Food Research International 157 (2022) 111288

Contents lists available at ScienceDirect

Food Research International

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

Thermal death kinetics of *Salmonella* Enteritidis PT30 in peanut butter as influenced by water activity

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ARTICLE INFO

Keywords:
Low-moisture food
Protective effect
Thermal resistance
First-order kinetics
Pathogen control
Thermal processing

ABSTRACT

It has been a challenge in developing effective thermal pasteurization processes for foods with high-fat and low-moisture contents like peanut butter, due to a general lack of reliable data on thermal resistance of pathogens in those food matrices. Recent studies on low-moisture foods like wheat flour and almond flour suggest that temperature and water activity (at the process temperatures) are two key factors that influence thermal inactivation of bacteria. In this study, we measured high-temperature water activities of peanut butter of two moisture content (MC), 3.1% and 5.6% (dry basis), and investigated the thermal death kinetics of *Salmonella enterica* Enteritidis PT 30 (*S.* Enteritidis) in those samples at 70, 80, 90, and 100 °C. The results indicated that the water activity of peanut butter increased with increasing temperature, e.g., from 0.33 and 0.53 at 23 °C, up to 0.39 and 0.59 at 100 °C, respectively. The thermal death of *S.* Enteritidis in peanut butter followed the first-order kinetics. Overall, higher moisture content and a higher treatment temperature led to a smaller *D*-value (decimal reduction time of the survival population) of *S.* Enteritidis. The maximum *D*-value was 102.6 ± 15.2 min at MC 3.1% and 70 °C, and the minimum *D*-value was 0.3 min (predicted) at MC = 5.6% and 100 °C. The log *D*-value reduced linearly with temperature at a given a_w, with *Z*-values equal to 15.4 °C (for MC = 3.1%) and 12.6 °C (for MC = 5.6%). Based on this study, the first-order kinetic model can be employed for developing and validating thermal pasteurization processes for peanut butter. The moisture content of peanut butter and the process temperature are two key parameters that need to be controlled for sufficient lethality.

1. Introduction

Food-borne Salmonellosis from consuming low-moisture foods remains to be a health risk to the public, a potential economic burden for the food industry, and may lead to criminal penalties for the responsible individuals in USA. For instance, a recent outbreak of *Salmonella* from contaminated cashew in brie caused 20 cases of illnesses and triggered a recall of the product (U.S. CDC, 2021). The worst outbreak associated with low-moisture foods happened in 2008–2009, causing 714 illnesses across the United States due to *Salmonella*-contaminated peanut butter (U.S. CDC, 2009). The peanut butter sale plummeted 24% immediately after the recall and the total industry loss was estimated to be $1 billion (Mallove, 2010).

Peanut butter is a high-fat low-moisture (HFLM) food paste that is made from grinding roasted peanuts. Tree-nut butter and tahini (sesame paste) are made in a similar manner. These three HFLM food products have been involved in multiple *Salmonella* outbreaks over the past two decades (Yang, 2020). Traditionally, these products were considered safe due to their low water activity (<0.6) which inhibits the metabolism and propagation of microorganisms (Barbosa-Canovas, et al., 2007). However, it has been reported that when pathogen contamination occurred before or during handling and processing and if the subsequent processing failed to deliver a sufficient lethality, the food matrix would cause desiccation of bacterial pathogens and enhance their ability to survive in the product (Kataoka, et al., 2014). Since it is impossible to totally prevent microbial contamination of the ingredients in the supply chain (Nascimento, et al., 2018), a valid pasteurization process is needed to reduce the risk to consumers.

Thermal processing has been considered as the most promising option for inactivating *Salmonella* in peanut butter. Early studies included

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https://doi.org/10.1016/j.foodres.2022.111288
Received 22 February 2022; Received in revised form 20 April 2022; Accepted 21 April 2022
Available online 25 April 2022

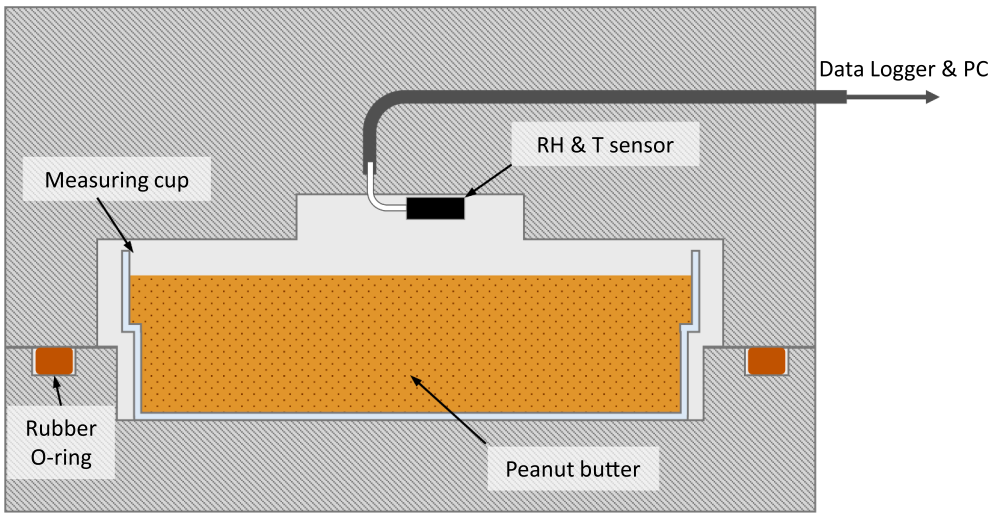

Fig. 1. High-temperature Cell (HTC) used to determine the water activity of peanut butter at elevated temperatures. The dimensions are in mm. (Please use color).

Table 1
Experimental conditions for isothermal treatment.

Moisture content (dry basis)	Water activity (at 23 °C)	Treatment temperature (°C)	Treatment time (min)	Number of independent replicates
3.1%	0.33	70	0, 5, 60, 120, 180, 240, 300, 360	3
		80	0, 5, 20, 35, 50, 65	4
		90	1 replicate at: 0, 5, 8, 11, 14, 17, 20, 23 2 replicates at: 0, 5, 10, 15, 20, 25	3
		100	0, 1, 2, 3, 4, 5, 6, 7	3
5.6%	0.53	70	0, 1, 31, 61, 91, 121, 151, 181	4
		80	0, 1, 11, 21, 31, 41, 51, 61	4
		90	0, 1, 3, 5, 7, 9, 11	4

temperature, water activity, product formulation (e.g., fat content and sugar content), and the "protective effect of oil" as the major factors influencing the thermal resistance of *Salmonella* in peanut butter (He et al., 2011; Kataoka et al., 2014; Li, Huang, & Chen, 2014; Ma et al., 2009; Shachar & Yaron, 2006). A recent study explored the possibility of pasteurizing HFLM foods (peanut butter, almond butter, and hazelnut butter) in jars using a boiling water bath (Wright et al., 2018). But without sufficient thermal death kinetic data for the target pathogens, it is difficult to properly design and control effective processes that take into account ingredients, moisture content, and the size of jars.

