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Evaluation of *Enterococcus faecium* NRRL B-2354 as a surrogate for *Salmonella* during cocoa powder thermal processing

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ABSTRACT

Salmonella is capable of surviving in a low moisture environment for long periods. Once adapted to the xeric conditions, the thermal resistance of *Salmonella* is enhanced. Cocoa powder is a low water activity (a_w) food (La_wF) that is an essential component in a wide variety of desserts and ready-to-eat (RTE) foods and drinks. The aim of this study was to evaluate the desiccation and thermal resistance of *Salmonella* in cocoa powder, as well as to examine the suitability of *Enterococcus faecium* NRRL B-2354 as a surrogate for *Salmonella* during cocoa powder thermal processing. Natural unsweetened cocoa powder was inoculated with a 3-strain *Salmonella* cocktail or *E. faecium* and was equilibrated to a_w 0.30 and 0.45 at room temperature, then subjected to isothermal treatments at 70–80 °C or 12-month storage at RT (room temperature, 22.0 ± 0.5 °C, a_w 0.30). At 70 and 80 °C, D-values of both *Salmonella* and *E. faecium* increased with decreasing a_w. D-values of *Salmonella* at a_w 0.30 cocoa powder were 46.2 ± 4.7, 20.5 ± 1.7, and 11.5 ± 0.9 min at 70, 75 and 80 °C, respectively. Higher heat resistance of *E. faecium* in a_w 0.30 cocoa powder was observed with D-values of 59.9 ± 5.0, 28.9 ± 1.8, and 16.1 ± 1.4 min at 70, 75, and 80 °C, respectively. However, *E. faecium* demonstrated less heat resistance than *Salmonella* when a_w was increased to 0.45. D-values for *Salmonella* at a_w 0.45 were 31.6–7.0 min at 70–80 °C compared to 25.8–4.7 min for *E. faecium*. During 12 months of storage at RT, surviving *E. faecium* population in a_w 0.30 cocoa powder was higher than that of the *Salmonella* cocktail; the population decreased by 1.39 and 3.75 logs, respectively. These findings indicate that the suitability of *E. faecium* as a surrogate organism for *Salmonella* is influenced by a_w of cocoa powder. The a_w correlates with thermal inactivation rates in both *Salmonella* and *E. faecium*, and should be considered as a significant contributor to the thermal resistance of *Salmonella* in cocoa powder.

1. Introduction

Salmonella is one of the most challenging foodborne pathogens in the food industry, due to its virulence and thermal resistance in low moisture foods. Infections by this pathogen have led to numerous illnesses and deaths (Fabrega and Vila, 2013). According to the United States Centers for Disease Control and Prevention (CDC, 2016), between 1998 and 2016, there were 3260 reported outbreaks of salmonellosis resulting in 78,158 illnesses and 113 deaths (CDC, 2016). Combined with non-reported food outbreaks and illnesses, it has been estimated that *Salmonella* is responsible for approximately 19,000 hospitalizations and 380 deaths annually in the U.S. alone (Scallan et al., 2011). In Canada, 50% of bacterial foodborne outbreaks were directly associated with *Salmonella* infection (Kozak et al., 2013). The number of outbreaks and recalls due to *Salmonella* contamination has increased over last two decades (CDC, 2013; Crim et al., 2014; Huang et al., 2016; Marder et al., 2017).

Low water activity (a_w) foods (La_wF) and food ingredients are characterized by $a_w < 0.60$, a a_w low enough to inhibit the growth of most microbes. However, low a_w doesn't guarantee microbial safety of food products. Outbreaks of salmonellosis caused by consumption of La_wF such as chocolate (Craven et al., 1975; D'Aoust, 1977; D'Aoust et al., 1975), tree nuts and their products (CDC, 2007; Kirk et al., 2004), powdered milk (CDC, 1993), cereal (CDC, 1998; Rushdy et al., 1998), and other foods (Imanishi et al., 2014; Koch et al., 2005) have occurred all over the world. *Salmonella* can survive in a low-a_w environment for

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several years, which is equivalent to or in excess of the recommended shelf-life of many La_wF (Abd et al., 2012; Blessington et al., 2013; Kimber et al., 2012; Santillana Farakos et al., 2013; Uesugi et al., 2006). The adaptation of *Salmonella* in response to xeric stress yields an increased propensity for survival when exposed to thermal treatment. As a result, *Salmonella* has been recognized as a major threat to La_wF microbial safety. The U.S. Food and Drug Administration (FDA) has requested data and models on survival of *Salmonella* to develop a quantitative assessment of the risk of human salmonellosis in tree nuts (Santillana Farakos et al., 2016).

Cocoa is the fermented dried seed of the cocoa tree, *Theobroma cacao* (McShea et al., 2009). Cocoa powder is rich in polyphenols with known health benefits and inherent antimicrobial properties (Calatayud et al., 2013; Kajiya et al., 2004). Cocoa powder is a key ingredient in chocolate confections and drink mixes. *Salmonella* might be introduced during processing, storage or transportion steps of cocoa powder production (Lima et al., 2012). *Salmonella* survives in the chocolate filling of cookie and cracker sandwiches (Beuchat and Mann, 2015) or chocolate (Tamminga et al., 1977) stored at ambient temperature for long durations. The D-values of *Salmonella* in molten milk chocolate products at 70 °C were up to 17.5 h (Goepfert and Biggie, 1968), highlighting the need to understand the inactivation kinetics of *Salmonella* in cocoa powder.

Salmonella contamination is a significant concern for cocoa powder due to the ability of this organism to survive in La_wF as well as a large number of salmonellosis outbreaks linked to La_wF, including chocolate products. Surrogate organisms, which are similar but avirulent (Sinclair et al., 2012), are commonly utilized to evaluate the effectiveness of thermal processing. *Enterococcus faecium* NRRL B-2354 (*E. faecium* is used hereafter) has been widely used as a surrogate for *Salmonella* in validation of La_wF thermal processing, such as thermal treatments for almonds (ABC, 2014), extrusion of carbohydrate-protein meal (Bianchini et al., 2014), and radio-frequency treatment of wheat flour (Liu et al., 2018b). The survival of microbes in La_wF during thermal processing is complex and varies with food matrix, a_w, and micro-environments of La_wF (Beuchat et al., 2013; Koseki et al., 2015; Li et al., 2014; Tiganitas et al., 2009; Vogel et al., 2010). It is imperative to systematically evaluate and compare the thermal resistance of *E. faecium* and *Salmonella* over different a_w and temperatures to validate thermal processing procedures of La_wF.

This study aimed to evaluate impacts of a_w on the survival of *Salmonella* in cocoa powder at three different process temperatures (70, 75, and 80 °C) and two a_w levels (0.30 and 0.45, at 22 °C), and to further examine the suitability of *E. faecium* as a surrogate for *Salmonella* during thermal processing of cocoa powder. Additionally, the survival of both strains during extended storage at ambient temperature was further examined.

