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Thermal inactivation of *Salmonella* spp. in commercial tree nut and peanut butters in finished packaging

Daniel G. Wright | Joseph Minarsich | Mark A. Daeschel | Joy Waite-Cusic 💿

Department of Food Science and Technology, Oregon State University, Corvallis, Oregon

Correspondence

Joy Waite-Cusic, Department of Food Science and Technology, 100 Wiegand Hall, Oregon State University, Corvallis, OR 97331.

Email: joy.waite-cusic@oregonstate.edu

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Abstract

Peanut and tree nut butters have been implicated in *Salmonella* outbreaks in recent years. Previous studies investigated efficacy of thermal treatments to reduce *Salmonella* in multiple peanut butter formulations; however, evidence is lacking to support thermal treatment of tree nut butters. This study evaluated thermal treatments to reduce *Salmonella* in commercial nut butter formulations in final package. Formulations (n = 6) were inoculated with a *Salmonella* cocktail and packaged in glass jars before thermal treatment (boiling waterbath, holding times: 0–90 min, >90 °C). *Salmonella* survivors were enumerated using standard dilution and plating on Hektoen Enteric Agar (37 °C, 24–48 hr). Low levels of survivors (<1 CFU/g) were quantified using a one-tube most probable number technique. Thermal treatments at >90 °C with a 30-min hold time effectively reduced >5 log CFU/g of *Salmonella* in tree nut formulations; however, holding times >60 min were required to achieve similar reductions in peanut butter.

Practical applications

This study was designed to provide evidence to support a reconditioning proposal for the processing of tree nut butters that were linked to an outbreak of *Salmonella* Paratyphi L(+)tartrate(+). There were no previous reports in the scientific literature to demonstrate the efficacy of thermal treatment to inactivate *Salmonella* spp. in tree nut butters, including nut butters with more complex formulations. Ideally, reconditioning of contaminated product would not require repackaging of the products; therefore, this study was designed to determine treatment parameters that could be easily achieved with the product remaining in its original packaging. This report supports thermal treatment as an option for reconditioning contaminated tree nut butter products using a thermal process that is less intense than what would be necessary to reduce *Salmonella* spp. in peanut butter products.

1 | INTRODUCTION

Various formulations of commercial peanut and tree nut butters have been associated with multiple outbreaks of *Salmonella* spp. both domestically and internationally. Two large U.S. outbreaks (>500 reported illnesses each) were caused by peanut butter and peanut products contaminated with *Salmonella* Tennessee and *Salmonella* Typhimurium in 2007 and 2008–2009, respectively (Centers for Disease Control and Prevention, 2007, 2009). Additional smaller *Salmonella* outbreaks (<50 cases) occurred in 2012 and 2014 involving peanut and tree nut butters (Palumbo, Beuchat, Danyluk, & Harris, 2015). In December 2015, the Centers for Disease Control and Prevention identified raw sprouted tree nut butters as the food source of a multistate outbreak of *Salmonella* Paratyphi B variant L(+) tartrate (+) (Centers for Disease Control and Prevention, 2016). The manufacturer voluntarily recalled all of their products and the firm's remaining inventory was embargoed until such a time as it would be destroyed or satisfactorily reconditioned in accordance with state and federal regulatory guidelines (Food and Drug Administration, 2016).

In response to the large peanut butter outbreaks, studies were conducted to identify processes that could be used to reduce *Salmonella* spp. contamination in peanut butter. The high fat content and low water activity of these products provides substantial protection to microorganisms from what would otherwise be effective food processing technologies (He et al., 2013). Thermal processing has been evaluated as an option for inactivating *Salmonella* spp. in peanut butters,

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spreads, and pastes using conventional thermal inactivation study approaches (He et al., 2013; He, Guo, Yang, Tortorello, & Zhang, 2011; Keller et al., 2012; Li, Huang, & Chen, 2014; Shachar & Yaron, 2006). Time and temperature were consistently verified as critical parameters; however, heat resistance of Salmonella spp. was significantly influenced by product formulation (He et al., 2011; Kataoka et al., 2014; Li et al., 2014; Ma et al., 2009; Shachar & Yaron, 2006). Reduced fat formulations with increased carbohydrate content required significantly longer treatment times (15-45 min) at 90 °C to achieve a 5-log reduction of Salmonella spp. compared to standard formulations (10-25 min) (He et al., 2011; Li et al., 2014). Given the variability in thermal resistance of Salmonella spp. in different peanut butter product formulations and the diversity of commercial nut butter products in the marketplace, there is a lack of evidence to critically evaluate processing or reconditioning proposals for products other than basic peanut butter formulations.

