Food Control 113 (2020) 107160

Contents lists available at ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

Water activity influence on the thermal resistance of *Salmonella* in soy protein powder at elevated temperatures

Yuqiao Jin[a], Juming Tang[a,*], Mei-Jun Zhu[b]

[a] Department of Biological Systems Engineering, Washington State University, Pullman, WA, 99163, USA
[b] School of Food Science, Washington State University, Pullman, WA, 99163, USA

ARTICLE INFO

Keywords:
Soy protein powder
High-temperature water activity
Low-moisture foods
Salmonella

ABSTRACT

Salmonella is a leading cause of foodborne illness associated with low-moisture foods. In addition to being able to survive in low-moisture environments during long storage, *Salmonella* has shown sharply increased thermal tolerance making it difficult to control in low-moisture foods. This research utilized soy protein powder as a food career to study the thermal resistance of *Salmonella* under a wide range of temperatures and a_w. *Salmonella* inoculated soy protein powder samples were pre-equilibrated to a_w from 0.13 to 0.82 at room temperature, then subjected to heat treatment (60–99 °C) under isothermal conditions. The a_w of soy protein powders at 25 to 99 °C were measured using high-temperature a_w cells with humidity sensors. The *D*-values as a function of high-temperature a_w from 0.25 to 0.70 showed a semi-log relationship under each treatment temperature level from 70 to 99 °C. Slightly downward trends were observed when the high-temperature a_w was above 0.70, showing increased effectiveness of thermal inactivation. Results from this study provide insights to assist the design of thermal treatments for control of *Salmonella* in intermediate- and low-moisture foods.

1. Introduction

Salmonella is a common cause of foodborne illness in the U. S. (CDC, 2016). Water activity (a_w) below 0.83 is a natural barrier for *Salmonella* growth, due to the lack of sufficient available water to sustain growth. But *Salmonella* can survive for years in intermediate-moisture (a_w 0.60 to 0.83) and low-moisture foods (a_w below 0.60) (Erickson, 1982; Gould, 1996; Haas & Herman, 1978; Karel, 1973; Ozturk, Kong, & Singh, 2020; Sperber, 1983). *Salmonella* outbreaks in low-moisture foods such as almonds, peanut butter, rice cereal, spices, infant formula, and oat cereal have caused significant public health concern (CDC, 1998; CDC, 2004; CDC, 2008; CDC, 2009; Chen, Wei, Irmak, Chaves, & Subbiah, 2019; Portela et al., 2019).

Previous published studies related the thermal resistance of *Salmonella* to the a_w of food samples measured at room temperature, although the food products were treated at elevated temperatures (Syamaladevi, Tang, & Zhong, 2016a). Smith, Hildebrandt, Casulli, Dolan, and Marks (2016) modeled the effect of room-temperature a_w (0.30–0.70) influence on the thermal resistance of *Salmonella* in wheat flour at three temperatures (75, 80, and 85 °C). Garces-Vega, Ryser, and Marks (2019) studied the relationships of room temperature a_w and moisture content to the inactivation kinetics of *Salmonella* in almonds. The a_w at treatment temperature was seldom taken into consideration when evaluating the inactivation efficiency of *Salmonella* in low-moisture foods. Recent studies show that the a_w at treatment temperatures is one of key factors influencing the thermal resistance of *Salmonella* in low-moisture foods such as wheat flour, almond flour, and whey protein (Liu, Tang, Tadapeneni, Yang, & Zhu, 2018; Xu et al., 2019). The value of a_w equals to the vapor pressure of a food sample to the vapor pressure of pure water under the same pressure and temperature (Scott, 1953). It is a thermodynamic value that varies in different foods and temperatures (Jin, Tang, & Sablani, 2019; Scott, 1953; Syamaladevi et al., 2016a). For example, a_w of high-carbohydrate corn starch increased from initial 0.52 to approximately 0.67 as temperature increased from 25 to 80 °C, while a_w of high-fat coconut powder remained constant at 0.52 over the same temperature change (Jin et al., 2019). It has been hypothesized that when bacterial cells were exposed to the micro-environment in the food during thermal treatments, the cells rapidly adjusted their moisture content in response to their micro-environment a_w (Syamaladevi et al., 2016). Thus, the a_w of food at treatment temperatures, rather than the a_w of the food at room temperature, closely reflects the micro-environmental conditions influencing the thermal resistance of *Salmonella* in intermediate- and low-moisture foods. Quantifying the a_w change at elevated temperatures and studying the influence of high-temperature a_w on thermal resistance of *Salmonella* in food systems is highly desirable for future

* Corresponding author.
E-mail address: jtang@wsu.edu (J. Tang).

https://doi.org/10.1016/j.foodcont.2020.107160
Received 25 October 2019; Received in revised form 8 February 2020; Accepted 10 February 2020
Available online 11 February 2020
0956-7135/

development of effective thermal treatments.

Liu et al. (2018) reported a semi-log trend between the *D*-values (time required to achieve one log reduction of microorganism at a given temperature) of *Salmonella* and the high-temperature a_w of wheat flour and silicon dioxide at 80 °C. Although wheat flour and silicon dioxide represented two totally different low-moisture matrices, the *D*-values of *S*. Enteritidis in these two matrices were not significantly different at the selected high-temperature a_w (Liu et al., 2018). Xu et al. (2019) reported a similar log-linear relationship between logarithmic *D*-values of *S*. Enteritidis and high-temperature a_w in wheat flour, almond flour and whey protein powder. The influence of food compositions on the obtained *D*-values was likely caused by the different high-temperature a_w change in those food samples.

This research selected the soy protein powder as the carrier to investigate the relationships between the high-temperature a_w and *D*-values for *Salmonella* over a wide range of treatment temperatures. Data from this study should provide insights on processing safety of intermediate and low-moisture foods under thermal treatments.

2. Material and method

2.1. Food material

The soy protein (GNC, Pittsburgh, PA) was specifically selected to represent a high-protein model food. It consisted of 93.1 g protein, 4.5 g ash, and 2.4 g fat per 100 g dry solids (Jin et al., 2019). The absence of *Salmonella* in the source soy protein was assessed by taking ten 10 g samples randomly and soaking into 90 mL tryptic soy broth with 0.6% yeast extract (TSBYE; Difco, BD, Franklin Lakes, NJ) for 1 h. Dilutions were plated onto tryptic soy agar with 0.6% yeast extract (TSAYE; Difco, BD) with 0.05% ammonium ferric citrate (Sigma-Aldrich, St. Louis, MO) and 0.03% sodium thiosulfate pentahydrate (Sigma-Aldrich) (Smith et al., 2016). *Salmonella* can show black colonies on this differential media (sTSAYE).