Most of the previously published studies on thermal death kinetics of *Salmonella* in peanut butter reported non-first-order thermal death behavior which was attributed by the authors to protective effect of oil (Li et al., 2014; Ma et al., 2009; Shachar & Yaron, 2006). Thermal resistance of *Salmonella* in oil was indeed found to be much higher than in other food matrices, which was attributed to a sharp reduction of water activity of lipid oil when heated to a high temperature (Yang et al., 2020a; 2020b). This finding is important as it clarified that the protection from oil was caused by the physical movement of water vapor between microorganisms and the surrounding oil, rather than an unknown chemical or biological reaction. However, in peanut butter, oil may not be a major ingredient that controls the water activity of the matrix, as the other components that are hydrophilic (i.e., starch and proteins) have much higher moisture capacity than oil (Yang et al., 2020). It is likely that the oil in emulsions gain moisture from the surroundings and lose the ability to keep bacteria dehydrated during thermal treatments, in contrast to the desiccated bacterial cells in heated oils (Yang et al., 2021). Our recent study indicated that non-hermetically sealed sample holders resulted in concave thermal death curves of *Salmonella* in peanut butter, due to surface drying of the sample during thermal treatments (Yang et al., 2022). This study suggests that by selecting proper sample holders, we should be able to obtain more accurate thermal death kinetic data of *Salmonella* in an HFLM food-matrix like peanut butter with fixed moisture contents.

The objectives of this study were to investigate the thermal death kinetics of *Salmonella* in peanut butter under the influence of water activity and to establish a mathematical model for process development and validation.

2. Material and methods

The experiments were conducted in three phases: 1) investigate the relationship between water activity and moisture content of peanut butter at room temperature; 2) measure the change of water activity in peanut butter samples of different moisture contents when heated to 70–100 °C; and 3) conduct thermal death kinetic studies on *Salmonella* inoculated peanut butter of fixed moisture contents at 70–100 °C.

2.1. Correlating water activity and moisture content of peanut butter

Organic creamy peanut butter in 16 oz jars was purchased from a local grocery in Pullman, WA, USA. The samples contained peanuts, palm oil, and sea salt as ingredients with a total fat content of 56% (wet basis). Peanut butter was homogenized using a sterile mud-mixer shaft driven by a hand drill, then weighted for 400 g and transferred into a 1500-ml home-blender jar. The sample was hermetically sealed to measure water activity by tracking the equilibrium relative humidity (RH) in the headspace using a humidity sensor (Sensirion SHT35, Chicago, IL). A 5-watt fan was used to improve convection in the headspace of the jar. To measure the water activity of peanut butter with higher moisture contents, deionized water was added to 400 g of peanut butter, for 7 consecutive times, each with 4 g. After each water addition, the sample was homogenized by turning on the blender for 30 s, left for two hours, and blended again for another 30 s, then the sample was kept in the blender jar for 22 h to allow a complete water vapor equilibration

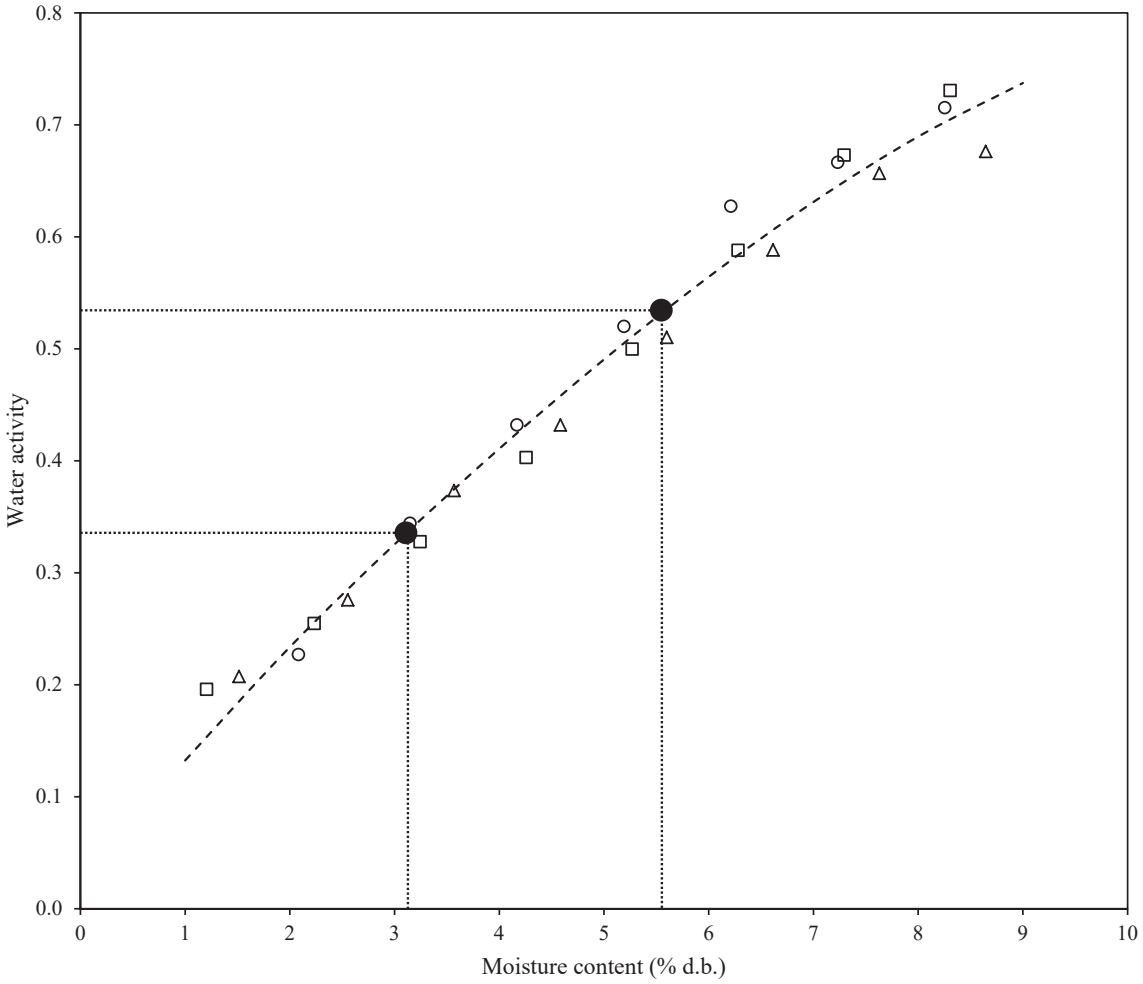

Fig. 2. The water activity of organic creamy peanut butter of different moisture content (dry basis) measured at 23 °C. The prediction line was fitted using Equation (1) and the model can be found in Table 2.

