Effect of water activity on the thermal inactivation kinetics of *Salmonella* in milk powders

Xinyao Wei,¹ Soon Kiat Lau,^{1,2} Byron D. Chaves,¹ Mary-Grace C. Danao,^{1,3} Shantanu Agarwal,⁴ and Jeyamkondan Subbiah^{5*}

¹Department of Food Science and Technology, University of Nebraska, Lincoln 68588

²Department of Biological Systems Engineering, University of Nebraska-Lincoln, Lincoln 68583

³The Food Processing Center, University of Nebraska, Lincoln 68588

⁴Mars Wrigley Confectionery, Chicago, IL 60560

⁵Department of Food Science and Technology, University of Arkansas, Fayetteville 72704

ABSTRACT

Persistence of *Salmonella* in milk powders has caused several foodborne outbreaks. The determination of proper pasteurization processing conditions requires an understanding of the thermal inactivation kinetics of *Salmonella* in milk powders. However, there is a lack of knowledge related to the effects of water activity (a_w) and fat content on *Salmonella* inactivation in milk powder during thermal processing. Two types of milk powders, nonfat dry milk and whole milk powder, with different fat contents (0.62 and 29.46% wt/wt, respectively) were inoculated with a 5-strain cocktail of *Salmonella* and equilibrated to 3 a_w levels (0.10, 0.20, and 0.30) for isothermal treatments at 75, 80, and 85°C to obtain D -values (the time required to achieve a 10-fold reduction of the bacteria at the isothermal treatment temperature) and z -values (the increase in temperature required to achieve a 90% reduction of the decimal reduction time D). Stability tests showed that the inoculation method used in this study provided a high and stable population of *Salmonella* for thermal inactivation studies. A moisture sorption isotherm was measured to understand the relationship between a_w and moisture content of milk powders. The thermal resistance of *Salmonella* was found to significantly increase as a_w decreased, which suggested that a higher temperature or longer processing time would be required at low a_w to achieve the desired inactivation of *Salmonella*. The microbial inactivation kinetics were not significantly different for the 2 milk powders; therefore, data were combined to develop a universal model. A response surface model was compared with a modified Bigelow model. The modified Bigelow model performed well to predict D -values [root mean square error (RMSE) =

1.47 min] and log reductions (RMSE = 0.48 log cfu/g). The modified Bigelow model developed here could be used to estimate D -value as a function of water activity and temperature to design a thermal pasteurization system for milk powders.

Key words: heat resistance, dry milk powder, moisture sorption isotherm, low-moisture food

INTRODUCTION

Annually, nontyphoidal *Salmonella* causes approximately 1.2 million illnesses and 450 deaths in the United States (Johnson et al., 2014). Historically, several foodborne illness outbreaks have been caused by *Salmonella*-contaminated milk powders, such as powdered infant formula (Rowe et al., 1987; Park et al., 2004; Brouard et al., 2007; Angulo et al., 2008; Rodríguez-Urrego et al., 2010), powdered milk products (CDC, 1993), infant milk powders (Jourdan-da Silva et al., 2018), skim milk powder, and dry milk powder (el-Gazzar and Marth, 1992). Milk powders are produced by spray drying of liquid milk. Although spray drying reduces the total bacterial population, it is not a pasteurization process, and *Salmonella* may remain viable after the process (LiCari and Potter, 1970). Additionally, after spray drying, opportunities exist for milk powders to be cross-contaminated, including poor environmental sanitation practices and inadequate storage conditions (Podolak et al., 2010). Currently, no processing step follows spray drying to pasteurize milk powders (LiCari and Potter, 1970). Considering that milk powders are commonly used as ingredients in many ready-to-eat foods (that do not undergo further cooking steps before consumption), such as confectionery, drink mixes, seasoning, nutritional bars, and dry blend infant formula, it is therefore necessary to identify an effective pasteurization process to improve the food safety of milk powders (Reij and Den Aantrekker, 2004).

Milk powders are considered low-water-activity (a_w) foods, with typical a_w values <0.70, at which *Salmo-*

Received January 31, 2020.

Accepted March 22, 2020.

*Corresponding author: jsubbiah@uark.edu

nella is unable to multiply (Blessington et al., 2013). Although milk powders do not support the growth of *Salmonella*, higher survival of *Salmonella* is observed with declining a_w (Lian et al., 2015). However, *Salmonella* has been shown to survive for a long period in low- a_w food commodities (Keller et al., 2013; Tsai et al., 2019). Furthermore, *Salmonella* may possess higher thermal resistance with decreasing a_w (Villa-Rojas et al., 2013; Smith et al., 2016; Syamaladevi et al., 2016).

The enhanced heat resistance of *Salmonella* at lower a_w makes the elimination of *Salmonella* in milk powders a serious challenge for the food industry. Unlike pasteurization of high- a_w foods, the thermal processing of low- a_w food is a dynamic process. As moisture evaporates during the thermal process, a_w is expected to decrease, which could affect inactivation of *Salmonella* because of its enhanced heat resistance at reduced a_w . To account for this dynamic thermal resistance during thermal pasteurization, the effect of a_w on *Salmonella* inactivation in milk powders needs to be evaluated. In the dairy industry, the moisture contents of milk powders are monitored as part of the process controls (Hutson, 2017). Therefore, the development of moisture sorption isotherms of milk powder will be helpful to clarify the relationship between a_w and moisture content and subsequently link moisture content to the inactivation of *Salmonella*. Additionally, the different fat and carbohydrate contents of different types of milk powders could contribute to the variation in bacterial inactivation in milk powders. Relatively high fat content has been shown to have a protective effect on *Salmonella* during thermal processing (Ahmed et al., 1995; Verma et al., 2018). Thus, it is also important to evaluate the effect of fat content on the microbial inactivation kinetics of *Salmonella* in milk powders. A better understanding of the thermal inactivation kinetics of *Salmonella* in milk powders will be helpful to guide the dairy industry in designing thermal pasteurization processes for milk powders.

The objectives of this study were to (1) determine the moisture sorption isotherm of milk powders; (2) evaluate the effect of a_w on microbial inactivation kinetics of *Salmonella* in milk powders; and (3) assess the effect of fat content on microbial inactivation kinetics.

MATERIALS AND METHODS

Milk Powder Samples

For this study, 3 production batches of 2 different milk powders, grade A pasteurized nonfat dry milk (NFD) and grade A pasteurized whole milk powder (WMP) were provided by Mars Inc. (McLean, VA). Upon receiving the samples, total plate counts were

performed to quantify the background microflora in milk powders following standard procedures (Villa-Rojas et al., 2017; Liu et al., 2018). Three random 10-g samples were removed from each batch and diluted in 90 mL of 0.1% buffered peptone water (BPW; Becton, Dickinson and Co., Sparks, MD). The diluted samples were blended for 1 min in a paddle mixer (Neutec Group Inc., Farmingdale, NY), serially diluted in 9 mL of 0.1% BPW blanks, plated onto tryptic soy agar (TSA) supplemented with 0.6% (wt/vol) yeast extract (TSAYE), and incubated for 24 h at 37°C for enumeration.