2.2. Determination of a_w at different temperatures

Soy protein powder was vacuum dried at 25 °C under 10 kPa for ten days in a vacuum oven to reach the final a_w of less than 0.1 at 25 °C. Dried powders were then conditioned in a humidity controlled glove box (Smith et al., 2016) pre-equilibrated to relative humidity of 10%, 20%, 30%, 40%, 50%, 60%, 70%, and 80% at room temperature. The value of a_w in the conditioned food samples equaled to the value of relative humidity divided by 100. The a_w of samples at 25 °C were measured by a calibrated a_w meter (Aqualab, Meter Group, Inc., Pullman, WA) to confirm the equilibrium. The moisture content (X_w) of samples at each initial a_w level was measured by a halogen moisture analyzer (Mettler-Toledo, LLC, Columbus, OH). The a_w of samples at different temperatures between 25 and 99 °C were measured by a high-temperature water activity test cell in about 5 °C increment (METER Group, Inc., Pullman, WA). Details of the cell are described in Tadapaneni, Yang, Carter, and Tang (2017). Briefly, a high-temperature cell consisted of an aluminum cup, and a capacitance-based relative humidity and temperature sensor (Honeywell HumidIcon™, Morristown, NJ) installed in a lid. This cell provided reliable high-temperature values of a_w in food samples in an enclosed environment during thermal treatments (Tadapaeni et al., 2017).

In the current study, each conditioned sample was sealed into the high-temperature water activity cell, and the cell was immersed into the glycol bath (Isotemp 5150 H11, Fisher Scientific, Waltham, MA) setting at different temperatures to reach an equilibrium state (approximately 30 min). Two replicates were performed at each conditioned a_w.

2.3. Bacterial strain

Salmonella Agona 447967 was chosen for its high thermal resistance and frequent outbreak in intermediate and low-moisture foods (Taylor, Barnett, del Rosario, Williams, & Barth, 1998; CDC, 2008; Jin et al., 2018; Verma et al., 2018; Dangel et al., 2019). This strain was obtained from U. S. Food and Drug Administration Arkansas Regional Laboratory (Jefferson, AR), and stored as frozen culture at - 80 °C. The working culture was streaked on TSAYE at 37 °C for 24 h and then kept at 4 °C.

2.4. Inoculum preparation

One single colony of *S.* Agona from the working culture was aseptically inoculated into 10 mL TSBYE and incubated at 37 °C for 24 h. A 100 μL of culture was spread plated on TSAYE and incubated at 37 °C for 24 h. The bacterial lawn was harvested by adding 1 mL of buffered peptone water (BPW, Difco, BD) per plate.

2.5. Sample preparation

S. Agona was inoculated into vacuum dried soy protein with a ratio of 1 mL (*Salmonella*, ~11 log CFU/mL): 300 g (soy protein). The inoculated soy protein powder was mixed by alternately hand massaging for 3 min and stomaching (Stomacher 400 Circulator, Seward Laboratory System Inc., Norfolk, UK) for 3 min with 230 strokes/min, three times each. The inoculated soy protein powder was then conditioned for 2 days in the humidity controlled glove box pre-equilibrated to the target relative humidity of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, respectively, at room temperature. The equilibrium a_w of inoculated food samples after conditioning was measured at 25 °C by a water activity meter (Aqualab, Meter Group, Inc., Pullman, WA, USA).

2.6. Thermal treatment

A custom test cell with a thermocouple (Type T, OMEGA Engineering, INC., Stamford, CT) secured in the sample center was immersed into a glycol bath to measure the come-up time of a soy protein sample at each target temperature. The improved design of TDT cells, as described in Jin and Tang (2019), were loaded with inoculated soy protein powder (0.5 g per cell) in the humidity control glove box, and sealed by a tightening fixture. The test cells were treated in the glycol bath at minimum four different temperatures, depending on sample initial a_w (see Table 1). At least five time intervals after the come-up time were selected for each temperature. After different intervals, three test cells were immediately immersed into the ice water to cool down the food samples in the cells. The soy protein samples were serially diluted in BPW. Aliquots were spread plated on sTSAYE in duplicate. The plates were incubated at 37 °C for 24 h. The tests were replicated three times.

2.7. Data analyses

The microbial counts were expressed as log CFU/g. Statistical analysis was performed in Microsoft Excel (16.10, Microsoft, WA, USA). Linear regression was used to fit the *Salmonella* inactivation data to a log-linear model:

$$\log\left(\frac{N}{N_o}\right) = \frac{-t}{D} \tag{1}$$

where N and N_o are surviving population (CFU/g) at times t (min) and 0 (after come-up time), respectively. D is the time (min) required to kill 90% of a microorganism in a population at a specific temperature (°C).

The z_T-value for a fixed room-temperature a_w was calculated using the Bigelow secondary model:

$$\log D = \log D_{ref} - \frac{1}{z_T}(T - T_{ref}) \tag{2}$$

where D_{ref} is the time needed to achieve 1-log reduction of microbial population at reference temperature T_{ref}, z_T is temperature change

Table 1
D-values and z_T-values of *Salmonella* at different temperature and a_w.

a_w at 25 °C	a_w at treatment temperature	Treatment temperature (° C)	*D*-value (min)	95% CI lower limit	95% CI upper limit	RMSE	z_T-value (° C)	95% CI lower limit	95% CI upper limit
0.13	0.25	80	155.2	140.6	170.8	0.56	12.5	11.8	13.3
0.13	0.27	85	50.2	46.4	53.8	0.36			
0.13	0.30	90	18.2	17.1	19.4	0.24			
0.13	0.31	95	8.3	8.0	9.0	0.23			
0.13	0.32	99	4.7	4.2	5.0	0.34			
0.22	0.36	80	93.9	90.5	104.1	0.30	13.2	12.7	13.7
0.22	0.38	85	33.4	31.5	35.7	0.27			
0.22	0.40	90	15.9	14.5	16.6	0.29			
0.22	0.42	95	6.4	5.9	6.6	0.24			
0.22	0.43	99	3.5	3.2	3.6	0.25			
0.30	0.47	80	65.1	61.7	68.6	0.22	13.1	12.2	14.2
0.30	0.48	85	21.1	19.8	22.3	0.26			
0.30	0.50	90	7.8	7.3	8.1	0.21			
0.30	0.51	95	4.2	3.9	4.5	0.25			
0.30	0.52	99	2.2	2.0	2.3	0.30			
0.41	0.52	75	99.9	97.3	107.1	0.25	11.6	11.0	12.2
0.41	0.53	80	48.7	46.7	51.5	0.25			
0.41	0.55	85	14.6	13.56	15.2	0.24			
0.41	0.57	90	4.9	4.6	5.2	0.28			
0.41	0.58	95	2.2	2.1	2.3	0.19			
0.50	0.58	70	152.6	140.8	159.8	0.26	11.2	10.5	12.1
0.50	0.59	75	77.2	72.7	81.8	0.29			
0.50	0.60	80	28.6	26.8	30.7	0.29			
0.50	0.61	85	7.5	7.1	7.9	0.23			
0.50	0.62	90	2.9	2.7	3.1	0.24			
0.61	0.68	70	90.5	85.9	94.8	0.22	10.8	10.2	11.6
0.61	0.68	75	39.1	38.0	44.1	0.33			
0.61	0.69	80	11.1	10.5	11.7	0.24			
0.61	0.70	85	4.0	3.7	4.2	0.27			
0.70	0.74	70	71.4	67.6	77.8	0.26	8.0	7.9	8.2
0.70	0.75	75	17.5	16.6	18.3	0.20			
0.70	0.76	80	4.0	3.8	4.3	0.28			
0.70	0.76	85	1.0	0.9	1.1	0.31			
0.82	0.84	60	161.7	152.3	171.8	0.23	6.7	6.3	7.0
0.82	0.85	65	39.9	37.6	42.3	0.23			
0.82	0.85	70	6.3	5.9	6.8	0.32			
0.82	0.86	75	1.0	0.9	1.0	0.24			

