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High temperature water activity as a key factor influencing survival of *Salmonella* Enteritidis PT30 in thermal processing

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ABSTRACT

Salmonella in low-moisture foods has enhanced thermal tolerance and is difficult to control. The objective of this research was to study relationship between thermal tolerance of *Salmonella* Enteritidis PT30 and water activity (a_w) of food matrices measured at elevated temperatures during thermal processing. Three different foods were selected for this study. They were wheat flour (WF), almond flour (AF) and whey protein (WP), representing carbohydrate-, fat-, and protein-rich food systems, respectively. Pre-equilibrated powders were inoculated independently with *S.* Enteritidis PT30 and conditioned to a_w of 0.25, 0.45, 0.60, and 0.80 at room temperature (~20 °C). Aluminum thermal death time test cells (TDT cells) and newly designed thermal a_w cells (TAC, with controlled a_w) were heated at 80 °C to determine *D*-values (the time needed to active 90% of target bacteria) of *S.* Enteritidis PT30 in the three powders. Water activities of powders in the TDT cells at 80 °C were calculated to be between 0.41 and 0.89, while in the TAC were controlled to 0.32, 0.50, 0.63, and 0.81, respectively. Results showed that $D_{80°C}$-values of *S.* Enteritidis PT30 decreased exponentially with increasing a_w of foods at the treatment temperature 80 °C regardless of the food matrices and the testing methods. Thus, it is critical to understand how a_w of a food matrix changes with temperature when selecting appropriate treatment conditions for thermal control of *Salmonella* in low-moisture foods.

1. Introduction

Outbreaks caused by contaminated low-moisture foods have brought public, regulatory, and industry attentions to food safety of products such as pistachios (FDA, 2016b), flours (FDA, 2016a), and peanut butter (CDC, 2007). Low-moisture foods are defined as having a room temperature water activity (a_w) well below 0.85, usually lower than 0.6 (Lang et al., 2016). *Salmonella* spp. is the most common pathogen causing low-moisture foods outbreaks, since a low number of cells can cause illness (Haas, 1983; Jarvis et al., 2016). Although *Salmonella* does not multiply in the low-moisture environment, it maintains viability over prolonged storage (Farakos, Frank, & Schaffner, 2013; Podolak, Enache, Stone, Black, & Elliott, 2010). Control of pathogens in food matrices in reduced a_w environments is challenging since these pathogens are much more tolerant to heat compared with those in aqueous environments (Bari et al., 2009; Commission, 2015; Villa-Rojas et al., 2017).

Low a_w has generally been considered to play a role in enhancing thermal resistance of microorganisms in low-moisture foods (Beuchat, Mann, Kelly, & Ortega, 2017; Murrell & Scott, 1966; Syamaladevi, Tang, Villa-Rojas et al., 2016). It has been shown that the thermal resistance of bacteria, as evaluated by *D*-values (the time needed to inactivate 90% of target bacteria at a given temperature), increased significantly with decrease in a_w measured at room temperature in the same food system (Farakos et al., 2013; Goepfert, Iskander, & Amundson, 1970; He et al., 2013; Villa-Rojas et al., 2013). For instance, *D*-value of *Salmonella* Enteritidis PT30 (*S.* Enteritidis PT30) increased 36 times when sample a_w measured at room temperature decreased from 0.95 to 0.60 in almond flour (Villa-Rojas et al., 2013). But the literature has reported vastly different thermal resistances for the same pathogens when treated in different food matrices having the same room temperature a_w. For example, *D*-values of *S.* Enteritidis PT30 in all-purpose flour and peanut butter with a_w of 0.45 at 20 °C were 6.9 ± 0.7 min and 17.0 ± 0.9 min, respectively (Syamaladevi, Tadapaneni et al., 2016).

In a recent study, Liu, Tang et al. (2018) reported an exponential relationship between $D_{80°C}$ -values of *S.* Enteritidis PT30 and *Enterococcus faecium* NRRL B-2354 and a_w measured at high temperature

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(80 °C), using specially designed thermal a_w cell (TAC) that provided a wide range of stable a_w during the thermal treatments. However, the above study was conducted on a non-food carrier, silicon dioxide. This relationship was only validated with several data points from the literature for wheat flour (Liu, Tang, Tadapaneni, Yang, & Zhu, 2018). The objective of this research was to systematically validate such relationship using three food powders that represented three different categories of food matrices.

It is well known that a_w of a food system changes with temperature, the level of the change depends on food composition (Chen & Grant, 1998; Labuza, 1968; Syamaladevi, Tang, Villa-Rojas et al., 2016; Tadapaneni, Yang, Carter, & Tang, 2017). In most previous studies, a_w of food samples was not controlled in isothermal inactivation tests, and the thermal resistance of *Salmonella* under the influence of a_w was assessed only by measured a_w at room temperature, instead of at treatment temperatures (He et al., 2013; Villa-Rojas et al., 2013). This was due to a lack of a_w measurement devices for high temperatures. To fill this gap, a high-temperature cell was recently developed by Syamaladevi et al. (2016) and commercialized by Meter Group, Inc. (Pullman, WA, USA). Tadapaneni, Yang et al. (2017) used this cell to study changes of a_w of three different food powders between 20 and 80 °C, and developed a model based on the Clausius Clapeyron equation (CCE) to predict a_w as function of temperature and moisture content. In addition, the same group has developed an improved version of TAC which is able to control a_w of inoculated food samples during isothermal tests (Tadapaneni, Xu, Yang, & Tang, 2018). In this research, a multifactorial experiment was designed to study the influence of a_w at treatment temperature on thermal resistance of *Salmonella*. We considered four a_w levels (0.25, 0.45, 0.60, and 0.80 at 20 °C), three different powder products (wheat flour, almond flour, whey protein, showing wide varieties in both physical structure and chemical composition). We selected 80 °C as the treatment temperature to allow direct comparison of our results with that of Liu, Tang et al. (2018). We used two different testing methods to determine the thermal resistance (*D*-values) of *S.* Enteritidis PT30. They were: 1) the TDT cell method by which powders were treated in small closed test cells, and sample moisture was maintained constant while their a_w changed with temperatures according to their intrinsic isothermal sorption behaviors (Chung, Birla, & Tang, 2008; Durakova & Menkov, 2005; Syamaladevi, Tadapaneni et al., 2016; Tham, Wang, Yeoh, & Zhou, 2016); 2) the TAC method by which high-temperature water activities of the inoculated samples were controlled by LiCl solutions during the isothermal treatments (Tadapaneni et al., 2018). *D*-values of *S.* Enteritidis PT30 as influenced by high temperature a_w was studied in both cases. Major experimental steps included: i) obtaining the characteristics of three powder products in terms of their chemical composition, particle size, and microstructure; ii) determining the thermal resistance of *S.* Enteritidis PT30 at 80 °C in three powder products pre-equilibrated to a_w from 0.25 to 0.80 at room temperature (~20 °C); iii) studying correlations between thermal resistance of *S.* Enteritidis PT30 and a_w at 80 °C associated with different powders.