Industrial application of thermal processing is further complicated by the necessity to apply the treatment to the product in its final package. The total processing time, including the come-up time, will be significantly influenced by the heat transfer properties of the food, the packaging, and the heating medium (Silva & Gibbs, 2012). Finished product quality and safety will be impacted by the total process and is an important consideration for process design and acceptability.

The main objective of this study was to determine the efficacy of a proposed in-package thermal process to reduce *Salmonella* spp. in a variety of commercial nut butter formulations.

2 | MATERIALS AND METHODS

2.1 Nut butters

Five formulations of commercial raw tree nut butters were provided by the manufacturer associated with the recall. A commercial, unsalted, creamy peanut butter was purchased locally and included for comparison with previously published studies. Ingredient and product characteristics are included in Table 1. All products were stored at ambient temperature (22 °C) prior to experimentation. Water activities were measured using a water activity meter (Rototronics HygroPalm 23-AW, Hauppauge, NY).

2.2 | Bacterial strains

Salmonella enterica strains were selected from previous association with tree nuts, peanuts, and/or nut-related outbreaks to create a seven-strain cocktail (Table 2). Individual strains were revived by transferring 10 μ L from a -80 °C stock culture of tryptic soy broth (TSB; Neogen, Lansing, MI) supplemented with 40% glycerol into freshly prepared TSB (10 ml) and incubated at 37 °C for 24 hr. Cultures were streaked for isolation on Hektoen Enteric Agar (HE; Neogen) and incubated at 37 °C for 24–48 hr prior to inoculation into fresh TSB. Cultures were stored at 4 °C for up to 1 week prior to use.

2.3 | Inoculum preparation

TSB cultures of each *Salmonella* strain were spread plated (300 μ L) onto large format Petri dishes (150 mm) containing Tryptic Soy Agar (TSA; Neogen) and incubated at 37 °C for 24 hr to create a lawn culture. Lawns were harvested by applying 8 ml of commercial almond oil (The Hain Celestial Group, Inc, Lake Success, NY) per plate followed by scraping the agar surface with a sterile cell spreader. Inoculated oil for each strain was transferred to a seperate 15 ml conical tube and thoroughly vortexed for distribution of the cells in the oil. Inoculated oil samples of the individual strains were combined in equal volumes (\sim 9 log CFU/ml) and used immediately for inoculation of the almond-based butters. Identical inocula were prepared using hazelnut oil (Huileries de Lapalisse, Lapalisse, France) and peanut oil (Signature Kitchens, Better Living Brands, LLC, Pleasanton, CA) for hazelnut and peanut butters, respectively.

2.4 | Inoculation of nut butters

Approximately 1700 g of nut butter was aseptically transferred from the commercial packaging into a 5-L stainless steel mixing bowl and the respective oil-based *Salmonella* cocktail (10 ml) was added and mixed thoroughly (2 min) with the wire whip attachment using the "stir" setting on a tilt-head stand mixer (KitchenAid, Benton Harbor, Ml) in a Class 2 biological safety cabinet. Following mixing, the sides of the bowl were scraped with a silicone spatula and mixed to uniformity. The contents of the bowl were transferred to a 3.7 L plastic bag and glass jars (8 cm height \times 6 cm diameter; Olshen's Bottle Supply, Portland, OR) were refilled by piping with approximately 170 g of

TABLE 1 Ingredients and characteristics of commercial nut butters used in this study

			Composition based on product label (wt %)			
Formulation	Ingredients ^a	A _w	Carbohydrate	Fat	Protein	Other
Almond Butter A	Almonds, cashews, coconut sugar, lucuma, vanilla, cardamom	0.38	46.7	30.0	13.3	10.0
Almond Butter B	Almonds, coconut, palm sugar, lucuma, vanilla, cardamom, spices	0.38	26.7	46.7	20.0	6.6
Almond Butter C	Almonds, coconut sugar, maca, ground cinnamon, vanilla	0.31	50.0	30.0	13.3	10.0
Almond Butter D	Almonds, coconut sugar, maqui berry, lucuma, vanilla, camu	0.35	50.0	30.0	13.3	6.7
Hazelnut Butter	Hazelnuts, coconut sugar, cacao nibs, vanilla	0.36	40.0	43.3	10.0	6.7
Peanut Butter	Peanuts	0.38	18.7	50.0	25.0	6.3

^aIngredients listed in order presented on product label. All product formulations indicated a sodium content of 0 mg.

