Food Control 124 (2021) 107935

Contents lists available at ScienceDirect

Food Control

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

Thermal inactivation of *Salmonella* Enteritidis PT30 in ground cinnamon as influenced by water activity and temperature

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ARTICLE INFO

Keywords:
Salmonella enteritidis PT 30
Water activity
Ground cinnamon
Thermal resistance
Antimicrobial effect

ABSTRACT

Reported outbreaks and recalls related to spices, including an on-going recall of cinnamon involved apple chips, reveal a need to understand thermal inactivation of *Salmonella* in spices. Recent studies have documented quantitative relationships between water activity (a_w) and thermal resistance of *Salmonella* in a wide range of low-moisture foods. Such quantitative data are useful in developing effective thermal treatments. However, the influence of a_w on thermal inactivation of *Salmonella* in spices has not been systematically studied. Cinnamon is known for its antimicrobial effect on pathogenic bacteria. We hypothesized that the synergetic effect of heat and the natural antimicrobial compounds in cinnamon would reduce the intensity of thermal treatments for cinnamon compared to that for other low-moisture foods. This study investigated the thermal resistance of *Salmonella* Enteritidis PT 30 in ground cinnamon at three inactivation temperatures (70, 75 and 80 °C). The $\log_{10}$ *D*-values of *S.* Enteritidis PT 30 in ground cinnamon decreased linearly with increasing a_w and treatment temperature. By comparing the $\log_{10}$ *D*-values obtained in ground cinnamon with the reported $\log_{10} D_{80^\circ C}$-values of *S.* Enteritidis PT 30 in other low-moisture foods, we found that the thermal treatments at 70 °C for *S.* Enteritidis PT 30 in cinnamon powder was roughly equivalent to the treatments at 80 °C for the same bacterial strain in other low-moisture foods, such as wheat flour and egg powders. Thus, milder thermal treatments can be used for the control of *Salmonella* in cinnamon powder, and perhaps other spices or herbs that contain antimicrobial compounds, for better retention of product quality.

1. Introduction

Low-moisture spices are used worldwide in food preparation and processing to enhance flavor. The majority of spices, such as garlic (allicin), black pepper (piperine) and cinnamon (cinnamaldehyde), are reported to have natural phytochemicals to inhibit the growth of foodborne pathogens and spoilage bacteria (Lu et al., 2011; Nabavi et al., 2015; Zou, Hu, & Chen, 2015). Yet, spices as low-moisture food ingredients for seasoning are able to cause large-scale outbreaks and recalls due to potential foodborne pathogen contamination. *Salmonella* spp. are one of the most contaminated microbial pathogens that have been found in spices, in which up to 80 serotypes were reported in implication with imported spices from 2007 to 2009 (CFSAN, 2017; ASTA, 2017). Between 2009 and 2010, 272 individuals from 44 states in the United States were implicated by *Salmonella* due to the consumption of salami associated with contaminated black and red pepper (CDC, 2010). Recalls of low-moisture foods have also been connected to cinnamon due to contamination with *Salmonella*, including apple chips (FDA, 2020b), granola cereal (FDA, 2017) and baked goods (FDA, 2018, 2020a).

In the United States, the majority of spices are imported from countries with tropical climates. Cross-contamination of spices from the suppliers is attributed to the lack of good agricultural environment and hygiene practices (Duncan et al., 2017). Pathogen contamination may occur at multiple points throughout the complicated supply chains of spices, including import, processing, packaging and retail (Van Doren et al., 2013). The manufacturers in the United States are encouraged to have a pathogen reduction step before delivering the spice products to the consumers (ASTA, 2017). This step is important because pathogens can survive in contaminated spices for long periods. For example,

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https://doi.org/10.1016/j.foodcont.2021.107935
Received 3 December 2020; Received in revised form 22 January 2021; Accepted 24 January 2021
Available online 29 January 2021

Salmonella can survive in black pepper for a year under dry conditions (Keller, VanDoren, Grasso, & Halik, 2013). Pathogen reduction treatments applied to spices include the use of heat (steam), irradiation, and chemical fumigant (ethylene oxide) (ASTA, 2017; Duncan et al., 2017). Because of the chemical residue risks in fumigation and a lack of international harmonization of regulation and slow acceptance from consumers for irradiated foods, thermal treatments are still the main approach in foodborne pathogen control for spices (Duncan et al., 2017; Loaharanu & Ahmed, 1991). Given the recent outbreaks and recalls related to spices, including the on-going recall of cinnamon involved apple chips (FDA, 2020a), it is of great importance to understand, verify or assess if thermal treatments could effectively inactivate *Salmonella* (achieving a 5-log or greater reduction) in order to deliver microbial-safe spices to consumers.

Cinnamon cassia, also called Chinese cinnamon, is widely used for many ready-to-eat food products and snacks. It is known for its antimicrobial activity against foodborne pathogens (Nabavi et al., 2015). Cinnamaldehyde, one of the main constituents of cinnamon, is responsible for its antimicrobial activity against bacteria, fungus and the formation of biofilms (Kawatra & Rajagopalan, 2015; Kumar, Kumari, & Mishra, 2019). Published studies investigated its antimicrobial effect by analyzing the minimal inhibitory concentration of its essential oil/-extracts at room temperature (Cui et al., 2016; Ju et al., 2018; Nabavi et al., 2015; Tsai, Sheng, & Zhu, 2017; Zhang et al., 2017). Of particular interest to the research community and the food industry is a better understanding of the synergetic effect between heat and natural antimicrobial constituents during thermal inactivation of the pathogen.

Water activity (a_w) is an important factor in designing an effective pathogen inactivation step for low moisture foods (Garces-Vega, Ryser, & Marks, 2019; Steinbrunner et al., 2019). *Salmonella* are more thermal resistant in foods with a lower a_w (Smith & Marks, 2015; Tsai et al., 2019; Wei et al., 2020). Several recent studies have shown that the thermal-death time (D_T-values, the time required to inactivate 90% of the microbial population at a given temperature) increases exponentially with a reduction in a_w at treatment temperatures ($a_{w,\ treatment\text{-}temperature}$) (Alshammari, Xu, Tang, Sablani, & Zhu, 2020; Liu, Tang, Tadapaneni, Yang, & Zhu, 2018; Perez-Reyes, Tang, Zhu, & Barbosa-Cánovas, 2020; Tsai et al., 2019; Xie et al., 2020; Xu et al., 2019). However, the influence of a_w on thermal inactivation of *Salmonella* in spices has not been systematically studied.