2. Materials and methods

2.1. Bacterial strains and lawn preparation

Outbreak strains *Salmonella* Enteritidis PT30 (raw almond), *S.* Tennessee K4643 (peanut butter), and *S.* Agona 447967 (toasted oats cereal) were used in the formulation of a 3-strain cocktail. *S.* Enteritidis PT30 was acquired from Dr. Linda Harris at the University of California, Davis. *S.* Tennessee K4643 and *S.* Agona were obtained from Dr. Nathan Anderson at Food and Drug Administration, Illinois. *E. faecium* NRRL B-2354 was obtained from the culture collection of the National Center for Agricultural Utilization Research (NRRL), USDA Agricultural Research service (Peoria, IL). All strains were maintained at −80 °C in Trypticase Soy Broth (TSB, Becton, Dickinson and Company, Sparks, MD) supplied with 0.6% Yeast Extract (Fisher Scientific, Pittsburgh, PA) (TSBYE) and 20% (v/v) glycerol. Each strain was cultured in TSBYE individually by static incubation at 35 ± 2 °C for 24 h. The overnight bacterial cultures were 1:1000 subculture in TSBYE at 35 ± 2 °C for additional 24 h, statically. Three hundred microliters of the bacterial sub-cultures were plated on 100 × 15 mm TSAYE plates (TSBYE with 1.5% agar) and incubated at 35 ± 2 °C for 24 h. Bacterial lawns from TSAYE plates were washed and collected using sterile Phosphate Buffered Saline (PBS, pH7.4) and centrifuged at 8000 × *g*, 4 °C for 15 min. The resulting pellet was suspended in sterile PBS to achieve 10^{11} CFU/ml. The prepared *Salmonella* cultures were combined in equal volumes to obtain the 3-strain cocktail. The population of the inoculum (3-strain *Salmonella* cocktail or *E. faecium*) was confirmed by enumeration.

2.2. Cocoa powder inoculation and water activity (a_w) equilibration

Natural unsweetened 100% cocoa powder (The Hershey Company, USA) was purchased from a local grocery store, which contains 57.36 ± 0.68% carbohydrate, 25.91 ± 0.09% protein, 6.50 ± 0.48% fat, 6.22 ± 0.03% ash and 4.00 ± 0.62% moisture as analyzed per AOAC standard methods. Detection of cocoa background flora was performed by taking three random 1.0 g samples diluted in 9.0 ml of sterile PBS, plating appropriate serial dilutions on TSAYE then incubating at 35 ± 2 °C for 48 h. Forty grams of cocoa powder was inoculated with 400 μL of a 3-strain *Salmonella* cocktail or *E. faecium* strain to achieve ~10^9 CFU/g of cocoa powder inside a stomacher bag (Fischer Scientific), which was then mixed by hand vigorously to ensure homogenous dispersion of inoculum.

Inoculated cocoa powder was divided into two 150 mm petri dishes (Fisher Scientific), in an even layer before being placed in a_w-equilibration chamber (Custom-designed at Michigan State University) set at the target a_w (0.30 and 0.45), and equilibrated for a minimum of 4 days at room temperature (RT, 22.0 ± 0.5 °C). The a_w of the respective cocoa powder samples was measured by a a_w meter (Aqualab Series 3, Decagon Devices, Inc., Pullman, WA). Samples were used for thermal inactivation after reaching the target a_w ± 0.02. All a_w was measured at RT.

Cocoa powders inoculated with individual *E. faecium* or 3-strain *Salmonella* cocktails were enumerated immediately following inoculation and a 4 days post-equilibration. Enumeration was achieved by combining 1.0 g of inoculated cocoa powder, in a 2 oz. Whirl-Pak® bag (Nasco, Ft. Atkinson, WI), with 9.0 ml sterile PBS, homogenizing for 2 min at 230 rpm in a stomacher (Seward Stomacher® Circulator 400, Worthing, UK), and serially diluting 10-fold in sterile PBS. The appropriate serial dilutions were then plated in duplicate onto TSAYE plate and incubated at 35 ± 2 °C for 24–48 h.

2.3. Thermal inactivation

Following equilibration to target a_w, 0.4 0 ± 0.02 g of inoculated cocoa powder was loaded into thermal death treatment (TDT) aluminum test cells designed by Washington State University (Chung et al., 2008). The TDT test cells were hermetically sealed and subjected to isothermal treatments (70, 75, or 80 °C) in an ethylene glycol bath (Isotemp Heat Bath Circulator, Fisher Scientific). The temperature of the ethylene glycol bath was recorded by Omega Precision RTD temperature recorder (OM-CP-RTDTemp2000, Omega Engineering Inc., Norwalk, CT). TDT test cells with T-type thermocouples at the geometrical center were used to measure heat penetration and come up time (CUT, the time required for sample core temperature to reach with 0.5 °C of the set temperature). The resulting CUT for cocoa powder was 1.5 min, and heat treatment timing started after reaching CUT. Test cells from respective treatments were collected at the selected time points, then immediately chilled in an ice-water bath for 2 min. All tests were conducted in triplicate and repeated three times independently.

2.4. Enumeration of viable bacteria

Heat-treated cocoa powder was transferred from TDT test cells to a

2 oz. Whirl-Pak® bag (Nasco, Ft.) and diluted 1:10 with sterile PBS, then homogenized for 2 min at 230 rpm in a stomacher. The recovered bacterial suspensions were 10-fold serially diluted and appropriate dilutions were plated on TSAYE plates in duplicate, and then incubated at 35 ± 2 °C for 24–48 h.

2.5. Salmonella and E. faecium storage survival in cocoa powder

Following equilibration at a_w 0.30 ± 0.02, inoculated cocoa powder was aliquoted and sealed in moisture barrier bags (Dri-Shield 3000®, Desco Industries, Inc) and held at RT for up to 1 year. Survival was analyzed on a bi-weekly or monthly basis per the method described above. The a_w of samples inside each moisture barrier bag was monitored at each sampling day. Two sets of biologically independent inoculated cocoa powder were prepared. For each independent set, there were three samples at each storage sampling time.

2.6. D-value and z-value analysis

Two primary models, the first-order kinetic model/log-linear model (Equation (1)) and Weibull model (Equation (2)), were used for analysis and comparison of the thermal inactivation curve (Peleg, 2006).

$$\mathrm{Log}\left(\frac{N}{N_0}\right) = -t/D \quad (1)$$

$$\mathrm{Log}\left(\frac{N}{N_0}\right) = -\left(\frac{t}{\delta}\right)^{\alpha} \quad (2)$$

where N_0 is the initial bacterial population, N is the bacterial population at time (t); t is the time of the isothermal treatment (min) after the come-up to the specified treatment temperature; D is the time in min required to reduce the microbial population by 90% at a selected temperature (°C); δ is a scale parameter in the Weibull model; and α is the shape parameter, with $\alpha = 1$ for linear or $\alpha \neq 1$ for non-linear.

D-value, thermal resistance in log-linear model, was estimated from the thermal inactivation curve using a log-linear regression analysis and is reported in min. The parameter δ and α were estimated from Weibull model (Equation (2)). The z-values were determined from the regression of log D-value versus temperature and were calculated as $z = \mathrm{slope}^{-1}$ for the linear trend lines. Data were analyzed through the Integrated Pathogen Modeling Program (IPMP) (Huang, 2014).

