to reach the target temperature (79.5°C) was measured in six replicates for each sample type, using a test cell with a T-type thermocouple probe positioned at the geometric center of the sample; this was averaged for use in all further experiments. After reaching the come-up time (2.0 ± 0.1 min for almond meal, 2.8 ± 0.1 min for almond butter, 1.3 ± 0.1 min for wheat meal, 1.4 ± 0.3 min for wheat flour, and 2.5 ± 0.1 min for date paste), the initial (time zero) sample was removed, and subsequent samples were pulled at predetermined time points and were immediately cooled in an ice bath to halt further bacterial inactivation.

Recovery and enumeration. Samples were aseptically removed from the test cells, diluted (1:10 dilution) in 0.1% peptone water, and homogenized by stomaching for 3 min (model 1381/471, Neutec Group Inc., Farmingdale, NY). Serial dilutions in 0.1% peptone water were plated in duplicate on TSAYE supplemented with 0.05% ammonium ferric citrate and 0.03% sodium thiosulfate pentahydrate (Fisher Chemical, Fair Lawn, NJ), which was a nonselective differential medium. The plates were incubated for 48 h at 37°C prior to counting the black colonies as *Salmonella*. Preliminary tests with uninoculated samples yielded no such colonies for any of the materials used in this study.

Statistical analyses. Initial *Salmonella* populations and initial a_w values from the pre- and postfabrication methods were compared using the paired *t* test (Microsoft Excel 2013 software, Microsoft Inc., Seattle, WA). For the prefabrication method, a_w and *Salmonella* populations on the initial inoculated samples (kernels or fruits) and final samples (meal, butter, paste, or flour) also were compared via paired *t* test.

Reproducibility for each product was determined by calculating the standard error of replication as follows:

$$\sigma_{rep} = \sqrt{\frac{\sum_{j=1}^{m}\sum_{i=1}^{n}(y_{ij}-\bar{y}_i)^2}{m\cdot n-m}} \quad (1)$$

where *m* is the number of data points over time for each survival curve, *n* is the number of replications for each observation point, and *y* is the *Salmonella* population (log CFU/g).

After pooling all triplicate data within each treatment, the inactivation model parameters were estimated using nlinfit (nonlinear regression routine in the statistical toolbox) in MATLAB (version R2016a, MathWorks Inc., Natick, MA) for the log-linear and Weibull models. The log-linear model was estimated by the following equation:

$$\log\frac{N}{N_0} = -\frac{t}{D(T)} \quad (2)$$

where N and N_0 are the populations (CFU/g) at times t and 0, respectively, t is the time of the isothermal treatment (min), and $D(T)$ is the time (min) required to reduce the microbial population by 90% at a specified temperature (T, °C).

The Weibull model parameters were estimated, according to the following equation *(28)*:

$$\log\frac{N}{N_0} = \left(\frac{-t}{\delta}\right)^p \quad (3)$$

where p is the shape factor, and δ is the location factor (min). The estimated time for a 1-log reduction (min) in each sample was calculated by the following equation *(28)*:

$$t = \delta\cdot(-\ln(10^{-d})^{\frac{1}{p}} \quad (4)$$

where d is the number of decimal reductions (i.e., $d = 1$ for a 1-log reduction).

The corrected Akaike information criterion (AIC_c) *(18)* was calculated to select the most-likely-correct model, with the lower AIC_c indicating the more-likely-correct model:

$$AIC_c = n\cdot\ln\left(\frac{SS}{n}\right) + 2K + \frac{2K(K+1)}{n-K-1} \quad (5)$$

where n is the number of data points, SS is the sum of squares of

TABLE 1. Salmonella *population and water activity of almond meal, almond butter, date paste, wheat meal, and wheat flour subjected to prefabrication and postfabrication inoculation protocols before heating*[a]

Product	*Salmonella* population (log CFU/g)		Water activity	
	Prefabrication protocol	Postfabrication protocol	Prefabrication	Postfabrication
Almond meal	8.0 ± 0.3 A	9.2 ± 0.2 B	0.410 ± 0.014 A	0.393 ± 0.003 A
Almond butter	7.7 ± 0.2 A	9.3 ± 0.3 B	0.414 ± 0.012 A	0.406 ± 0.004 A
Date paste	7.7 ± 0.2 A	7.6 ± 0.2 A	0.450 ± 0.015 A	0.456 ± 0.019 A
Wheat meal	8.8 ± 0.1 A	9.7 ± 0.1 B	0.406 ± 0.009 A	0.405 ± 0.005 A
Wheat flour	9.0 ± 0.1 A	9.7 ± 0.1 B	0.392 ± 0.017 A	0.400 ± 0.012 A