Our preliminary results suggest that *Salmonella* can survive in cinnamon powder for months in storage. Therefore, the objectives of this study were to 1) study the a_w changes with treatment temperature in ground cinnamon, 2) determine the effect of a_w (0.20, 0.30, 0.40 and 0.50, at room temperature) on the thermal inactivation of *S.* Enteritidis PT 30 in ground cinnamon at three treatment temperatures (70, 75 and 80 °C), and 3) assess the synergistic effects between the antimicrobial activity of cinnamon and thermal treatment on the inactivation of *S.* Enteritidis PT 30 by comparing *D*-values from this study with that for the same bacteria strain in other non-spicy low-moisture foods.

2. Materials and methods

2.1. Physiochemical properties of ground cinnamon

Ground cinnamon (*Cinnamon cassia*) of a major brand was purchased from a local grocery in Pullman, WA. The a_w of ground cinnamon at room temperature (~21 °C) was determined in triplicates by a water activity meter (Aqualab, Meter Group, Inc., Pullman, WA, USA). Subsamples were sent to the Particle Technology Labs (Chicago, IL, USA) and Northern California Laboratory (Silliker, Inc., Salida, CA, USA) for the analysis of particle size distribution and proximate compositions, respectively. The proximate analysis included the determination of ash, carbohydrates (including fiber), fat, moisture and protein contents, according to the standard methods of the American Spices Trade Association (ASTA) and Association of Official Agricultural Chemists (AOAC) (AOAC, 2012, 1994a, 1994b; ASTA, 1997a, 1997b). The particle size distribution was measured by sieve analysis.

2.2. Measuring changes in a_w of ground cinnamon with temperatures

2.2.1. Sample preparation and measurement of a_w

The a_w changes of ground cinnamon at elevated temperatures were determined using the high-temperature cell (HTC, Meter Group, Inc., Pullman, WA, USA) designed by Tadapaneni, Yang, Carter, and Tang (2017), and a_w was measured by a capacitance-based relative humidity (RH) and temperature sensor (Honeywell HumidIcon™, Morristown, NJ, USA) installed in the center of the lid. Calibrations were carried out using standard solutions with different a_w.

Before the measurements, powdered cinnamon samples were conditioned in air-tight jars under different RH levels at room temperature for 5 days to reach the equilibrium. The saturated salt solution of LiCl, CH_3COOK, $MgCl_2$, K_2CO_3, $Mg(NO_3)_2$, $NaNO_2$, NaCl and KCl in a closed system generated a consistent RH of 11.3, 22.5, 32.8, 43.2, 52.9, 65.8, 75.3 and 84.3% at 25 °C, respectively (Lewis Greenspan, 1977). The equilibrated samples were used for experiments after reaching the target $a_w \pm 0.02$. The equilibrium procedures followed the descriptions in Tadapaneni et al. (2017).

The conditioned samples were placed in HTCs and sealed tightly to prevent any leakage. HTCs were firstly put at room temperature (~21 °C), and then heated from 30 to 85 °C at an increment of 5 or 10 °C in an oil bath (Isotemp™ 5150 H24, Fisherbrand™, PA, USA). After each increment of temperature in the oil bath, RH and temperature of the headspace in HTCs were recorded every minute, and the equilibrium state at the respective temperature was achieved when constant RH values were obtained for at least 10 records (up to 10 min). These constant RH values at each isothermal temperature, regarding the corresponding a_w values, were collected (Tadapaneni et al., 2017). After isothermal treatments, HTCs were cooled to room temperature, and then the moisture content of ground cinnamon was determined using a halogen moisture analyzer (HB43–S, Mettler Toledo, Columbus, OH, USA). All the experiments were carried out in triplicate.

2.2.2. Modeling a_w changes of cinnamon powder with increasing temperature

In order to obtain the relationship between a_w and moisture content with temperatures, the experimental a_w data were fitted by a modified Clausius-Clapeyron equation (CCE) according to Tadapaneni et al. (2017), which could be expressed as Eq. (1):

$$a_{w2} = a_{w1} exp\left(\frac{q_{st}}{R}\left(\frac{1}{T_1} - \frac{1}{T_2}\right)\right) \tag{1}$$

where a_{w1} and a_{w2} are the water activity values of a sample with the same moisture content at temperature T_1 and T_2 (K), respectively; R is the universal gas constant (8.314×10^{-3} kJ mol^{-1} K^{-1}); q_{st} is the net isosteric heat of sorption (kJ/mol), which can be determined from the slope of plotted data (ln(a_w) versus 1/T). At a specific dry basis moisture content (M_d, g water/100g dry solids), q_{st} can be obtained from an empirical relation as shown in Eq. (2), where a and b are constants (Corrêa, Goneli, Jaren, Ribeiro, & Resende, 2007).

$$q_{st} = a\ exp(-b * M_d) \tag{2}$$

2.3. Thermal inactivation of S. Enteritidis PT 30 in ground cinnamon

2.3.1. Bacteria strain and inoculation preparation

S. Enteritidis PT 30 was used in the isothermal inactivation tests, which has been implicated in worldwide outbreaks of low-moisture foods. *S.* Enteritidis PT 30 was obtained from the University of California, Davis. It was kept in at −80 °C in tryptic soy broth (TSB, Difco™, Sparks, MD, USA) supplemented with 0.6% (w/v) yeast extract (TSBYE) and 20% (v/v) glycerol.

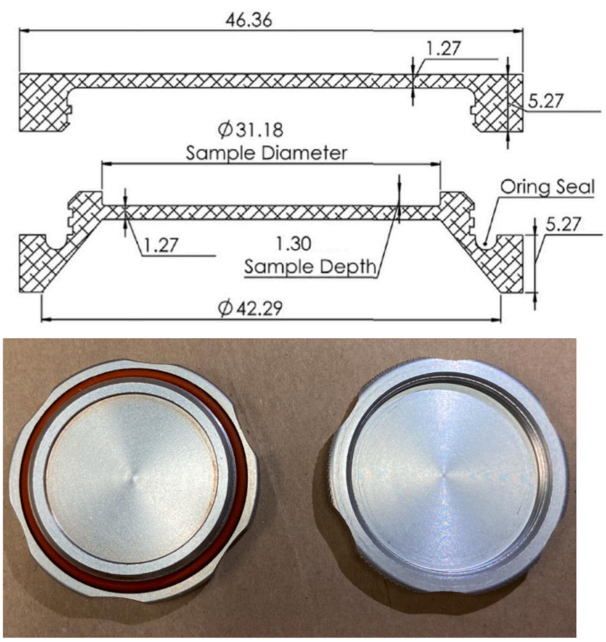

Fig. 1. The schematics of the isothermal treatment test cell (TDT II).