z_T-value: the temperature change required to cause a 90% reduction in *D*-value. The z_T-values were estimated based on the treatment temperatures at each a_w levels at 25 °C. CI: confidence interval. RMSE: root mean square error.

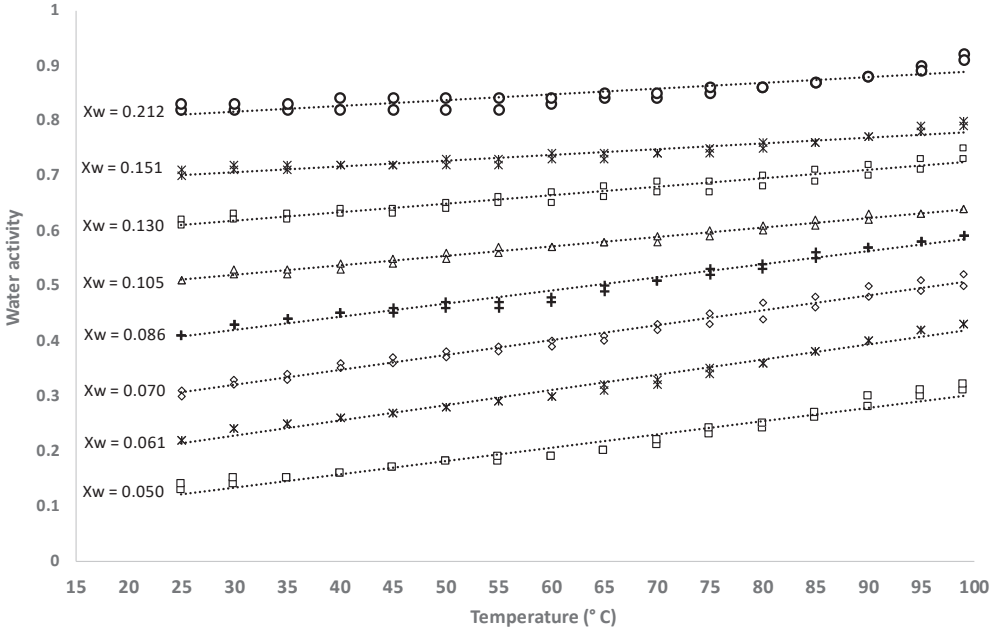

Fig. 1. The a_w values of soy protein powder at different temperatures and different moisture content X_w (g H_2O/g dry solid). n = 2, lines are the linear regressions of the data.

required to cause a 90% reduction in D-value. The z_T-values were estimated based on the treatment temperatures at each a_w levels at 25 °C.

The z_{aw} for a fixed temperature was calculated by using the flowing equation:

$$\log D = \log D_{ref} - \frac{1}{z_{aw}}(a_w - a_{w\,ref}) \quad (3)$$

where D_{ref} is the time needed to achieve a 1-log reduction of microbial population at $a_{w\,ref}$, z_{aw} is a_w change required to cause a 90% reduction in D-value. The z_{aw}-values at each treatment temperature levels were estimated based on the room-temperature (25 °C) a_w and high-temperature a_w, respectively.

The root mean square error (RMSE) was used to estimate model error:

$$RMSE = \sqrt{\frac{\sum (\log(N/N_0)_{\text{predicted}} - \log(N/N_0)_{\text{observed}})^2}{n - p}} \quad (4)$$

where N and N_o are microbial populations at times t and 0, n is the total number of observations, and p is the number of model parameters. The RMSE and confidence interval (CI) were analyzed through the Minitab (19.2.0.0, Minitab Inc., PA, USA).

3. Results and discussion

3.1. a_w at different temperatures

The a_w changes in soy protein powder samples at different temperatures are shown in Fig. 1. The a_w increased with increasing temperature at each fixed moisture content (X_w, g H_2O/g solids). The sample of low X_w had more significant a_w increase with temperature. For example, the a_w of the sample with X_w 0.050 increased from 0.13 to 0.32 as temperature increased from 25 to 99 °C, while the a_w of the sample with X_w 0.212 increased from 0.82 to 0.92. Similar trends showing the temperature influence on the a_w have also been reported for wheat flour, non-fat milk powder, corn starch, and cheese powder (Jin et al., 2019; Tadapaneni et al., 2017).

3.2. Thermal resistance of Salmonella

The *Salmonella* in the source soy protein was lower than the detectable limit of 1.7 log CFU/g in this study. The initial inoculation of *Salmonella* was larger than 7 log CFU/g. Homogeneity was achieved with a standard deviation of inoculated *Salmonella* population less than 0.3 log CFU/g. The come-up time for the protein powder in the new TDT cells when heated to the temperature ranges from 60 to 99 °C was in each case less than 1 min, and was conservatively chosen as 1 min.

Representative results from isothermal inactivation of *Salmonella* in soy protein powders are shown in Figs. 2 and 3. The trend lines are fitted curves based on the log-linear model (Eq. (1)). As expected, high temperature resulted in more rapid inactivation of *Salmonella*. For example, the inactivation of *Salmonella* increased with increasing thermal temperature from 80 to 99 °C for the inoculated soy protein powder conditioned to a_w 0.22 at room temperature (Fig. 2). High a_w could also led to a large microbial reduction, as the survival of *Salmonella* decreased from high-temperature a_w 0.27 to 0.76 at the same thermal temperature of 85 °C (Fig. 3). Similar phenomena were also reported in wheat flour, almond flour, and silicon dioxide (Liu et al., 2018; Villa-Rojas et al., 2013; Xu et al., 2019).

