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Comparative study of thermal inactivation kinetics of *Salmonella* spp. in peanut butter and peanut butter spread☆

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ABSTRACT

Peanut butter has been implicated in multi-state outbreaks of salmonellosis in recent years. Studies have shown that *Salmonella* exhibited increased thermal resistance in peanut butter. However, little is known about the effect of product formulation on the kinetics of survival of *Salmonella* during thermal treatment. Therefore, the objective of this research was to compare the thermal resistance of *Salmonella* in four commercially available peanut butter and spread products, and evaluate the effect of product formulation on the survival of this pathogen during heating.

Four peanut butter and spread samples, including Omega 3 (A), regular fat (B), reduced sugar (C), and reduced fat (D), inoculated with a 6-strain cocktail of *Salmonella* spp., were heated at 70, 75, 80, 85, and 90 °C. Experimental results showed that the highest thermal resistance of *Salmonella* was found in the samples with reduced fat, while the least in the samples with Omega 3 formulation. No significant difference in the bacterial thermal resistance was observed in the regular fat and reduced sugar formulations. The Weibull survival model was used to describe the survival curves. Results showed that the average exponent (shape factor) of the model ranged from 0.38 to 0.662, suggesting progressively decreased rate of inactivation during heating. The scale (rate) coefficients of the model increased linearly with temperature. The calculated minimum lethal temperature for *Salmonella* was 54.8, 59.8, 59.5, and 63.9 °C in samples A, B, C, and D, respectively. No tail effect was observed. The results of this study suggest that proper formulation of peanut butter and spread may enhance thermal inactivation of *Salmonella*.

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1. Introduction

In the United States, *Salmonella* accounts for an estimated 1,027,561 cases of foodborne illness, causing 19,336 hospitalizations and 378 deaths annually (CDC, 2011). While the outbreaks of salmonellosis are more commonly associated with the consumption of animal products (such as poultry, meat, and eggs) and fresh produce, several serious outbreaks of *Salmonella* infections have been linked to low water activity and high fat foods such as chocolate (Werber et al., 2005), peanut butter, and peanut butter-related products in recent years. While the first documented outbreak of peanut butter-related salmonellosis was reported in 1996 (Burnett, Gehm, Weissinger, & Beuchat, 2000), there have been at least 3 recorded outbreaks of salmonellosis involving peanut butter contaminated with *Salmonella* in the U.S. since 2006. In late 2006 to early 2007, *Salmonella* Tennessee from a leaking roof of a processing plant caused 628 infections in 47 states (CDC, 2007). Between 2008 and 2009, an outbreak of salmonellosis involving *Salmonella* Typhimurium in peanut butter and peanut butter-containing products occurred, leading to 714 cases of infection and 9 deaths in 46 states (CDC, 2009). The latest multi-state outbreak associated with peanut butter was caused by *Salmonella* Bredeney, in which a total of 42 people were infected and 28% of the ill persons were hospitalized (CDC, 2012). Peanut butter has been identified as a new food vehicle for salmonellosis in the U.S. (Sheth et al., 2011). Once contaminated, *Salmonella* can survive in peanut butter and peanut butter spread during storage (Burnett et al., 2000; Park, Oh, & Kang, 2008).

In the U.S., peanut butter is consumed by approximately 90% of the households, with a total spending of $800 million per year

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(National Peanut Board, 2014). In response to the recurring *Salmonella* outbreaks related to peanut butter and other peanut butter-containing products, the U.S. Food and Drug Administration (FDA) issued guidance for the industry, recommending at least a 5-log reduction in *Salmonella* for peanut and peanut-derived products (FDA, 2009). Although some emerging technologies, such as high pressure (Grasso, Somerville, Balasubramaniam, & Lee, 2010) and irradiation (Ban & Kang, 2014; El-Rawas et al., 2012), have been reported for use to control *Salmonella* in peanut butter, the scale-up feasibility and efficacy of these technologies remains uncertain and needs further examination. Thermal processing is still one of the most common and effective methods that can be used to inactivate spoilage and pathogenic microorganisms in foods, including peanut butter.

Heat treatment has been explored to inactivate *Salmonella* in peanut butter. However, it has been demonstrated that the thermal resistance of *Salmonella* is significantly higher in peanut butter than in other foods. Ma et al. (2009) investigated the thermal resistance of three strains of *Salmonella* Tennessee previously associated with an outbreak and other *Salmonella* strains in peanut butter. This study reported that the outbreak-associated *Salmonella* strains were more heat resistant and heating at 90 °C for >30 min was needed to achieve a 5-log reduction of *Salmonella* in highly contaminated peanut butter products. Shachar and Yaron (2006) also reported that *Salmonella* withstood heating at 90 °C and only 3.2 log reductions were observed in peanut butter. However, no significant difference was observed in the thermal resistance among *Salmonella enterica* serovars Agona, Enteritidis, and Typhimurium in artificially inoculated peanut butter. Keller et al. (2012) studied the effect of growth media on the thermal resistance of *Salmonella* Tennessee and Oranienburg in peanut butter and suggested that thermal death curves obtained from sessile cultures exhibited greater linearity than those from planktonic cells. He, Guo, Yang, Tortorello, and Zhang (2011) evaluated the survival and heat resistance of *Salmonella* and *Escherichia coli* O157:H7 in peanut butters with different formulations at two temperatures (70 and 90 °C) and reported the survival and heat resistance of the bacteria were significantly affected by the product formulation. This study also reported that the bacteria survived better, but were less heat-resistant in peanut butter with higher carbohydrate contents.