A loop of the above strain was twice activated in TSBYE at 37 °C for 24 h. The activated *S*. Enteritidis PT 30 was then plated on tryptic soy agar (TSA) supplemented with 0.6% (w/v) yeast extract (TSAYE) and incubated at 37 °C for 24 h. The bacterial lawn was harvested from three plates by using sterile buffered peptone water (BPW), and then centrifuged at 8000×g, 4 °C for 10 min. The supernatant was discarded, and the pellet was resuspended in 1 mL sterile BPW to achieve ~10^{11} CFU/mL for inoculation, the population of which was confirmed by enumeration.

The above inoculum was inoculated into ground cinnamon at a ratio of 1 mL (inoculum): 50 g (cinnamon). In brief, 5 g of cinnamon sample was inoculated with 1 mL of above resuspended inoculum (11.3 ± 0.1 log_{10} CFU/mL) and mixed manually in a 4 oz. Whirl-Pak® bag (Nasco™, Madison, WI, USA) for up to 5 min until no visible clump was observed. Then, another 5 g cinnamon powder was added into the bag, manually massaged the sample bag and then stomached it at 230 rpm for 3 min (Stomacher® 400 Circulator, Seward Laboratory System Inc., Norfolk, UK). The above homogenous inoculated sample (10 g) was further transferred into a 10 oz. Whirl-Pak® bag with 40 g uninoculated cinnamon, manually shaken and massaged for 3 min. The inoculated cinnamon was divided and spread in two 150 mm × 15 mm Petri dishes and the uniformity was determined by the difference in population from 5 randomly selected locations. Then, the Petri dishes were placed in a humidity/temperature-controllable chamber (Memmert HCP 50 humidity chamber, Germany) for 3 days at ~21 °C until conditioned to the target $a_{w,\ room\text{-}temperature}$ ± 0.02 (0.20, 0.30, 0.40 and 0.50). The a_w of conditioned cinnamon was confirmed in triplicates before isothermal treatments. The population of *S*. Enteritidis PT 30 in the conditioned samples was also evaluated.

2.3.2. Isothermal treatments

The improved version of aluminum test cells (Thermal-Death-Time cell, TDT II) designed by Jin and Tang (2019) was used for the isothermal treatments. The schematic diagram of a TDT II cell is shown in Fig. 1. The TDT II cell has a relatively short come-up-time (CUT, min) because of a larger thermo-contact surface and a smaller sample depth compared to the traditional TDT cells (Chung, Birla, & Tang, 2008; Jin & Tang, 2019). The samples were hermetically sealed in thermal test cells during the isothermal treatments in which the moisture content of the samples remained constant while their a_w changed with temperature (Syamaladevi et al., 2016).

Come-up time (CUT) is the time required for samples to reach the target temperature within 0.5 °C. The CUT for the cinnamon powder samples in TDT II cells was measured using a special TDT II cell installed with a 0.5 mm-diameter thermocouple (Type T, OMEGA Engineering, Inc., Stamford, CT, USA) through the center of the lid. Around 0.6 g of cinnamon was filled into the cell and hermetically sealed, and then immersed the cell in the pre-heated oil bath. The core temperature was recorded every 0.02 s by a data logger (LR8402-20, HIOKIE. E. Corporation, Nagano, Japan). In this study, the CUT for cinnamon to reach the target isothermal treatment temperatures (70, 75 and 80 °C) were from 52 s to 1 min 10 s. In order to simplify the experiments, the CUT for isothermal treatments was normalized to 1 min.

Prior to isothermal treatments, each TDT II cell was filled with 0.60 ± 0.01 g of conditioned cinnamon powder and hermetically sealed. Isothermal treatments (at 70, 75 and 80 °C) were conducted by immersing these test cells in the pre-heated glycol bath circulator (Isotemp™ 5150 H24, Fisher Scientific™ PA, USA). The isothermal treatment time started (regarded as 0 min in thermal death curves as shown in Fig. 4) after the CUT. The test cells from respective treatment were collected at 5 sampling points (in duplicates at each point) and immediately cooled down in the ice water for 2 min. Experiments were repeated in triplicate independently.

2.3.3. Enumeration of S. Enteritidis PT 30 in thermally treated ground cinnamon

Thermal-treated cinnamon powder (~0.6 g) was transferred from the test cell to a 15-mL sterile conical centrifuge tube with 5.4 mL of sterile BPW to achieve a 10-fold dilution, then homogenized using a vortex for 1 min and further 10-fold serially diluted. Appropriated dilutions were spread on TSAYE plates supplemented with 0.05% (w/v) ferric ammonium citrate and 0.03% (w/v) sodium thiosulfate (m-TSAYE) in two technical replicates and incubated at 37 °C for 48 h.

2.3.4. Thermal inactivation parameters

The first-order kinetic model was applied for the analysis of the isothermal inactivation data, which can be expressed as Eq. (3):

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

where t (min) is the thermal treatment time; N (CFU/g) is the bacterial population at treatment time t ; N_0 (CFU/g) is the initial bacterial population; D (min) is the time required to inactive the microbial population by 90% at the treatment temperature.

Thermal decimal time (D-value) was estimated from the slope of the thermal inactivation curve using a log-linear regression; the goodness of fit was evaluated by R^2 coefficient and root mean square error (RMSE). The smaller the RMSE, the more effective the model fitness.

The z_T-value (°C), which represents the temperature change required to achieve a 10-fold change in D-value, was determined from the regression of log D-value versus treatment temperature. It can be calculated as the reciprocal value of the slope for the above linear regression line, which can be determined by Eq. (4).

$$z_T = \frac{T_2 - T_1}{log(D_1/D_2)} \tag{4}$$

Similarly, the effect of treatment-temperature a_w on the thermal resistance of *S*. Enteritidis PT30 in ground cinnamon, as represented by z_{aw}-value, could be determined as Eq. (5).

$$z_{a_w} = \frac{a_{w,2} - a_{w,1}}{log(D_1/D_2)} \tag{5}$$

2.4. Statistical Analyses

Thermal inactivation data were analyzed by the Prism8 (GraphPad

Table 1
The proximate analysis composition of ground cinnamon (n = 3).