Table 2
Fitting equations.

#	Modeled variable	Fitting equation	R^2
1	Relationship between water activity and moisture content of peanut butter at 23 °C (Fig. 1)	$a_w = 0.132442 \bullet (100 \bullet MC)^{0.820003} - 0.0000049 \bullet (100 \bullet MC)^{4.324904}$	0.982
2	Net isosteric heat of water absorbed in peanut butter	$q_{st,n} = -1.255 \bullet ln(MC) - 2.2659$	0.576
3	log *D*-value vs. temperature (MC = 3.1%)	$logD = -0.0659 \bullet T + 6.6721$	0.959
4	log *D*-value vs. temperature (MC = 5.6%)	$logD = -0.0788 \bullet T + 7.439$	0.983
5	Prediction line (Fig. 6)	$logD_{80} = -3.0818 \bullet T + 2.7423$	0.916
6	Upper prediction line of 95% confidence (Fig. 6)	$logD_{80} = -3.0944 \bullet T + 3.1144$	N/A
7	Lower prediction line of 95% confidence (Fig. 6)	$logD_{80} = -3.0692 \bullet T + 2.3702$	N/A

among different food contents and the air space. Changes in the RH of the headspace in the jar were continuously monitored during the 22 h. The RH at the equilibrium was recorded as the water activity of the sample. The moisture content of the initial sample was measured by the weight change from vacuum drying 3–5 g of the sample at 90 °C and about 100 mm Hg absolute pressure for 24 h. The experiment was replicated for three times.

2.2. Study high-temperature water activity of peanut butter

The water activities of peanut butter samples of 5 different moisture contents (0.94%, 1.50%, 2.55%, 4.25%, and 5.53%, dry basis) were measured at 23, 70, 80, 90, and 100 °C using high-temperature cells (HTC) which were modified in Tadapaneni et al. (2017) based on the first version developed by Syamaladevi et al. (2016a). A schematic diagram of an aluminum HTC is shown in Fig. 1. During a test, the RH and temperature sensors installed in the lid provided measurement data at pre-set intervals. The RH sensors were calibrated with three lithium chloride solutions (molalities equal to 13.95, 8.59, and 5.61 mol/kg) at the target temperatures before the measurements.

Before tests, the moisture contents of peanut butter samples were adjusted by either adding water (method described in section 2.2) or drying in a vacuum oven (60 °C, 100 mm Hg, 12 h) and then stored at room temperature in sealed containers for 24 h. For the measurement of water activity change with temperature, a 6-g sample was loaded into each of three high-temperature water activity cells, tightly sealed, and moved into an oven (Binder ED53, Bohemia, NY) for temperature control. The samples occupied over 80% of the cell cavities, leaving small headspaces to minimize measurement errors of RH caused by the air. The relative humidity sensors (Honeywell, HumidIcon™ HIH8120)

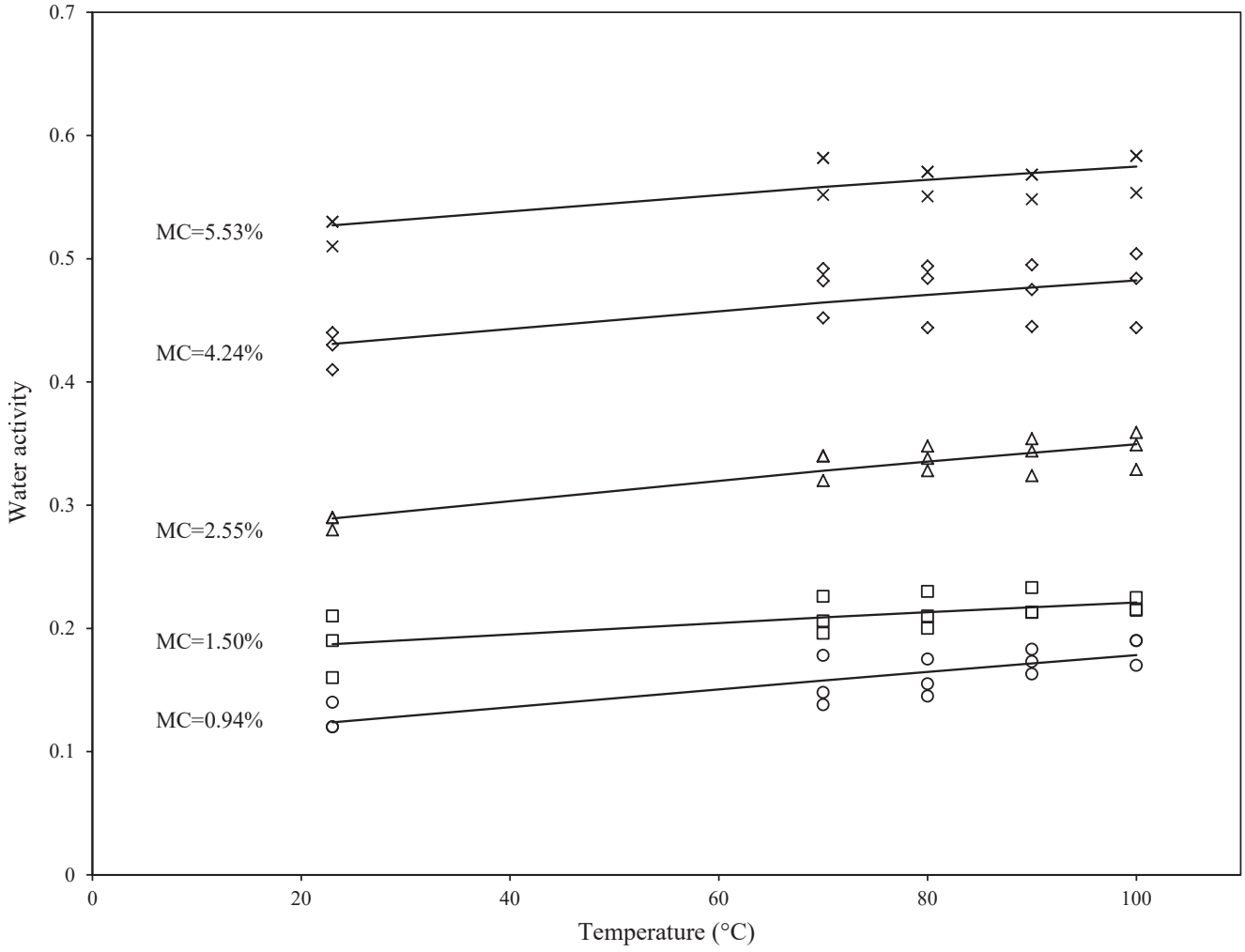

Fig. 3. Temperature-dependent water activity of peanut butter with different moisture content levels (dry basis). Prediction curves were fitted using Equation (2).

monitored the RH in the headspace in high-temperature water activity cells over the time when the cells were held consecutively at room temperature (about 23 °C), 70, 80, 90, and 100 °C each for 3 h. The equilibrium RH inside each cell at each temperature was recorded as the water activity of the sample corresponding to that temperature.