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TABLE 2 Salmonella enterica strains included in the inoculation cocktail for this study

Serotype	Isolate identifier	Description	Source
Salmonella Braenderup	JWC-1808	Hazelnut isolate	Dr. Joy Waite-Cusic Oregon State University
Salmonella Enteritidis	ATCC BAA-1045	Almond isolate	American Type Culture Collection (ATCC)
Salmonella Tennessee	MDD319	Clinical isolate from 2007 outbreak associated with peanut butter	Dr. Larry Beuchat University of Georgia
Salmonella Montevideo	GRC1	Pistachio isolate	Food and Drug Administration
Salmonella Oranienburg	MDD317	Pecan isolate	Dr. Michelle Danyluk University of Florida
Salmonella Saintpaul	UH-1311-1	Walnut isolate	Dr. Linda Harris University of California, Davis
Salmonella Paratyphi B variant L(+) tartrate(+)	OSPHL 15092808070	Clinical isolate from 2015 outbreak associated with raw sprouted nut butters	Oregon State Public Health Labs

inoculated product (<1 cm of headspace). Mixing the inoculum resulted in a reduction in the viscosity of these products (shear-thinning) that allowed them to be easily piped into the jars without the formation of air pockets. The observation of shear-thinning during mixing was confirmed by the manufacturer as a typical behavior of these products. Jars were sealed with metal twist closures (Olshen's Bottle Supply). This process was repeated to yield 20 jars of each inoculated nut butter (~7 log CFU/g). Inoculated products were stored at ambient temperature (22 °C) for 24 hr prior to thermal treatment. Upon visual observation at 24 hr, the nut butters viscosity had recovered to that of typical finished product.

2.5 | Thermal treatment of nut butters

Thermal treatments were conducted by submerging sealed jars into boiling water in a 9 L Dutch oven (Cascade Meadows Kitchenware, Eugene, OR) heated on an electric range (Hotpoint, Rapid City, SD). A single treatment consisted of 15 jars in a single Dutch oven: two jars of each inoculated nut butter and three uninoculated nut butters (temperature controls randomized across treatments). Holding times (two times per Dutch oven) were randomized across the two Dutch ovens for three independent replicates of each thermal treatment time for each nut butter. Internal product temperature was recorded throughout the process using a USB Temperature Data Logger with a Type K General Purpose Thermocouple Probe (ThermoWorks, American Fork, UT) inserted through a rubber septum in the lid to reach the geometric center of a jar of uninoculated product. Real-time temperature readings were monitored using a secondary differential thermocouple meter equipped with two type K Penetration Thermocouple Probes (Thermo-Works). One probe was submerged in the boiling water bath and the other probe was inserted into the geometric center of a single jar of uninoculated product. Treatment temperatures and holding times were based on the real-time readings and confirmed using the data logger results. All product formulations were consistent in their thermal

profiles and achieved a central product temperature of >90°C after 42–47 min of submersion in the water bath. Treatments were designed to evaluate the survival of *Salmonella* spp. in products heated to >90°C and held at or above that temperature for 0, 30, 60, and 90 min. Upon completion of the treatment, samples were immediately removed from the Dutch oven and sampled as quickly as possible (<5 min from end of treatment time).

2.6 | Microbiological analyses

Salmonella spp. were enumerated in treated and untreated nut butters using standard plating techniques. For low levels of survivors, samples were also enumerated for Salmonella spp. using a one-tube most probable number (MPN) method (Jarvis, 2016). To sample the product, the lid was removed, the product was stirred to thoroughly mix the entire contents of the jar, and a 10-g sample was transferred to a sterile WhirlPak bag (Nasco, Salida, CA). Sterile 0.85% saline solution containing 0.1% Tween 80 was added to the WhirlPak bag at a 1:10 ratio and homogenized by stomaching (150 rpm, 2 min). Serial dilutions were prepared in 0.85% saline and plated using a combination of spread and spiral plating techniques (AutoPlater 4000, Advanced Instruments, Norwood, MA) on TSA and HE agar. Plates were incubated at 37°C for 24–48 hr prior to enumeration.

For the one-tube MPN method, 1-, 10-, and 100-g samples of product were transferred to sterile WhirlPak bags, mixed at a 1:10 ratio with Lactose Broth (LB) with 0.1% Tween 80, homogenized by stomaching (150 rpm, 30 s), and incubated at $37 \,^{\circ}$ C for 24 hr. Following incubation, 1 ml of LB culture was transferred to 5 ml of tetrathionate (TT) Broth and incubated at $37 \,^{\circ}$ C for 24 hr. Incubated TT cultures were streaked for isolation onto HE agar and incubated at $37 \,^{\circ}$ C for 24 hr. Plates with at least one typical *Salmonella* colony were calculated using the Thomas approximation of MPN/g (Swanson, Petran, & Hanlin, 2001):

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 $MPN/g = P/\sqrt{NT}$

where *P* is the number of positive tubes, *N* is total grams of sample in all negative tubes, and *T* is total grams of samples in all tubes. The detection limit for the one-tube MPN method was calculated to be 0.009 MPN/g with a score of 1, 0, 0 for the 1, 10, and 100 g samples, respectively.