2. Materials and methods

Three powder products with different physiochemical properties were used in this study. Soft white wheat organic flour (WF) (Eden Foods, Clinton, MI, USA), organic blanches almond flour (AF) (Nuts.com, Cranford, NJ, USA), and whey protein powder (WP) (Nuts.com, Cranford, NJ, USA) were specifically selected and represented as a carbohydrate-, fat-, and protein-rich product, respectively.

2.1. Physiochemical properties of powders

2.1.1. Initial moisture content and a_w

The initial moisture content (% g H_2O/100 g sample) and initial a_w of three powder products at room temperature (~20 °C) were determined by a halogen moisture analyzer (HB43-S; Mettler Toledo, Columbus, OH, USA) and a water activity meter (Aqualab, Meter Group, Inc., Pullman, WA, USA), respectively. The results were based on three independent measurements.

2.1.2. Physical structure analysis

The variation in the physical structures of foods may affect the thermal susceptibility of microorganisms (Liu, Snoeyenbos, & Carlson, 1969; Syamaladevi, Tang, Villa-Rojas et al., 2016). Thus, particle size and microstructure of powdered granulates were analyzed. For the particle size analysis, 3 g of each powder sample was sieved in a sonic sifter (Model L3P, ATM Corporation, Milwaukee, WI, USA). The granulates were fractioned and separated by six pre-selected precision sieves. After sieving, each size interval was weighted, and its relative weight amount (%) was determined by dividing to its total weight. The average value of weight in percentage was based on results from an independent experiment in duplicate.

The microstructure of three powdered granules was examined using a scanning electron microscope (Quanta 200F, FEI company, Hillsboro, OR, USA). Powder particles were thinly spread onto double coated carbon conductive tabs (Ted Pella Inc., Redding, CA, USA) and the excess was removed with compressed air blown over the tab. All samples were gold coated with a thin layer (6 nm in thickness) in a vacuum-evaporator (Technics Hummer V Sputter Coater, Technics, San Jose, CA, USA). Images at different magnifications were captured by a digital camera (Quartz Imaging Corporation, Vancouver, British Columbia, Canada). Representative micrographs were presented at 1000 × and 5000 × magnifications, respectively. At least five specimens of each sample were observed to obtain representative micrographs of samples.

2.1.3. Chemical composition analysis

Powders were sent to the Northern California Laboratory of Silliker Inc. (Salida, CA, USA) to analyze the composition based on standard analytical methods (Latimer, 2012), including moisture content, ash, carbohydrate, fat, and protein. The measurement was conducted in duplicate.

2.2. Measurement of a_w changes of samples in thermal treatment cells

Dimensions of the TDT cells used for isothermal treatments of the inoculated samples are shown in Fig. 1, A. These cells were used by several researchers in determining thermal resistance of *Salmonella* in low-moisture foods (Liu, Ozturk et al., 2018; Xu, Liu, Song et al., 2018; Xu, Liu, Tang et al., 2018). In those sealed cells, moisture contents of the samples would be constant while their water activities would change with temperature (Syamaladevi, Tang, Villa-Rojas et al., 2016).

2.2.1. A_w changes of samples in TDT cells

In this study, changes in a_w of the three food samples when heated from 20 to 60 °C were measured directly by a vapor sorption analyzer (VSA) (AquaLab, Meter Group, Inc., Pullman, WA, USA). At higher temperatures (> 60 °C), a_w was estimated by a newly developed method (Tadapaneni, Yang et al., 2017) in our laboratory. In brief, powders were vacuum dried in an oven (10 kPa) for 2 days at 50 °C before measurement. Samples were then conditioned under different relative humidities (11.3%, 22.5%, 32.8%, 43.2%, 52.9%, 65.8%, 75.3%, 84.3%) in airtight containers for 14 days at 20 °C. Two grams of the equilibrated samples were placed in the high-temperature cells (HTC) with relative humidity sensors (Honeywell HumidIcon™, Morristown, NJ, USA) (Tadapaneni, Yang et al., 2017) and heated in an oil bath. HTC was thermally treated at a 10°C-temperature interval from 10 to 100 °C, and a_w changes were recorded until the readings were stable. When the thermal cells were cool down to 20 °C, the moisture contents of the samples inside the cells were determined using a halogen moisture analyzer (HB43-S; Mettler Toledo, Columbus, OH, USA).

q_{st} is the net isosteric heat of sorption of a certain food at a given

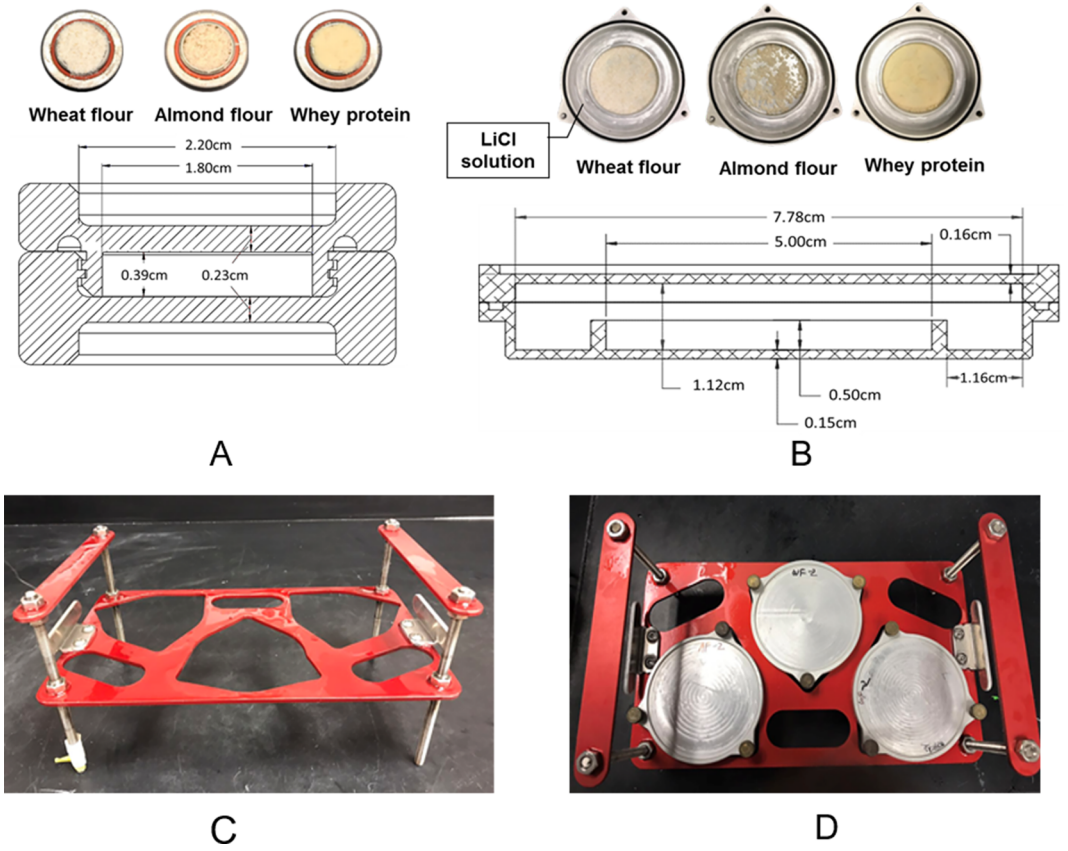

Fig. 1. Representative images of thermal inactivation test cells and TAC rack. A: TDT cells, and B: TAC; C: a specific designed rack for holding TAC during treatment; D: three TAC can be treated at a time. TDT cell: aluminum thermal death time test cell, TAC: thermal a_w cell.