The goodness-of-fit of the models was quantified by the root mean square error (RMSE) obtained from IPMP, accuracy factor (A_f) (Equation (3)) and bias factor (B_f) (Equation (4)) (Baranyi et al., 1999):

$$A_f = 10^{\frac{\sum_1^n \left| lg \frac{\mathrm{Log}\left(\frac{N}{N_0}\right)_{pred}}{\mathrm{Log}\left(\frac{N}{N_0}\right)_{data}} \right|}{n}} \quad (3)$$

$$B_f = 10^{\frac{\sum_1^n lg \frac{\mathrm{Log}\left(\frac{N}{N_0}\right)_{pred}}{\mathrm{Log}\left(\frac{N}{N_0}\right)_{data}}}{n}} \quad (4)$$

where $\mathrm{Log}\,(N/N_0)_{pred}$ is the predicted log reduction from IPMP, $\mathrm{Log}\,(N/N_0)_{dara}$ is the measured reduction of bacteria during treatment, and n is the total number of observations. The smaller the A_f and B_f value, the more effective is the model fitness (Baranyi et al., 1999).

3. Results

3.1. Stability of Salmonella and E. faecium during equilibration and extended storage

To evaluate strain variability, the survival of three individual *Salmonella* strains and *E. faecium* during desiccation was determined. Following four days post-inoculation at RT, 0.68, 0.75, 0.58 and 0.56 Log_{10} CFU/g reductions were determined for *S.* Enteritidis PT30, *S.* Tennessee K4643, *S.* Agona 447967 and *E. faecium*, respectively, indicating these strains had a similar stability in a_w 0.30 cocoa powder during 4 days of equilibration in the moisture chamber (Fig. 1).

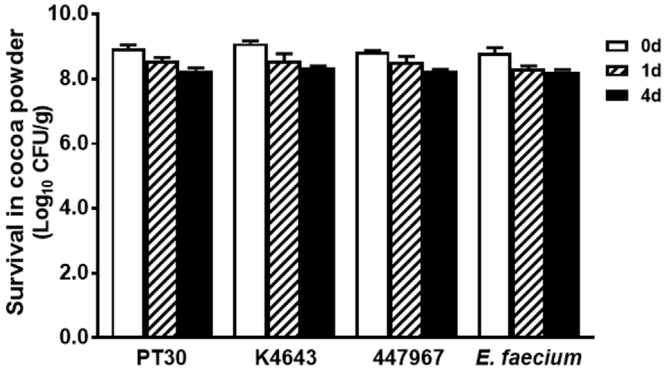

Fig. 1. The survival of *Salmonella* and *E. faecium* NRRL B-2354 strains in a_w 0.30 cocoa powder stored at room temperature. PT30: *Salmonella* Enteritidis PT30; K4643: *S.* Tennessee K4643; 447967: *S.* Agona 447967. Mean ± SEM, n = 3.

During 4-day equilibration at a_w 0.30, there were 0.87 and 0.29 Log CFU/g reductions of *Salmonella* cocktail and *E. faecium*, respectively (Fig. 2A). The population of *Salmonella* cocktail and *E. faecium* remained at similar levels as the initial inoculation level during the first month of RT storage. Both *Salmonella* cocktail and *E. faecium* survived in a_w 0.30 cocoa powder during 1-year RT storage with ~1.39 Log

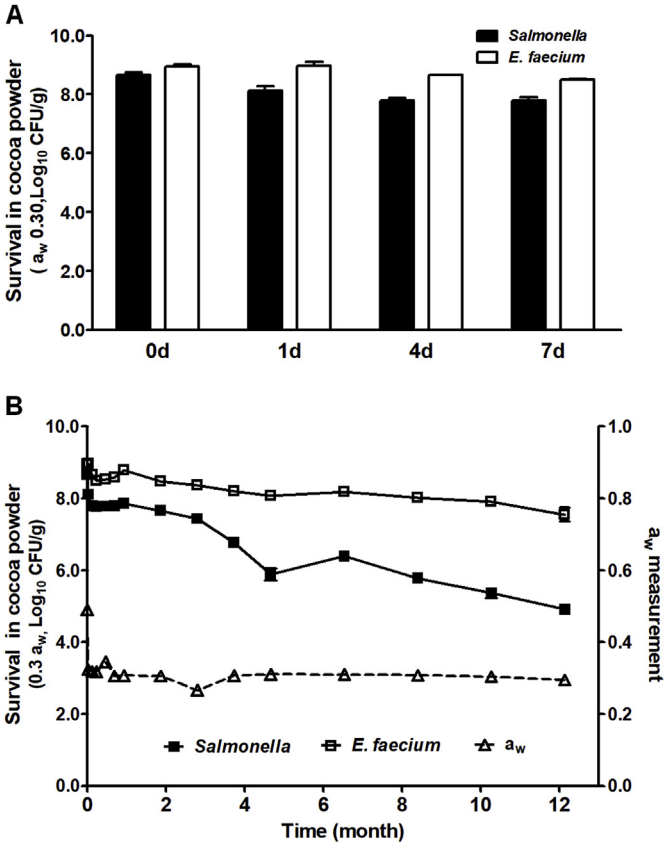

Fig. 2. The survival of the *Salmonella* cocktail and *E. faecium* NRRL B-2354 in aw 0.30 cocoa powder during 12-month of storage at room temperature. A. 7-day post-inoculation; B. Survival and a_w measurement over 12-month. The average a_w of cocoa powder during 12-month storage is 0.31 ± 0.02. Experiments were repeated independently twice.

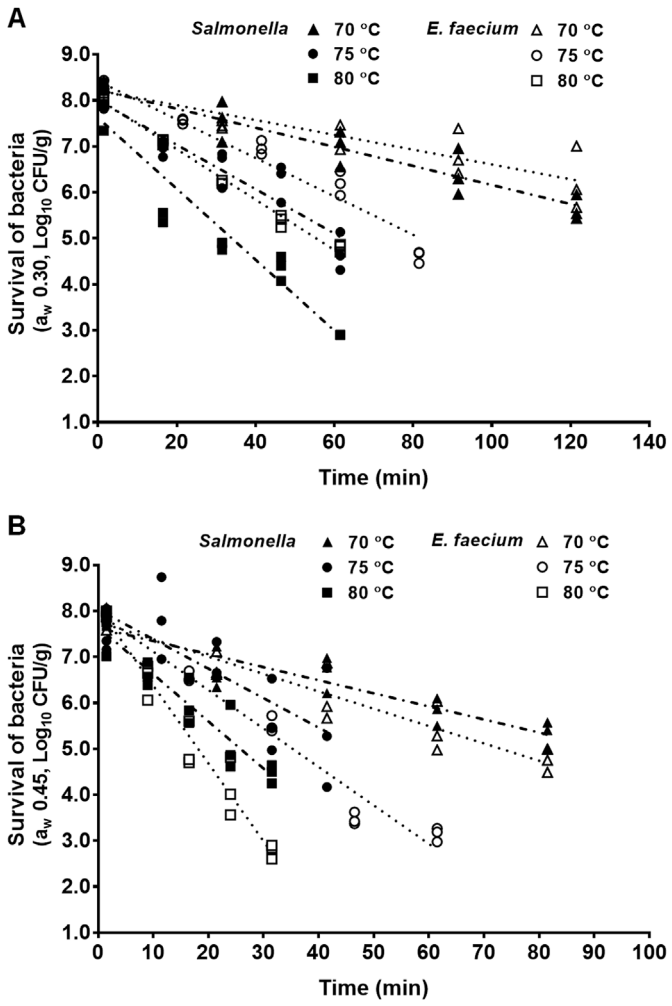

Fig. 3. The death curves of the *Salmonella* cocktail and *E. faecium* NRRL B-2354 in cocoa powder at a_w 0.30 (A) and 0.45 (B) at selected temperatures. Experiments were independently repeated thrice. The a_w was measured at RT.