2.7 Data analysis

Salmonella spp. populations were converted to log CFU/g prior to all statistical analyses. Survival curves were plotted as log-reductions of bacterial counts (Log N/N₀). The impact of product, treatment time, and replicate on changes in surviving populations were evaluated using the Mixed Model function in JMP Pro software version 12.0.1 (SAS Institute, Inc., Cary, NC). Tukey honest significant difference tests were used to evaluate pairwise comparisons between products at each treatment temperature.

3 | RESULTS

All six product formulations displayed comparable temperature profiles in all replicates (Figure 1). The increase in product temperature during the linear phase of heating was $2.3 \,^{\circ}$ C/min. Average time for the geometric center of the all products to reach >90 $^{\circ}$ C was 45 min (range: 42–47 min). Product temperature continued to increase slowly during the holding time. Final average temperatures with a 30, 60, and 90 min holding time were 98.0, 98.7, and 99.0, respectively.

Salmonella spp. survival in thermally treated nut butters is shown in Figure 2. Initial inoculation levels in the various products ranged from an average of 6.47 log CFU/g in peanut butter to 7.46 log CFU/g in almond butter D. After the internal product temperature reached >90 °C (Holding time = 0), average reductions ranged from 1.04 log CFU/g in the peanut butter to 4.64 log CFU/g in the hazelnut butter. Surviving populations in the hazelnut butter were significantly lower



FIGURE 1 Representative curves of internal product temperature as a function of residence time in the boiling water bath. Additional vertical lines are included to indicate the relative assignments of come-up time (CUT; 0 hold time), 30, 60, and 90 min for interpretation of processing times



FIGURE 2 Reduction of a *Salmonella* spp. cocktail in nut butters treated in a boiling water bath with a product temperature of >90 °C for up to 90 min. Internal product temperatures of >90 °C were achieved after 45 min of submersion in the boiling water bath; therefore, a hold time of 45 min represents untreated samples (initial counts) and a hold time of 0 min represents treatments in which the product was heated to 90 °C and immediately sampled (come-up time [CUT]; no hold). Data is presented as the mean (n = 3) with error bars indicating the standard error

(*p* < .05) than in all other product formulations at this time point. After holding internal product temperature >90 °C for 30, 60, and 90 min, reductions of *Salmonella* spp. populations in the peanut butter were significantly lower than in all tree nut butters at 2.55 log CFU/g, 4.24 log CFU/g, and 5.89 log CFU/g, respectively. *Salmonella* spp. was not detected in any samples of the hazelnut butter with holding times of ≥30 min (detection limit −2.05 log CFU/g). After the 30 min holding time, reductions of *Salmonella* spp. in tree nut butters ranged from 6.43 log CFU/g (almond butter B) to 9.34 log CFU/g (almond butter C). Holding times of 60 and 90 min reduced *Salmonella* survivors to below the detection limit in all tree nut butters with the exception of one replicate of the almond butter B at 60 min.

4 | DISCUSSION

While several studies have evaluated the efficacy of thermal treatments to inactivate *Salmonella* spp. in peanut butter, this is the first study to use a variety of tree nut butters as the food matrices. Despite the similarities in product composition and identical heating profiles, *Salmonella* spp. inactivation differed significantly based on product formulation. *Salmonella* spp. were significantly more resistant to thermal treatment (>90 °C) in commercial peanut butter (single ingredient, no salt) compared to the tree nut butters used in this study. During the 60-min hold time (time 0–60 min) at >90 °C, the *Salmonella* cocktail was reduced in this peanut butter by an average of 3.2 log CFU/g. This reduction was comparable to the 3.7–4.6 log CFU/g reduction of *Salmonella* spp. in peanut butter formulation D (organic, no salt) treated at Journal of Food Safety