Analyte	Percentage (%, w/w)
Moisture	9.61 ± 0.11
Protein	3.80 ± 0.17
Fat	2.43 ± 0.05
Ash	3.42 ± 0.06
Carbohydrate[a]	80.7 ± 0.26
Fiber	59.5 ± 1.28

[a] The carbohydrate content in this table includes fiber.

Software, San Diego, CA), generating linear models. Means and standard deviations were reported for each independent experiment.

3. Results and discussion

3.1. Chemical composition and particle size distribution of ground cinnamon

The results from the proximate analysis for cinnamon are summarized in Table 1. The initial a_w of ground cinnamon was around 0.5 at room temperature (~21 °C). The background mesophilic microflora count in this ground cinnamon product was below the detection limit (<100 CFU/g), which should not interfere with the inactivation study. Ground cinnamon had carbohydrate content as high as 80.7% (Table 1) due to the significant contribution of plant fiber content (59.5%). Based on this proximate composition analysis, this ground cinnamon can be considered as a carbohydrate-rich low-moisture food.

Generally, the particle sizes of the cinnamon powder were fine, with the majority in the range of less than 355 μm (over 98.59%) (Fig. 2). The particles were relatively evenly distributed from the diameter 75–355 μm. There was about 27% of cinnamon particle size less than 75 μm (Fig. 2). The particle size of spices, determining the specific surface area exposed to the bacteria, may influence the microbial inhibitory activity. In theory, the smaller the particle size, the higher the antimicrobial compounds releasing rate. According to Kuang et al. (2011), ultra-fine cinnamon and clove powders with proper particle size could be equivalent to essential oils as effective antibacterials.

3.2. a_w changes of ground cinnamon at elevated temperatures

As shown in Fig. 3, the a_w of ground cinnamon increased with the increasing treatment temperature at each of the eight moisture contents. For instance, the a_w of cinnamon at room temperature was 0.15, 0.43 and 0.75 for moisture content of 0.057, 0.099 and 0.149 g water/g dry solids, respectively; when heated to 80 °C in sealed containers (without change in moisture content), it increased to 0.32 0.67 and 0.84, respectively. This trend is similar to other reported high-carbohydrate low-moisture foods, such as wheat flour (Tadapaneni et al., 2017) and corn starch (Jin, Tang, & Sablani, 2019). The a_w of organic wheat flour at 20 °C was 0.44 (with the moisture content of 0.116 g water/g dry solids), and it increased to 0.69 at 80 °C (Tadapaneni et al., 2017). For corn starch with a moisture content of 0.089 g water/g dry solids, the a_w increased from 0.12 to 0.30 when heated from 25 to 80 °C (Jin et al., 2019).

For oil-rich food systems, the a_w may not increase as much with the increasing treatment temperature. For example, the a_w of coconut milk powder (a high-fat product with a fat content up to ~64% d. b.) was relatively stable with increasing temperature when its moisture content higher than 0.021 g water/g dry solids (Jin et al., 2019). However, in pure peanut oil, the a_w sharply decreased with increasing temperature (Yang, Xu, Lombardo, Ganjyal, & Tang, 2020).

Equation (1) was fitted to the data in Fig. 3 for each moisture content. The values for net isosteric heat of sorption q_{st} (kJ/mol) were related to moisture content M_d (g water/100 g dry solids) using Equation

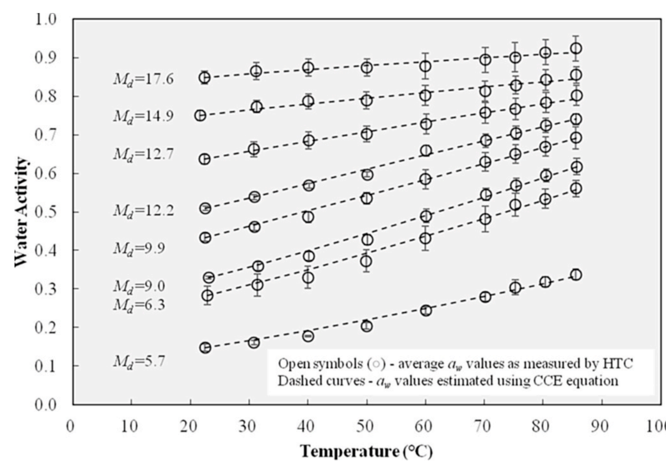

Fig. 3. Water activity changes of ground cinnamon at different temperatures (n = 3). M_d refers the moisture content in dry basis (g water/100g dry solids).

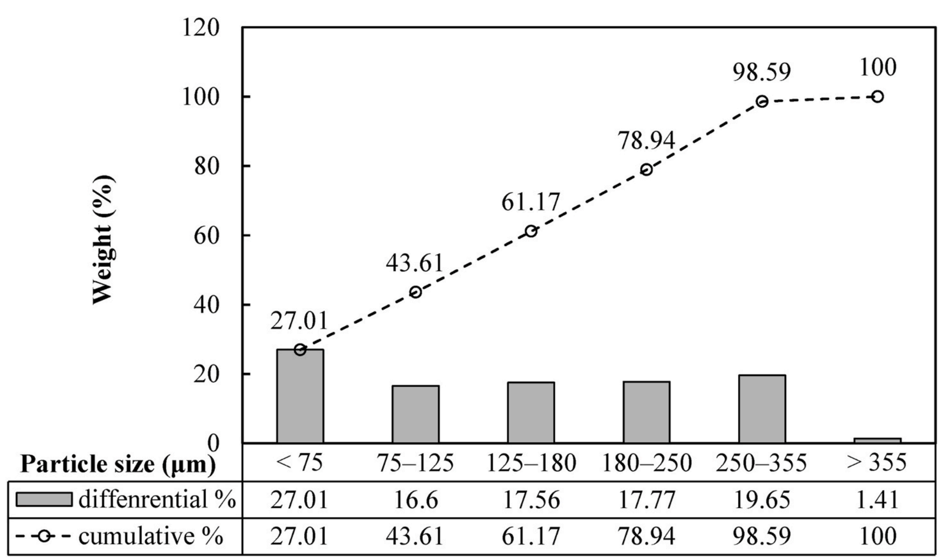

Fig. 2. Particle size distribution of ground cinnamon in this study.