CFU/g reduction in *E. faecium* and 3.75 Log CFU/g reduction in *Salmonella* (Fig. 2B).

3.2. Thermal inactivation of Salmonella cocktail and E. faecium in cocoa powder

Microbial survival data from isothermal inactivation (Fig. 3) were analyzed using both Weibull and log-linear models (Table 1). Overall, both models fit *Salmonella* and *E. faecium* thermal inactivation data with similar RMSE, A_f and B_f. Given that the Weibull model yielded no significant improvement in fit over the log-linear model, the log-linear model was used for subsequent analyses and comparisons among treatments.

The resulting D-values for *Salmonella* determined in cocoa powder at a_w 0.30, calculated from the slope of the trend lines of 70, 75, and 80 °C, were 46.2 ± 4.7, 20.5 ± 1.7, and 11.5 ± 0.9 min, respectively (Table 1 & Fig. 3A). The resistance of *E. faecium* to thermal inactivation in cocoa powder at a_w 0.30 was higher when compared to *Salmonella*. The D-values for *E faecium* in a_w 0.30 cocoa powder at 70, 75, and 80 °C were 59.9 ± 5.0, 28.9 ± 1.8, and 16.1 ± 1.4 min, respectively (Table 1 & Fig. 3A). Moreover, as temperature increased, D-values for *Salmonella* decreased at a greater rate when compared to *E. faecium* (Fig. 3A).

Salmonella in a_w 0.45 cocoa powder exhibited a higher resistance to thermal inactivation at all inactivation temperatures comparative to *E. faecium*. The D-values for *Salmonella* in a_w 0.45 cocoa powder at 70, 75, and 80 °C were 31.6 ± 3.3, 16.9 ± 2.0, and 7.0 ± 0.9 min, respectively (Fig. 3B), compared to the D-values of 25.8 ± 1.3, 13.7 ± 0.6, and 4.7 ± 0.6 min for *E. faecium*, respectively (Fig. 3B). At all three temperatures used for heat inactivation, both *Salmonella* and *E. faecium* showed a higher thermal resistance in cocoa powder equilibrated at a_w 0.30 than those equilibrated at a_w 0.45.

The z-values for *Salmonella* in cocoa powder at a_w 0.30 to 0.45 were 16.9 ± 1.9 and 17.5 ± 2.7 °C, respectively (Table 1, Fig. 4). The z-values of *E. faecium* decreased from 15.2 ± 2.5 to 13.0 ± 1.3 °C as a_w increased from 0.30 to 0.45 (Table 1, Fig. 4).

4. Discussion

La_wF are traditionally considered safe, yet there are significant occurrences of foodborne pathogen outbreaks linked to La_wF. According to CDC reports, from 2006 to 2017, there were 59 *Salmonella* outbreaks linked to a variety of fruits, shelled eggs, sprouts, tree nuts, organic shakes and meals, peanut butter products, frozen and raw fish, chicken, turkey, cheese, ground beef, pork, and cereals (CDC, 2017). Amongst these outbreaks, approximately one third of reported *Salmonella* outbreaks were associated with La_wF (CDC, 2017). Multiple *Salmonella* strains, including *S.* Eastbourne (Craven et al., 1975), *S.* Napoli (Gill et al., 1983), *S.* Nima (Hockin et al., 1989), *S.* Typhimurium (Kapperud et al., 1990), and *S.* Oranienburg (Werber et al., 2005), were linked to chocolate outbreaks worldwide, clearly demonstrating a need to evaluate and validate thermal processing of cocoa powder against potential *Salmonella* contamination and outbreaks.

Pathogen reduction procedures for the manufacture of cocoa powder includes alkalization, heating up to 100 °C, and roasting of whole cocoa beans or nibs for 5–35 min at temperatures between 110 and 140 °C, which are considered the main kill steps for *Salmonella* (Beckett, 2008; ICMSF, 2005; Lima et al., 2012). However, pathogenic bacteria including *Salmonella* might survive during roasting, or be introduced post process, during storage or transportation steps of cocoa powder production. In current industry practice, after roasting, no other heat treatment steps are applied to cocoa powder until it is used as an ingredient for chocolate, chocolate drinks, and other formulated products containing chocolate (Beckett, 2008; Lima et al., 2012). Conching at 50–70 °C during chocolate manufacturing alone is not sufficient to eliminate *Salmonella* contamination (Krapf and Gantenbein-Demarchi, 2010; Nascimento Mda et al., 2012). To sufficiently develop and implement appropriate procedures as part of a risk-based preventative control measure, it is key to understand the thermal inactivation kinetics of *Salmonella* at common thermal processing conditions and further validate *E. faecium* as a surrogate for thermal processing of cocoa powder.

4.1. Salmonella thermal resistance in cocoa powder

The survival of *Salmonella* in cocoa powder during thermal inactivation is inversely related to a_w. Similar phenomena were reported in previous studies on the thermal inactivation kinetics of *Salmonella* in La_wF such as wheat flour (Liu et al., 2018a), pet foods (Ceylan and Bautista, 2015), skim milk powder (Laroche et al., 2005), and peanut paste (Kataoka et al., 2014). A similar relationship between a_w and thermal resistance has also been observed in *Listeria monocytogenes* in wheat flour (Taylor et al., 2018). The underlying mechanisms of the increased thermal resistance of *Salmonella,* once adapted to low a_w environments, have yet to be elucidated.

Besides a_w, thermal resistance of bacteria is also affected by many other factors including bacteria strain (Goepfert and Biggie, 1968), bacterial growth stage (Ng et al., 1969; Rolfe et al., 2012), food physical structure (Mogollon et al., 2009), fat content (Juneja and Eblen, 2000), and carbohydrates (He et al., 2011). D-values of *S.* Typhimurium in molten milk chocolate at 70–90 °C were nearly 2-fold of those of *S.*

Table 1
Thermal inactivation data for *Salmonella* and *E. faecium* NRRL B-2354 in cocoa powder.

Initial sample a_w		Log-Linear Model				Weibull Model							
		Temp (°C)	D-value (min)	95% CI upper limit of D-value	RMSE	z-value (°C)	A_f	B_f	δ (min)	α	RMSE	A_f	B_f
0.30	*Salmonella*	70	46.2 ± 4.7	55.5	0.86	16.9 ± 1.9	1.70	0.82	19.3 ± 0.0	0.60 ± 0.18	0.82	1.57	1.17
		75	20.5 ± 1.7	23.9	0.91		1.51	0.84	12.4 ± 0.1	0.76 ± 0.18	0.91	1.52	1.14
		80	11.5 ± 0.9	13.3	0.82		1.44	0.91	3.2 ± 0.0	0.58 ± 0.15	0.78	1.32	1.00
	E. faecium	70	59.9 ± 5.0	69.9	0.56	15.2 ± 2.5	1.99	0.76	72.9 ± 9.7	1.39 ± 0.25	0.55	2.20	0.63
		75	28.9 ± 1.8	32.4	0.51		1.61	0.81	38.6 ± 0.4	1.37 ± 0.20	0.50	1.23	1.04
		80	16.1 ± 1.4	19.0	0.66		1.44	0.91	11.1 ± 0.1	0.79 ± 0.19	0.65	1.32	1.00
0.45	*Salmonella*	70	31.6 ± 3.3	38.2	1.13	17.5 ± 2.7	2.15	0.77	27.8 ± 0.3	0.91 ± 0.23	1.13	1.99	0.79
		75	16.9 ± 2.0	20.9	1.10		2.00	0.85	11.3 ± 0.0	0.78 ± 0.26	1.10	1.78	1.08
		80	7.0 ± 0.9	8.8	0.78		2.00	1.01	4.8 ± 0.0	0.79 ± 0.37	0.79	1.52	0.88
	E. faecium	70	25.8 ± 1.3	28.4	0.67	13.0 ± 1.3	1.66	1.09	30.9 ± 0.1	1.13 ± 0.13	0.67	1.66	1.03
		75	13.7 ± 0.6	14.9	0.48		1.38	0.83	12.0 ± 0.1	0.92 ± 0.10	0.48	1.36	0.86
		80	4.7 ± 0.6	5.8	1.11		1.82	1.45	2.6 ± 0.0	0.76 ± 0.34	1.11	1.48	1.25