moisture content and its value was determined from the following equation:

$$d \ln a_w = \frac{q_{st}}{RT^2} dT \quad (1)$$

where R is the universal gas constant (8.314 J mol^{-1} K^{-1}), T is the temperature (K). For a given moisture content, q_{st} can be determined from the slope of the plot (ln a_w vs. $1/T$), which was obtained from the method described previously.

The a_w of food samples at 80 °C was determined from the following equation (Tadapaneni, Yang et al., 2017):

$$a_{w.\,80\,°C} = a_{w,\,ref} \exp\left(\frac{q_{st}}{R}\left(\frac{1}{T_{ref}} - \frac{1}{T_{80\,°C}}\right)\right) \quad (2)$$

where $a_{w,\,ref}$ is a_w-value of the same sample at a fixed moisture content at a reference temperature (T_{ref}). The results of measurement data were based on the average data of duplicated independent replicates.

2.2.2. Water activity of powders in TAC

LiCl solutions with different molalities were prepared and used as an a_w-controlling solution during thermal treatments of the inoculated samples. The molalities of LiCl solutions having a_w 0.25, 0.45, 0.60, 0.80 at 20 °C (corresponding to an equilibrium relative humidity of 25%, 45%, 60%, and 80%) were 13.35, 9.37, 7.10, 4.15 mol kg^{-1}, respectively (Tadapaneni, Syamaladevi et al., 2017). The proper amount of LiCl granulates was weighted and dissolved into distilled water before use, and the a_w of prepared LiCl solutions was measured by a water activity meter (Aqualab, Meter Group, Inc., Pullman, WA, USA).

The TAC used in this study were manufactured by Washington State University, the detailed design can be referred to a previous paper (Tadapaneni et al., 2018). A TAC has a lid and a base machined from aluminum blocks. The base consisted of two parts: a central well and a ring groove. A rubbery ring between tightly fitted lid and the base in each TAC prevented leakage of moisture during thermal treatments (Fig. 1, B). 0.7 g of each inoculated powders was thinly spread as a single layer on the bottom of the central well, and the surrounded groove was filled with 3 mL of LiCl solution prepared previously. The TAC loaded with samples and LiCl solution was sealed completely with high vacuum grease (Dow Corning, MD, USA) applied to the O-ring before treatment. In a TAC, the powder was in a 0.6–0.9 mm thin layer and fully exposed to the headspace with relative humidity generated by the LiCl solution.

2.3. Isothermal treatment of S. Enteritidis PT30 in three powder products

2.3.1. Bacteria strain

S. Enteritidis PT30 was used in this study because of its relatively high tolerance to heat and its association with international outbreaks of raw almond contamination (Isaacs et al., 2005; Podolak et al., 2010; Villa-Rojas, 2015). *S.* Enteritidis PT30 (ATCC-1045) was obtained from University of California, Davis. The culture was kept as a stock in tryptic soy broth supplemented with 0.6% (w/v) yeast extract (TSBYE) and 20% (v/v) glycerol at −80 °C. A loopful of *S.* Enteritidis PT30 from the stock was subjected to 9 mL TSBYE for two consecutive transfers and incubated at 37 °C for 24 h. Then, 1 mL of incubated culture was plated on trypic soy agar supplemented with 0.6% (w/v) yeast extract (TSAYE) plate (150 mm × 15 mm) (dia x height). After incubation at 37 °C for 24 h, bacterial lawns were harvested from the plates with 20 mL of 0.1% (w/v) peptone water and centrifuged at 6000 × *g* at 4 °C for 15 min. After discarding the supernatant, bacterial pellets were further re-suspended in 3 mL of 0.1% (w/v) peptone water for inoculation (10 $\log_{10}$ CFU/mL).

2.3.2. Inoculation preparation

Samples from three different powders were firstly conditioned to the same a_w levels at room temperature (~20 °C) to avoid the influence of initial a_w on the results. Different a_w levels (0.25, 0.45, 0.60, 0.80 at 20 °C) were obtained by conditioning powders for 4–5 days at 20 °C in an equilibration chamber (EW- 34788-00, Cole Parmer, Vernon Hills, IL, USA) with a humidity control system built at Michigan State University (Hildebrandt et al., 2016). Then, 50 g of pre-conditioned powder was inoculated with 1 mL of the above-prepared inoculum (10 $\log_{10}$ CFU/mL) and mixed manually in sealed plastic bags for at least 5 min until all visible clumps were disappeared. Inoculated powders were then spread evenly in a 150 mm × 15 mm (dia x height) sterilized Petri dish and placed in the above-mentioned environmental chamber for another 4–5 days at 20 °C until equilibrated to the target a_w. The a_w of the inoculated powders after equilibration was measured three times at 20 °C before isothermal treatments. The population level of the inoculated sample after equilibrium was measured by the method described in 2.3.5.

2.3.3. Come-up time measurement

To determine the *D*-values of *S*. Enteritidis PT30 in powders, aluminum thermal death time test cells (TDT cells) (Chung et al., 2008; Xu, Liu, Song et al., 2018) and thermal a_w cells (TAC) (Fig. 1, B) (Tadapaneni, 2018) were used in parallel to treat the conditioned powders at isothermal condition. To measure the come-up time (CUT), which was the time necessary for samples to reach within 0.5 °C of target temperature (80 °C), the temperature changes in a sample within a TDT cell was recorded by a thermometer (Digiense DuaLogR 99100-50, Cole-Parmer Instruments Co., Vernon Hills, IL, USA) connected with a T-type thermocouple with the tip located at the geometric center of the sample. Similarly, the temperature changes in a TAC was measured by a data logger (DL2e, Delta-T Devices Ltd., Cambridge, UK) connected with a pre-calibrated thermocouple probe located at the geometric center of the sample. The measurement of CUT was conducted in triplicate. The conservative CUTs for powders to reach 79.5 °C in TDT cells and TAC were 1.5 min and 3.0 min, separately. Detailed description on the high heat transfer coefficient of both test cells as lumped systems during isothermal treatment were previously analyzed and discussed (Tadapaneni et al., 2018; Villa-Rojas et al., 2013).