2.3. Thermal death kinetic studies

For the thermal death kinetic studies, four temperatures, 70, 80, 90, and 100 °C, were selected. Seventy degrees Celsius is a common temperature for peanut butter that came out from a grinder. Temperatures above 100 °C may cause rapid dehydration in open systems. Two water activity levels were selected, one at 0.33 (at 23 °C) which is a common water activity of peanut butter, another at 0.53 (at 23 °C) which may help reduce the thermal resistance of *Salmonella* and therefore reduce thermal processing time. The water activity of 0.53 may reduce the crispness of roasted peanuts thus cannot be used for "crunchy" peanut butter (Lee & Resurreccion, 2004), but could be employed for the creamy types.

Salmonella enterica Enteritidis PT 30 (ATCC BAA-1045) (*S.* Enteritidis) was selected as the inoculation bacterium for this study. This serovar was reported to have greater thermal resistance in peanut butter compared to other serovars, like *S.* Typhimurium and *S.* Tennessee (He et al., 2013). The peanut butter used in this study contained <1000 CFU/g (standard aerobic plate count) background microflora, thus were directly used for inoculation as the low initial population won't affect the results obtained from inoculated samples with very high populations ($10^{8\text{-}10}$ CFU/g). The peanut butter samples of moisture content (dry basis) 3.1% ($a_{w.23}$ = 0.33) and 5.6% ($a_{w.23}$ = 0.53) were used for inoculation and the thermal death study.

The inoculation procedures followed a lawn-based method (Hildebrandt et al., 2016; Yang et al., 2022). In brief, for each independent inoculation, a single colony of *S.* Enteritidis was collected from a streak plate of trypticase soy agar (BD Difco, Franklin Lakes, NJ) with 0.6% yeast extract (BD Difco) (TSAYE) and subjected to two consecutive growths in 9 ml trypticase soy broth (TSB, BD Difco) with 0.6% yeast extract (TSBYE) at 37 °C each for 24 h. Then 1 ml of the culture was incubated on a 150 × 15 mm plate of TSAYE at 37 °C for 24 h. The bacteria lawn was scraped off with a sterile L-spreader and washed into a 50 ml centrifugal tube with 18 ml of sterile 2% buffered peptone water (BPW, BD Difco), subjected to vortex (20 s), and centrifuged at 3,000 × g for 15 min. The supernatant was discarded, the bacteria pellet was transferred into 50 g of peanut butter with a sterile loop and homogenized thoroughly in a stomacher bag from the outside using a dough scraper and hand-massage. Inoculated samples were stored for 4–6 days in two different jars of constant relative humidity, one at 33% controlled with saturated magnesium chloride, and another at 53% controlled with saturated magnesium nitrate.

The 1.5 g samples were loaded in each of the thin aluminum thermal death test (TDT) cells developed in Jin & Tang (2019). The inner cavity of each test cell was 1.3 mm in height and 31.2 mm in diameter (see Jin & Tang, 2019). The samples were flattened with an L-spreader to get rid