3.3. Changes of D-values with high-temperature a_w

The D-values of *Salmonella* obtained from the isothermal inactivation experiments using Eq. (1) are summarized in Table 1. Both the low temperatures and low high-temperature a_w led to large D-values. For example, the D-value for the same high-temperature a_w of 0.58 was 2.2 min at 95 °C, and it increased almost 70 times (152.6 min) at 70 °C. The D-value at 85 °C was 4.0 min at high-temperature a_w of 0.70; it increased 12.5 times (50.2 min) at high-temperature a_w of 0.27. High temperature combined with high a_w could more readily cause protein unfolding, enzyme inactivation, ribosomal RNA and DNA destruction, and cytoplasmic membrane damage of *Salmonella* (Mackey, Miles, Parsons, & Seymour, 1991; Nguyen & Corry, 2006). Thus, the thermal inactivation of bacteria cells is more effective under high a_w environment (Lee & Kaletunc, 2002; Mackey et al., 1991).

Bacterial spores had higher thermal resistance than vegetative cells, due to the lower molecular flexibility of protein structures in spores (Potts, 1994; Sunde, Setlow, Hederstedt, & Halle, 2009). Similar to spores, it was hypothesized that the vegetative bacterial cells in low a_w environment also had similar adaptation mechanisms (Esbelin, Santos, & Hébraud, 2018). For example, some non-spore forming bacteria in low a_w environment had lower molecular mobility (Csonka & Hanson, 1991; Finn, Condell, McClure, Amézquita, & Fanning, 2013), more stabilized ribosomal units (Gruzdev et al., 2012), and filaments to against thermal damage (Stackhouse, Faith, Kaspar, Czuprynski, & Wong, 2012), compared with the cells in the intermediate and high a_w environment. When bacterial cells are heated in the intermediate and high a_w environment, water molecules could vibrate, break disulfide and hydrogen bonds in the surrounding protein, alter the three-dimensional configuration, prevent protein functioning, and lead to a rapid inactivation of cells (Earnshaw, Appleyard, & Hurst, 1995). Further research on the thermal resistance of bacteria as influenced by the molecular mobility is desired.

3.4. z_T-values and z_{aw}-values

The influence of treatment temperature on D-values of *Salmonella* in soy protein samples preconditioned to different room-temperature a_w levels are summarized in Fig. 4. The sensitivity of D-value to temperature change is characterized by z_T using Eq. (2). The z_T is the inverse of the slope for each curve in Fig. 4. As shown in Table 1, the z_T-value decreased from 8.0 to 6.7 °C as room-temperature a_w increased from 0.70 to 0.82. Similar trends were found in Villa-Rojas et al. (2013) that the z_T-value for *S.* Enteritidis PT 30 decreased from 8.4 to 6.6 °C with increasing room-temperature a_w from 0.72 to 0.89 in almond flour.

The z_T-values between room-temperature a_w 0.13 to 0.61 were all above 10 °C, and were much larger than the z_T-values between a_w 0.70 to 0.82 (below 10 °C) (Table 1). This trend was reflected in Fig. 4, the slopes of the curves became less steep between room-temperature a_w 0.13 and 0.61, indicating an increase in z_T-value at low a_w. Similar result was found in Jin et al. (2018) that the z_T-value for *S.* Agona in a cracker dough matrix at room temperature a_w of 0.50 was 11.5 °C. Villa-Rojas et al. (2013) reported the z_T-value of 10.4 °C for *S.* Enteritidis PT 30 in the almond kernel flour which was conditioned at a_w 0.60 at room temperature. Tsai et al. (2019) reported that the z_T-values of *Salmonella* cocktail (*S.* Enteritidis PT 30, *S.* Tennessee K4643, and *S.* Agona 447967) were 16.9 and 15.2 °C in cocoa powder at room-temperature a_w 0.3 and 0.45, respectively. *Salmonella* cells in low a_w environment showed similar z_T-value to spores, such as *Clostridium botulinum*, which had an approximate z_T-value of 10 °C or larger (Diao, André, & Membré, 2014). It was hypothesized that vegetative cells such as *Salmonella* in a low a_w environment may behave like spores (Syamaladevi et al., 2016b). The desiccated *Salmonella* cells had lower molecular mobility and were less sensitive to temperature change. When a_w increased from 0.70 to 0.82, which was in the intermediate-moisture state, the food environment could provide relatively more free water for vegetative cells than low-moisture foods. It is possible that vegetative cells had higher molecular mobility in the intermediate-moisture environment. This explained the lower z_T-values (below 10 °C) of *Salmonella* at higher a_w levels (above 0.70). Similarly, the z_T-value for *Salmonella* Typhimurium in chocolate syrup with the a_w of 0.84 was