2.3.4. Isothermal treatment

For the TDT cell method, each cell (4 mm thick) was filled with inoculated powders (0.7 g for WF and AF, 0.5 g for WP) and sealed (Fig. 1, A). For the TAC method (Fig. 1, B), powders were further conditioned in the TAC for at least 8 h before treatments. The changes of *S*. Enteritidis PT30 population after 8 h-condition in TAC were assessed, and less than 0.5 $\log_{10}$ CFU/g of the population has been observed (Tadapaneni, Syamaladevi et al., 2017). For isothermal treatment, a specifically designed 6 cm-height holding rack was used to keep TAC horizontally loaded during the treatment (Fig. 1, C). This rack also allowed easy removal of TAC after a pre-set treatment time. Three TAC with each powder sample were treated simultaneously (Fig. 1, D).

Isothermal treatment was conducted by immersing TDT cells and TAC (with rack) in a well-stirred oil bath (Isotemp 5150 H11, Fisher 180 Scientific, Inc., PA, USA) preheated to 80 °C. After the treatment, the test cells were removed at five pre-determined time intervals, cooled down in an ice-water bath for 3 min. The rack was gently removed from the oil bath, and the TAC were kept horizontally during the whole treatment and cooling to avoid inside-cell contamination with LiCl.

2.3.5. Survived bacteria enumeration

After isothermal treatment, the appearance (e.g. color, structure) of the powder products were not changed. To obtain the survivor counts of *S*. Enteritidis PT30, heat-treated samples were scraped from the test cells and transferred into 0.1% (w/v) peptone water to reach 10-fold dilution and stomached at 230 rpm for 3 min (Stomacher® 400 Circulator, Seward Laboratory Systems Inc, Norfolk, UK). Samples were 10-fold serially diluted, and proper dilutions were plated in duplicate on TSAYE plates supplemented with 0.05% (w/v) ferric ammonium citrate (Sigma-Aldrich, St Louis, MO, USA), and 0.03% (w/v) sodium thiosulfate (Sigma-Aldrich, St Louis, MO, USA) and incubated at 37 °C for 48 h (Mattick et al., 2001; McLaughlin & Balaa, 2006). Colonies had a dark center and clear circles were identified as typical *Salmonella* cells. The average number of survivors (CFU/g) was based on three independent biological replicates of each powder sample in each test cell.

2.4. Statistic analysis

Thermal decimal time (*D*-value) at 80 °C of *S*. Enteritidis PT30 in each powder sample was calculated by a linear regression model using USDA integrated predictive modeling program (IPMP) tools (Huang, 2014).

The mean *D*-values and standard deviation for each product were based on three independent replicates. Two-way ANOVA followed by Duncan's post-hoc means comparison ($P < 0.05$) test was performed to evaluate the *D*-values with the effect of two investigated factors: test cells and food type. All statistical results were performed in IBM SPSS statistics 22.

Inactivation curves of *S*. Enteritidis PT30 in three types of powder with changes in a_w levels (at 20 °C) were plotted separately for each of the two testing methods. The relationship of thermal resistance data with a_w at 80 °C was also plotted for each test cell or food matrice.

The effect of a_w on thermal resistance of bacteria is represented by Z_{aw}, which is the a_w change required to change the *D*-value of the target pathogen by a factor of 10. The value of Z_{aw} can be determined from the following equation:

$$\log D_{80°C} = -\frac{1}{z_{a_w}} \times (a_w - a_{w,\,ref}) + \log D_{ref} \quad (3)$$

3. Results

3.1. Comparison of powder products

3.1.1. Chemical composition

The proximate chemical compositions of the three powder products are listed in Table 1. Moisture content and a_w measured at 20 °C of the three powders are also shown in Table 1. Almond flour (AF) had the

Table 1
Initial moisture content, a_w, and proximate compositions of three powders.

	Moisture[a] % (gH_2O/100 g sample)	a_w at 20 °C	Ash[b] (w/w) %	Carbohydrate (w/w) %	Fat (w/w) %	Protein (w/w) %
Wheat flour	8.34 ± 0.17	0.32 ± 0.05	1.55 ± 0.04	78.9 ± 0.16	3.28 ± 0.12	7.92 ± 0.48
Almond flour	3.66 ± 0.33	0.56 ± 0.05	3.22 ± 0.08	23.3 ± 0.88	48.8 ± 0.33	21.1 ± 0.30
Whey protein	5.00 ± 0.13	0.22 ± 0.05	2.97 ± 0.13	8.60 ± 0.68	6.36 ± 0.14	77.1 ± 0.28

[a] The measurements of moisture content and a_w were based on three independent replicates.
[b] The measurements of proximate compositions were based on two independent replicates.

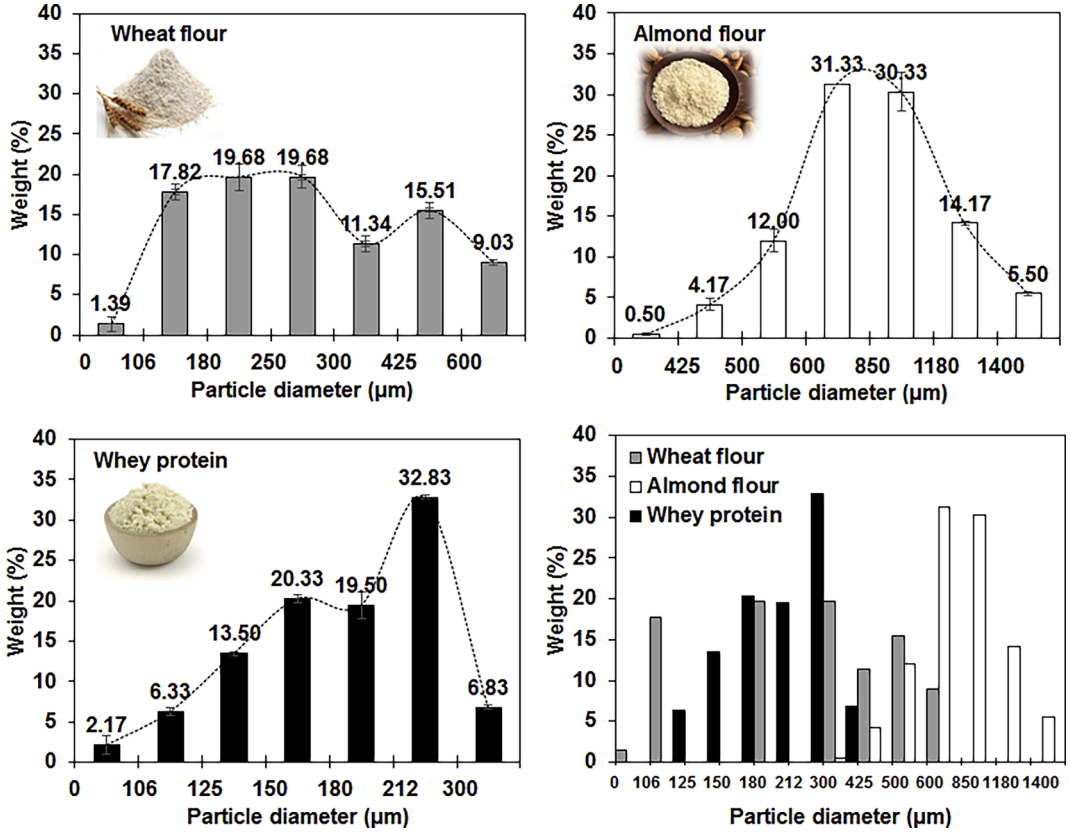

Fig. 2. Particle size distribution of three powder products.