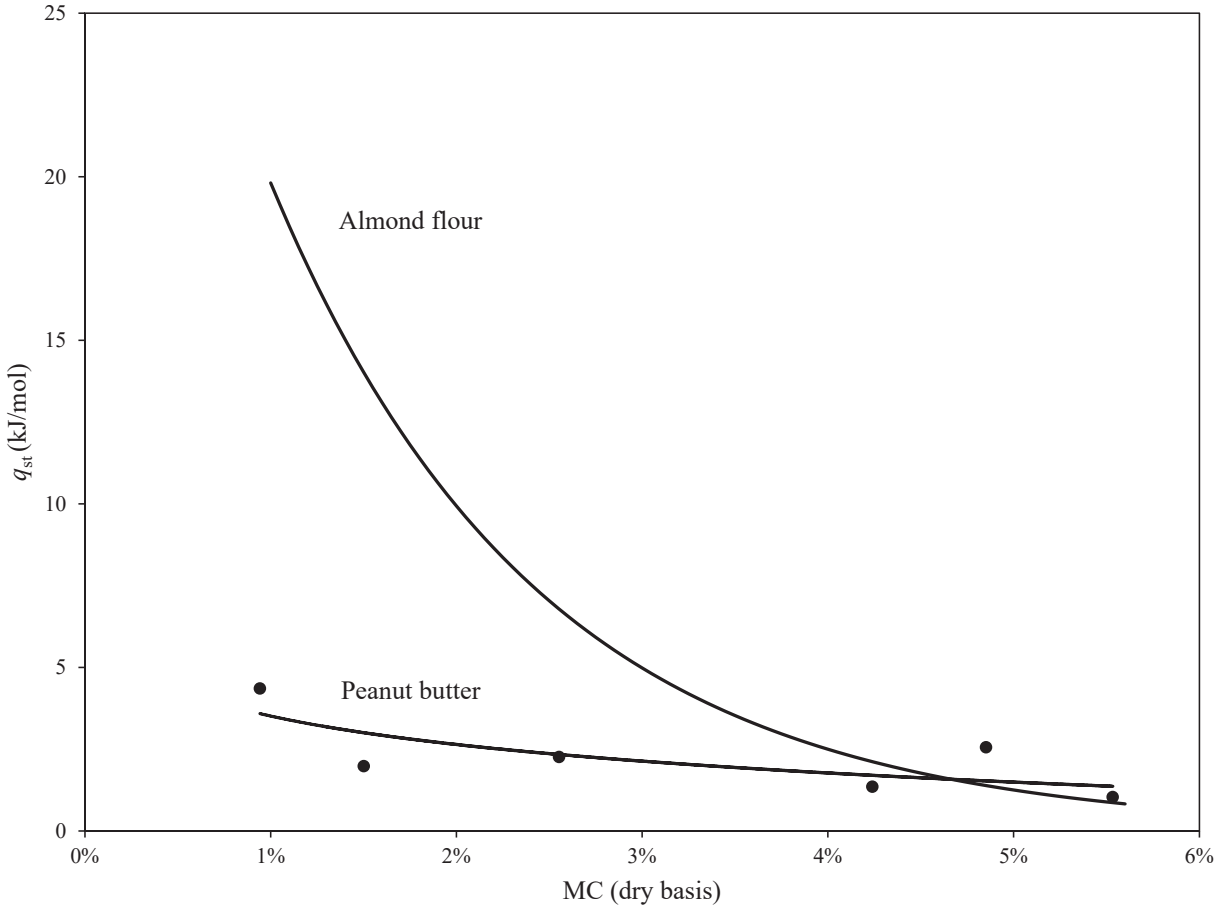

Fig. 4. Net isosteric heat of water in peanut butter and almond flour (Tadapaneni et al., 2017).

of air pockets and make good contact with the lid. The TDT cells were tightly closed with the lids and then subjected to thermal treatments in pairs with an oil bath at a constant temperature for different amounts of time. The treatments were terminated by transferring the samples into an ice-water bath and chilled down for at least 2 min to <1 °C. The treatment time for each experimental condition was determined from preliminary experiments to obtain the thermal death curves that covered at-least 3-log reductions. For each experimental condition of certain water activity and treatment temperature, thermal death experiments were replicated 3–4 times using independently inoculated samples. The experimental conditions are presented in Table 1.

After the thermal treatment, 1 g sample was transferred from each TDT cell into a 4-oz stomacher bag (WHIRL-PAK, Madison, WI), then mixed with 9 ml sterile 2% BPW for a 1:10 dilution using hand-massage, followed by further massaging in a stomacher (Stomacher 400, Seward Laboratory Systems Inc., Bohemia, NY) cycle at 260 rpm for 5 min. The diluted sample was subjected to further serial dilutions, when necessary, to achieve a countable concentration for platting. The appropriate dilution levels were estimated through preliminary experiments. Each dilution was sampled by taking 0.1 ml, spreading on 100 mm TSAYE plates supplemented with 0.05% ferric ammonium citrate (Sigma-Aldrich, St Louis, MO) and 0.03% (w/v) sodium thiosulfate (Sigma-Aldrich), and incubating for 48 h at 37 °C. Then the plates were counted for any colonies with a dark center and the numbers were calculated for the population of *S.* Enteritidis (CFU/g) in peanut butter based on the dilution levels.

2.4. Data analyses

The relationship between water activity (at 23 °C) and moisture content of peanut butter was modeled using the following empirical equation:

$$a_w = A \bullet MC^C + B \bullet MC^D \tag{1}$$

where a_w and MC are the water activity and moisture content of peanut butter, respectively. A, B, C (<1), and D (>1) are constants determined through the least sum-of-square technique.

The measured temperature-dependent water activities of five peanut butter samples of different moisture contents were modeled using Clausius Clapeyron Equation (Tadapaneni et al., 2017):

$$ln(a_{w.T}) = \frac{q_{st,n}}{\mathrm{R}}\left(\frac{1}{T_r} - \frac{1}{T}\right) + ln(a_{w.T_r}) \tag{2}$$

where $q_{st,n}$, is the net isosteric heat of absorbed water (in J/mol) which equals to the difference between the specific latent heat of water vaporization and the total enthalpy change for the sorption process; R is the universal gas constant (8.312 J mol^{-1} K^{-1}); T and T_r are the temperature and the reference temperature in K.

The net isosteric heat was fitted using a standard logarithmic model. Then the net isosteric heat of the two peanut butter samples (MC = 3.1% & 5.6%) that were subjected to thermal death study were calculated and substituted into Equation (2) to obtain the temperature-dependent water activity of these two samples.

For each independently repeated set of experiments, the populations of *S.* Enteritidis in peanut butter at different treatment times were fitted with the first-order model (Peleg, 2006):