[a] Values are means ± standard deviations. Within a row (and same measurement), means followed by a common letter were not significantly different ($\alpha = 0.05$).

residuals, and K is the number of parameters plus 1. The relative probability of each model being the correct model also was calculated as follows *(18)*:

$$\text{Relative likelihood of log} - \text{linear over Weibull model} = \frac{e^{\left(\frac{AIC_{c,\ \log-\text{linear model}} - AIC_{c,\text{Weibull model}}}{2}\right)}}{1 + e^{\left(\frac{AIC_{c,\ \log-\text{linear model}} - AIC_{c,\text{Weibull model}}}{2}\right)}} \quad (6)$$

Model parameters for pre- and postfabrication samples of each product were also compared using the paired t test (Microsoft Excel 2013).

RESULTS AND DISCUSSION

Sample preparation and a_w control. For the prefabrication methods, *Salmonella* Enteritidis PT 30 populations on the products after fabrication (i.e., meal, butter, flour, paste) were not significantly different from the populations on the intact products prior to fabrication (i.e., almonds, wheat kernels, date pieces) ($P > 0.05$). Additionally, the pre- and postfabrication products had similar a_w ($P > 0.05$).

In a comparison of initial *Salmonella* Enteritidis PT 30 populations between the pre- and postfabrication protocols before heating (Table 1), initial populations in date paste were statistically equivalent for the pre- and postfabrication methods ($P > 0.05$, 7.6 to 7.7 log CFU per sample). Additionally, separate subsampling tests yielded good homogeneity for both date preparation methods (±0.2 and ±0.3 log CFU/g for pre- and postfabrication, respectively).

The bacterial populations for almond and wheat products in the prefabrication method were significantly lower ($P < 0.05$) than those for the postfabrication method (Table 1), because the *Salmonella* Enteritidis PT 30 concentration in the pellet inoculum for postfabrication was higher than in the liquid inoculum for prefabrication. For date paste, the initial pre- and postfabrication populations of *Salmonella* Enteritidis PT 30 were similar ($P > 0.05$) and were lower than the other product types because the inoculum contained fewer cells. However, prior results have shown that initial inoculation level does not affect thermal resistance of *Salmonella* Enteritidis PT 30 in low-moisture products *(10)*; therefore, comparisons of thermal resistance between pre- and postfabrication samples should not be affected by these differences in initial population.

Model selection. Model parameters (Table 2) for the log-linear and Weibull models were estimated using *Salmonella* Enteritidis PT 30 survival data (Fig. 1). AIC_c analysis (Table 2) gave the most-likely-correct model for