lowest moisture content but had the highest initial a_w compared to wheat flour (WF) and whey protein powder (WP). This is the result of the high-fat content in AF. Based on chemical composition, WF is considered as a starch-rich food product since its high composition in carbohydrate (78.9 ± 0.16%). AF and WP are considered as fat- or protein-rich foods, respectively, since the percentage of fat (48.8 ± 0.33%) or protein (77.1 ± 0.28%) was the highest compared with other components.

3.1.2. Particle size analyses

The mean particle size distribution of the three powders are shown in Fig. 2. The three powders used in this study had different size-distributions (Fig. 2). In WF, powder particles were relatively evenly distributed over particle diameter between 106 and 600 μm. In AF, particle size was peaked in the ranges of 600 and 1180 μm. In WP, 32.83% of the total sample lied between the particle sizes 212 and 300 μm. The overall particle size distribution of three powders on the same x-scale is provided in Fig. 2. The average particle size among three powders was in the following order: AF > WF > WP.

3.1.3. Microstructure of powder particles

Micrographs of the three powders at the same magnification level are shown in Fig. 3. In general, three powder granulates showed different characteristics in granulate shape and surface topography. In WF, round starch granules with a smooth surface were identified. Proteinaceous endosperm tissue was attached around the starch grains (Fig. 3, A, D). In AF, constituent starch-lipid complex granules were found in the grit particles with a porous morphology (Fig. 3, B, E). The crater-like structure surface was caused by the broken oil globules resulting in high levels of surface fat (Drusch & Berg, 2008). The similar microstructure of AF was also observed in a previous study (Zhang et al., 2009). The whey protein particles were an agglomeration of primary particles produced during spray drying (Fig. 3, C, F). The typical WP

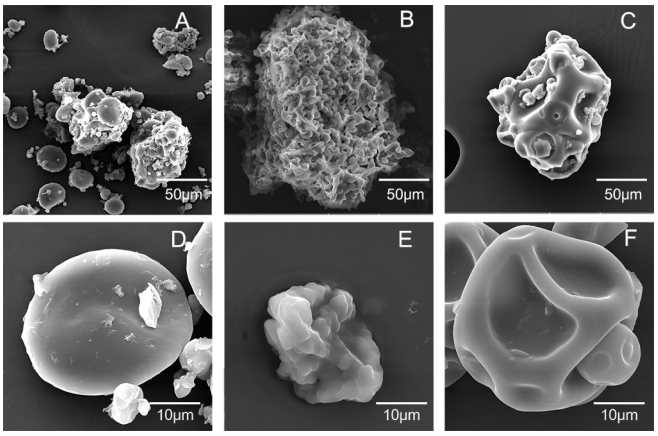

Fig. 3. Scanning electron microscope (SEM) images of wheat flour (A, D), almond flour (B, E), and whey protein (C, F) at 1000 × and 5000 × magnifications, respectively. Images at 1000 × magnification gave an overview of powder and images at 5000 × magnification gave the morphology of powder particles.

particles were characterized as having a spherical shape with concavities and surface deflations with no evidence of cracks and fissures. Similar micro-characterization has also been identified previously (Drapala, Auty, Mulvihill, & O'Mahony, 2017; Soukoulis, Behboudi-Jobbehdar, Yonekura, Parmenter, & Fisk, 2014).

3.2. Thermal resistance of S. Enteritidis PT30 in TDT cells

3.2.1. Water activity changes of food powders in TDT cells

In general, a_w of all the tested powders increased with the increase of temperature at constant moisture contents, but the extent of the

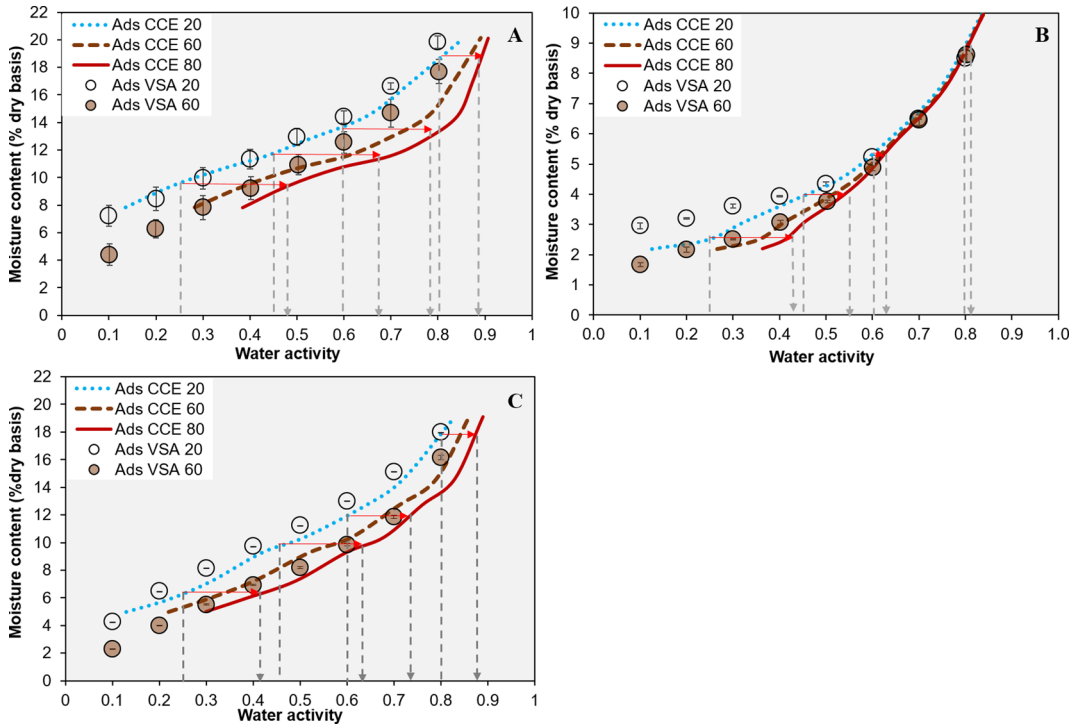

Fig. 4. Moisture sorption isotherms of three powder products (A: wheat flour, B: almond flour, C: whey protein) measured by vapor sorption analyzer at 20 °C and 60 °C (dash points) and estimated by CCE equation at 80 °C (continuous curves).