$$\log\left(\frac{N}{N_0}\right) = -\frac{t}{D} \tag{3}$$

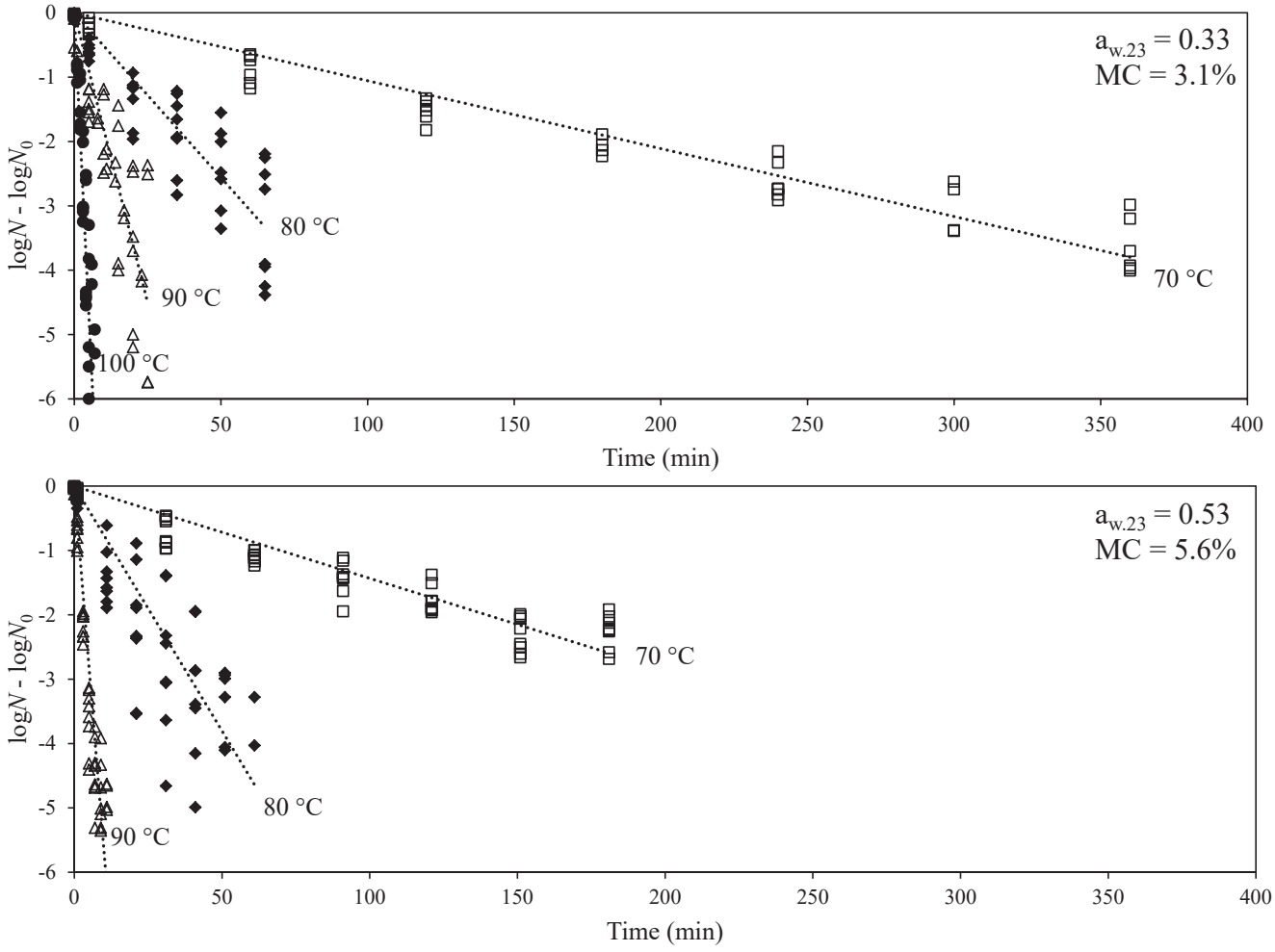

Fig. 5. Log-reductions of *S.* Enteritidis in peanut butter of two different moisture content (3.1% and 5.6%, dry basis) from thermal death at 70, 80, 90, and 100 °C.

Table 3
Thermal death parameters of *S.* Enteritidis in peanut butter.

MC (dry basis)	a_w (23 °C)	Treatment temperature (°C)	a_w during treatment	D_{80}-value (min)	logD_{80}-value	RMSE (logCFU/g)	Z_T-value (°C)
3.1%	0.33	70	0.37	102.6 ± 15.2	2.01 ± 0.06^{A}	0.16	15.4
		80	0.38	23.3 ± 7.6	1.35 ± 0.14^{B}	0.23	
		90	0.39	6.9 ± 3.1	0.81 ± 0.19^{D}	0.26	
		100	0.39	1.1 ± 0.3	0.02 ± 0.10^{F}	0.33	
5.6%	0.53	70	0.57	77.0 ± 6.1	1.89 ± 0.04^{A}	0.22	12.6
		80	0.58	14.5 ± 4.7	1.14 ± 0.16^{C}	0.35	
		90	0.59	2.0 ± 0.2	0.30 ± 0.05^{E}	0.47	
		100	0.59	0.3*	−0.48*	–	

Difference in grouping letters indicate a significant difference (95% confidence)
*Predicted data from mathematical model

where N and N_0, are the populations of *S.* Enteritidis (in log CFU/g) corresponding to treatment time t (in min) and time 0, respectively. D, in min, is the thermal death time or decimal reduction time of the bacterial population.

The goodness of fit was quantified for each experimental condition using the root mean squared error (RMSE) (Motulsky & Christopoulos, 2004):

$$\mathrm{RMSE} = \sqrt{\frac{\sum_{i=1}^{n}\left[log\left(\frac{N}{N_0}\right)_{\mathrm{data,\ i}} - log\left(\frac{N}{N_0}\right)_{\mathrm{model,\ i}}\right]^2}{n-p}} \quad (4)$$

where $\log(N/N_0)_{\mathrm{data},\ i}$ and $\log(N/N_0)_{\mathrm{model},\ i}$ are the calculated and modeled log reduction of survival population of *Salmonella*, n is the number of data points, and p is the number of degree of freedom.

The model fitting of this study was performed using the Solver function or the Linest function in Microsoft Excel 16.0. The means of D-values were compared between every two experimental conditions using one-way ANOVA and Fisher's LSD testes with a significance threshold of 5%. The statistical analysis was conducted using a computer software, Minitab 18.1.