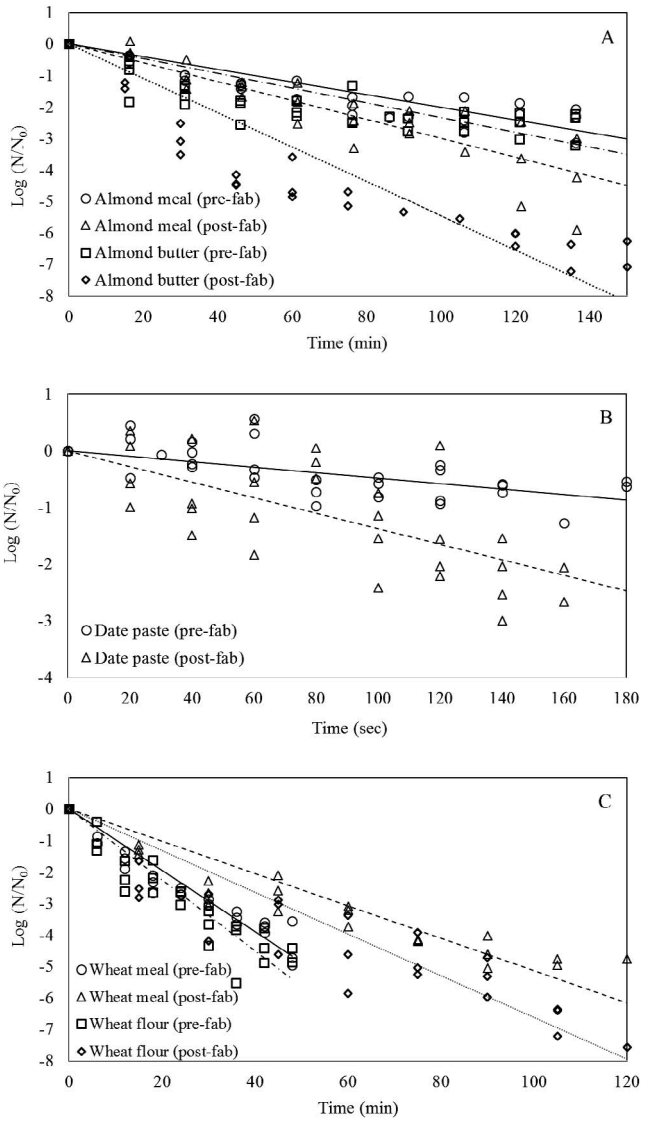

FIGURE 1. *Isothermal (80°C)* Salmonella *survival curves and log-linear model fit after prefabrication and postfabrication inoculation of (A) almond meal and almond butter at 0.4 a_w, (B) date paste at 0.45 a_w, and (C) wheat meal and wheat flour at 0.4 a_w.*

TABLE 2. *Standard errors of replications, $D_{80°C}$-values determined by nonlinear regression of the* Salmonella *survivor curves, and δ and* p *Weibull parameters for the almond meal, almond butter, date paste, wheat meal, and wheat flour subjected to prefabrication and postfabrication inoculation protocols*[a]

		Log-linear model			Weibull model					
Products	SE of replications (log CFU/g)	*D*-value (min)	RMSE (log CFU/g)	AIC_c	δ (min)	*p*	RMSE (log CFU/g)	AIC_c	Estimated time for 1-log reduction (min)	Relative likelihood of log-linear over Weibull model (per AIC)
Almond meal										
Prefabrication	0.33	49.8 ± 2.1 A	0.418	−54.1	29.6 ± 4.5 A	0.61 ± 0.07 A	0.308	−72.9	29.6 ± 5.3 A	0.0001
Postfabrication	0.85	33.4 ± 1.7 B	0.729	−18.6	34.1 ± 6.4 A	1.02 ± 0.15 B	0.740	−14.4	34.1 ± 6.8 A	0.8870
Almond butter										
Prefabrication	0.90	42.9 ± 2.6 A	0.694	−20.7	8.5 ± 3.5 A	0.37 ± 0.06 A	0.390	−57.4	8.5 ± 3.0 A	∼0.0000
Postfabrication	0.49	18.3 ± 1.0 B	1.132	2.0	4.7 ± 1.5 A	0.57 ± 0.06 A	0.477	−32.0	3.4 ± 0.9 A	∼0.0000
Date paste										
Prefabrication	0.31	3.5 ± 0.5 A	0.322	−72.5	3.3 ± 0.6 A	1.11 ± 0.38 A	0.327	−66.8	3.3 ± 0.4 A	0.9436
Postfabrication	0.79	1.2 ± 0.1 B	0.696	−20.5	1.1 ± 0.2 B	1.30 ± 0.37 A	0.699	−18.8	1.4 ± 0.3 B	0.6995
Wheat meal										
Prefabrication	0.80	10.3 ± 0.3 A	0.422	−59.7	5.8 ± 0.7 A	0.69 ± 0.05 A	0.279	−64.0	5.8 ± 0.8 A	0.1043
Postfabrication	0.33	19.5 ± 0.8 B	0.652	−35.7	7.5 ± 1.6 A	0.60 ± 0.05 A	0.373	−42.3	7.5 ± 1.6 A	0.0367
Wheat flour										
Prefabrication	0.54	8.9 ± 0.4 A	0.619	−36.4	5.1 ± 1.2 A	0.71 ± 0.09 A	0.524	−28.6	5.1 ± 1.2 A	0.9802
Postfabrication	0.74	15.1 ± 0.7 B	0.978	−7.6	4.7 ± 1.7 A	0.59 ± 0.08 A	0.726	−10.9	4.7 ± 1.4 A	0.1572