RMSE: the root mean square error; a_w: water activity. Upper limit of the 95% prediction interval (PI) for the D-value was calculated from the regression line equation + 2 RMSE. Mean ± SEM, n = 3.

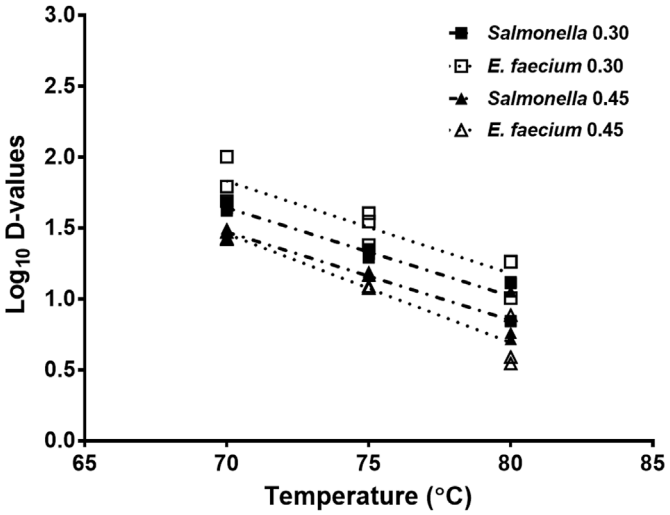

Fig. 4. Log D (decimal reduction time to achieve 90% population reduction at the selected temperatures) values of the *Salmonella* cocktail and *E. faecium* NRRL B-2354 in a_w 0.30 and 0.45 cocoa powder at different temperature. The a_w was measured at RT.

Senftenberg 775 W (Goepfert and Biggie, 1968). D_{70}- and D_{80}- values of *Salmonella* in molten chocolate were much higher than respective D-values of *Salmonella* strains in cocoa powder obtained in this study (Goepfert and Biggie, 1968). The observed difference could be due to combination of strain, food matrix, a_w, and food physical state. *E. faecium* showed a much higher resistance in wheat flour compared to cocoa powder at both a_w 0.30 and 0.45; D_{75}- and D_{80}- in wheat flour were about 2 times those in cocoa powder (Liu et al., 2018a). At a_w 0.30 and 0.45, D_{75}- and D_{80}- values for *Salmonella* in cocoa powder were comparable to those in wheat flour (Liu et al., 2018a). Higher fat content can also increase thermal tolerance of *Salmonella* (Hiramatsu et al., 2005; Juneja and Eblen, 2000). However, *Salmonella* adapted to peanut butter with lower fat content and higher carbohydrate content had higher heat resistance compared to those in peanut butter with high fat and low carbohydrate levels (He et al., 2013). Given that cocoa powder has a higher fat content than wheat flour (3.3% fat) (Liu et al., 2018a), other factors besides fat might be responsible for the difference in thermal resistance.

4.2. Suitability of *E. faecium* as a surrogate for *Salmonella*

The Food Safety Modernization Act final rule for Preventive Controls for Human Food (FDA, 2015) mandates that a food company must implement and validate interventions to prevent or control identified significant hazards, such as *Salmonella* in many La_wF. Due to its non-pathogenic characteristics and higher heat and acid resistance (Kopit et al., 2014), *E. faecium* has been used extensively as a surrogate for *Salmonella* in La_wF thermal validation. When compared to *Salmonella, E. faecium* exhibits a higher heat resistance during the processing of various La_wF, such as the extrusion processing of carbohydrate-protein meal (Bianchini et al., 2014), thermal heating of low-a_w pet food (Ceylan and Bautista, 2015) and peanut paste (Kataoka et al., 2014), and radio-frequency processing (Liu et al., 2018b) or thermal treatment of wheat flour (Liu et al., 2018a).

In cocoa powder with a_w 0.30, D-values of *E. faecium* at all test temperatures were higher than those of *Salmonella*. Ratios of D-values of *E. faecium* and *Salmonella* ranged between 1.3 and 1.4, indicating *E. faecium* is a suitable surrogate for *Salmonella* during thermal processing of cocoa powder at a_w 0.30. However, when the initial a_w is increased to 0.45, the D-value of *Salmonella* exhibited a higher resistance than those of *E. faecium* in cocoa powder at all target temperatures, with the ratios of D-value of *E. faecium* and *S.* Enteritidis at 0.67–0.81. This indicates that *E. faecium* may not be an ideal surrogate for *Salmonella* when a_w is increased beyond a certain level in cocoa powder. D_{80}-values of *Salmonella* in a confectionery formulation (87.5% carbohydrate, 1% fat and 3% protein) with a_w 0.57 were higher than that of *E. faecium* (Rachon et al., 2016). In the Log_{10} D-values versus temperature plot of this study, the line of *Salmonella* intersects with the line of *E. faecium* at approximately 70 °C at a_w 0.45, indicating the point at which the suitability of *E. faecium* as bacterial surrogate changes. Temperatures below this point such as 60 °C, showed a higher thermal resistance in *E. faecium* (data not shown).

4.3. *Salmonella* stability in unsweetened cocoa powder

During 1-year RT storage in a_w 0.30 cocoa powder, a 3.75 log reduction of *Salmonella* in cocoa powder during storage was observed. Similarly, during a 12-month RT storage, *Salmonella* inoculated on almond kernels and in shell pistachios at a_w 0.40 decreased approximately 2.4 and 1.8 log, respectively (Kimber et al., 2012). A 2.5 log reduction of *Salmonella* also occurred on inoculated almond kernel stored at RT for 1 year (Uesugi et al., 2006). In peanut paste with 47% fat and a_w 0.30, a 1.3 to 1.8 log reduction of *Salmonella* was observed