Proximate Composition and Calorie Estimation

Official methods (AOAC International, 2000) were used to determine ash (method 985.01), fat (method 989.05), and protein (method 990.03) of NFD and WMP. The moisture contents of both milk powders were measured by a halogen moisture analyzer (HR73, Mettler Toledo Laboratory and Weighing Technologies, Greifensee, Switzerland), and a_w values at 25°C were determined with a dew point water activity meter (Aqualab Series 4TE, Meter Group, Pullman, WA). Carbohydrates were determined by subtracting other compositions from the total weight. Calories were calculated using the Atwater general factors, which are 4 kcal/g for protein, 9 kcal/g for fat, and 4 kcal/g for carbohydrate.

Moisture Sorption Isotherm

The moisture sorption isotherm of each milk powder product was generated with an AquaLab Vapor Sorption Analyzer (Meter Group Inc.) at 20°C. Approximately 1 g of sample was spread uniformly on a stainless steel sample cup for measurement of both absorption and desorption curve at a single temperature. The dynamic vapor sorption method was used to measure the sorption isotherm (Garbalińska et al., 2017). For the sorption isotherm development, the moisture content at equilibrated points was determined in triplicate at a_w levels from 0.10 to 0.90 with 0.05 intervals. The trigger (% dm/dt ; percent change in mass over a change in time for determination of the equilibrated point) was set at 0.020%/h with 3 events, which indicated that 3 readings in a row must be less than the set trigger value.

Bacterial Strain and Inoculation

The 5 strains of *Salmonella enterica* used in this study have been associated with different low a_w foodborne outbreaks. *Salmonella enterica* subsp. *enterica* serovar

Agona 447967, *Salmonella* Montevideo 488275, and *Salmonella* Mbandaka 698538 were obtained from the Food and Drug Administration (Office of Regulatory Affairs Regional Laboratory, Jefferson, AR). *Salmonella* Reading 180418 and *Salmonella* Tennessee K4643 were obtained from the University of Georgia (Athens). The frozen bacteria cultures were kept in trypticase soy broth (TSB) with 0.6% (wt/vol) yeast extract (TSBYE) supplemented with 20% glycerol and stored at -80°C until used.

Procedures to prepare the *Salmonella* inoculum were similar to those described in Hildebrandt et al. (2016) and Liu et al. (2018). To prepare the 5-strain *Salmonella* cocktail, one cryovial of frozen culture of each *Salmonella* strain was thawed at 22°C for 10 min. Then, 1 mL of each bacterial culture was transferred to 10 mL of TSBYE and incubated for 24 ± 2 h at 37°C . From the overnight culture, 1 loopful of culture was streaked onto the surface of TSAYE. After incubation at 37°C for 24 ± 2 h, the agar plate with isolated colonies was wrapped with parafilm and stored at 4°C for up to 1 mo. One isolated colony of each strain was transferred to 10 mL of TSBYE and incubated for 24 h at 37°C for a second enrichment. To prepare the inoculum lawn, 0.1 mL of the overnight culture was spread plated onto TSAYE plates for incubation (24 ± 2 h at 37°C). The overnight lawns grown on the plate were harvested by adding 3 mL of 0.1% BPW and agitating the lawns with a sterile L-shaped spreader. Equal amounts of the dissolved lawns of each *Salmonella* strain were transferred to a sterile centrifuge tube and vortexed for 10 s to prepare the *Salmonella* cocktail. The initial bacterial population of the *Salmonella* cocktail was approximately $10.5 \log \text{cfu/mL}$.

The prepared *Salmonella* cocktail (10 mL) was sprayed onto 100 ± 0.1 g of WMP or NFDM samples in a sterile Whirl-Pak bag (23×12 cm; Whirl-Pak, Nasco, Fort Atkinson, WI). The inoculated sample was hand-massaged to exfoliate all powder from the inner surface of the bag and then the sample was homogenized in a paddle mixer for 6 min.

Sample Equilibration and Stability Tests

Inoculated samples were placed uniformly on a sanitized aluminum tray ($230 \times 300 \times 15$ mm) and transferred to a custom-designed humidity-controlled chamber. To evaluate the effect of a_w on thermal inactivation of *Salmonella*, the relative humidity in the chamber was set to 10.0, 20.0, or 30.0% to equilibrate the inoculated sample to a target a_w of 0.10, 0.20, and 0.30, respectively. During the inoculation process, the addition of moisture from the inoculum caused the powders to cake and form clumps; therefore, additional

steps were taken to reduce the clumps back into powder. Upon reaching the target a_w , inoculated samples were packed into sterile Whirl-Pak bags and homogenized in a paddle mixer for 3 min to break the dried-up clumps. The homogenized samples were transferred back to the humidity-controlled chamber for re-equilibration.

Stability tests were conducted for both powder samples at an a_w of 0.20 to evaluate the inoculation method used in this study. At d 0, 1, 2, 3, 4, 5, 8, 12, 16, and 20, 2 ± 0.1 g of each inoculated powder sample from each batch were transferred to a sterile Whirl-Pak bag for enumeration. The samples were first diluted 10-fold with 18 mL of 0.1% BPW and then stomached for 1 min. Blended samples were serially diluted in 9 mL of 0.1% BPW and 3 dilutions of all samples were plated in duplicate onto TSAYE supplemented with 0.05% (wt/vol) ammonium iron citrate (Sigma-Aldrich Co., St. Louis, MO), and 0.03% (wt/vol) sodium thio-sulfate (mTSA; Fisher Chemical, Lenexa, KS) and incubated for 24 h at 37°C (Smith et al., 2016; Liu et al., 2019). After incubation, colonies with a black center were enumerated as *Salmonella*. Homogeneity of inoculated samples was evaluated by counting colonies on 6 randomly selected subsamples (2 ± 0.1 g) from a 100-g batch of inoculated powder on d 1. Inoculated samples were considered to be homogeneous when the standard deviation of the 6 enumerated samples was $<0.3 \log \text{cfu/g}$.

Isothermal Inactivation

In this study, we conducted the isothermal treatment of inoculated milk powder using a custom-designed thermal-death-time sandwich (Lau et al., 2019). Two grams of inoculated sample was packed into an aluminum-laminated plastic pouch ($75 \times 75 \times 1$ mm, Impak Corp., Los Angeles, CA). To measure the temperature of the sample, a T-type thermocouple probe was inserted into the center of the aluminum pouch. The come-up time at each temperature was defined as the time required for the sample temperature from room temperature (22°C) to reach 0.5°C below the target temperature. For each target temperature, the average of 3 measurements of come-up time plus 2 standard deviations was used as time zero for the subsequent isothermal treatment. No thermocouples were inserted into the inoculated samples during the actual isothermal treatments.