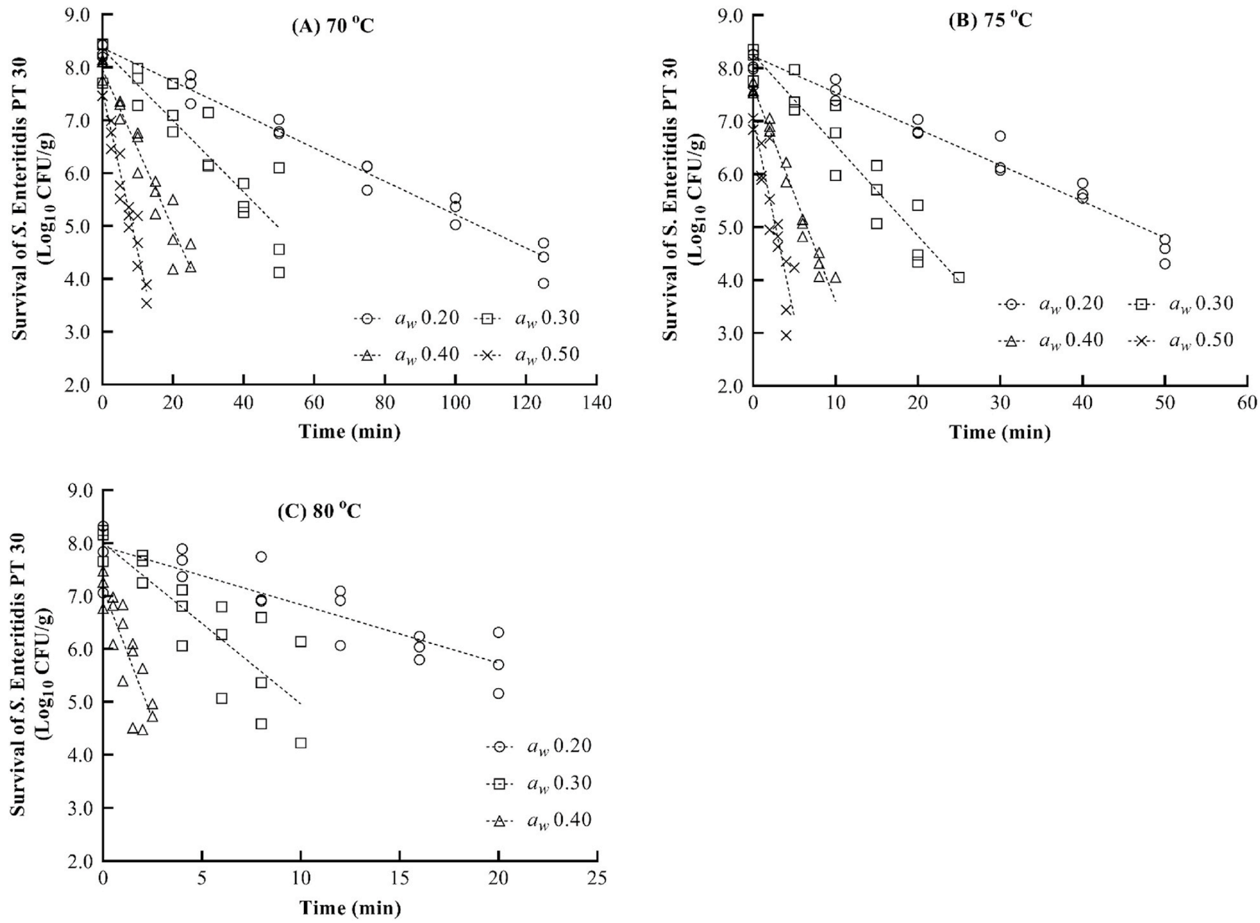

Fig. 4. Thermal death curves for *S.* Enteritidis PT 30 in ground cinnamon equilibrated to room-temperature a_w of 0.20, 0.30, 0.40 and 0.50 at (A) 70 °C, (B) 75 °C and (C) 80 °C. Experiments were carried out in triplicates independently.

(2), as $q_{st} = 43.13\,exp(-\ 0.20 * M_d)$. In general, the q_{st} values were inversely related to the moisture content of powdered cinnamon. For instance, the cinnamon sample of moisture content 9.9 g water/100 g dry solids, the q_{st} was 6.0 kJ/mol; when the moisture content of cinnamon sample increased to 12.7 g water/100 g dry solids, the q_{st} value was reduced to 3.4 kJ/mol. The higher q_{st} values obtained at lower moisture contents suggest stronger bonds between water molecules and macromolecules that require considerably more energy to break than at higher moisture contents (Lim, Tang, & Hw, 1995). Along with Eq (1), this relationship serves as a useful tool to calculate the change in a_w of ground cinnamon when thermally treated in a closed system.

Published exponential relationships between q_{st} and moisture content for other low a_w matrices were summarized in Table 2. The range of moisture content and q_{st} for high-carbohydrate ground cinnamon (~81% carbohydrate) is similar to other reported carbohydrate-rich or protein-rich low moisture foods, including wheat flour (~79% carbohydrate), corn starch (~98% carbohydrate), and soy protein (~93% protein) (Jin et al., 2019; Tadapaneni et al., 2017). The values of q_{st} depend largely on food compositions due to the influence of macromolecules in binding ability (Tadapaneni et al., 2017; Xie et al., 2020).

3.3. Thermal inactivation of S. Enteritidis PT 30 in ground cinnamon

The population of inoculated cinnamon was 8.6 ± 0.1 $\log_{10}$ CFU/g before conditioning to different a_w. The deviations of the inoculated sample from random locations for each batch were less than 0.2 $\log_{10}$ CFU/g, indicating the homogeneity of the inoculated cinnamon. Equilibrium in the humidity chamber at different a_w/RH levels caused less than 1 $\log_{10}$ CFU/g reduction. Thus, the initial population of *S.* Enteritidis PT30 in the samples when subjected to the isothermal treatments was 7–8 $\log_{10}$ CFU/g.

Generally, the survival of *S.* Enteritidis PT 30 in ground cinnamon decreased linearly with treatment time on a semi-log scale at respective treatment temperatures (Fig. 4A, B and C). *S.* Enteritidis PT 30 in ground cinnamon was more tolerant to heat at a lower a_w environment. In other words, less log reduction in population was observed at a lower room-temperature a_w when thermally treated at the same conditions. For example, when inoculated ground cinnamon was isothermally treated at 75 °C for 10 min, around 4-log reduction of *S.* Enteritidis PT 30 was achieved in the sample with a room-temperature a_w of 0.40; while around 2-log reduction was observed for the cinnamon sample with a $a_{w,\ room\text{-}temperature}$ of 0.30; and less than 1-log reduction in population was obtained in the sample at a $a_{w,\ room\text{-}temperature}$ of 0.20 (Fig. 4B). Similar results were also observed at other treatment temperatures (Fig. 4A and C).