over 12-month RT storage dependent on *Salmonella* strain (Kataoka et al., 2014). However, a 7-week 25 °C storage resulted in a 6.0-log reduction of *S.* Montevideo or *S.* Heidelberg inoculated on a_w 0.43 cocoa powder using slant-grown inoculum (Juven et al., 1984), much less stable than *Salmonella* cocktail in a_w 0.30 cocoa powder determined in this study. These data highlight important roles of a_w in *Salmonella* survival in cocoa powder. Additionally, strain variability, inoculation method, food matrix, and possibly cocoa powder source might also contribute to the difference in desiccation stability of *Salmonella* in cocoa powder during prolonged ambient storage. *Salmonella* was more stable in pine nuts compared to pecan and hazelnuts at each a_w and storage temperature combination (Farakos et al., 2017). Lawn-grown *Salmonella* displayed much higher stability in cocoa butter oil than those inoculated with liquid inoculum; within the same inoculation, *S.* Oranienburg was more stable than *S.* Enteritidis PT30 in cocoa butter oil over 21 day storage at both 5 and 21 °C (Komitopoulou and Penaloza, 2009). The ability of *Salmonella* to survive in La_wF is also dependent on storage temperature. *Salmonella* survived better in sucrose stored at 5 °C than those stored at 25 °C regardless of a_w (Beuchat et al., 2017). *Salmonella* were more stable in pine nuts, pecan and hazelnuts stored at 4 and 10 °C than those stored at 25 °C, and there was less than1.5-log CFU/g reduction over 1-year storage at 4 and 10 °C (Farakos et al., 2017). *Salmonella* retains a higher viability in dried fruits (Beuchat and Mann, 2014), almond (Kimber et al., 2012; Uesugi et al., 2006), pistachios (Kimber et al., 2012), infant formula (Koseki et al., 2015) stored at 4 or 5 °C compared to ambient storage temperature. Similarly, Shiga toxin producing *E. coli* retains a higher viability in chocolate (~a_w 0.40) stored at 10 °C compared to RT storage (Baylis et al., 2004). Our data suggest that *E. faecium* is an appropriate surrogate for *Salmonella* in cocoa powder during RT storage; there was a 3.75 and 1.39 Log reduction of *Salmonella* and *E. faecium* in a_w 0.30 cocoa powder, respectively, over 1-year RT storage.

5. Conclusions

Both *Salmonella* and *E. faecium* survived in a_w 0.30 cocoa powder over 1-year RT storage. *E. faecium* had a higher desiccation stability in a_w 0.30 cocoa powder compared to *Salmonella*. There was 1.39 log reduction of *E. faecium* during 12 months of storage at RT compared to a 3.75 log reduction of the *Salmonella* cocktail stored at the same condition and environment. Thermal resistance, as indicated by the D-values, of both *Salmonella* and *E. faecium* increased as sample a_w decreased from 0.45 to 0.30. However, *E. faecium* showed higher D-values in a_w 0.30 cocoa power compared to *Salmonella*, but at a_w 0.45 cocoa powder, *E. faecium* had lower D-values than *Salmonella*. These data provide technical information for *Salmonella* inactivation validation during cocoa powder production. Data also highlight that precaution is needed when adopting *E. faecium* as a *Salmonella* surrogate for La_wF thermal process validation.

Conflicts of interest

The authors have no known conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fm.2019.01.005.

References

ABC, 2014. Guidelines for Using *Enterococcus Faecium* NRRL B-2354 as a Surrogate Microorganism in Almond Process Validation.
Abd, S.J., McCarthy, K.L., Harris, L.J., 2012. Impact of storage time and temperature on thermal inactivation of *Salmonella* Enteritidis PT 30 on oil-roasted almonds. J. Food Sci. 77, M42–M47.
Baranyi, J., Pin, C., Ross, T., 1999. Validating and comparing predictive models. Int. J. Food Microbiol. 48, 159–166.
Baylis, C.L., MacPhee, S., Robinson, A.J., Griffiths, R., Lilley, K., Betts, R.P., 2004. Survival of *Escherichia coli* O157:H7, O111:H- and O26:H11 in artificially contaminated chocolate and confectionery products. Int. J. Food Microbiol. 96, 35–48.
Beckett, S.T., 2008. The Science of Chocolate, second ed. .
Beuchat, L.R., Komitopoulou, E., Beckers, H., Betts, R.P., Bourdichon, F., Fanning, S., Joosten, H.M., Ter Kuile, B.H., 2013. Low-water activity foods: increased concern as vehicles of foodborne pathogens. J. Food Protect. 76, 150–172.
Beuchat, L.R., Mann, D.A., 2014. Survival of *Salmonella* on dried fruits and in aqueous dried fruit homogenates as affected by temperature. J. Food Protect. 77, 1102–1109.
Beuchat, L.R., Mann, D.A., 2015. Survival of Salmonella in cookie and cracker sandwiches containing inoculated, low-water activity fillings. J. Food Protect. 78, 1828–1834.
Beuchat, L.R., Mann, D.A., Kelly, C.A., Ortega, Y.R., 2017. Retention of viability of *Salmonella* in sucrose as affected by type of inoculum, water activity, and storage temperature. J. Food Protect. 80, 1408–1414.
Bianchini, A., Stratton, J., Weier, S., Hartter, T., Plattner, B., Rokey, G., Hertzel, G., Gompa, L., Martinez, B., Eskridge, K.M., 2014. Use of *Enterococcus faecium* as a surrogate for *Salmonella enterica* during extrusion of a balanced carbohydrate-protein meal. J. Food Protect. 77, 75–82.
Blessington, T., Theofel, C.G., Harris, L.J., 2013. A dry-inoculation method for nut kernels. Food Microbiol. 33, 292–297.
Calatayud, M., Lopez-de-Dicastillo, C., Lopez-Carballo, G., Velez, D., Hernandez Munoz, P., Gavara, R., 2013. Active films based on cocoa extract with antioxidant, antimicrobial and biological applications. Food Chem. 139, 51–58.
CDC, 1993. Salmonella Serotype Tennessee in Powdered Milk Products and Infant Formula – Canada and United States, 1993. pp. 516–517. Retrieved from. https://www.cdc.gov/mmwr/preview/mmwrhtml/00021081.htm. Morb. Mortal. Wkly. Rep. 42.
CDC, 1998. Multistate Outbreak of Salmonella Serotype Agona Infections Linked to Toasted Oats Cereal – United States, April-may, 1998. pp. 462–464. Retrieved from: https://www.cdc.gov/mmwr/preview/mmwrhtml/00053368.htm. Morb. Mortal. Wkly. Rep. 47.
CDC, 2007. Multistate Outbreak of Salmonella Serotype Tennessee Infections Associated with Peanut Butter — United States, 2006–2007. pp. 521–524. Retrieved from: https://www.cdc.gov/mmwr/preview/mmwrhtml/mm5621a1.htm. Morb. Mortal. Wkly. Rep. 56.
CDC, 2013. Incidence and Trends of Infection with Pathogens Transmitted Commonly through Food — Foodborne Diseases Active Surveillance Network,. 10 U.S. Sites, 1996–2012.
CDC, 2016. Foodborne Outbreak Online Database (FOOD Tool). Retrieved from. https://wwwn.cdc.gov/foodborneoutbreaks/.
CDC, 2017. Reports of Selected Salmonella Outbreak Investigations. Retrieved from: https://www.cdc.gov/salmonella/outbreaks.html.
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 Protect. 78, 934–939.
Chung, H.J., Birla, S.L., Tang, J., 2008. Performance evaluation of aluminum test cell designed for determining the heat resistance of bacterial spores in foods. LWT - Food Sci. Technol. (Lebensmittel-Wissenschaft -Technol.) 41, 1351–1359.
Craven, P.C., Mackel, D.C., Baine, W.B., Barker, W.H., Gangarosa, E.J., 1975. International outbreak of *Salmonella* Eastbourne infection traced to contaminated chocolate. Lancet 1, 788–792.
Crim, S.M., Iwamoto, M., Huang, J.Y., Griffin, P.M., Gilliss, D., Cronquist, A.B., Cartter, M., Tobin-D'Angelo, M., Blythe, D., Smith, K., Lathrop, S., Zansky, S., Cieslak, P.R., Dunn, J., Holt, K.G., Lance, S., Tauxe, R., Henao, O.L., 2014. Incidence and Trends of Infection with Pathogens Transmitted Commonly through Food — Foodborne Diseases Active Surveillance Network. 10 U.S. Sites, 2006–2013.
D'Aoust, J.Y., 1977. *Salmonella* and the chocolate industry. A review. J. Food Protect. 40, 718–727.
D'Aoust, J.Y., Aris, B.J., Thisdele, P., Durante, A., Brisson, N., Dragon, D., Lachapelle, G., Johnston, M., Laidley, R., 1975. *Salmonella* eastbourne outbreak associated with chocolate. Can. Inst. Food Sci. Technol. J. 8, 181–184.
Fabrega, A., Vila, J., 2013. Salmonella enterica serovar Typhimurium skills to succeed in the host: virulence and regulation. Clin. Microbiol. Rev. 26, 308–341.
Farakos, S.M.S., Pouillot, R., Keller, S.E., 2017. *Salmonella* survival kinetics on pecans, hazelnuts, and pine nuts at various water activities and temperatures. J. Food Protect. 80, 879–885.
FDA, 2015. FSMA Final Rule on Preventive Controls for Human Food. https://www.fda.gov/food/guidanceregulation/fsma/ucm334115.htm.
Gill, O.N., Sockett, P.N., Bartlett, C.L., Vaile, M.S., Rowe, B., Gilbert, R.J., Dulake, C., Murrell, H.C., Salmaso, S., 1983. Outbreak of *Salmonella* Napoli infection caused by contaminated chocolate bars. Lancet 1, 574–577.
Goepfert, J.M., Biggie, R.A., 1968. Heat resistance of Salmonella typhimurium and Salmonella senftenberg 775W in milk chocolate. Appl. Microbiol. 16, 1939–1940.
He, Y., Guo, D., Yang, J., Tortorello, M.L., Zhang, W., 2011. Survival and heat resistance of *Salmonella enterica* and *Escherichia coli* O157:H7 in peanut butter. Appl. Environ. Microbiol. 77, 8434–8438.