The isothermal treatment temperatures used to obtain thermal death curves for *Salmonella* in both WMP and NFDM were 75, 80, and 85°C . The target isothermal temperatures were determined based on our preliminary experiments. Temperatures $>85^{\circ}\text{C}$ can result in discoloration of samples, and temperatures $<75^{\circ}\text{C}$

result in a long pasteurization time. Six time points, including time zero, with equal time intervals were used for isothermal treatment to achieve at least a 3-log reduction of *Salmonella*. After each isothermal treatment, the treated aluminum pouch was immediately transferred to an ice-water bath for about 1 min to stop further thermal inactivation. The treated samples were enumerated using the same method as described previously in the sample equilibration and stability tests section. Three biological replicates (batches of milk powder samples from different production lots inoculated with independently grown bacterial cultures) were used in the experiments. Untreated inoculated samples from each batch were randomly selected and enumerated as the control.

Determination of *D*- and *z*-Values

The thermal inactivation kinetics of *Salmonella* in milk powders were modeled with the log-linear model to obtain the *D*-value:

$$D = \frac{t}{\log_{10} N_0 - \log_{10} N},$$

where N_0 (cfu/g) is the bacteria population at come-up time, N (cfu/g) is the bacteria population at time t , t (min) is the length of isothermal treatment, and D (min) is the time required to achieve a 10-fold (decimal) reduction of the bacteria at the isothermal treatment temperature. In addition to R^2 values, the goodness-of-fit of the models were quantified by the root mean square error (**RMSE**; log cfu/g):

$$RMSE = \sqrt{\frac{\sum_{i=1}^n [\log_{10}(N)_{\text{observed}, i} - \log_{10}(N)_{\text{predicted}, i}]^2}{n}},$$

where $\log_{10}(N)_{\text{observed}, i}$ is the observed log population, $\log_{10}(N)_{\text{predicted}, i}$ is the predicted log population from the model, and n is the total number of observations.

The *z*-value is defined as the increase in temperature required to achieve a 90% reduction of the decimal reduction time *D* and was determined as follows:

$$z = \frac{T_2 - T_1}{\log_{10} D_{T_1} - \log_{10} D_{T_2}},$$

where D_{T_1} and D_{T_2} are the *D*-values at temperatures T_1 and T_2 , respectively.

Secondary Model Fitting

A split-plot analysis (where a_w is the whole plot and temperature is the subplot) was used to determine the effect of temperature and a_w on the *D*-value and reduction of *Salmonella* in WMP and NFD. The first secondary model evaluated in this study was a response surface model (**RSM**), and only factors with significant effects ($P < 0.05$) were considered. The parameters for the RSM were estimated using ANOVA and response surface method functions in the open-source statistical software R (<https://www.R-project.org/>; Lenth, 2009).

The second secondary model evaluated in this study was a modified version of a Bigelow-type relationship (Gaillard et al., 1998):

$$D(T, a_w) = D_{\text{ref}} \cdot 10^{\frac{a_{w, \text{ref}} - a_w}{z_{a_w}}} \cdot 10^{\frac{T_{\text{ref}} - T}{z_T}},$$

where D_{ref} is the time (min) needed to achieve a 10-fold reduction in the population at T_{ref} and $a_{w, \text{ref}}$, T is the temperature ($^{\circ}\text{C}$), T_{ref} and $a_{w, \text{ref}}$ are the optimized reference temperature and water activity (Dolan et al., 2013), and z_{a_w} or z_T is the a_w or temperature increment needed to decrease the *D*-value by 10-fold, respectively. All parameters for the Bigelow-type model were estimated using ordinary least squares (OLS) minimization with `nlinfit` in MATLAB 2019 (MathWorks, Natick, MA).

The corrected Akaike information criterion (**AIC_c**) was calculated to evaluate each model (Motulsky and Christopoulos, 2004):

$$\text{AIC}_c = n \ln \left(\frac{SS}{n} \right) + 2K + \frac{2K(K+1)}{n-K-1},$$

where n is the total number of the data points, SS is the sum of squares of the residuals, and K is the number of parameters being estimated plus 1. Lower AIC_c values indicate that the determined model is more likely to be correct for the data.

RESULTS AND DISCUSSION

Moisture Sorption Isotherm

The moisture sorption isotherm results are shown in Figure 1. For both milk powders, the moisture contents slowly increased with increasing a_w . After a_w reached 0.40, the moisture content was found to increase sharply with increasing a_w until it reached 0.55, which may

be attributed to capillary condensation (Tham et al., 2016). When the powder started to absorb moisture, part of the powder became crystalline and part of it remained amorphous. Compared with the corresponding pure amorphous substance, powders containing a mixture of amorphous and crystalline components have greater water sorption capacity (Hartmann and Palzer, 2011). Consequently, a higher absorption rate of moisture was observed after the initial uptake of moisture.

After a_w reached 0.60, the moisture contents of both milk powders declined slightly, which may be due to lactose crystallization (Tham et al., 2016). According to McCarthy et al. (2013), a sudden decrease in water content is observed because the amorphous lactose has lower water sorption before lactose crystallization. Subsequent increases in a_w are followed by an increase in the moisture content. At high a_w , lactose crystallization is an irreversible change (Berggren and Alderborn, 2004); therefore, the trends observed from 0.4 to 0.6 a_w in the absorption curve were not observed in the desorption curve. At the same a_w level, NFDM had a higher moisture content than WMP (Figure 1), which was due to the higher protein content of NFDM (Table 1). Proteins have been shown to increase moisture absorption in milk powder, by binding more water molecules at the same a_w level (Ibach and Kind, 2007; Shrestha et al., 2007). Therefore, NFDM was found to have higher moisture content than WMP at the same a_w level due to interactions between moisture and protein.

Stability Tests of Powder Inoculation

The aerobic bacteria counts of both types of uninoculated milk powders were estimated to be <100 cfu/g. As the inoculated population of *Salmonella* was considerably higher and differential medium (mTSA) was used for enumeration, the overall microbial results should not be affected by the background microflora. The detection limit of the enumeration method used in this study was <10 cfu/g.