The survival of *S.* Enteritidis PT 30 from isothermal treatments was analyzed using the log-linear model. The statistic parameters are summarized in Table 3. At a given isothermal treatment temperature, the *S.* Enteritidis PT 30 showed enhanced resistance to heat (a larger *D* value) in ground cinnamon at a lower room-temperature or treatment-temperature a_w. For instance, when thermally treated at 70 °C, the $D_{70^\circ C}$-values increased from 3.3 min to 31.3 min with $a_{w,\ room\text{-}temperature}$ reducing from 0.5 to 0.2 ($a_{w,\ treatment\text{-}temperature}$ ranging from 0.68 to 0.35) (Table 3). The inverse relationship between survival of *S.* Enteritidis PT 30 and a_w was also observed in thermal inactivation of *Salmonella* in other low-moisture foods, such as wheat flour (Liu, Rojas, Gray, Zhu, & Tang, 2018), cocoa powder (Tsai et al., 2019), and honey powder (Alshammari et al., 2020). Similar observations have also been reported

Table 2
The prediction equations for isosteric heat of sorption (q_{st}, kJ/mol) as function of moisture content (M_d, g water/100 g dry solids) in different low moisture matrices. The range of moisture content was derived from published studies and range of q_{st} was calculated based on the reported equations, respectively.

Low moisture matrices	Prediction equation	R^2	Range of M_d (g water/100 g dry solids)	Range of q_{st} (kJ/mol)	Reference
Ground cinnamon	$q_{st} = 43.13 \exp(-0.20^*M_d)$	0.93	5.7–17.6	13.9–1.3	This study
Organic wheat flour	$q_{st} = 87.8 \exp(-0.26^*M_d)$	0.99	7.8–20.1	11.6–0.5	Tadapaneni et al. (2017)
Almond flour	$q_{st} = 39.5 \exp(-0.69^*M_d)$	0.96	2.2–9.9	8.7–0.0	
Non-fat milk powder	$q_{st} = 26.7 \exp(-0.30^*M_d)$	0.98	2.8–15.9	11.5–0.2	
Corn starch	$q_{st} = 85.6 \exp(-0.21^*M_d)$	0.97	8.9–21.5	13.2–0.9	Jin et al. (2019)
Soy protein	$q_{st} = 31.6 \exp(-0.24^*M_d)$	0.99	4.5–21.4	10.7–0.2	
Cheddar cheese powder	$q_{st} = 30.9 \exp(-0.50^*M_d)$	0.91	2.8–32.8	7.6–0.0	
Coconut milk powder	$q_{st} = 10826 \exp(-4.43^*M_d)$	0.97	1.5–9.4	14.1–0.0	
Freeze-dried S. Enteritidis PT 30	$q_{st} = 17.85 \exp(-0.10^*M_d)$	0.99	6.1–22.3	9.7–1.9	Xie et al. (2020)

for other strains, such as *Listeria monocytogenes* (Taylor, Tsai, Rasco, Tang, & Zhu, 2018) and *Enterococcus feacium* (Liu, Rojas, et al., 2018).

The $D_{70^\circ C}$-values for *S.* Enteritidis PT 30 were 31.3 ± 2.0, 15.3 ± 2.9, 6.4 ± 0.4 and 3.2 ± 0.6 min in ground cinnamon with $a_{w,\ room\text{-}temperature}$ of 0.20, 0.30, 0.40 and 0.50, respectively (Table 3). The $D_{75^\circ C}$-values were 14.1 ± 1.4, 5.5 ± 0.7, 2.4 ± 0.1 and 1.3 ± 0.4 min in ground cinnamon with $a_{w,\ room\text{-}temperature}$ of 0.20, 0.30, 0.40 and 0.50, respectively (Table 3). At each a_w level, the *D*-values for *S.* Enteritidis PT 30 at 70 °C were more than twice of that at 75 °C. The $D_{80^\circ C}$-values for *S.* Enteritidis PT 30 were 8.8 ± 1.5, 2.5 ± 0.4 and 0.9 ± 0.1 min at $a_{w,\ room\text{-}temperature}$ of 0.20, 0.30 and 0.40, respectively (Table 3). The $D_{80^\circ C}$-values for *S.* Enteritidis PT 30 at $a_{w,\ room\text{-}temperature}$ of 0.20 was almost 10 times that for $a_{w,\ room\text{-}temperature}$ of 0.40. In summary, *Salmonella* are more thermal resistant in dry environments, resulting in larger *D*-values under a lower a_w level when thermally inactivated at a specific temperature.

The $\log_{10}$ *D*-values for *S.* Enteritidis PT 30 in ground cinnamon decreased linearly with increasing treatment temperatures at respective $a_{w,\ room\text{-}temperature}$ (Fig. 5). The z_T-values for *S.* Enteritidis PT 30 were 18.2 ± 1.5, 12.8 ± 1.2, 11.9 ± 0.6 and 12.0 ± 1.3 °C in ground cinnamon with $a_{w,\ room\text{-}temperature}$ of 0.20, 0.30, 0.40 and 0.50, respectively (Table 3). The z_T-value of *S.* Enteritidis PT 30 in ground cinnamon at $a_{w,\ room\text{-}temperature}$ of 0.20 was observed significantly larger than that of other room-temperature a_w levels ($P < 0.05$), while z_T-values for *S.* Enteritidis PT 30 in ground cinnamon with $a_{w,\ room\text{-}temperature}$ of 0.30, 0.40 and 0.50 were not significantly different. According to Liu, Rojas, Gray, Zhu, and Tang (2018), the z_T-value for *S.* Enteritidis PT 30 in wheat flour at $a_{w,\ room\text{-}temperature}$ of 0.30 was 16.9 °C, which is larger than that in ground cinnamon at the same room-temperature a_w (12.8 °C, Table 3). Similarly, the z_T-value for a five-strain *Salmonella* cocktail in milk powder was ~15 °C at $a_{w,\ room\text{-}temperature}$ of 0.30 (Wei et al., 2020). However, the z_T-value for a five-strain *Salmonella* -cocktail in milk powder equilibrated to a_w of 0.20 at room temperature was ~17 °C, which is slightly smaller than the value for *S.* Enteritidis PT 30 in ground cinnamon (Lau et al., 2020).