He, Y., Li, Y., Salazar, J.K., Yang, J., Tortorello, M.L., Zhang, W., 2013. Increased water activity reduces the thermal resistance of Salmonella enterica in peanut butter. Appl. Environ. Microbiol. 79, 4763–4767.
Hiramatsu, R., Matsumoto, M., Sakae, K., Miyazaki, Y., 2005. Ability of Shiga toxin-producing *Escherichia coli* and *Salmonella* spp. to survive in a desiccation model system and in dry foods. Appl. Environ. Microbiol. 71, 6657–6663.
Hockin, J.C., Daoust, J.Y., Bowering, D., Jessop, J.H., Khanna, B., Lior, H., Milling, M.E., 1989. An international outbreak of *Salmonella* Nima from imported chocolate. J. Food Protect. 52, 51–54.
Huang, J.Y., Henao, O.L., Griffin, P.M., Vugia, D.J., Cronquist, A.B., Hurd, S., Tobin-D'Angelo, M., Ryan, P., Smith, K., Lathrop, S., Zansky, S., Cieslak, P.R., Dunn, J., Holt, K.G., Wolpert, B.J., Patrick, M.E., 2016. Infection with Pathogens Transmitted Commonly through Food and the Effect of Increasing Use of Culture-independent Diagnostic Tests on Surveillance — Foodborne Diseases Active Surveillance Network. 10 U.S. Sites, 2012–2015.
Huang, L., 2014. IPMP 2013–a comprehensive data analysis tool for predictive microbiology. Int. J. Food Microbiol. 171, 100–107.
ICMSF, 2005. Cocoa, chocolate, and confectionery. In: Microorganisms in Foods 6: Microbiological Ecology of Food Commodities, second ed. Kluwer Academic/Plenum Publishers, New York, pp. 467–479 International Commission on Microbiological Specifications for Foods.
Imanishi, M., Rotstein, D.S., Reimschuessel, R., Schwensohn, C.A., Woody Jr., D.H., Davis, S.W., Hunt, A.D., Arends, K.D., Achen, M., Cui, J., Zhang, Y., Denny, L.F., Phan, Q.N., Joseph, L.A., Tuite, C.C., Tataryn, J.R., Behravesh, C.B., 2014. Outbreak of Salmonella enterica serotype Infantis infection in humans linked to dry dog food in the United States and Canada, 2012. J. Am. Vet. Med. Assoc. 244, 545–553.
Juneja, V.K., Eblen, B.S., 2000. Heat inactivation of Salmonella typhimurium DT104 in beef as affected by fat content. Lett. Appl. Microbiol. 30, 461–467.
Juven, B.J., Cox, N.A., Bailey, J.S., Thomson, J.E., Charles, O.W., Shutze, J.V., 1984. Survival of *Salmonella* in dry food and feed. J. Food Protect. 47, 445–448.
Kajiya, K., Hojo, H., Suzuki, M., Nanjo, F., Kumazawa, S., Nakayama, T., 2004. Relationship between antibacterial activity of (+)-catechin derivatives and their interaction with a model membrane. J. Agric. Food Chem. 52, 1514–1519.
Kapperud, G., Gustavsen, S., Hellesnes, I., Hansen, A.H., Lassen, J., Hirn, J., Jahkola, M., Montenegro, M.A., Helmuth, R., 1990. Outbreak of *Salmonella* Typhimurium infection traced to contaminated chocolate and caused by a strain lacking the 60-megadalton virulence plasmid. J. Clin. Microbiol. 28, 2597–2601.
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 Protect. 77, 1252–1259.
Kimber, M.A., Kaur, H., Wang, L., Danyluk, M.D., Harris, L.J., 2012. Survival of *Salmonella, Escherichia coli* O157:H7, and *Listeria monocytogenes* on inoculated almonds and pistachios stored at -19, 4, and 24 degrees C. J. Food Protect. 75, 1394–1403.
Kirk, M.D., Little, C.L., Lem, M., Fyfe, M., Genobile, D., Tan, A., Threlfall, J., Paccagnella, A., Lightfoot, D., Lyi, H., McIntyre, L., Ward, L., Brown, D.J., Surnam, S., Fisher, I.S.T., 2004. An outbreak due to peanuts in their shell caused by *Salmonella* enterica serotypes Stanley and Newport–sharing molecular information to solve international outbreaks. Epidemiol. Infect. 132, 571–577.
Koch, J., Schrauder, A., Alpers, K., Werber, D., Frank, C., Prager, R., Rabsch, W., Broll, S., Feil, F., Roggentin, P., Bockemühl, J., Tschäpe, H., Ammon, A., Stark, K., 2005. *Salmonella* Agona outbreak from contaminated aniseed, Germany. Emerg. Infect. Dis. *11*, 1124–1127.
Komitopoulou, E., Penaloza, W., 2009. Fate of *Salmonella* in dry confectionery raw materials. J. Appl. Microbiol. 106, 1892–1900.
Kopit, L.M., Kim, E.B., Siezen, R.J., Harris, L.J., Marco, M.L., 2014. Safety of the surrogate microorganism *Enterococcus faecium* NRRL B-2354 for Use in thermal process validation. J. Appl. Environ. Microbiol. 80, 1899–1909.
Koseki, S., Nakamura, N., Shiina, T., 2015. Comparison of desiccation tolerance among *Listeria monocytogenes, Escherichia coli* O157:H7, *Salmonella enterica*, and *Cronobacter sakazakii* in powdered infant formula. J. Food Protect. 78, 104–110.
Kozak, G.K., MacDonald, D., Landry, L., Farber, J.M., 2013. Foodborne outbreaks in Canada linked to produce: 2001 through 2009. J. Food Protect. 76, 173–183.
Krapf, T., Gantenbein-Demarchi, C., 2010. Thermal inactivation of *Salmonella* spp. during conching. Lwt-Food Sci. Technol. 43, 720–723.
Laroche, C., Fine, F., Gervais, P., 2005. Water activity affects heat resistance of microorganisms in food powders. Int. J. Food Microbiol. 97, 307–315.
Li, H., Fu, X., Bima, Y., Koontz, J., Megalis, C., Yang, F., Fleischman, G., Tortorello, M.L., 2014. Effect of the local microenvironment on survival and thermal inactivation of *Salmonella* in low- and intermediate-moisture multi-ingredient foods. J. Food Protect. 77, 67–74.