The initial *Salmonella* populations after inoculation ranged from 7.7 to 7.9 and 8.1 to 8.5 log cfu/g, in WMP and NFDM, respectively (Figure 2). One day after the samples were transferred to the equilibration chamber,

Figure 1. Moisture sorption isotherm of whole milk powder (WMP) and nonfat dry milk (NFDM) at 20°C. The moisture content at equilibration point was determined in triplicate at a water activity (a_w) level from 0.10 to 0.90 at 0.05-unit intervals. The error bar indicates 1 SD.

the a_w levels of WMP and NFDM were adjusted to the target a_w . About 1.1 and 0.5 log cfu/g reductions of *Salmonella* in WMP and NFDM, respectively, were observed at the end of the a_w adjustment (Figure 2).

Table 1. Proximate composition (%; mean $\pm$ SD, unless otherwise noted) of whole milk powder (WMP) and nonfat dry milk (NFDM), $n = 3$ samples

Milk powder	Ash	Protein	Lipid	Carbohydrate	Moisture	Water activity (-)	Calories (kcal/kg)
WMP	4.91 $\pm$ 0.53	25.50 $\pm$ 0.12	29.46 $\pm$ 0.52	37.62 $\pm$ 1.27	2.51 $\pm$ 0.43	0.175 $\pm$ 0.001	5,176.2 $\pm$ 17.10
NFDM	7.97 $\pm$ 0.24	36.41 $\pm$ 0.24	0.62 $\pm$ 0.03	51.77 $\pm$ 0.52	3.23 $\pm$ 0.29	0.158 $\pm$ 0.002	3,583.0 $\pm$ 14.10

The *Salmonella* populations in WMP and NFDM were then monitored during the following 20 d, and a <0.2 log cfu/g reduction of *Salmonella* population was found in both samples. The inoculation method used in this study was shown to provide stable *Salmonella* populations in both types of milk powders prepared for thermal treatment. Different inoculation methodologies have been shown to significantly influence the thermal inactivation of *Salmonella* in low a_w foods (Hildebrandt et al., 2016, 2017; Liu et al., 2019). A proper inoculation method that simulates the real route of sample contamination could help yield more reliable inactivation kinetics. Hildebrandt et al. (2016) suggested that a lawn-based pelletized inoculum, which was used in this study, could provide a stable *Salmonella* population before isothermal treatment and yield consistent D -values. Several studies have shown that this lawn-based inoculation method provides consistent and stable *Salmonella* inoculated samples of different low- a_w foods, such as spices (Chen et al., 2019; Wei et al., 2018, 2019), oat and wheat flour (Liu et al., 2018; Verma et al., 2018), and egg white powder (Wei et al., 2020). Also, this wet inoculation method could simulate a common contamination scenario of milk powders, in which *Salmonella*-contaminated liquid drips from the roof of a processing plant into the powders being processed.

The 20-d stability tests indicated that *Salmonella* could survive in milk powders for a long time, and no considerable decrease in *Salmonella* population was found after the inoculated samples were stabilized. Several studies (Abd et al., 2012; Keller et al., 2013; Lian

et al., 2015) have shown that *Salmonella* could survive in low- a_w foods for a long period, and higher survival of *Salmonella* was found at a lower a_w level.

During the a_w adjustment of milk powders and the corresponding desiccation stress, thermal resistance of *Salmonella* bacteria would begin to increase (Gruzdev et al., 2011). Therefore, the inoculated samples were equilibrated for at least 5 d before isothermal treatment to allow the inoculated *Salmonella* to fully adapt to the low- a_w environment and presumably attain a high thermal resistance.

Thermal Resistance of *Salmonella* in WMP and NFDM

This study aimed to determine the effect of a_w on the thermal inactivation kinetics of *Salmonella* in WMP and NFDM. At 75, 80, and 85°C, WMP had come-up times of 63, 74, and 75 s, respectively. Similarly, the come-up times of NFDM were 87, 87, and 88 s at 75, 80, and 85°C, respectively. The thermal-death-time sandwich has a proportional-integral-derivative (PID) control system to achieve the target temperature. When 2 standard deviations were added to the mean time to reach the target temperature, the come-up time values were similar for all isothermal temperatures. The isothermal inactivation curves of *Salmonella* in WMP and NFDM at a_w of 0.10, 0.20, and 0.30 are shown in Figure 3.

Thermal resistance parameters of WMP and NFDM are reported in Table 2. According to Liu et al. (2019), the D -value of *Salmonella* in NFDM with a_w of 0.25 at 85°C was 16.05 min. Michael et al. (2014) reported that D -values of *Salmonella* in NFDM were 23.02, 10.45, 8.63, and 5.82 min at 75, 80, 85, and 90°C, respectively (a_w data were not reported). In this study, at a_w of 0.20, the D -values of *Salmonella* at 75, 80, and 85°C, respectively, were 22.70, 12.11, and 6.17 min for WMP and 26.76, 10.46, and 5.60 for NFDM (Table 2). The minor differences in D -values for similar products could be attributed to the use of different bacterial strains (Quintavalla et al., 2001; Ma et al., 2009), different inoculation methods (Bowman et al., 2015; Liu et al., 2019), or different a_w (Villa-Rojas et al., 2013; Syamaladevi et al., 2016; Tsai et al., 2019). For both types of milk powders, at all temperatures and a_w levels, the isothermal inactivation curves were found to follow a log-linear trend with high R^2 values ($R^2 > 0.94$).

The effect of temperatures and a_w on $\log_{10}$ D -values are shown in Figure 4. The z_T values of *Salmonella* in both milk powder at different a_w levels ranged from 14.75 to 17.68°C, and the z_{aw} values ranged from 0.44 to 0.66 (Table 2). Liu et al. (2019) reported that the

Figure 2. Stability tests of *Salmonella* in whole milk powder (WMP) and nonfat dry milk (NFDM). Samples from each independent batch were collected and enumerated at d 0, 1, 2, 3, 4, 5, 8, 12, 16, and 20. The error bar indicates 1 SD.

Figure 3. Thermal inactivation kinetics of *Salmonella* in whole milk powder (WMP) and nonfat dry milk (NFDM) equilibrated to the different water activity (a_w ; 0.10, 0.20 and 0.30) levels at various temperatures. Each isothermal treatment condition was performed in triplicate.

z -values of *Salmonella* in NFDM at a_w of 0.25 varied between 16.0 and 16.5°C, which were similar to the z -values of *Salmonella* in NFDM found in this study. No significant difference ($P < 0.05$) was found for the z -values of *Salmonella* in NFDM at different a_w levels. The estimated z -values could be used to predict the D -values at different temperatures, which could assist

in determining the desired thermal processing temperature. If a food manufacturer wants to estimate a D -value simultaneously in water activity and temperature, these independent z -values will not be sufficient. Therefore, the RSM and Bigelow model were developed.