changes depended on product composition. The shift in a_w of the treated samples in a closed system (TDT cell) from 20 °C to 80 °C for different initial a_w (0.25, 0.45, 0.60, and 0.80) are indicated with arrows in Fig. 4. For wheat flour (WF), a_w at 20 °C was 0.25, 0.45, 0.60, and 0.80, and when heated to 80 °C, a_w increased to 0.47, 0.68, 0.78, and 0.89, respectively. For almond flour (AF) with initial a_w of 0.25, 0.45, 0.60, and 0.80 at 20 °C, the a_w increased to 0.43, 0.54, 0.63, and 0.81 at 80 °C, respectively. The a_w of the whey protein (WP) increased from 0.25, 0.45, 0.60, 0.80 at 20 °C to 0.41, 0.62, 0.74, and 0.87 at 80 °C, respectively. As shown in Fig. 4, the changes in a_w with temperature in the three matrices were in order: WF > WP > AF. This order was not correlated to particle size distributions (see section 3.1.2) but appeared to be inversely related to the fat content of the samples, in order: AF > WP > WF (see Table 1).

3.2.2. Inactivation kinetics of S. Enteritidis PT30

Inactivation curves of *S.* Enteritidis PT30 at 80 °C in three powder products with different a_w levels (measured at 20 °C) obtained from TDT cells are shown in Fig. 5. Since a_w at 80 °C was different for different powders at the same room temperature a_w, a_w measured at 20 °C was used for illustration for now to avoid confusion. For instance, AF-0.25 referred to almond flour with 20 °C a_w of 0.25. In general, survival populations of *S.* Enteritidis PT30 decreased linearly with treatment times on a semi-log chart. The inactivation rates, as characterized by the slopes of those linear lines, increased with increasing a_w. The smallest and the largest inactivation rates corresponded to AF-0.25 and AF-0.80, respectively. The inactivation curves showed no regularity of either powder type or a_w level measured at room temperature.

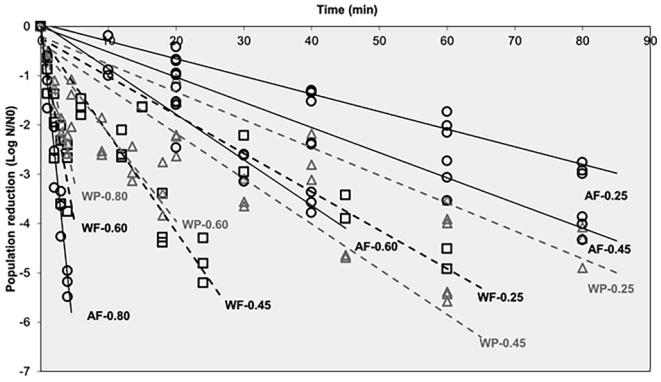

Fig. 5. Inactivation curves of *S.* Enteritidis PT30 at 80 °C in TDT cells. WF: wheat flour; AF: almond flour; WP: whey protein. Inactivation study was based on three independent replicates.

3.3. Thermal resistance of S. Enteritidis PT30 in TAC

3.3.1. Water activity changes of food powders in TAC

At equilibrium, a_w of food samples in the TAC was equal to its surrounding relative humidity, which was created by a_w-controlling solution (LiCl). The a_w-controlling effect of LiCl in TAC has been explained previously (Tadapaneni, Syamaladevi et al., 2017). The a_w of LiCl at 80 °C determined from Fig. 6 was used as the corresponded a_w for the powders at the treatment temperature (80 °C). From 20 °C to 80 °C, a_w of LiCl with initial values of 0.25, 0.45, 0.60, 0.80 increased to 0.32, 0.50, 0.63, and 0.81, respectively (Fig. 6).

3.3.2. Inactivation kinetics of S. Enteritidis PT30

Since the come-up time for samples in TAC was twice (3.0 min) as long as samples treated in TDT cells (1.5 min), the influence of different CUT on calculated *D*-values can be significantly reduced by normalizing the survival data using the bacteria population at CUT as the denominator for calculating population reduction (Fig. 7). Moreover, the heat transfer performance of both test cells has been discussed in previous studies based on lumped system analysis (Tadapaneni, Syamaladevi et al., 2017; Villa-Rojas et al., 2013). In this case, we assumed that the difference in CUT of two test cells would not influence the results during isothermal treatment.

Similar to the observed inactivation curves from TDT cells shown in Fig. 5, *S.* Enteritidis PT30 were rapidly inactivated at high a_w levels for

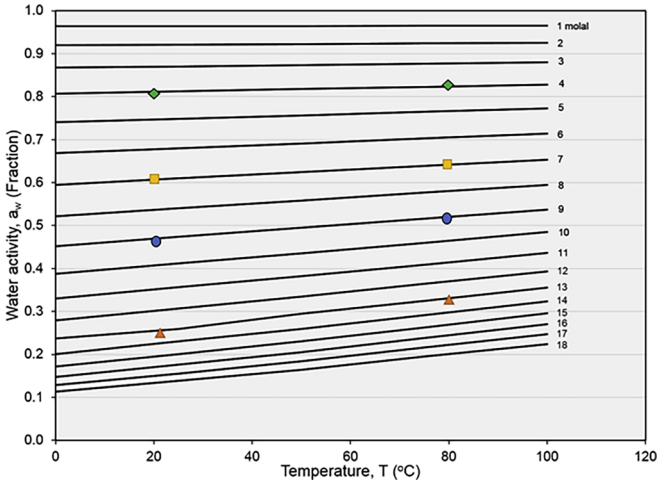

Fig. 6. Prediction of a_w of LiCl of different molarities at temperatures from 0 to 100 °C (Gibbard & Scatchard, 1973; Tadapaneni et al., 2017).