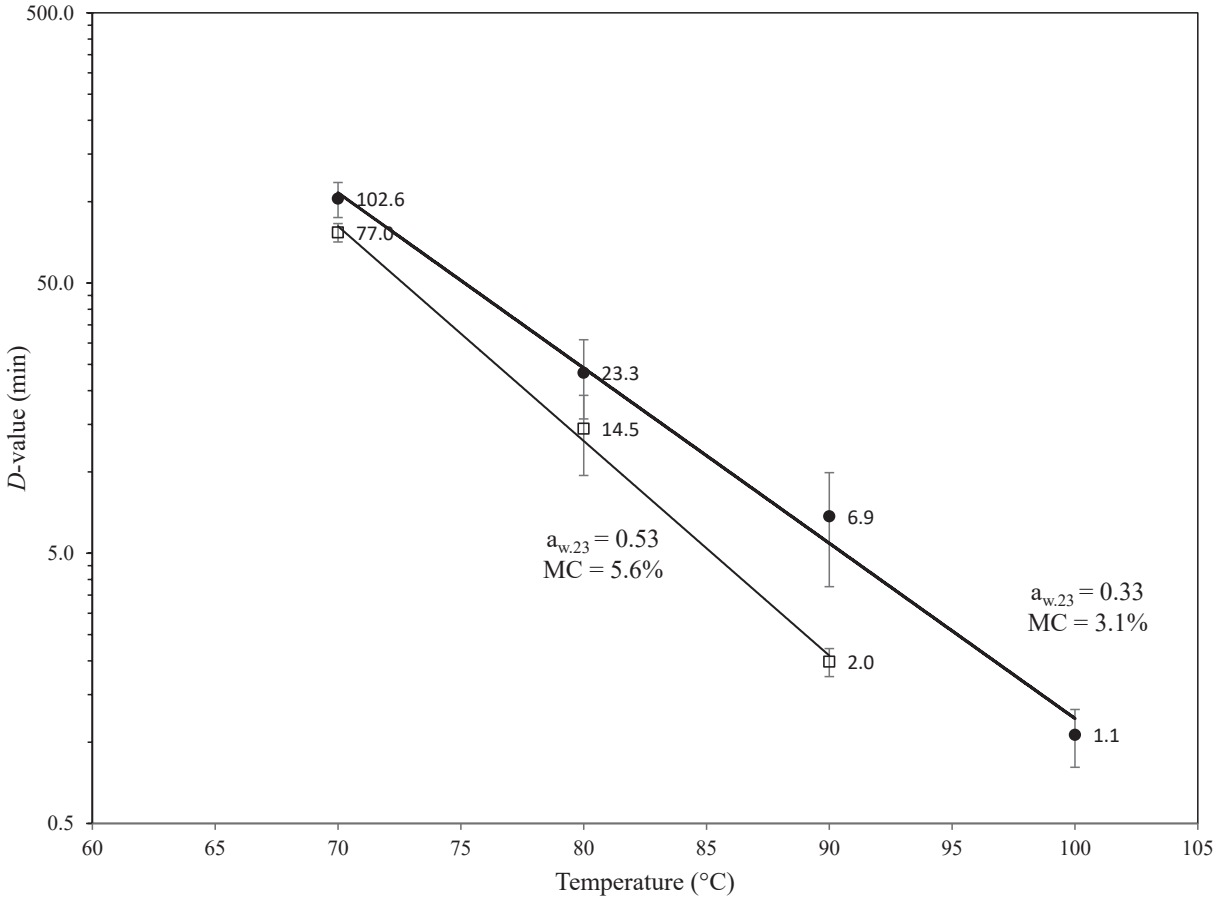

Fig. 6. *D*-value of *S.* Enteritidis in peanut butter of two different moisture content (3.1% and 5.6%, dry basis) vs. the treatment temperature.

3. Results and discussion

3.1. Sorption isotherm of peanut butter at 23 °C

Fig. 2 shows the water activities of peanut butter of different moisture contents measured at 23 °C. The water activity of peanut butter increased with increasing moisture content in a nearly linear trend. This type of curve is common in other low-moisture foods like peanuts and almond flour at water activity range between 0.1 and 0.7 (Chen, 2000; Tadapaneni et al., 2017). The fitted line was generated using Equation (1) and the fitted parameters are presented in Table 2.

3.2. High-temp water activity of peanut butter

Fig. 3 shows the change of water activity with temperature in peanut butter samples of five different moisture contents. The water activity of all samples increased slightly with temperature. For example, the water activity of samples with the lowest moisture content, 0.94%, increased from 0.13 ± 0.01 at 23 °C to 0.15 ± 0.02 at 70 °C, and 0.18 ± 0.01 at 100 °C. The water activity of the sample with the highest moisture content, 5.53%, increased from 0.52 ± 0.01 at 23 °C to 0.57 ± 0.02 at 100 °C. The increase of water activity with temperature in peanut butter is significantly less than in wheat flour of the same initial water activity (Tadapaneni et al., 2017), likely due to the high oil content in peanut butter.

The a_w changes were modeled for each sample using Equation (2). The fitted net isosteric heats (q_{st}) are presented in Fig. 4, in comparison with the q_{st} of almond flour (obtained from Tadapaneni et al., 2017). The net isosteric heat represents the difference in latent heat of evaporation between absorbed water and pure water (Yang et al., 2020a). As shown in Fig. 4, the q_{st} was similar in peanut butter and almond flour in the moisture content range between 4.3% and 5.6%. As the moisture content was reduced to below 4.3%, the q_{st} of almond flour raised sharply to about 20 kJ/mol at moisture content of 1%, but the q_{st} of peanut butter remained low (<5 kJ/mol). This can be explained by their structural differences. Even though almond flour has similar oil content as peanut butter, it takes further grinding to turn almond flour into a butter form where oil is released from the broken lipid bodies in the plant cells (Young & Schadel, 1990). The released oil in butter could reduce the availability of hydrophilic binding sites by filling the capillaries and covering the surface of protein and starch granules (Yang et al., 2020a).

3.3. Thermal death kinetics of S. Enteritidis in peanut butter

Fig. 5 shows the thermal death curves of *S.* Enteritidis in peanut butter of two moisture contents at different temperatures. The survival populations of *S.* Enteritidis in peanut butter (log CFU/g) reduced log-linearly ($P < 0.05$) with treatment time in all circumstances. Temperature played an important role in the thermal resistance of *Salmonella* as the slope of the curve decreased with the increase of temperature. These results show that the thermal death of *Salmonella* followed the first-order kinetics in peanut butter (Yang et al., 2022).

The fitted parameters for the first-order thermal death model (Equation (3)) are presented in Table 3. The small RMSE (0.16–0.47) between measured and modeled data indicates the goodness of fit of the model. The mean *D*-values, each with 3 or 4 independent replicates (Table 1), were compared using the one-way ANOVA test. The results are shown in the grouping letters (Table 3). All the means were significantly different from each other, except for the comparison between two groups, which are both at 70 °C but with different moisture