The RSM were fit for D -values of *Salmonella* in WMP and NFDM under isothermal treatment at different a_w

Table 2. Thermal resistance of *Salmonella* in whole milk powder (WMP) and nonfat dry milk (NFDM) at different water activities (a_w)¹

Milk powder	a_w	Temperature (°C)	<i>D</i> -value (min)	RMSE ² (log cfu/g)	<i>z</i> -value ³ (°C)
WMP	0.10	75	41.10 ± 2.69 ^a	0.17	16.41 ± 0.62 ^{ab}
		80	22.83 ± 2.51 ^d	0.27	
		85	10.11 ± 0.97 ^g	0.22	
	0.20	75	22.79 ± 1.68 ^c	0.20	17.68 ± 0.95 ^a
		80	12.12 ± 0.41 ^f	0.25	
		85	6.17 ± 0.06 ^{ij}	0.35	
	0.30	75	16.97 ± 0.05 ^e	0.24	14.75 ± 0.26 ^c
		80	8.11 ± 0.62 ^{hj}	0.18	
		85	3.56 ± 0.10 ^k	0.10	
NFDM	0.10	75	40.58 ± 0.51 ^a	0.28	16.01 ± 0.97 ^{bc}
		80	19.69 ± 0.59 ^d	0.17	
		85	9.63 ± 0.76 ^{gh}	0.31	
	0.20	75	26.87 ± 2.09 ^b	0.29	14.90 ± 0.10 ^{bc}
		80	10.46 ± 0.22 ^{fg}	0.20	
		85	5.65 ± 0.65 ^j	0.31	
	0.30	75	16.95 ± 0.53 ^e	0.18	15.02 ± 1.45 ^{bc}
		80	9.82 ± 0.66 ^{gh}	0.18	
		85	3.64 ± 0.48 ^k	0.18	

^{a-k}Within a column, values with differing letters are significantly different ($P < 0.05$).

¹Values are mean ± SD.

²RMSE = root mean square error.

³A single *z*-value of each a_w was determined based on the 3 temperatures.

and temperatures. The resulting *D*-value models were as follows:

For WMP:

$$D = 1,162.581479 - 24.321946T - 896.135507a_w \\ + 8.788976Ta_w + 0.128314T^2 + 303.089547a_w^2; \\ RMSE = 1.70 \text{ min}; AIC_c = 119.20.$$

For NFDM:

$$D = 1,347.962075 - 28.889744T - 867.512273a_w \\ + 8.825606Ta_w + 0.5587T^2 + 239.108313a_w^2; \\ RMSE = 1.36 \text{ min}; AIC_c = 107.32.$$

where *D* = *D*-values of WMP and NFDM, *T* = isothermal treatment temperature (°C), and a_w = water activity of the sample.

The fitted modified Bigelow models were as follows:

$$\text{WMP: } D = 33.13 \cdot 10^{\frac{0.12-a_w}{0.54}} \cdot 10^{\frac{75.8-T}{15.4}}; \\ RMSE = 1.57 \text{ min}, AIC_c = 31.33;$$

$$\text{NFDM: } D = 32.58 \cdot 10^{\frac{0.12-a_w}{0.47}} \cdot 10^{\frac{75.7-T}{16.65}}; \\ RMSE = 1.25 \text{ min}, AIC_c = 18.81.$$

For both RSM models, temperature and a_w showed significant linear and quadratic effects and a significant interaction between temperature and a_w . In these 2 models, the estimated values of each parameter were not different from each other ($P > 0.05$). Therefore, data were combined for both milk powders and the following universal RSM and modified Bigelow model were generated for both WMP and NFDM:

$$D = 1,255.271777 - 26.605845T - 881.82389a_w \\ + 8.807291Ta_w + 0.1421T^2 + 271.09893a_w^2; \\ RMSE = 1.61 \text{ min}; AIC_c = 218.43;$$

$$D = 32.58 \cdot 10^{\frac{0.12-a_w}{0.47}} \cdot 10^{\frac{75.7-T}{16.65}}; \\ RMSE = 1.47 \text{ min}, AIC_c = 47.40.$$

The universal models also provided a reasonable prediction of *D*-values for both types of milk powders and may be more convenient for use as a reference for the food industry. The predictions and residual analyses of both models are shown in Figure 5. In general, the RMSE of both models are within an acceptable range. However, for the predictions of lower *D*-values (at the higher temperature and a_w), the residuals indicate high percent errors with up to ±41.8% for RSM and ±24.0% for the modified Bigelow model. The fat content did not have a significant effect on the *D*-values of the

Figure 4. The z -values for temperature (top) and water activity (a_w ; bottom) of *Salmonella* in whole milk powder (WMP) and nonfat dry milk (NFDM).

RSM and therefore was not included in the final model. Although the protective effect of fat on *Salmonella* inactivation has been observed in many high-moisture foods (Ahmed et al., 1995; Verma et al., 2018), a similar protective effect was not found in milk powders. A protective effect of fat was not observed in milk powder during this short storage time (5 d).

For the convenience of application of the RSM, an RSM to directly predict log reductions was generated by including time (t) as an independent variable as follows for both WMP and NFDM:

$$\begin{aligned} \log(N_o/N) = & 106.5685 - 2.585471T - 57.91383a_w \\ & - 0.6847655t + 0.714746Ta_w + 0.008980569Tt \\ & + 0.2262141a_wt + 0.01567976T^2 + 6.369736a_w^2 \\ & + 0.00004763879t^2; \\ RMSE = & 0.48 \log \text{ cfu/g}, AIC_c = 617.19; \end{aligned}$$

where $\log(N_o/N)$ is the log reduction of *Salmonella* in WMP and NFDM, and t is the holding time of isothermal treatment. This model could predict *Salmonella*

reduction directly for both types of milk powders, which is more convenient for industrial applications. To compare its performance with the combined Bigelow model, the log reduction could also be predicted by using D -values generated from the universal Bigelow model, which gave an RMSE of 0.47 log cfu/g and AIC_c of -456.93 . The predictions and residual analyses of both models are shown in Figure 6.

Based on the AIC_c and RMSE values, the modified Bigelow model was a better fit for the range of conditions evaluated than the RSM, which agreed with Smith et al. (2016). Although the RSM is an empirical (data-driven) model, the Bigelow model is a mechanistic model with phenomenological meaning and is preferable.

Influence of Temperature and a_w on the Thermal Resistance of *Salmonella*

The contour plots based on the developed universal model for predicting D -values are shown in Figure 7. According to the results, temperature had a significant positive effect ($P < 0.05$) on the inactivation of *Salmo-*

nella in both milk powders. A significant negative effect ($P < 0.05$) of a_w was observed on the inactivation of *Salmonella* in both milk powders based on the models. Therefore, a higher temperature would be required to obtain similar inactivation kinetics at lower a_w levels, supporting the observation that *Salmonella* thermal resistance increases at lower a_w values.