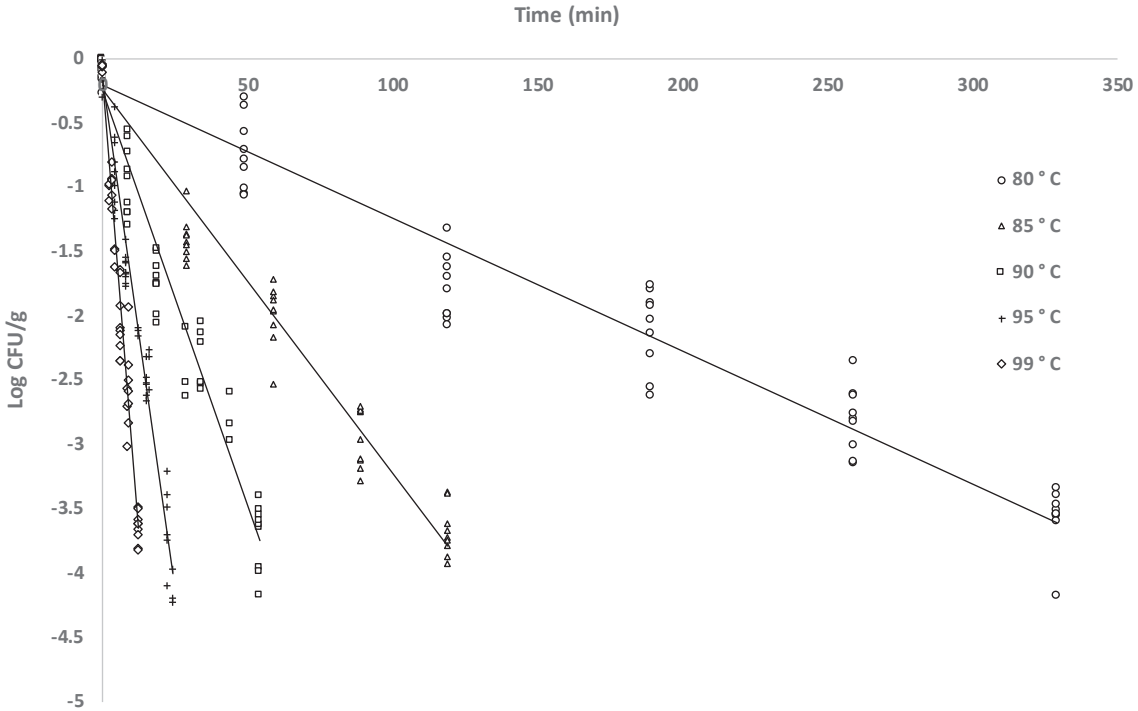

Fig. 2. Thermal inactivation of *Salmonella* in soy protein powder equilibrated to a_w 0.22 at 25 ˚C, and treated at 80, 85, 90, 95, and 99 ˚C.

7.6 ˚C, which was also lower than 10 ˚C (Sumner, Sandros, Harmon, Scott, & Bernard, 1991).

The *D*-values of *Salmonella* as function of high-temperature a_w exhibited a semi-log relationship between the high-temperature a_w from 0.25 to 0.70 (Fig. 5). Similar semi-log phenomena of the *D*-values of *Salmonella* were also reported in wheat flour, almond flour, and whey protein at 80 ˚C (Xu et al., 2019). A slightly downward trend was found above the high-temperature a_w of 0.70. This suggested that *Salmonella* were more easily inactivated when the product high-temperature a_w was larger than 0.70. The high-temperature a_w below 0.70 and above 0.70 might be considered differently, when designing thermal treatments. For example, the *D*-value of *Salmonella* decreased almost a hundred times from 99.9 to 1.0 min, if the high-temperature a_w increased from 0.52 to 0.86 at 75 ˚C. Thus, increasing high-temperature a_w of a food product or the relative humidity of the treatment environment could lead to a more rapid inactivation of bacteria cells. But

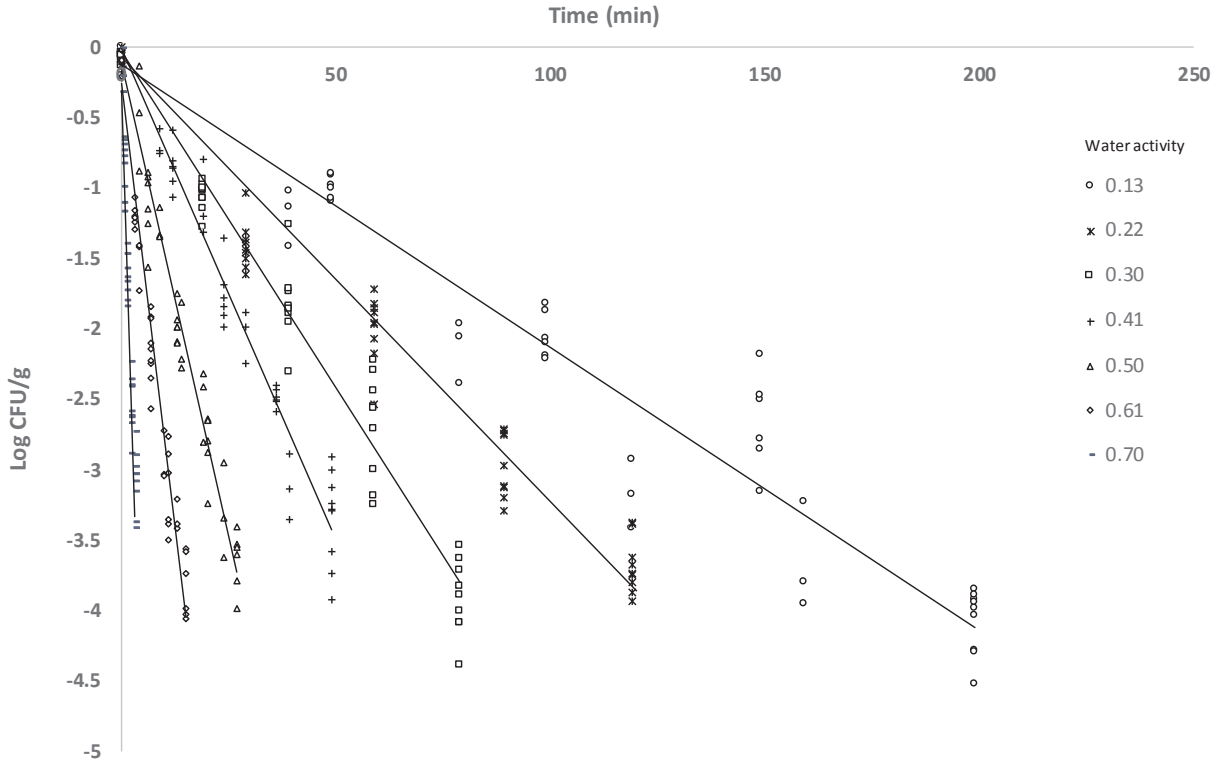

Fig. 3. Thermal inactivation of *Salmonella* in soy protein powder equilibrated to a_w 0.13, 0.22, 0.30, 0.41, 0.50, 0.61, and 0.70 at 25 ˚C, and treated at 85 ˚C.