3.4. Thermal resistance of S. Enteritidis PT 30 in ground cinnamon as influenced by treatment-temperature a_w

Log$_{10}$ *D*-values for *S.* Enteritidis PT 30 in ground cinnamon decreased linearly with increasing $a_{w,\ treatment\text{-}temperature}$ at each temperature, with

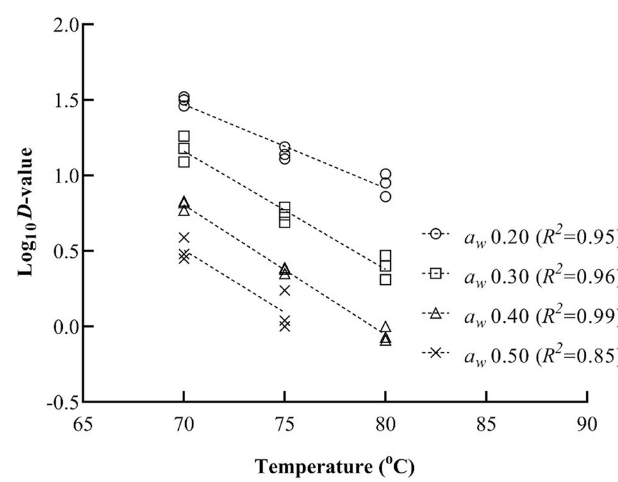

Fig. 5. Logarithm *D*-values of *S.* Enteritidis PT 30 in ground cinnamon under different a_w levels (at room temperature) and inactivation temperatures.

Table 3
Thermal inactivation data for *S.* Enteritidis PT 30 in ground cinnamon according to log-linear model.

Room-temperature $a_w \pm 0.02$	Treatment temperature (°C)	Treatment-temperature $a_w \pm 0.02$	*D*-value (min)	R^2	*RSME*	z_T-value (°C)
0.2	70	0.35	31.3 ± 2.0	0.98	0.23	18.2 ± 1.5
	75	0.37	14.1 ± 1.4	0.96	0.37	
	80	0.39	8.8 ± 1.5	0.70	0.52	
0.3	70	0.51	15.3 ± 2.9	0.90	0.28	12.8 ± 1.2
	75	0.53	5.5 ± 0.7	0.89	0.46	
	80	0.56	2.5 ± 0.4	0.93	0.24	
0.4	70	0.59	6.4 ± 0.4	0.97	0.18	11.9 ± 0.6
	75	0.61	2.4 ± 0.1	0.98	0.22	
	80	0.63	0.9 ± 0.1	0.89	0.28	
0.5	70	0.68	3.3 ± 0.6	0.94	0.25	12.0 ± 1.3
	75	0.70	1.3 ± 0.4	0.85	0.40	
	80	0.72	NA	NA	NA	

Mean ± SD. n = 3. Treatment-temperature a_w were estimated by CCE from this study. NA: not available (population below the detection limit, < 250 CFU/g, n = 2).

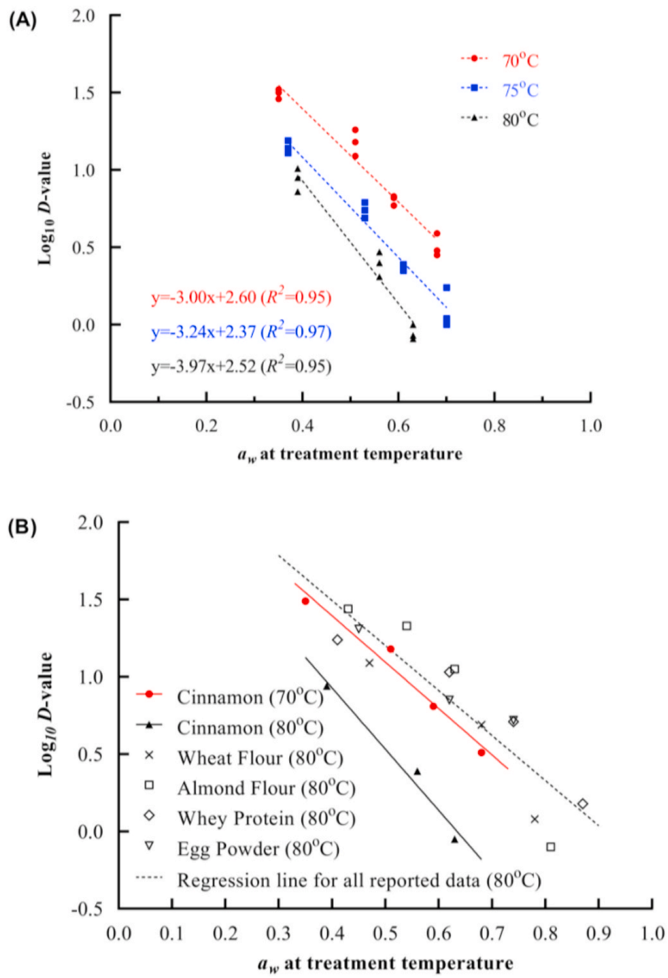

Fig. 6. Relationship between $\log_{10}$ *D*-values of *S.* Enteritidis PT 30 and treatment-temperature a_w in ground cinnamon and other low-moisture foods. (A) ground cinnamon treated at 70, 75 and 80 °C. Dashed lines represent the linear regression. (B) Comparison of $\log_{10}$ *D*-values for *S.* Enteritidis PT 30 in cinnamon and other low-moisture foods using TDT test cells.

the goodness of fit more than 0.95 ($R^2 \geq 0.95$, Fig. 6A). At respective isothermal inactivation temperature, the resistance to heat was determined by the value of $a_{w,\ treatment\text{-}temperature}$. The effect of a_w at thermal treatment temperature on the thermal inactivation of *Salmonella* has been reported in multiple low-moisture foods, including wheat flour, almond flour, soy protein, egg powder, and honey powder (Alshammari et al., 2020; Jin, Tang, & Zhu, 2020; Perez-Reyes et al., 2020; Xu et al., 2019). Similar conclusions could also be obtained in other strains or serotypes, such as *E. faecium* and *S.* Agona (Jin et al., 2020; Liu, Tang, et al., 2018; Yang, Xie, Lombardo, & Tang, 2020).