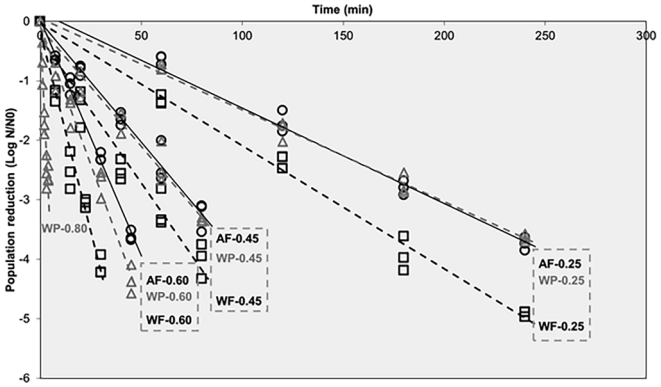

Fig. 7. Inactivation curves of *S.* Enteritidis PT30 at 80 °C in TAC. WF: wheat flour; AF: almond flour; WP: whey protein. Inactivation study was based on three independent replicates.

all powders. At a_w 0.25 and 0.45, the inactivation curves of AF and WP were mostly overlapped, indicating the similar thermal resistance of *S.* Enteritidis PT30 in these conditions. However, *S.* Enteritidis PT30 in WF was more sensitive to heat with a sharper slope of inactivation curves compared with *S.* Enteritidis PT30 in AF and WP at all a_w levels (Fig. 7). The inactivation curves of *S.* Enteritidis PT30 at the same a_w levels were mostly grouped regardless of the food types.

3.4. Variation of D-values with high temperature a_w

D-values of *S.* Enteritidis PT30 in both TDT cells and TAC calculated by a linear regression model (Huang, 2014) are summarized in Table 2. At a_w of 0.80 measured at 20 °C, *D*-values of WF and AF in TAC, and WF in TDT cells were not available (NA) since *S.* Enteritidis PT30 in these conditions was too sensitive to heat, and the survivor counts for several interval treatment times were below our detection limit (< 2 $\log_{10}$ CFU/mL). In this case, there were no complete data points to calculate the corresponding *D*-values. *S.* Enteritidis PT30 in the three different powder products showed different thermal resistance (*D*-values) with the same initial a_w at 20 °C. In contrast, *D*-values were similar among different powders with identical a_w at treatment temperature (80 °C). For example, *D*-values of AF and WP at 80 °C a_w of 0.50 were 24.9 ± 1.9, 23.2 ± 0.1 min, respectively. No significant difference ($P > 0.05$) in *D*-values of *S.* Enteritidis PT30 were observed between AF and WP at other high temperature a_w levels.

Changes of *D*-values of *S.* Enteritidis PT30 in three powders with a_w

Table 2
D-values of *S.* Enteritidis PT30 in different powders.

Powder product	a_w at 20 °C	TDT cell		TAC	
		a_w at 80 °C	*D* (min)	a_w at 80 °C	*D* (min)
Wheat flour	0.25	0.47	12.2 ± 0.7	0.32	46.8 ± 1.0
	0.45	0.68	4.9 ± 0.5	0.50	18.2 ± 1.6
	0.60	0.78	1.2 ± 0.2	0.63	6.6 ± 1.0
	0.80	0.89	NA[a]	0.81	NA
Almond flour	0.25	0.43	27.3 ± 0.3	0.32	62.0 ± 2.0
	0.45	0.54	21.2 ± 0.9	0.50	24.9 ± 1.9
	0.60	0.63	11.1 ± 0.8	0.63	12.1 ± 0.2
	0.80	0.81	0.8 ± 0.1	0.81	NA
Whey protein	0.25	0.41	17.5 ± 1.3	0.32	63.7 ± 1.4
	0.45	0.62	10.6 ± 0.2	0.50	23.2 ± 0.1
	0.60	0.74	5.1 ± 0.4	0.63	9.6 ± 0.0
	0.80	0.87	1.5 ± 0.1	0.81	1.2 ± 0.1

[a] NA means not available.

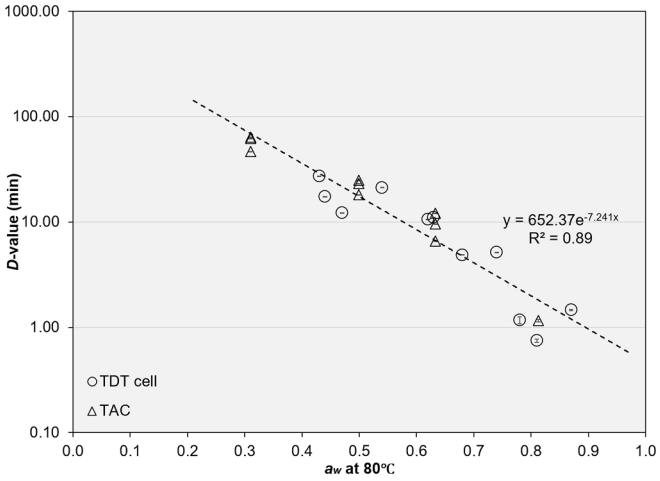

Fig. 8. The log-scale *D*-values of *S.* Enteritidis PT 30 with a_w (at 80 °C) for different test cells at different a_w. TDT cell: aluminum thermal death time test cell, TAC: thermal a_w cell.

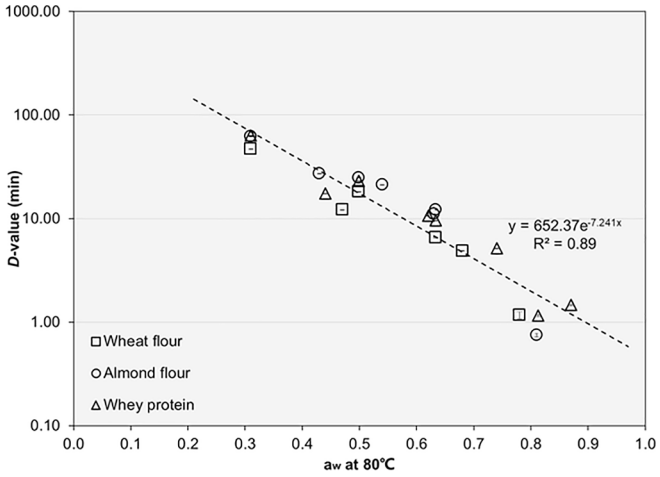

Fig. 9. The log-scale *D*-values of *S.* Enteritidis PT 30 with a_w (at 80 °C) for three powder products at different a_w.

at 80 °C are shown in Fig. 8 and Fig. 9 regarding test methods or food products, respectively. As shown in Fig. 8, *D*-values of *S.* Enteritidis PT30 obtained by both test cells changed linearly with a_w at 80 °C on a semi-log scale, with trend goodness of fit $R^2 = 0.89$. In Fig. 9, regardless of powder types, the inactivation data points were distributed

evenly around the semi-log linear curve. These data suggest that the overall thermal resistance of bacteria was dominated by a_w at treatment temperature, especially when a_w was controlled (as in a TAC). The standard deviation of the data point in Figs. 8 and 9 could not be shown correctly on the semi-log scale in the direction of the y-axis. The information on standard deviation for each *D*-value is included in Table 2.

The corresponding Z_{aw} value (0.32) was calculated from the negative inverse of the slope in Fig. 9. That is, a 0.32 change in a_w at 80 °C would reduce 1 log reduction in *D*-value of *S.* Enteritidis PT30.