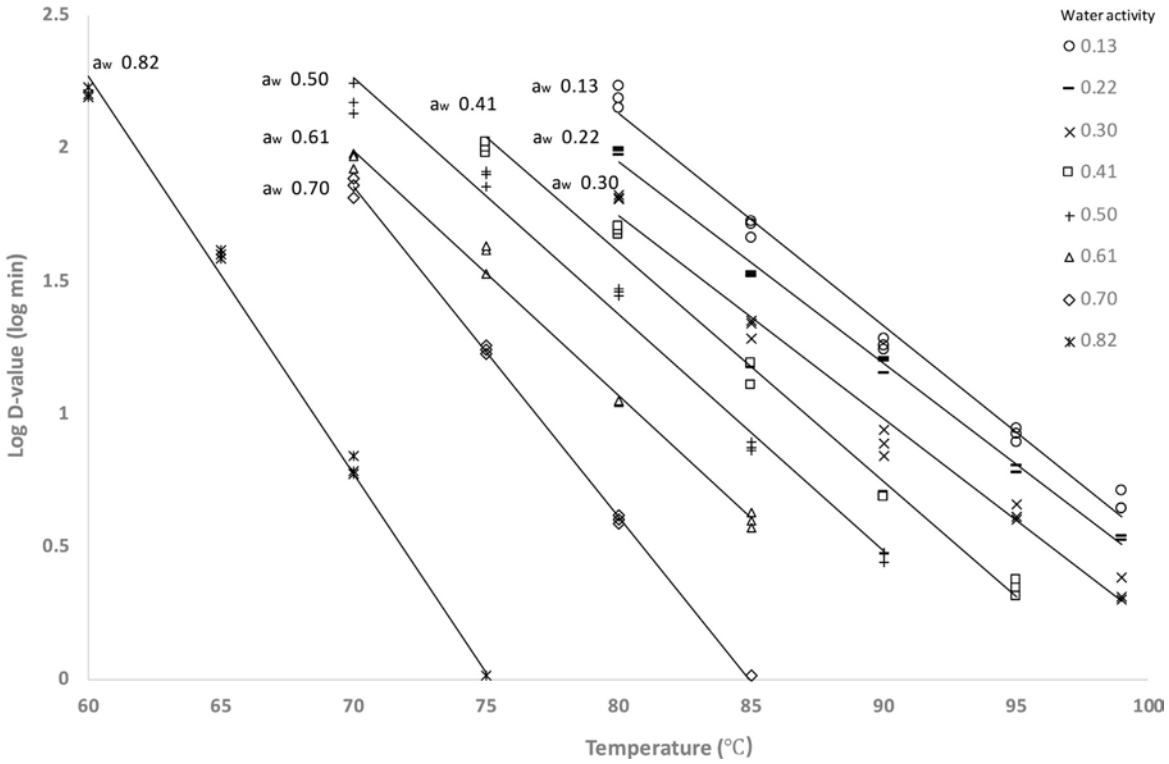

Fig. 4. Log *D*-value of *Salmonella* in soy protein powder equilibrated to a_w 0.13, 0.22, 0.30, 0.41, 0.50, 0.61, 0.70, and 0.82 at 25 °C, and treated under a wide range of temperatures.

it is worth noting that additional dehydration process may be needed after the thermal process to maintain the quality and shelf life of low-moisture foods.

As the small vegetative cells are exposed in the high-temperature a_w micro-environment during heat treatment, the high-temperature a_w, instead of room-temperature a_w, should be more closely related to the thermal resistance of *Salmonella* cells in the intermediate- and low-moisture foods. It is consistent with the results of larger z_T-value under lower room-temperature a_w in Table 1. For example, at room-temperature a_w of 0.61, the high-temperature a_w was between 0.68 and 0.70 under the heating temperature from 70 to 85 °C. And at room-temperature a_w of 0.70, the high-temperature a_w was between 0.74 and

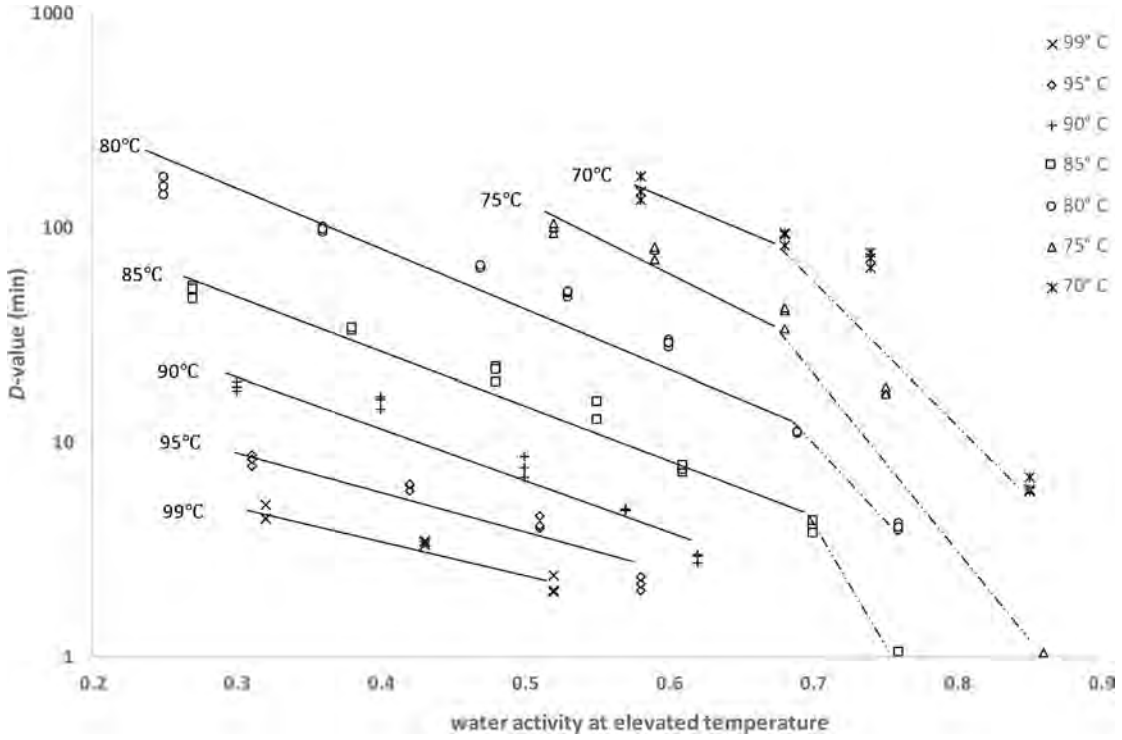

Fig. 5. The log-scale *D*-values of *Salmonella* and high-temperature a_w at 70, 75, 80, 85, 90, 95, and 99 °C.

Table 2
z_{aw}-values between 70 and 99 °C based on room-temperature (25 °C) a_w from 0.13 to 0.61 and its corresponding high-temperature a_w from 0.25 to 0.70, respectively.

Temperature (° C)	Room-temperature (25 °C) a_w				High-temperature a_w			
	z_{aw}	95% CI lower limit	95% CI upper limit	R^2	z_{aw}	95% CI lower limit	95% CI upper limit	R^2
70	0.49	0.34	0.90	0.90	0.44	0.30	0.82	0.90
75	0.48	0.39	0.64	0.93	0.39	0.31	0.50	0.94
80	0.45	0.41	0.50	0.97	0.41	0.36	0.47	0.94
85	0.44	0.41	0.47	0.99	0.39	0.35	0.44	0.96
90	0.45	0.40	0.50	0.97	0.40	0.34	0.47	0.94
95	0.48	0.43	0.54	0.97	0.49	0.40	0.62	0.91
99	0.51	0.42	0.66	0.94	0.61	0.49	0.80	0.93

z_{aw}-value: the a_w change required to cause a 90% reduction in *D*-value. R^2: coefficient of determination.