90°C for 60 min previously reported by He et al. (2011). Previous studies have found Salmonella spp. to be significantly more resistant to thermal inactivation in peanut butter formulations with lower water activity (A_w 0.2–0.4) compared to those with higher water activity (A_w 0.6-0.8) (He et al., 2013, 2011). Additional studies found that reduced fat (33%)-higher carbohydrate (41%) peanut butters were more protective of Salmonella spp. to thermal inactivation than higher fat (50%) formulations (He et al., 2013; Li et al., 2014). Minor differences in water activity in product formulations used in this study (0.31-0.38) did not correlate with Salmonella inactivation. However, in contrast to the previous studies, higher fat (>45%), lower carbohydrate (<30%), and higher protein (>15%) formulations provided more protection to Salmonella spp. The data most strongly suggests that the relative efficacy of the thermal treatment may differ based on the primary nut (hazelnut > almond > peanut) used as well as the complexity of the formulation (complex formulation > simple formulation). Care should be exercised in using this data to form broad conclusions about the impact of nutritional composition on relative efficacy of thermal treatments as this study used commercial product formulations and was not designed for this purpose.

Previous studies evaluating thermal inactivation of Salmonella spp. in peanut butter have maximized the surface area to volume ratio of the product to minimize the come-up time required to achieve the target lethal temperature (He et al., 2013, 2011; Keller et al., 2012; Li et al., 2014; Ma et al., 2009; Shachar & Yaron, 2006). The current study sought to demonstrate the inactivation of Salmonella spp. in a finished package subjected to thermal treatment. Using boiling water (100°C) as the heating medium allows for a consistent reference point for any processor and demonstrated a consistent temperature profile for all formulations of tree nut butters. Due to the bulk of the product in commercial packaging, it was not surprising that there was significant lethality attributed to the come-up-time. Thermal treatment of all tree nut formulations achieved a 5-log reduction of Salmonella spp. with a holding time of 30 min; however, to achieve the same reduction in the peanut butter formulation would require a holding time at >90 °C of >60 min. Changes in texture and viscosity were apparent after thermal treatments for all product formulations. Sensory research and/or formula modifications may be necessary to quantify and mitigate these changes.

The National Advisory Committee on Microbiological Criteria for Foods (NACMCF) has emphasized the importance of appropriate strain selection for process validation studies (NACMCF, 2010). Ma et al. (2009) demonstrated that clinical isolates of *Salmonella* Tennessee associated with the 2006–2007 peanut butter outbreak were significantly more resistant to thermal treatment in peanut butter compared to *Salmonella* Tennessee strains not associated with outbreaks. The strains (n = 7) used in the inoculation cocktail in this study included a clinical isolate of *Salmonella* Tennessee from the same peanut butter outbreak as well as a clinical isolate of *Salmonella* Paratyphi B L(+)tartrate(+) associated with tree nut butters evaluated in this study. To round out the 7-strain cocktail, additional *Salmonella* strains were selected that had been previously isolated from a variety of tree nuts.

Inoculum preparation conditions can have a significant impact on subsequent thermal inactivation studies. Salmonella spp. inocula in this study were prepared by suspending sessile cells in oil prior to inoculating the commercial product. Li et al. (2014) previously established that Salmonella spp. were significantly more resistant to thermal inactivation (70 °C) in peanut butter when the inocula were prepared in corn oil versus peptone water. Additional studies have used peanut oil as the inoculum suspending medium for thermal treatment of peanut butter (He et al., 2013, 2011). To minimize the impact of the inoculum on the product formulation, inocula were prepared with the oil type that matched the dominant nut type of the product (i.e., peanut oil was used for peanut butter, almond oil was used for almond butter, hazelnut oil for hazelnut butter). Thermal inactivation profiles were significantly different for products inoculated with different oil types. It is possible that the respective oil used for inoculation had an impact on thermal resistance; however, this was not evaluated in this study. Following inoculation, the products were held at ambient temperature for 24 hr prior to heat treatments. Previous studies by He et al. (2013, 2011) stored inoculated peanut butters for extended times (>30 days) prior to thermal processing. Extended storage could lead to increased thermal resistance as the cells adapt to the food matrix; however, Keller et al. (2012) found no difference in thermal inactivation of Salmonella spp. in peanut butters treated immediately after inoculation and those inoculated 2 weeks prior to treatment.

Thermal treatment in a boiling water bath was an effective method to reduce *Salmonella* spp. in various commercial formulations of tree nut butters in finished packaging; however, product texture and viscosity was impacted and would be an important consideration for processors. Thermal resistance of *Salmonella* spp. was significantly greater in peanut butter compared to nut butter products despite all matrices having similar water activities. Additional research is needed to determine compositional or formulaic components that contribute to enhanced protection of *Salmonella* spp. in peanut butter formulations compared to the tree nut products.

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ORCID

Joy Waite-Cusic (b) http://orcid.org/0000-0002-4556-2942

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