[a] RMSE, root mean square error. *D*, δ, and *p* values are means ± standard errors. Products have ∼0.40 to 0.45 a_w. Within a column (and within the same product), means followed by common letters were not significantly different ($\alpha = 0.05$).

each product type. The Weibull model was more likely correct for prefabrication almond meal (% likelihood > 99.99%), prefabrication almond butter (% likelihood > 99.99%), postfabrication almond butter (% likelihood > 99.99%), prefabrication wheat meal (% likelihood > 90%), prefabrication wheat flour (% likelihood > 96%), and postfabrication wheat flour (% likelihood > 84%). However, the log-linear model was more likely correct for postfabrication almond meal, pre- and postfabrication date paste, and postfabrication wheat meal (% likelihood ~70 to 98%). Because the Weibull model was not the most-likely-correct model for all products and was dependent on product type and inoculation protocol, both the $D_{80°C}$-value and the Weibull-estimated time for a 1-log reduction were calculated and compared for all products (Table 2).

Replication error. Replication errors (Table 2) for each product were calculated to quantify consistency of the experiments. The highest standard error of replication (0.90 log CFU/g) was for prefabrication almond butter, which may have been affected by oil separation during the equilibration process.

Product effects. Based on the prefabrication $D_{80°C}$ values, *Salmonella* Enteritidis PT 30 thermal resistance in almond products was approximately four times greater ($P < 0.05$) than in wheat products, which was approximately three times greater ($P < 0.05$) than in date products. For the postfabrication results, the same general rank ordering was true ($P < 0.05$), except for a smaller difference between almond and wheat products. This observation is consistent with prior *Salmonella* Enteritidis PT 30 studies, which have reported larger *D*-values for high fat products (e.g., $D_{83°C}$ of 16 min for peanut butter *(16)* compared with a $D_{80°C}$ of 5 min for wheat flour *(22)*).

Structure effects. *Salmonella* Enteritidis PT 30 thermal resistance was significantly greater in almond butter than in almond meal ($P < 0.05$) for the postfabrication protocol. In addition, *Salmonella* Enteritidis PT 30 thermal resistance in wheat meal was significantly ($P < 0.05$) greater than in wheat flour for both inoculation protocols. Surface interactions between product particles and *Salmonella* cells during fabrication may have impacted *Salmonella* Enteritidis PT 30 attachment differently in almond and wheat products (due to significantly different composition between these products), resulting in different impacts on thermal resistance; however, the fundamental mechanisms causing these differences are not yet conclusively known.

Almond products. The $D_{80°C}$ for prefabrication almond meal (49.8 min) was higher ($P < 0.05$) than that for postfabrication almond meal (33.4 min). Villa-Rojas et al. *(29)* reported a much lower $D_{80°C}$ (1.63 min for almond meal at 0.6 a_w) compared with this study, which would be expected due to the differences in a_w. Additionally, this may have been impacted by differences in inoculation preparation: the prior study used phosphate buffer as the liquid suspension.

In almond butter, the prefabrication $D_{80°C}$ for *Salmonella* Enteritidis PT 30 (42.9 min) was two times greater than for postfabrication (18.3 min). During the milling process, almond oil was expressed, and bacteria were presumably forced into the oil droplets. It can be assumed that the internal shear force during hand mixing (postfabrication) was much lower than for mechanical stomaching (prefabrication); therefore, the fraction of *Salmonella* Enteritidis PT 30 cells entrained in the oil phase likely increased during fabrication, leading to greater thermal resistance in pre- as opposed to postfabrication almond butter. This enhanced survival is supported by the published literature, which indicates that high fat content protects bacterial cells at high temperature *(20)*.

Thermal resistance of *Salmonella* has been assessed in peanut butter but not in almond butter. Based on the log-linear model, Ma et al. *(16)* and He et al. *(8, 9)* reported a $D_{83°C}$ of *Salmonella* Tennessee in regular peanut butter of 16 min at 0.45 a_w, and a $D_{90°C}$ of *Salmonella* cocktail in regular and low-fat peanut butter of 3.5 and 2.6 min, respectively, at 0.4 a_w *(8, 9, 16)*. Therefore, *Salmonella* strain, temperature, and fat content can be assumed to affect thermal resistance of *Salmonella* in nut butter products during processing *(8, 16, 20)*.