0.76 at the same temperature range. Higher high-temperature a_w could provide more free water in the food environment. *Salmonella* thus showed less thermal resistance with a lower z_T-value of 8.0 °C at room-temperature a_w of 0.70, compared with a higher thermal resistance and z_T-value of 10.8 °C at room-temperature a_w of 0.61.

The z_{aw}-values based on room-temperature (25 °C) a_w from 0.13 to 0.61 and its corresponding high-temperature a_w from 0.25 to 0.70, respectively, were calculated using Eq. (3) and summarized in Table 2. The z_{aw}-values based on high-temperature a_w were relatively unchanged (between 0.39 and 0.41) with treatment temperature increasing from 75 to 90 °C. Similar phenomena was also found in the z_{aw}-values based on room-temperature a_w (between 0.44 and 0.48) under the same temperature range. Different *Salmonella* serotypes had different high-temperature z_{aw}-values. For example, the z_{aw}-value of *S.* Enteritidis PT 30 in wheat flour and whey protein powder was 0.32 at 80 °C (Xu et al., 2019). It was lower than the z_{aw}-value of 0.45 for *S.* Agona in soy protein powder at the same temperature, indicating a more significant a_w change influence on the thermal resistance of *S.* Enteritidis PT 30 compared with *S.* Agona.

This research provides insights into the impact of a_w at treatment temperature on thermal resistance of *Salmonella*. The experimentally determined relationships between thermal resistance of *Salmonella* and a_w at treatment temperature can be used for the prediction of *Salmonella* inactivation in intermediate- and low-moisture foods during heat process.

4. Conclusions

Thermal resistance of *Salmonella* is highly dependent on treatment temperature and the high-temperature a_w of the food environment. The *D*-values and high-temperature a_w showed a semi-log relationship between the high-temperature a_w from 0.25 to 0.70 at any given temperature between 70 and 99 °C. *D*-values decreased more sharply when a_w increased above 0.70. The high-temperature a_w at 0.70 might therefore be a threshold above which a thermal treatment for *Salmonella* became more effective. Understanding the correlation between the high-temperature a_w and thermal resistance of *Salmonella* could lead to better design of heat treatments for pathogen control in low-moisture foods.

CRediT authorship contribution statement

Yuqiao Jin: Conceptualization, Methodology, Writing - original draft, Data curation. **Juming Tang:** Funding acquisition, Conceptualization. **Mei-Jun Zhu:** Conceptualization.

Acknowledgements

This work was supported by the USDA Agricultural and Food Research Initiative (AFRI) CAP grant 2015-68003-23415. We thank the WSU Engineering Shop for the fabrication of the new design of aluminum test cells.

References

Centers for Disease Control and Prevention (1998). *Multistate outbreak of Salmonella serotype Agona infections linked to toasted oats cereal – United States, April – may, 1998.* Retrieved from https://www.cdc.gov/mmwr/preview/mmwrhtml/00053368.htm Accessed 19 October 2019 .

Centers for Disease Control and Prevention. Outbreak of Salmonella Serotype Enteritidis infections associated with raw almond. (2004). Retrieved from https://www.cdc.gov/mmwr/preview/mmwrhtml/mm5322a8.htm Accessed 11 August 2019.

Centers for Disease Control and Prevention. Multistate outbreak of Salmonella Agona infections linked to rice and wheat puff cereal. (2008). Retrieved from https://www.cdc.gov/salmonella/2008/rice-wheat-puff-cereal-5-13-2008.html Accessed 11 August 2019.

Centers for Disease Control and Prevention. Multistate outbreak of Salmonella Typhimurium infections linked to peanut butter. (2009). Retrieved from https://www.cdc.gov/salmonella/2009/peanut-butter-2008-2009.html Accessed 11 August 2019.

Centers for Disease Control and Prevention. Salmonella. (2016). Retrieved from https://www.cdc.gov/dotw/salmonella/index.html Accessed 1 February 2020.

Chen, L., Wei, X., Irmak, S., Chaves, B. D., & Subbiah, J. (2019). Inactivation of *Salmonella enterica* and *Enterococcus faecium* NRRL B-2354 in cumin seeds by radiofrequency heating. *Food Control, 103*, 59–69.

Csonka, L. N., & Hanson, A. D. (1991). Prokaryotic osmoregulation: Genetics and physiology. *Annual Review of Microbiology, 45*, 569–606.

Dangel, A., Berger, A., Messelhäußer, U., Konrad, R., Hörmansdorfer, S., Ackermann, N., et al. (2019). Genetic diversity and delineation of *Salmonella* Agona outbreak strains by next generation sequencing, Bavaria, Germany, 1993 to 2018. *Euro Surveillance, 24*(18), 46–58.

Diao, M. M., André, S., & Membré, J. M. (2014). Meta-analysis of D-values of proteolytic *Clostrdium botulinum* and its surrogate strain *Clostridium sporogenes* PA 3679. *International Journal of Food Microbiolgoy, 174*, 23–30.

Earnshaw, R. G., Appleyard, J., & Hurst, R. M. (1995). Understanding physical inactivation processes: Combined preservation opportunities using heat, ultrasound and pressure. *International Journal of Food Microbiology, 28*(2), 197–219.

Erickson, L. E. (1982). Recent developments in intermediate moisture foods. *Journal of Food Protection, 45*, 484–491.

Esbelin, J., Santos, T., & Hébraud, M. (2018). Desiccation: An environmental and food industry stress that bacteria commonly face. *Food Microbiology, 69*, 82–88.

Finn, S., Condell, O., McClure, P., Amézquita, & Fanning, S. (2013). Mechanisms of survival, responses, and sources of *Salmonella* in low-moisture environments. *Frontiers in Microbiology, 4*, 331.

Garces-Vega, F. J., Ryser, E. T., & Marks, B. P. (2019). Relationships of water activity and moisture content to the thermal inactivation kinetics of *Salmonella* in low-moisture foods. *Journal of Food Protection, 82*(6), 963–970.

Gould, G. W. (1996). Methods for preservation and extension of shelf life. *International Journal of Food Microbiology, 33*, 51–64.

Gruzdev, N., Mcclelland, M., Porwollik, S., Ofaim, S., Pinto, R., & Saldinger-Sela, S. (2012). Global transcriptional analysis of dehydrated *Salmonella enterica* serovar Typhimurium. *Applied and Environmental Microbiology, 78*, 7866–7875.