Various factors may affect the thermal resistance and survival of *Salmonella* in peanut butter during thermal inactivation. Few studies have systematically investigated the combined effect of temperature and product composition on the survival of *Salmonella* in peanut butter. Therefore, the main objective of this research was to compare the thermal inactivation kinetics of *Salmonella* spp. in commercially available peanut butter products, and evaluate the effect of composition in peanut butter on the survival of this pathogen during heating.

2. Materials and methods

2.1. Peanut butter and peanut butter spread

Four commercially processed peanut butter products with different formulations (fat, carbohydrate, protein, and sodium, Table 1) were purchased from a local grocery store. These products differed mainly in fat, carbohydrate, and protein contents. Three of the products (A, B, and C) selected for study contained approximately 50% fat, while product D contained only 33% fat. Product A contained extra Omega-3 fatty acids. Product B was labeled by the manufacturer as regular peanut butter. Product C was labeled as a reduced sugar product, while the last one, Product D, was a reduced fat formulation. Products A, B, and C, containing at least 90% peanuts, were categorized as peanut butter, according to 21CFR164.150 (FDA, 2013a). Product D contained 60% peanuts. Therefore, it was a peanut butter spread, according to 21CFR102.23 (FDA, 2013b).

Table 1
Formulations of four commercially available peanut butter and peanut butter spread. The relative contents of each product were calculated from nutrient labels.

Product code	Ingredient content (w%)				
	Total fat	Total carbohydrate	Protein	Sodium	Other
Omega-3 (A)	48.5%	24.2% (9.1%)	21.2%	0.5%	5.6%
Regular (B)	50.0%	21.9% (9.4%)	25.0%	0.5%	2.7%
Reduced sugar (C)	53.1%	18.8% (6.3%)	25.0%	0.2%	2.9%
Reduced fat (D)	33.3%	41.7% (11.1%)	19.4%	0.6%	5.0%

2.2. Bacteria strains and preparation of inoculum

Six strains of *Salmonella*, including *S.* Thompson120, *S.* Newport H1073, *S.* Typhimurium TD104, *S.* Copenhagen 8457, *S.* Montevideo, and *S.* Heidelberg, were obtained from the microbiological culture collection of the Eastern Regional Research Center (ERRC), USDA Agricultural Research Service (ARS), located at Wyndmoor, PA. Each strain of the *Salmonella* cultures was induced to resist rifampicin (100 mg/L) by successively inoculating and transferring the cultures in brain heart infusion broth (BHI broth, BD/Difco Laboratories, Sparks, MD) containing 25, 50, 75, and 100 mg/L of rifampicin (Sigma, R 3501-5G, Sigma–Aldrich Co., MO). The bacterial cultures were incubated individually at 37 °C overnight on an orbital shaker (~100 rpm). Once the antibiotic resistance was induced and stabilized, each culture was streaked onto Tryptic Soy agar (TSA, BD/Difco Laboratories) plates supplemented with 100 mg/L rifampicin (TSA/R). To maintain the viability of the cells, the rifampicin-resistant *Salmonella* cultures were regularly propagated and maintained on TSA/R plates and stored in a refrigerator maintained at 8 °C. Using the rifampicin-resistant strains of *Salmonella* allowed recovery of the bacteria after heat treatments without using a selective medium, which may damage or kill thermally injured cells.

The working cultures were prepared by inoculating the rifampicin-resistant stock cultures individually into 10 mL BHI broth supplemented with 100 mg/L rifampicin. The bacterial cultures were incubated at 37 °C on an orbital shaker (~100 rpm) for approximately 19–20 h, harvested by centrifugation (2400 g, 15 min, at 4 °C), washed once with 10 mL 0.1% peptone water (PW, BD/Difco Laboratories), re-centrifuged, decanted, and re-suspended in 1.5 mL corn oil or 0.5 mL PW, and then combined to form a 9 mL oil suspension or 3 mL PW suspension of bacterial cocktail. In the preliminary experiment to determine the effect of culture preparation on the survival of *Salmonella*, both oil and PW suspensions of the bacterial cultures were used to inoculate samples. In the subsequent experiments, however, only the oil suspension was used. The final concentration of the cocktail was approximately $10^{9.0}$ colony-forming unit (CFU) per mL.

2.3. Sample preparation and inoculation

During inoculation, peanut butter and spread samples (1.00 ± 0.05 g each) were aseptically weighted into Whirl-Pak (7 oz/207 mL, Nasco-Fort Atkinson, WI) bags. Each sample was individually inoculated with 30 μL oil suspension or 10 μL PW suspension (Product B only) of the bacterial cocktail. The inoculated sample bags were gently massaged by hand for at least 3 min, and then flattened with a round bottle to a thin layer (<0.5 mm). To ensure uniform heating, the bags were vacuumed to evacuate the