The z_{aw}-values (as a function of $a_{w,\ treatment\text{-}temperature}$) is an important factor indicating the sensitivity of microbial resistance to heat as influenced by the change of a_w at treatment temperature (ranging from 0.35 to 0.72 among 70–80 °C in this study, Table 3). The z_{aw}-values for *S.* Enteritidis PT 30 in ground cinnamon (−1/Slope in Fig. 6A) were 0.33, 0.31 and 0.25 at the isothermal temperature of 70, 75 and 80 °C, respectively. The z_{aw}-values at 80 °C for *S.* Enteritidis PT 30 in wheat flour, almond flour and whey protein is 0.32 (Xu et al., 2019), and for that in silico dioxide was 0.31 (Liu, Tang, et al., 2018). The z_{aw}-values at 80 °C for *S.* Enteritidis PT 30 in ground cinnamon in this study (0.25) is lower than that in other reported low a_w systems (~0.32), indicating the thermal resistance of *S.* Enteritidis PT 30 in ground cinnamon is more sensitive to the change of a_w. The z_{aw}-values for *S.* Agona in soy protein were from 0.49 to 0.45 when treated from 70 to 80 °C, which is higher than the z_{aw}-values for *S.* Enteritidis PT 30 obtained in this study. It indicates that different serotypes or strains may lead to different z_{aw}-values.

3.5. *Synergistic effect of antimicrobial constituent and heat on the inactivation of S. Enteritidis PT 30*

The thermal resistance of *S.* Enteritidis PT 30 at 80 °C has been studied in several low a_w matrices (Alshammari et al., 2020; Liu, Rojas, et al., 2018; Perez-Reyes et al., 2020; Xu et al., 2019). Our recent study on freeze-dried *S.* Enteritidis PT 30 suggests that the thermal resistance of *S.* Enteritidis PT 30 at a certain temperature in different low moisture foods was determined by the moisture content of bacterial cells (Xie et al., 2020). Linear relationships were observed between $\log_{10} D_T$-values and moisture content of bacteria or a_w of food matrices (at treatment temperature) in different non-spicy low-moisture foods (Xie et al., 2020). Since the same test cells and procedures were also used in the thermal inactivation studies for *S.* Enteritidis PT 30 in wheat flour, almond flour, whey protein and egg powder as described in Section 2.3, a valid comparison could be made among the $\log_{10}$ *D*-values. The values of $\log_{10} D_{80^\circ C}$ for *S.* Enteritidis PT 30 at $a_{w,\ treatment\text{-}temperature}$ in high-carbohydrate and high-protein low-moisture foods derived from TDT cells were similar and scattered evenly around a linear regression line (Fig. 6B, dark dashed line). Interestingly, the above linear regression line for $\log_{10} D_{80^\circ C}$-values of *S.* Enteritidis PT 30 in reported non-spicy low-moisture foods was close to the regression line for $\log_{10}$ *D*-values of *S.* Enteritidis PT 30 in cinnamon treated at 70 °C (Fig. 6B, the red solid circle with regression line). The $\log_{10}$ *D*-values of *S.* Enteritidis PT 30 in cinnamon powder (Fig. 6B, the dark solid triangle with regression line) at 80 °C were about half to one order of magnitude lower than that in wheat flour, almond flour, whey protein and egg powders when treated at the same temperature. This is likely due to a synergistic effect of antimicrobial compounds in cinnamon powder and thermal lethality. Theoretically, the temperature has a positive correlation to the volatile release rate (Varga-Visi, Jócsák, Ferenc, & Végvári, 2019). This observation suggests that the inactivation of *S.* Enteritidis PT 30 in cinnamon powder can be achieved in a lower temperature treatment compared with the low-moisture foods without antimicrobial constituents. For example, according to Table 3, when powdered cinnamon with $a_{w,\ room\text{-}temperature}$ of 0.3 is exposed to 75 °C for 28 min (5 × 5.5 min), more than 5-log reduction of *S.* Enteritidis PT 30 would be achieved. Whilst according to Liu, Rojas, et al. (2018), in order to obtain a 5-log reduction of *S.* Enteritidis PT 30 in wheat flour with the same $a_{w,\ room\text{-}temperature}$, an exposure of around 30 min-treatment (5 × 5.9 min) to 85 °C (10 °C higher than that for ground cinnamon) would be required. Further studies on the synergistic effect of the bioactive compounds in other spices (with regard to different antimicrobial constituents) are needed to validate and reinforce this hypothesis. Moreover, quantitative analysis of antimicrobial constituents should be conducted in future studies.

4. Conclusion

The isothermal thermal inactivation of *S.* Enteritidis PT 30 in ground cinnamon under multiple $a_{w,\ treatment\text{-}temperature}$ at three treatment temperatures (70, 75 and 80 °C) revealed linear relationships between $\log_{10}$ *D*-values and isothermal temperature as well as a_w of the samples at any specific treatment temperature ($a_{w,\ treatment\text{-}temperature}$). The comparison of the regression lines for $\log_{10}$ *D*-values of *S.* Enteritidis PT 30 in ground cinnamon with that in other reported low-moisture foods at 80 °C suggested that the antimicrobial compounds in ground cinnamon facilitated thermal inactivation of *S.* Enteritidis PT 30. In particular, the inactivation efficacy of thermal treatments at 70 °C for *S.* Enteritidis PT 30 in cinnamon powder was similar to that of the treatments at 80 °C in other low-moisture foods, such as wheat four and egg powders. Thus, milder thermal treatments can be used for the control of *Salmonella* in cinnamon powder, and perhaps other spices that contain antimicrobial compounds, for better retention of product quality, including the color and volatiles.

CRediT authorship contribution statement

Yucen Xie: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation, Writing - original draft. **Teng Cheng:** Validation, Investigation, Writing - review & editing. **Lina Wei:** Validation, Writing - review & editing. **Mei-Jun Zhu:** Supervision, Writing - review & editing. **Shyam S. Sablani:** Writing - review & editing. **Juming Tang:** Conceptualization, Supervision, Project administration, Funding acquisition, Writing - review & editing.

Declaration of competing interest

The authors have no conflict of interest in this manuscript. This work is an original research that has not been published in whole or in part.

Acknowledgment

This study was supported by the USDA Agricultural and Food Research Initiative (AFRI) CAP grant (2015-68003-2341), the USDA National Institute of Food and Agriculture Hatch project (#1016366) and the Agriculture Research Center of Washington State University. The author Yucen Xie would like to appreciate the grant from China Scholarship Council (CSC) for supporting her Ph.D. study. The authors also appreciate Dr. Linda Harris from the University of California, Davis, for providing the stock culture. We acknowledge the assistance from Shuang Zhang during isothermal treatments.

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