During the thermal processing of low- a_w foods, a_w is a critical parameter to be controlled because it considerably affects the heat resistance of bacteria (Aljarallah and Adams, 2007; Podolak et al., 2010). Low a_w has been reported to have a protective effect on different

foodborne pathogens, such as *Staphylococcus epidermidis* (Verrips and Van Rhee, 1981), *Listeria monocytogenes* (Valdramidis et al., 2006), and *Salmonella enterica* (Villa-Rojas et al., 2013; Smith et al., 2016). In this study, D -values of *Salmonella* in both WMP and NFDM increased with decreasing a_w , which indicated that a longer thermal treatment time would be required to achieve the desired reduction of *Salmonella* for the milk powder with lower a_w .

Because of moisture evaporation during thermal processing, the a_w of milk powder is expected to decrease throughout thermal pasteurization. The reduced a_w

Figure 5. Comparison of the response surface model (RSM) and modified Bigelow model for predicting D -value of *Salmonella* in milk powders. AIC_c = corrected Akaike information criterion; RMSE = root mean square error.

could enhance the thermal resistance of *Salmonella*, which will thus require a more severe treatment. It is essential to understand the effect of a_w on thermal inactivation of *Salmonella* in milk powders when developing the proper pasteurization process. The models developed in this study will help the food industry to understand the effects of temperature and a_w on the thermal inactivation of *Salmonella* in milk powders and to identify the proper temperature-time combinations for the development and implementation of pasteurization process to ensure food safety.

CONCLUSIONS

The inoculation method used in this study achieved high and stable populations of *Salmonella* in both WMP and NFDM. The thermal resistance of *Salmonella* has been shown to increase significantly with decreasing a_w . However, different fat contents in WMP and NFDM did not significantly affect the thermal resistance of *Salmonella*. The universal models developed here could be a valuable tool to predict a reference D -value of *Salmonella* at selected a_w and temperature values for

Figure 6. Comparison of response surface model (RSM) for predicting log reduction and modified Bigelow model for predicting D -values by evaluating log reductions of *Salmonella* in milk powders. AICc = corrected Akaike information criterion; RMSE = root mean square error.

Figure 7. Contour surface plots of predicted *D*-values (min) of *Salmonella* in both types of milk powders as a function of temperature and water activity (a_w) by the response surface model (RSM) and modified Bigelow model.

the food industry. The thermal inactivation kinetics of *Salmonella* in milk powders could provide technical information for developing and validating thermal processing to control *Salmonella* in milk powders.

ACKNOWLEDGMENTS

This project was funded by the National Dairy Council (Rosemont, IL) and Mars Wrigley (Chicago,

IL). The milk powder samples were provided by Mars Wrigley. The author have not stated any conflicts of interest.

REFERENCES

- Abd, S. J., K. L. McCarthy, and L. J. Harris. 2012. Impact of storage time and temperature on thermal inactivation of *Salmonella* Enteritidis PT 30 on oil-roasted almonds. *J. Food Sci.* 77:M42–M47. <https://doi.org/10.1111/j.1750-3841.2011.02439.x>.
- Ahmed, N. M., D. E. Conner, and D. L. Huffman. 1995. Heat-resistance of *Escherichia coli* O157:H7 in meat and poultry as affected by product composition. *J. Food Sci.* 60:606–610. <https://doi.org/10.1111/j.1365-2621.1995.tb09838.x>.
- Aljarallah, K. M., and M. R. Adams. 2007. Mechanisms of heat inactivation in *Salmonella* serotype Typhimurium as affected by low water activity at different temperatures. *J. Appl. Microbiol.* 102:153–160. <https://doi.org/10.1111/j.1365-2672.2006.03054.x>.
- Angulo, F. J., S. M. Cahill, I. K. Wachsmuth, M. de L. Costarrica, and P. K. B. Embarek. 2008. Powdered infant formula as a source of *Salmonella* infection in infants. *Clin. Infect. Dis.* 46:268–273. <https://doi.org/10.1086/524737>.
- AOAC International. 2000. Official Methods of Analysis. 17th ed. AOAC International, Gaithersburg, MD.
- Berggren, J., and G. Alderborn. 2004. Long-term stabilisation potential of poly (vinylpyrrolidone) for amorphous lactose in spray-dried composites. *Eur. J. Pharm. Sci.* 21:209–215. <https://doi.org/10.1016/j.ejps.2003.10.005>.
- Blessington, T., C. G. Theofel, and L. J. Harris. 2013. A dry-inoculation method for nut kernels. *Food Microbiol.* 33:292–297. <https://doi.org/10.1016/j.fm.2012.09.009>.
- Bowman, L. S., K. M. Waterman, R. C. Williams, and M. A. Ponder. 2015. Inoculation preparation affects survival of *Salmonella enterica* on whole black peppercorns and cumin seeds stored at low water activity. *J. Food Prot.* 78:1259–1265. <https://doi.org/10.4315/0362-028X.JFP-14-483>.
- Brouard, C., E. Espié, F.-X. Weill, A. Kérouanton, A. Brisabois, A.-M. Forgue, V. Vaillant, and H. de Valk. 2007. Two consecutive large outbreaks of *Salmonella enterica* serotype Agona infections in infants linked to the consumption of powdered infant formula. *Pediatr. Infect. Dis. J.* 26:148–152. <https://doi.org/10.1097/01.inf.0000253219.06258.23>.
- CDC (Centers for Disease Control and Prevention). 1993. *Salmonella* serotype Tennessee in powdered milk products and infant formula—Canada and United States, 1993. Accessed April 27, 2020. <https://www.cdc.gov/mmwr/preview/mmwrhtml/00021081.htm>.
- Chen, L., X. Wei, S. Irmak, B. D. Chaves, and J. Subbiah. 2019. Inactivation of *Salmonella enterica* and *Enterococcus faecium* NRRL B-2354 in cumin seeds by radiofrequency heating. *Food Control* 103:59–69. <https://doi.org/10.1016/j.foodcont.2019.04.004>.
- Dolan, K. D., V. P. Valdramidis, and D. K. Mishra. 2013. Parameter estimation for dynamic microbial inactivation: Which model, which precision? *Food Control* 29:401–408. <https://doi.org/10.1016/j.foodcont.2012.05.042>.
- el-Gazzar, F. E., and E. H. Marth. 1992. Salmonellae, salmonellosis, and dairy foods: A review. *J. Dairy Sci.* 75:2327–2343. [https://doi.org/10.3168/jds.S0022-0302\(92\)77993-4](https://doi.org/10.3168/jds.S0022-0302(92)77993-4).
- Gaillard, S., I. Leguerinel, and P. Mafart. 1998. Model for combined effects of temperature, pH and water activity on thermal inactivation of *Bacillus cereus* spores. *J. Food Sci.* 63:887–889. <https://doi.org/10.1111/j.1365-2621.1998.tb17920.x>.
- Garbalińska, H., M. Bochenek, W. Malorny, and J. von Werder. 2017. Comparative analysis of the dynamic vapor sorption (DVS) technique and the traditional method for sorption isotherms determination—Exemplified at autoclaved aerated concrete samples of four density classes. *Cement Concr. Res.* 91:97–105. <https://doi.org/10.1016/j.cemconres.2016.11.001>.
- Gruzdev, N., R. Pinto, and S. Sela. 2011. Effect of desiccation on tolerance of *Salmonella enterica* to multiple stresses. *Appl. Environ. Microbiol.* 77:1667–1673. <https://doi.org/10.1128/AEM.02156-10>.