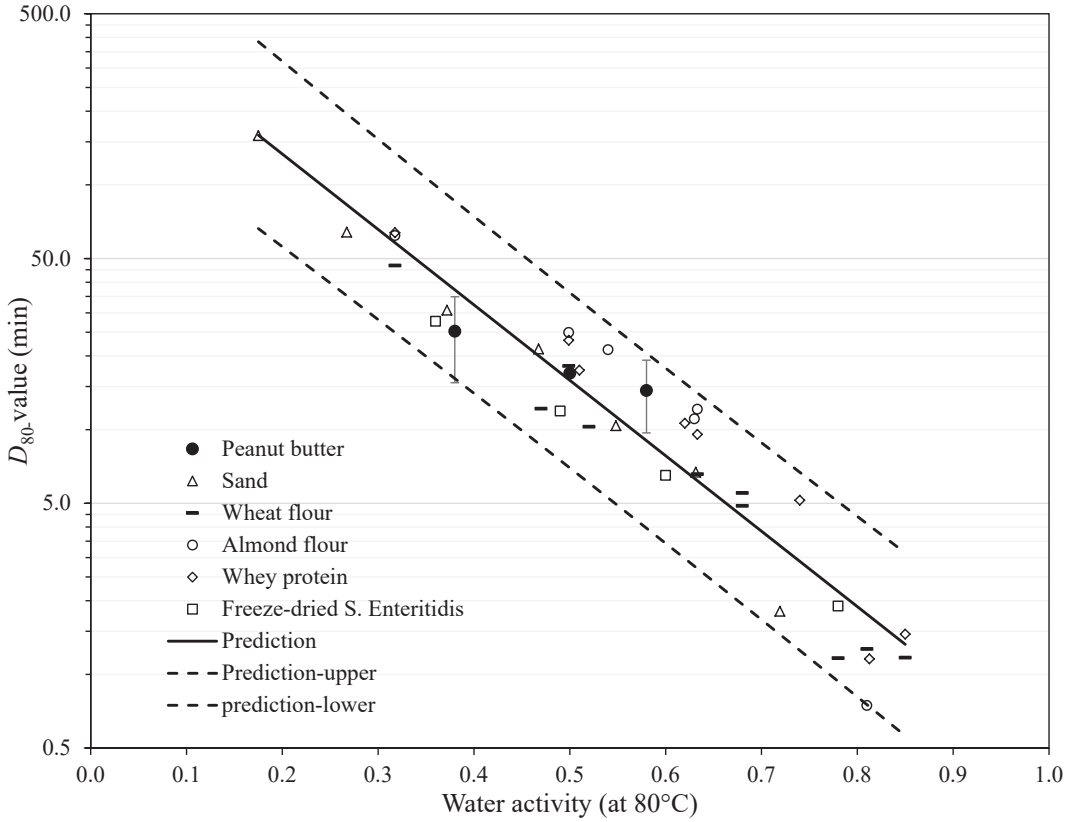

Fig. 7. D_{80}-value of *S.* Enteritidis in peanut butter and other matrices vs. the water activity during the process. A prediction line with 95% prediction interval (Equations can be found in Table 2) were made for general application purposes. Data source: the middle point of peanut butter group- Syamaladevi et al. (2016a); the sand group and one part of the wheat flour group- from Liu et al. (2018); the almond flour, whey protein groups, and the other part of wheat flour group- Xu et al. (2019); the freeze-dried *S.* Enteritidis group- Xie et al. (2021).

contents.

3.4. The effect of temperature

The *D*-values were plotted in a semi-log scale vs. the treatment temperature in Fig. 6. A linear relationship ($P < 0.05$) between *D*-value and temperature was found for both moisture content levels. A linear regression model was made for each group of data of the same moisture content. The fitted equations are shown in Table 2. The calculated Z_T-values (Table 3) show that the *D*-value would reduce 10-fold as temperature increases for 15.4 °C or 12.6 °C, respectively, in the peanut butter of moisture content 3.1% and 5.6%. The phenomenon that Z_T-value would be higher in a sample with a lower moisture content was also observed in Jin, Tang, & Zhu (2020).

3.5. The effect of water activity

Recent studies indicate that food matrix influences the thermal death rate of the bacteria by controlling the moisture content of the bacteria cells (Xie, et al., 2021). This happens because bacteria gain or lose moisture from the environment through the diffusion of water (vapor) (Syamaladevi et al., 2016b). Moisture may play a crucial role in the heat inactivation of critical components in the bacterial cells, like critical enzymes and ribosomes, whose destruction leads to bacteria death (Cebrián, Condón, & Mañas, 2017, Xie, et al., 2021). In low-moisture foods, the amount of moisture inside bacterial cells changes readily with relative humidity or water activity in the surrounding micro-environment during thermal treatments (Syamaladevi, Tang, & Zhong, 2016; Xu et al., 2021). To compare the thermal resistance of *S.* Enteritidis in peanut butter with other food matrices, the D_{80}-values from this work and several other studies were plotted in Fig. 7 on a semi-log scale against the water activity of the matrix measured at 80 °C. The middle point of the peanut butter group was obtained from Syamaladevi et al. (2016a), which falls right between the two points from this work after plotting against its calculated high-temperature water activity. Like other low-moisture foods, the D_{80}-values of *S.* Enteritidis fall in a band that decreases linearly with the increase of water activity. The similarities in thermal resistance of *S.* Enteritidis among different food matrices indicate that peanut butter had no special protective effect on bacteria from thermal processing. Overall, the common impression that *Salmonella* is more resistant in peanut butter than other low-moisture foods (e.g. wheat flour) can be attributed to the generally lower water activity in peanut butter (0.2–0.35) than that of other typical low-moisture foods (0.45–0.65). In addition, when heated to the treatment temperatures, the water activity of peanut butter doesn't increase as much as that of a low-moisture food with high starch but low fat content, like wheat flour.

A prediction line and interval of 95% confidence were drawn in Fig. 7, the corresponding equations are presented in Table 2. The upper prediction line could be used as a conservative estimation for the thermal resistance of *S.* Enteritidis in a food matrix that has not been studied before.

3.6. Recommendation for peanut butter pasteurization

With adequate thermal inactivation data for *Salmonella* in specific low moisture foods, effective industrial-scale thermal pasteurization processes can be developed to control *Salmonella* in those products. For example, the Almond Board of California (ABC) launched a mandatory pasteurization program in September 2007, and no outbreaks have been attributed to California Almonds thereafter (Birmingham, 2018). For

HFLM food pastes, roasting and grinding were considered as potential critical control points among all the processing stages (drying, shelling, sorting, blanching, roasting, and grinding) (Ma et al., 2009). However, it's been reported that a grinding process (up to 70 °C and 40 min) may not deliver sufficient reduction of *Salmonella* or *E. coli* O157:H7 (He et al., 2011, Ma et al., 2009), and a roasting process may also fail to achieve a 5-log reduction (recommended by FDA) of *Salmonella* under some process conditions (Prestes et al., 2019). Even if a sufficient roasting process was employed, it is in an early stage of the production line, and there is still a risk of cross-contamination in later processing operations (Borrell, 2009). A pasteurization step for the end-product (after grinding) could help reduce the risk of *Salmonella* in HFLM food pastes. Based on the results from this study, for peanut butter with a water activity of 0.33 at room temperature, it would take about 35 min exposure at 90 °C to achieve a 5-log reduction of *S.* Enteritidis. The holding time can be reduced to 5.5 min at 100 °C. If the initial water activity of the peanut butter could be increased to 0.55, it would take only 10 min or 1.5 min to achieve a 5-log reduction of *S.* Enteritidis at 90 °C or 100 °C, respectively.

4. Conclusion

In summary, this study observed that the thermal death of *S.* Enteritidis followed the first-order kinetics in peanut butter. The death rate of *S.* Enteritidis was correlated to two key factors: moisture content (or water activity) of the product and the process temperature. The results suggest that the low-water activity was the major cause of the high thermal resistance of bacteria in peanut butter, and peanut butter has no special protective effect on heat inactivation of bacteria compared to other low-moisture foods, like wheat flour. The thermal death kinetic model can be used to develop appropriate thermal pasteurization processes for high-fat low-moisture food like peanut butter. Temperature and moisture content (or water activity) need to be controlled for a thermal process to achieve the desired lethality.

CRediT authorship contribution statement

Ren Yang: Methodology, Conceptualization, Investigation, Writing – original draft, Project administration. **Lina Wei:** Methodology, Conceptualization, Investigation, Writing – review & editing. **Jianwu Dai:** Investigation, Writing – review & editing. **Juming Tang:** Conceptualization, Resources, Supervision, Writing – review & editing, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgment

This research was funded by the USDA-NIFA Agricultural and Food Research Initiative (AFRI) CAP grant 2015-68003-23415 and the USDA-NIFA AFRI SAS grant 2020-68012-31822.

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