4. Discussion

Thermal resistance of *Salmonella* as influenced by a_w has been studied experimentally by numerous investigators (Farakos et al., 2013; Lian, Zhao, Yang, Tang, & Katiyo, 2015; Smith, 2014; Smith, Hildebrandt, Casulli, Dolan, & Marks, 2016; Villa-Rojas, 2015; Villa-Rojas et al., 2013). Bacteria showed different resistance to heat in low-moisture foods even at the same a_w level (at the same treatment temperature), and thus, food composition has been considered as an influential factor on the thermal resistance of bacteria (Syamaladevi, Tadapaneni et al., 2016). In this study, three representative powder products with large variations in physio-chemical characteristics were chosen as model foods. Wheat flour (WF) is a carbohydrate-rich flour with medium particle size, almond flour (AF) is a fat-rich flour product with large particle size, and whey protein (WP) is a high-protein powder with fine and small particle granulates. The influence of a_w on the measured thermal resistance (*D*-values) of *Salmonella* cells was interpreted using the following assumptions: 1) a_w of *S.* Enteritidis PT30 cells was the same as that of the inoculated powders, since bacteria cells could reach equilibrium with its surrounding materials (food matrices) in seconds (Syamaladevi, Tang, & Zhong, 2016); and 2) at equilibrium, a_w of conditioned powders was equal to its surrounding relative humidity.

Based on the observation of thermal resistance data in Figs. 8 and 9, the difference in *D*-values of *Salmonella* was mainly caused by the variations in a_w of the treated samples during heating. Even though the three selected model foods were different in particle size and physical properties in the microstructure, a linear relationship between logarithmic *D*-values of *S.* Enteritidis PT30 and a_w (at treatment temperature) was observed with fair goodness of fit ($R^2 = 0.89$) across food matrices. A similar trend of *D*-values of *S.* Enteritidis PT30 and its surrogate *E. faecium* under the influence of a_w at treatment temperature have also been found in a non-food carrier (Liu, Tang et al., 2018). Even though WF showed lower *D*-values in comparison with AF and WP at the same a_w levels at 80 °C, the overall linear trend between thermal resistance and high temperature a_w in the semi-log graph did not change. In this study, AF had the smallest a_w changes when heated from 20 to 80 °C compared with WF and WP. Thus, *S.* Enteritidis PT30 in AF showed the most heat resistance at a_w levels from 0.25 to 0.60 (measured at 20 °C) compared to in other two food powders. This result is consistent with previous findings that fatty materials may have a protective effect on bacteria resulting in increased resistance of microorganism (He et al., 2013; Kataoka et al., 2014; Senhaji & Loncin, 1977). A significant reduced *D*-value of *S.* Enteritidis PT30 in AF at a_w of 0.80 was observed in this study, and further studies are needed to explore the reason.

Most importantly, our data suggest that the influential effect of food components on *D*-values in different food matrices was mainly caused by the differences in a_w changes at high temperatures. The commonly anticipated protective of fatty materials on microorganism could have been caused by the minimum a_w changes at elevated temperatures (from that of room temperature) compared with carbohydrates- or protein-rich products (He et al., 2013). Previous studies also attributed the heat protection phenomenon of food components (sucrose, fats, etc.) to the reduced a_w of treated substrates during heating (Li, Huang, & Chen, 2014; Moats, Dabbah, & Edwards, 1971; Senhaji, 1977). Also, the protected effect of low-a_w was found to be temperature-dependent, with apparent protective effect at high temperatures (> 70 °C) (Mattick et al., 2001). Since bacteria cells can immediately equilibrate to their surrounding environment, e.g., by losing moisture in a low-a_w environment, the enhanced thermal resistance of bacteria in low-moisture foods during heating might be related to the reduced mobility of available water molecules, and more rigid dimensional configuration of protein and enzymes resulting in thermal denaturation (Laroche, Fine, & Gervais, 2005; Syamaladevi, Tang et al., 2016). Losses of moisture from cells in microorganisms might have created stable ribosomes and proteins, making these cells more resistant to damage caused by thermal treatment (Syamaladevi, Tang, Villa-Rojas et al., 2016). For low-moisture foods, empirical correlations used for calculations of thermal resistance of *Salmonella* have considered other factors, such as pH, temperature or a_w (at room temperature) (Farakos et al., 2013; Smith et al., 2016). However, the accuracy of the model developed in this study was highly dependent on the precision of a_w measurement at high temperatures. Therefore, it is certainly desirable to have precise values of a_w at elevated temperature. Currently, a_w at high temperature (> 60 °C) cannot be determined directly by commercial devices such as vapor sorption analyzer. CCE equation for high temperature a_w estimation was product dependent and should be determined additionally for extended applications. Z_{aw} is an important parameter to indicate the sensitivity of thermal resistance of bacteria with respect to changing a_w (with an application range from 0.31 to 0.89 in this study). Z_{aw} value of *S.* Enteritidis PT30 (0.32) determined in the three real food systems in this study was very comparable to the reported Z_{aw} value (0.31) determined in silicon dioxide (Liu, Tang et al., 2018). But, further works are needed to validate this model on other low-moisture foods to check if Z_{aw} values were in the same magnitude (e.g., close to 0.32). Moreover, systematical studies using more temperature levels, in addition to 80 °C, should be conducted to further study interactions between treatment temperatures and water activities at those treatment temperatures.

This study provides useful insights for the food industry in designing appropriate pre-treatment and thermal treatment conditions for pathogens controls in low-moisture foods. For example, our previous studies have validated the inactivation effect of radio-frequency (RF) heating on *S.* Enteritidis PT30 and its surrogate in wheat flour (initial a_w 0.45 at room temperature) (Liu, Ozturk et al., 2018; Xu, Liu, Tang et al., 2018). Those studies demonstrated that 24–33 min of RF heating at 80 °C could achieve 4–5 log reduction of target microorganism (Liu, Ozturk et al., 2018; Xu, Liu, Song et al., 2018; Xu, Liu, Tang et al., 2018). It is clear from the new data in this study that the same thermal treatment conditions developed for wheat flour will not be as effective when applied to almond flour. For example, when almond flour with initial a_w of 0.45 (measured at 20 °C) is heated to 80 °C, its a_w would increase to 0.54 in sealed containers. According to the linear regression trend in Figs. 8 and 9, *D*-value of *S.* Enteritidis PT30 at 80 °C and a_w of 0.54 would be 20 min. A thermal process to achieve a six-log reduction of *S.* Enteritidis PT30 would require the almond flour to be fully exposed to 80 °C for 120 min. Either longer processing time or higher treatment temperature is needed to achieve similar log-reduction of bacteria cells in almond flours as compared to wheat flour of the same initial water activities (measured at 20 °C). Alternatively, almond flour could be pre-conditioned to higher initial moisture contents or humid hot air be used so that the corresponding water activities of the products at the treatment temperatures would be high enough to significantly reduce *D*-values of the target pathogens and ensure the effectiveness of the thermal treatments.

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