- Hartmann, M., and S. Palzer. 2011. Caking of amorphous powders—Material aspects, modelling and applications. *Powder Technol.* 206:112–121. <https://doi.org/10.1016/j.powtec.2010.04.014>.
- Hildebrandt, I. M., C. Hu, E. M. Grasso-Kelley, P. Ye, N. M. Anderson, and S. E. Keller. 2017. Dry transfer inoculation of low-moisture spices containing antimicrobial compounds. *J. Food Prot.* 80:338–344. <https://doi.org/10.4315/0362-028X.JFP-16-279>.
- Hildebrandt, I. M., B. P. Marks, E. T. Ryser, R. Villa-Rojas, J. Tang, F. J. Garces-Vega, and S. E. Buchholz. 2016. Effects of inoculation procedures on variability and repeatability of *Salmonella* thermal resistance in wheat flour. *J. Food Prot.* 79:1833–1839. <https://doi.org/10.4315/0362-028X.JFP-16-057>.
- Hutson, G. 2017. How to improve process control to ensure dairy product standardization. Accessed Mar. 24, 2019. <https://www.dairyfoods.com/articles/92213-how-to-improve-process-control-to-ensure-dairy-product-standardization>.
- Ibach, A., and M. Kind. 2007. Crystallization kinetics of amorphous lactose, whey-permeate and whey powders. *Carbohydr. Res.* 342:1357–1365. <https://doi.org/10.1016/j.carres.2007.03.002>.
- Johnson, N. B., L. D. Hayes, K. Brown, E. C. Hoo, K. A. Ethier, and Centers for Disease Control and Prevention (CDC). 2014. CDC National Health Report: Leading causes of morbidity and mortality and associated behavioral risk and protective factors—United States, 2005–2013. *MMWR Suppl.* 63:3–27.
- Jourdan-da Silva, N., L. Fabre, E. Robinson, N. Fournet, A. Nisavanh, M. Bruyand, A. Mailles, E. Serre, M. Ravel, V. Guibert, S. Issenhuth-Jeanjean, C. Renaudat, M. Tourdjman, A. Septfons, H. de Valk, and S. Le Hello. 2018. Ongoing nationwide outbreak of *Salmonella* Agona associated with internationally distributed infant milk products, France, December 2017. *Euro Surveill.* 23. <https://doi.org/10.2807/1560-7917.ES.2018.23.2.17-00852>.
- Keller, S. E., J. M. VanDoren, E. M. Grasso, and L. A. Halik. 2013. Growth and survival of *Salmonella* in ground black pepper (*Piper nigrum*). *Food Microbiol.* 34:182–188. <https://doi.org/10.1016/j.fm.2012.12.002>.
- Lau, S. K., X. Wei, and J. Subbiah. 2019. Performance of an improved thermal death time sandwich system for determining the thermal death kinetics of *Salmonella*. Page 1-09 in IAFP Annual Meeting. Louisville, Kentucky 2019. International Association for Food Protection, Des Moines, IA.
- Lenth, R. V. 2009. Response-surface methods in R, using rsm. *J. Stat. Softw.* 32:1–17. <https://doi.org/10.18637/jss.v032.i07>.
- Lian, F., W. Zhao, R. Yang, Y. Tang, and W. Katiyo. 2015. Survival of *Salmonella enteric* in skim milk powder with different water activity and water mobility. *Food Control* 47:1–6. <https://doi.org/10.1016/j.foodcont.2014.06.036>.
- LiCari, J. J., and N. N. Potter. 1970. *Salmonella* survival during spray drying and subsequent handling of skimmilk powder. II. Effects of drying conditions. *J. Dairy Sci.* 53:871–876. [https://doi.org/10.3168/jds.S0022-0302\(70\)86310-X](https://doi.org/10.3168/jds.S0022-0302(70)86310-X).
- Liu, S., S. Ozturk, J. Xu, F. Kong, P. Gray, M. Zhu, S. S. Sablani, and J. Tang. 2018. Microbial validation of radio frequency pasteurization of wheat flour by inoculated pack studies. *J. Food Eng.* 217:68–74.
- Liu, S., J. Xu, L. Xie, M.-J. Zhu, and J. Tang. 2019. Dry inoculation methods for nonfat milk powder. *J. Dairy Sci.* 102:77–86. <https://doi.org/10.3168/jds.2018-14478>.
- Ma, L., G. Zhang, P. Gerner-Smidt, V. Mantripragada, I. Ezeoke, and M. P. Doyle. 2009. Thermal inactivation of *Salmonella* in peanut butter. *J. Food Prot.* 72:1596–1601. <https://doi.org/10.4315/0362-028X-72.8.1596>.
- McCarthy, N. A., V. L. Gee, D. K. Hickey, A. L. Kelly, J. A. O'Mahony, and M. A. Fenelon. 2013. Effect of protein content on the physical stability and microstructure of a model infant formula. *Int. Dairy J.* 29:53–59. <https://doi.org/10.1016/j.idairyj.2012.10.004>.
- Michael, M., R. K. Phebus, H. Thippareddi, J. Subbiah, S. L. Birla, and K. A. Schmidt. 2014. Validation of radio-frequency dielectric heating system for destruction of *Cronobacter sakazakii* and *Salmonella* species in nonfat dry milk. *J. Dairy Sci.* 97:7316–7324. <https://doi.org/10.3168/jds.2013-7862>.
- Motulsky, H., and A. Christopoulos. 2004. Fitting Models to Biological Data using Linear and Nonlinear Regression. GraphPad Software., San Diego, CA.
- Park, J. K., W. S. Seok, B. J. Choi, H. M. Kim, B. K. Lim, S. S. Yoon, S. Kim, Y. S. Kim, and J. Y. Park. 2004. *Salmonella enterica* serovar London infections associated with consumption of infant formula. *Yonsei Med. J.* 45:43–48. <https://doi.org/10.3349/ymj.2004.45.1.43>.
- Podolak, R., E. Enache, W. Stone, D. G. Black, and P. H. Elliott. 2010. Sources and risk factors for contamination, survival, persistence, and heat resistance of *Salmonella* in low-moisture foods. *J. Food Prot.* 73:1919–1936. <https://doi.org/10.4315/0362-028X-73.10.1919>.
- Quintavalla, S., S. Larini, P. Mutti, and S. Barbuti. 2001. Evaluation of the thermal resistance of different *Salmonella* serotypes in pork meat containing curing additives. *Int. J. Food Microbiol.* 67:107–114. [https://doi.org/10.1016/S0168-1605\(01\)00430-5](https://doi.org/10.1016/S0168-1605(01)00430-5).
- Reij, M. W., and E. D. Den Aantrekker. 2004. Recontamination as a source of pathogens in processed foods. *Int. J. Food Microbiol.* 91:1–11. [https://doi.org/10.1016/S0168-1605\(03\)00295-2](https://doi.org/10.1016/S0168-1605(03)00295-2).
- Rodríguez-Urrego, J., S. Herrera-León, A. Echeita-Sarriondia, P. Soler, F. Simon, and S. Mateo. 2010. Nationwide outbreak of *Salmonella* serotype Kedougou associated with infant formula, Spain, 2008. *Euro Surveill.* 15:19582.
- Rowe, B., D. N. Hutchinson, R. J. Gilbert, B. H. Hales, N. T. Begg, H. C. Dawkins, M. Jacob, F. A. Rae, and M. Jepson. 1987. *Salmonella* Ealing infections associated with consumption of infant dried milk. *Lancet* 330:900–903. [https://doi.org/10.1016/S0140-6736\(87\)91384-5](https://doi.org/10.1016/S0140-6736(87)91384-5).
- Shrestha, A. K., T. Howes, B. P. Adhikari, B. J. Wood, and B. R. Bhandari. 2007. Effect of protein concentration on the surface composition, water sorption and glass transition temperature of spray-dried skim milk powders. *Food Chem.* 104:1436–1444. <https://doi.org/10.1016/j.foodchem.2007.02.015>.
- Smith, D. F., I. M. Hildebrandt, K. E. Casulli, K. D. Dolan, and B. P. Marks. 2016. Modeling the effect of temperature and water activity on the thermal resistance of *Salmonella* Enteritidis PT 30 in wheat flour. *J. Food Prot.* 79:2058–2065. <https://doi.org/10.4315/0362-028X.JFP-16-155>.
- Syamaladevi, R. M., R. K. Tadapaneni, J. Xu, R. Villa-Rojas, J. Tang, B. Carter, S. Sablani, and B. Marks. 2016. Water activity change at elevated temperatures and thermal resistance of *Salmonella* in all purpose wheat flour and peanut butter. *Food Res. Int.* 81:163–170. <https://doi.org/10.1016/j.foodres.2016.01.008>.
- Tham, T. W. Y., C. Wang, A. T. H. Yeoh, and W. Zhou. 2016. Moisture sorption isotherm and caking properties of infant formulas. *J. Food Eng.* 175:117–126. <https://doi.org/10.1016/j.jfoodeng.2015.12.014>.
- Tsai, H.-C., M. H. Taylor, X. Song, L. Sheng, J. Tang, and M.-J. Zhu. 2019. Thermal resistance of *Listeria monocytogenes* in natural unsweetened cocoa powder under different water activity. *Food Control* 102:22–28. <https://doi.org/10.1016/j.foodcont.2019.03.006>.
- Valdramidis, V. P., A. H. Geeraerd, J. E. Gaze, A. Kondjoyan, A. R. Boyd, H. L. Shaw, and J. F. Van Impe. 2006. Quantitative description of *Listeria monocytogenes* inactivation kinetics with temperature and water activity as the influencing factors; model prediction and methodological validation on dynamic data. *J. Food Eng.* 76:79–88. <https://doi.org/10.1016/j.jfoodeng.2005.05.025>.
- Verma, T., X. Wei, S. K. Lau, A. Bianchini, K. M. Eskridge, and J. Subbiah. 2018. Evaluation of *Enterococcus faecium* NRRL B-2354 as a surrogate for *Salmonella* during extrusion of low-moisture food. *J. Food Sci.* 83:1063–1072. <https://doi.org/10.1111/1750-3841.14110>.
- Verrips, C. T., and R. Van Rhee. 1981. Heat inactivation of *staphylococcus epidermidis* at various water activities. *Appl. Environ. Microbiol.* 41:1128–1131. <https://doi.org/10.1128/AEM.41.5.1128-1131.1981>.
- Villa-Rojas, R., J. Tang, S. Wang, M. Gao, D.-H. Kang, J.-H. Mah, P. Gray, M. E. Sosa-Morales, and A. Lopez-Malo. 2013. Thermal inactivation of *Salmonella* Enteritidis PT 30 in almond kernels as