Lima, L.J., van der Velpen, V., Wolkers-Rooijackers, J., Kamphuis, H.J., Zwietering, M.H., Nout, M.J., 2012. Microbiota dynamics and diversity at different stages of industrial processing of cocoa beans into cocoa powder. Appl. Environ. Microbiol. 78, 2904–2913.
Liu, S., Rojas, R.V., Gray, P., Yang, R., Zhu, M.J., Tang, J., 2018a. *Enterococcus faecium* as a *Salmonella* surrogate in the thermal processing of wheat flour: influence of water activity at high temperatures. Food Microbiol. 74, 92–99.
Liu, S.X., Ozturk, S., Xu, J., Kong, F.B., Gray, P., Zhu, M.J., Sablani, S.S., Tang, J.M., 2018b. Microbial validation of radio frequency pasteurization of wheat flour by inoculated pack studies. J. Food Eng. 217, 68–74.
Marder, E.P., Cieslak, P.R., Cronquist, A.B., Dunn, J., Lathrop, S., Rabatsky-Ehr, T., Ryan, P., Smith, K., Tobin-D'Angelo, M., Vugia, D.J., Zansky, S., Holt, K.G., Wolpert, B.J., Lynch, M., Tauxe, R., Geissler, A.L., 2017. Incidence and Trends of Infections with Pathogens Transmitted Commonly through Food and the Effect of Increasing Use of Culture-independent Diagnostic Tests on Surveillance — Foodborne Diseases Active Surveillance Network. 10 U.S. Sites, 2013–2016.
McShea, A., Leissle, K., Smith, M.A., 2009. The essence of chocolate: a rich, dark, and well-kept secret. Nutrition 25, 1104–1105.
Mogollon, M.A., Marks, B.P., Booren, A.M., Orta-Ramirez, A., Ryser, E.T., 2009. Effect of beef product physical structure on *Salmonella* thermal inactivation. J. Food Sci. 74, M347–M351.
Nascimento Mda, S., Brum, D.M., Pena, P.O., Berto, M.I., Efraim, P., 2012. Inactivation of *Salmonella* during cocoa roasting and chocolate conching. Int. J. Food Microbiol. 159, 225–229.
Ng, H., Bayne, H.G., Garibaldi, J.A., 1969. Heat resistance of *Salmonella*: the uniqueness of *Salmonella* senftenberg 775W. Appl. Microbiol. 17, 78–82.
Peleg, M., 2006. Advanced Quantitative Microbiology for Foods and Biosystems: Models for Predicting Growth and Inactivation. CRC Press.
Rachon, G., Penaloza, W., Gibbs, P.A., 2016. Inactivation of Salmonella, Listeria monocytogenes and Enterococcus faecium NRRL B-2354 in a selection of low moisture foods. Int. J. Food Microbiol. 231, 16–25.
Rolfe, M.D., Rice, C.J., Lucchini, S., Pin, C., Thompson, A., Cameron, A.D.S., Alston, M., Stringer, M.F., Betts, R.P., Baranyi, J., Peck, M.W., Hinton, J.C.D., 2012. Lag phase is a distinct growth phase that prepares bacteria for exponential growth and involves transient metal accumulation. Int. J. Bacteriol. 194, 686–701.
Rushdy, A.A., Stuart, J.M., Ward, L.R., Bruce, J., Threlfall, E.J., Punia, P., Bailey, J.R., 1998. National outbreak of *Salmonella* senftenberg associated with infant food. Epidemiol. Infect. 120, 125–128.
Santillana Farakos, S.M., Frank, J.F., Schaffner, D.W., 2013. Modeling the influence of temperature, water activity and water mobility on the persistence of Salmonella in low-moisture foods. Int. J. Food Microbiol. 166, 280–293.
Santillana Farakos, S.M., Pouillot, R., Anderson, N., Johnson, R., Son, I., Van Doren, J., 2016. Modeling the survival kinetics of *Salmonella* in tree nuts for use in risk assessment. Int. J. Food Microbiol. 227, 41–50.
Scallan, E., Hoekstra, R.M., Angulo, F.J., Tauxe, R.V., Widdowson, M.A., Roy, S.L., Jones, J.L., Griffin, P.M., 2011. Foodborne illness acquired in the United States–major pathogens. Emerg. Infect. Dis. 17, 7–15.
Sinclair, R.G., Rose, J.B., Hashsham, S.A., Gerba, C.P., Haas, 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.
Tamminga, S.K., Beumer, R.R., Kampelmacher, E.H., van Leusden, F.M., 1977. Survival of Salmonella eastbourne and Salmonella typhimurium in milk chocolate prepared with artificially contaminated milk powder. J. Hyg. 79, 333–337.
Taylor, M.H., Tsai, H., Rasco, B., Tang, J., Zhu, M.J., 2018. Stability of *Listeria Monocytogenes* in Wheat Flour during Extended Storage and Isothermal Treatment. Food Control (in press).
Tiganitas, A., Zeaki, N., Gounadaki, A.S., Drosinos, E.H., Skandamis, P.N., 2009. Study of the effect of lethal and sublethal pH and a(w) stresses on the inactivation or growth of Listeria monocytogenes and Salmonella Typhimurium. Int. J. Food Microbiol. 134, 104–112.
Uesugi, A.R., Danyluk, M.D., Harris, L.J., 2006. Survival of Salmonella enteritidis phage type 30 on inoculated almonds stored at -20, 4, 23, and 35 degrees C. J. Food Protect. 69, 1851–1857.
Vogel, B.F., Hansen, L.T., Mordhorst, H., Gram, L., 2010. The survival of *Listeria monocytogenes* during long term desiccation is facilitated by sodium chloride and organic material. Int. J. Food Microbiol. 140, 192–200.
Werber, D., Dreesman, J., Feil, F., van Treeck, U., Fell, G., Ethelberg, S., Hauri, A.M., Roggentin, P., Prager, R., Fisher, I.S., Behnke, S.C., Bartelt, E., Weise, E., Ellis, A., Siitonen, A., Andersson, Y., Tschape, H., Kramer, M.H., Ammon, A., 2005. International outbreak of *Salmonella* Oranienburg due to German chocolate. BMC Infect. Dis. 5, 7.