The Weibull distribution has also been used previously to model *Salmonella* inactivation in peanut butter. Ma et al. *(16)* and He et al. *(9)* also reported the Weibull parameters; they estimated times for a 1-log reduction of 1.92 min at 83°C *(16)* and 6.62 min at 90°C *(9)*. Weibull parameters from Li et al. *(15)* yield an estimated time for a 1-log reduction (80°C) of a *Salmonella* cocktail (Thompson, Newport, Typhimurium, Copenhagen, Montevideo, and Heidelberg) in regular peanut butter (0.45 a_w) of 1.9 min, which was lower than in prefabrication (8.5 min) and in postfabrication (3.4 min) almond butter in this study.

Date products. Thermal resistance of *Salmonella* Enteritidis PT 30 in postfabrication inoculated date paste was the lowest among all the products ($D_{80°C} \sim 1.2$ min). *Salmonella* cells originally inoculated onto the date surface (prefabrication protocol) were more thermally resistant than those inoculated directly into the date paste (postfabrication protocol). In the prefabrication method, the inoculated dates were equilibrated before grinding and reequilibrated after grinding, but the postfabrication samples were equilibrated in paste form. This slight difference in equilibration procedures, necessitated by the different fabrication procedures, may partially explain the observed differences in *Salmonella* Enteritidis PT 30 thermal resistance.

Date paste also has very high sugar content (~66%) *(25)*. Although previous studies on *Salmonella* thermal resistance in date paste are lacking, Mattick et al. *(17)* reported the Weibull parameters for high sugar content broths (0.65 a_w) at 80°C. Their estimated time for a 1-log reduction of *Salmonella* Typhimurium was 3.6 min, which was higher than that for postfabrication inoculated date paste (1.5 min) in this study but was on the same order of magnitude.

Wheat products. Thermal resistance of *Salmonella* Enteritidis PT 30 in wheat meal and wheat flour showed an opposite result from the almond and date products, with resistance greater in post- as opposed to prefabrication samples. In the prefabrication protocol, wheat meal and flour particle surfaces that previously were internal in the intact wheat kernel would have been cross-contaminated from the inoculated external surfaces during grinding and handling. However, in the postfabrication protocol, all wheat meal and flour particle surfaces had equal probability of being contaminated when the inoculum was added to the powders and mixed. This difference between the two protocols, therefore, may have influenced the extent of *Salmonella* Enteritidis PT 30 attachment to any given particle surface, which could have affected thermal resistance in a manner that would have been different from in the almond products, given the significantly different compositions.

According to Smith et al. *(22), Salmonella* Enteritidis PT 30, which was inoculated via a method similar to the present postfabrication protocol, exhibited a $D_{80°C}$ of 5.5 min in wheat flour at 0.43 a_w, which was lower than that for postinoculation wheat flour at 0.4 a_w (15.1 min). Smith et al. *(22)* used commercial white wheat flour, which may have altered the heat resistance because of differences in composition (i.e., lower lipids content) and particle-cell interactions. Syamaladevi et al. *(24)* also assessed thermal inactivation of a *Salmonella* cocktail in wheat flour at 80°C (inoculated postfabrication). At 0.45 a_w, the $D_{80°C}$ was 6.9 min, which was lower than for the postfabrication method used in this study (15.1 min). The Syamaladevi et al. *(24)* experiment was similar to this study, except for the inoculum preparation. These results support the premise that inoculation procedures impact thermal resistance of *Salmonella* in wheat flour *(10)*.

In conclusion, these results have shown that thermal resistance of *Salmonella* Enteritidis PT 30 depends on the inoculation protocol, product type, and product structure. In all known prior studies with fabricated products (e.g., peanut butter *(9, 16),* wheat flour *(10, 23),* and dried fruits *(1)*), postfabrication inoculation protocols were applied to inoculate products, determine inactivation kinetics, and validate the processes. This suggests that some published data may not accurately reflect actual scenarios in which a raw material is contaminated and then fabricated into an ingredient or finished product, which may influence thermal resistance. The results also suggest that prefabrication contamination events may be of greater concern in process validation. Additional tests are being conducted to quantify *Salmonella* thermal resistance in different product structures at various a_w levels and to model *Salmonella* behavior in a range of low-moisture foods.