Haas, F. J., & Herman, E. B. (1978). Bacterial growth in intermediate moisture food systems. *Lebensmittel-Wissenschaft & Technologie, 11*, 74–78.

Jin, Y., Pickens, S. R., Hilderbrandt, I. M., Burbrick, S. J., Grasso-Kelley, E. M., Keller, S. E., et al. (2018). Thermal inactivation of *Salmonella* Agona in low-water activity foods: Predictive models for the combined effect of temperature, water activity, and food component. *Journal of Food Protection, 81*(9), 1411–1417.

Jin, Y., & Tang, J. (2019). Improved design of aluminum test cell to study the thermal resistance of *Salmonella enterica* and *Enterococcus faecium* in low-water activity foods. *Food Control, 104*, 343–348.

Jin, Y., Tang, J., & Sablani, S. S. (2019). Food component influence on water activity of low-moisture powders at elevated temperatures in connection with pathogen control. *LWT-Food Science and Technology, 112*, 108257.

Karel, M. (1973). Recent research and development in the field of low-moisture and intermediate-moisture foods. *Critical Reviews In Food Technology, 3*, 329–373.

Lee, J., & Kaletunc, G. (2002). Evaluation of the heat inactivation of *Escherichia coli* and *Lactobacillus plantarum* by differential scanning calorimetry. *Applied and Environmental Microbiology, 68*(11), 5379–5386.

Liu, S., Tang, J., Tadapeneni, R. K., Yang, R., & Zhu, M. (2018). Exponentially increased thermal resistance of *Salmonella* spp. and *Enterococcus faecium* at reduced water activity. *Applied and Environmental Microbiology, 84*(8) 2742-2717.

Mackey, B. M., Miles, C. A., Parsons, S. E., & Seymour, D. A. (1991). Thermal denaturation of whole cells and cell components of *Escherichia coli* examined by differential scanning calorimetry. *Journal of General Microbiology, 137*, 2361–2374.

Nguyen, H. T., Corry, J. E., & Miles, C. A. (2006). Heat resistance and mechanism of heat inactivation in thermophilic Campylobacters. *Applied and Environmental Microbiology, 72*(1), 908–913.

Ozturk, S., Kong, F., & Singh, R. K. (2020). Evaluation of *Enterococcus faecium* NRRL B-2354 as a potential surrogate of *Salmonella* in packaged paprika, white pepper and cumin powder during radio frequency heating. *Food Control, 108*, 106833.

Portela, J. B., Coimbra, P. T., Cappato, L. P., Alvarenga, V. O., Oliveira, R. B. A., Pereira, K. S., et al. (2019). Predictive model for inactivation of salmonella in infant formula during microwave heating processing. *Food Control, 104*, 308–312.

Potts, M. (1994). Desiccation tolerance of prokaryotes. *Microbiological Reviews, 58*(4), 755–805.

Scott, W. J. (1953). Water relations of *Staphylococcus aureus* at 30 °C. *Australian Journal of Biological Sciences, 6*(4), 549–564.

Smith, D. F., Hildebrandt, I. M., Casulli, K. E., Dolan, K. D., & Marks, B. P. (2016). Modeling the effect of temperature and water activity on the thermal resistance of *Salmonella* Enteritidis PT 30 in wheat flour. *Journal of Food Protection, 79*(12), 2058–2065.

Sperber, W. H. (1983). Influence of water activity on foodborne bacteria - a review. *Journal of Food Protection, 46*(2), 142–150.

Stackhouse, R. R., Faith, N. G., Kaspar, C. W., Czuprynski, C. J., & Wong, A. C. L. (2012). Survival and virulence of *Salmonella enterica* serovar Enteritidis filaments induced by reduced water activity. *Applied and Environmental Microbiology, 78*(7), 2213–2220.

Sumner, S., Sandros, T. M., Harmon, M. C., Scott, V. N., & Scott, V. N. (1991). Heat resistance of *Salmonella typhimurium* and *Listeria monocytogenes* in sucrose solutions of various water activities. *Journal of Food Science, 6*, 1741–1743.

Sunde, E. P., Setlow, P., Hederstedt, L., & Halle, B. (2009). The physical state of water in bacterial spores. *Proceedings of the National Academy of Sciences, 106*(46), 19334–19339.

Syamaladevi, R. M., Tadapaneni, R. K., Xu, J., Villa-Rojas, R., Tang, J., Carter, B., et al. (2016a). Water activity change at elevated temperatures and thermal resistance of *Salmonella* in all purpose wheat flour and peanut butter. *Food Research International, 81*, 163–170.

Syamaladevi, R. M., Tang, J., Villa-Rojas, R., Sablani, S., Carter, B., & Campbell, G. (2016b). Influence of water activity on thermal resistance of microorganisms in low-moisture foods: A review. *Comprehensive Reviews in Food Science and Food Safety, 15*, 353–370.

Syamaladevi, R. M., Tang, J., & Zhong, Q. (2016). Water diffusion from a bacterial cell in low-moisture foods. *Journal of Food Science, 81*, 2129–2134.

Tadapaneni, R. K., Yang, R., Carter, B., & Tang, J. (2017). A new method to determifood ne the water activity and the net isosteric heats of sorption for low moisture foods at elevated temperatures. *Food Research International, 102*, 203–212.

Taylor, J. P., Barnett, B. J., del Rosario, L., Williams, K., & Barth, S. S. (1998). Prospective investigation of cryptic outbreaks of *Salmonella* Agona Salmonellosis. *Journal of Clinical Microbiology, 36*(10), 2861–2864.

Tsai, H., Ballom, K. F., Xia, S., Tang, J., Marks, B. P., & Zhu, M. (2019). Evaluation of *Enterococcus faecium* NRRL B-2354 as a surrogate for *Salmonella* during cocoa powder thermal processing. *Food Microbiology, 82*, 135–141.

Verma, T., Wei, X., Lau, S. K., Bianchini, A., Eskridge, K. M., & Subbiah, J. (2018). Evaluation of *Enterococcus faecium* NRRL B-2354 as a surrogate for *Salmonella* during extrusion of low-moisture food. *Journal of Food Science, 83*(4), 1063–1072.

Villa-Rojas, R., Tang, J., Wang, S., Gao, M., Kang, D. H., Mah, J. H., et al. (2013). Thermal inactivation of *Salmonella* Enteritidis PT 30 in almond kernels as influenced by water activity. *Journal of Food Protection, 76*(1), 26–32.

Xu, J., Tang, J., Jin, Y., Song, J., Yang, R., Sablani, S. S., et al. (2019). High temperature water activity as a key factor influencing survival of *Salmonella* Enteritidis PT 30 in thermal processing. *Food Control, 98*, 520–528.