- influenced by water activity. *J. Food Prot.* 76:26–32. <https://doi.org/10.4315/0362-028X.JFP-11-509>.
- Villa-Rojas, R., M.-J. Zhu, B. P. Marks, and J. Tang. 2017. Radiofrequency inactivation of *Salmonella* Enteritidis PT 30 and *Enterococcus faecium* in wheat flour at different water activities. *Biosyst. Eng.* 156:7–16. <https://doi.org/10.1016/j.biosystemseng.2017.01.001>.
- Wei, X., S. K. Lau, B. S. Reddy, and J. Subbiah. 2020. A microbial challenge study for validating continuous radio-frequency assisted thermal processing pasteurization of egg white powder. *Food Microbiol.* 85:103306. <https://doi.org/10.1016/j.fm.2019.103306>.
- Wei, X., S. K. Lau, J. Stratton, S. Irmak, A. Bianchini, and J. Subbiah. 2018. Radio-frequency processing for inactivation of *Salmonella enterica* and *Enterococcus faecium* NRRL B-2354 in black peppercorn. *J. Food Prot.* 81:1685–1695. <https://doi.org/10.4315/0362-028X.JFP-18-080>.
- Wei, X., S. K. Lau, J. Stratton, S. Irmak, and J. Subbiah. 2019. Radiofrequency pasteurization process for inactivation of *Salmonella* spp. and *Enterococcus faecium* NRRL B-2354 on ground black pepper. *Food Microbiol.* 82:388–397. <https://doi.org/10.1016/j.fm.2019.03.007>.

ORCIDS

- Xinyao Wei <https://orcid.org/0000-0002-1746-2653>
- Soon Kiat Lau <https://orcid.org/0000-0001-8264-7761>
- Mary-Grace C. Danao <https://orcid.org/0000-0001-7898-5004>
- Jeyamkondan Subbiah <https://orcid.org/0000-0002-8512-0735>