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REFERENCES

1. Beuchat, L. R., and D. A. Mann. 2014. Survival of *Salmonella* on dried fruits and in aqueous dried fruit homogenates as affected by temperature. *J. Food Prot.* 77:1102–1109.
2. Burnett, S. L., E. R. Gehm, W. R. Weissinger, and L. R. Beuchat. 2000. Survival of *Salmonella* in peanut butter and peanut butter spread. *J. Appl. Microbiol.* 89:472–477.
3. Centers for Disease Control and Prevention. 2012. Multistate outbreak of *Salmonella* Bredeney infections linked to peanut butter manufactured by Sunland, Inc., final update. Available at: http://www.cdc.gov/salmonella/bredeney-09-12/index.html. Accessed 30 November 2012.
4. Centers for Disease Control and Prevention. 2016. Multistate outbreak of *Salmonella* Paratyphi B variant L(+) tartrate(+) infections linked to JEM raw brand sprouted nut butter spreads, final update. Available at: http://www.cdc.gov/salmonella/paratyphi-b-12-15/index.html. Accessed 15 January 2016.
5. Chen, Y. H., V. N. Scott, T. A. Freier, J. Kuehm, M. Moorman, J. Meyer, T. Morille-Hinds, L. Post, L. Smoot, S. Hood, J. Shebuski, and J. Banks. 2009. Control of *Salmonella* in low-moisture foods III: process validation and environmental monitoring—part three of a three-part series. *Food Prot. Trends* 29:493–508.
6. Chung, H. J., S. L. Birla, and J. Tang. 2008. Performance evaluation of aluminum test cell designed for determining the heat resistance of bacterial spores in foods. *Lebensm-Wiss. Technol. Food Sci. Technol.* 41:1351–1359.
7. Danyluk, M. D., A. R. Uesugi, and L. J. Harris. 2005. Survival of *Salmonella* Enteritidis PT 30 on inoculated almonds after commercial fumigation with propylene oxide. *J. Food Prot.* 68:1613–1622.
8. He, Y. S., D. J. Guo, J. Y. Yang, M. L. Tortorello, and W. Zhang. 2011. Survival and heat resistance of *Salmonella enterica* and *Escherichia coli* O157:H7 in peanut butter. *Appl. Environ. Microbiol.* 77:8434–8438.
9. He, Y. S., Y. Li, J. K. Salazar, J. Y. Yang, M. L. Tortorello, and W. Zhang. 2013. Increased water activity reduces the thermal resistance of *Salmonella enterica* in peanut butter. *Appl. Environ. Microbiol.* 79:4763–4767.
10. Hildebrandt, I. M., B. P. Marks, E. T. Ryser, R. Villa-Rojas, J. Tang, F. J. Garces-Vega, and S. E. Buchholz. 2016. Effects of inoculation procedures on variability and repeatability of *Salmonella* thermal resistance in wheat flour. *J. Food Prot.* 79:1833–1839.
11. Isaacs, S., J. Aramini, B. Ciebin, J. A. Farrar, R. Ahmed, D. Middleton, A. U. Chandran, L. J. Harris, M. Howes, E. Chan, A. S. Pichette, K. Campbell, A. Gupta, L. Y. Lior, M. Pearce, C. Clark, F. Rodgers, F. Jamieson, I. Brophy, and A. Ellis, for the *Salmonella* Enteritidis PT30 Outbreak Investigation Working Group. 2005. An international outbreak of salmonellosis associated with raw almonds contaminated with a rare phage type of *Salmonella* Enteritidis. *J. Food Prot.* 68:191–198.
12. Kataoka, A., E. Enache, D. G. Black, R. Podolak, and M. Hayman. 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.
13. Keller, S. E., E. M. Grasso, L. A. Halik, G. J. Fleischman, S. J. Chirtel, and S. F. Grove. 2012. Effect of growth on the thermal resistance and survival of *Salmonella* Tennessee and Oranienburg in peanut butter, measured by a new thin-layer thermal death time device. *J. Food Prot.* 75:1125–1130.
14. Laroche, A., F. Fine, and P. Gervais. 2005. Water activity affects heat resistance of microorganisms in food powders. *Int. J. Food Microbiol.* 97:307–315.
15. Li, C. C., L. H. Huang, and J. Q. Chen. 2014. Comparative study of thermal inactivation kinetics of *Salmonella* spp. in peanut butter and peanut butter spread. *Food Control* 45:143–149.
16. Ma, L., G. D. Zhang, P. Gerner-Smidt, V. Mantripragada, I. Ezeoke, and M. P. Doyle. 2009. Thermal inactivation of *Salmonella* in peanut butter. *J. Food Prot.* 72:1596–1601.
17. Mattick, K. L., F. Jorgensen, P. Wang, J. Pound, M. H. Vandeven, L. R. Ward, J. D. Legan, H. M. Lappin-Scott, and T. J. Humphrey. 2001. Effect of challenge temperature and solute type on heat tolerance of *Salmonella* serovars at low water activity. *Appl. Environ. Microbiol.* 67:4128–4136.

18. Motulsky, H., and A. Christopoulos. 2004. Fitting models to biological data using linear and nonlinear regression: a practical guide to curve fitting. Oxford University Press, Oxford.
19. Russo, E. T., G. Biggerstaff, R. M. Hoekstra, S. Meyer, N. Patel, B. Miller, R. Quick, and *Salmonella* Agona Outbreak Investigation Team. 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.
20. Shachar, D., and S. Yaron. 2006. Heat tolerance of *Salmonella enterica* serovars Agona, Enteritidis, and Typhimurium in peanut butter. *J. Food Prot*. 69:2687–2691.
21. Silva, F. V. M., and P. A. Gibbs. 2012. Thermal pasteurization requirements for the inactivation of *Salmonella* in foods. *Food Res. Int*. 45:695–699.
22. Smith, D. F., I. M. Hildebrandt, K. E. Casulli, K. D. Dolan, and B. P. Marks. 2016. Modeling the effect of temperature and water activity on the thermal resistance of *Salmonella* Enteritidis PT 30 in wheat flour. *J. Food Prot*. 79:2058–2065.
23. Smith, D. F., and B. P. Marks. 2015. Effect of rapid product desiccation or hydration on thermal resistance of *Salmonella enterica* serovar Enteritidis PT 30 in wheat flour. *J. Food Prot*. 78:281–286.
24. Syamaladevi, R. M., R. K. Tadapaneni, J. Xu, R. Villa-Rojas, J. M. Tang, B. Carter, S. Sablani, and B. Marks. 2016. Water activity change at elevated temperatures and thermal resistance of *Salmonella* in all purpose wheat flour and peanut butter. *Food Res. Int*. 81:163–170.
25. U.S. Department of Agriculture, Agricultural Research Service. 2016. Basic report: 09421, dates, medjool. National nutrient database for standard reference, release 28. Available at: https://ndb.nal.usda.gov/ndb/foods/show/2424. Accessed 14 November 2016.
26. U.S. Food and Drug Administration. 2014. Perfect Bar & Company recalls Peanut Butter and Cranberry Crunch nutrition bars due to possible health risk. Available at: http://www.fda.gov/Safety/Recalls/ucm427672.htm. Accessed 19 December 2014.
27. U.S. Food and Drug Administration. 2015. Rocky Mountain Foods, Inc. voluntarily recalls Free Range Snack Co. brand 16 oz. Island Fruit and Nut Trail Mix and bulk macadamia nuts because of possible health risk. Available at: http://www.fda.gov/Safety/Recalls/ucm427672.htm. Accessed 26 June 2015.
28. van Boekel, M. 2002. On the use of the Weibull model to describe thermal inactivation of microbial vegetative cells. *Int. J. Food Microbiol*. 74:139–159.
29. Villa-Rojas, R., J. Tang, S. J. Wang, M. X. Gao, D. H. Kang, J. H. Mah, P. Gray, M. E. Sosa-Morales, and A. Lopez-Malo. 2013. Thermal inactivation of *Salmonella* Enteritidis PT 30 in almond kernels as influenced by water activity. *J. Food Prot*. 76:26–32.
30. Witthuhn, R. C., S. Engelbrecht, E. Joubert, and T. J. Britz. 2005. Microbial content of commercial South African high-moisture dried fruits. *J. Appl. Microbiol*. 98:722–726.