Evaluation of *Enterococcus faecium* NRRL B-2354 as a surrogate for *Salmonella enterica* in milk powders at different storage times and temperatures

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ABSTRACT

While the increase in thermal resistance of microorganisms at reduced water activity is demonstrated for low-moisture food products, the effect of storage time on the thermal resistance of microorganisms in low-moisture foods is not well established. As low-moisture foods are stored for long periods and are used as ingredients, cross-contamination can occur at any time period before the lethality step. Therefore, this study was designed to investigate the effect of storage time (30, 60, and 90 d) on the thermal resistance of *Salmonella* and *Enterococcus faecium* NRRL B-2354 in milk powders at a low water activity of 0.10 (conservative level). In this study, 2 milk powders, whole milk powder (WMP) and nonfat dry milk (NFDM), were inoculated with a 5-serotype *Salmonella* cocktail or *E. faecium* and equilibrated to a water activity of 0.10. The thermal resistance of *Salmonella* and *E. faecium* in WMP and NFDM were determined at different storage times (30, 60, and 90 d) at 85°C. The storage time had no effect on the thermal inactivation kinetics of *Salmonella* within 90 d of storage at 85°C. In the second part of this study, isothermal treatments were also conducted at higher temperatures (90 and 95°C) to evaluate the suitability of *E. faecium* as a surrogate for *Salmonella* in milk powders. The *D*-values of *Salmonella* in WMP with 30 d of storage at 85, 90, and 95°C were 7.98, 3.35, and 1.68 min. The corresponding values for *E. faecium* were 16.96, 7.90, and 4.16 min. Higher *D*-values of *E. faecium* indicates that it is a conservative surrogate. Similar results were found for NFDM. In general, *D*-values of both microorganisms are slightly higher in NFDM than WMP. Two primary models (log-linear and Weibull) were compared for their goodness-of-fit.

The Weibull model was found to be more appropriate than the log-linear model. This study provides valuable information for establishing process validation for the pasteurization of milk powders.

Key words: surrogate, Weibull, model analysis, process validation, low-moisture food

INTRODUCTION

Food products with water activities <0.70 are categorized as low water activity foods (Blessington et al., 2013). Most of the foodborne pathogens require water activity >0.9 for proper growth; thus, they were historically not a concern in low water activity foods. However, the survival of *Salmonella* in low water activity foods, such as almond (CDC, 2004), milk powder (CDC, 1993; Angulo et al., 2008), peanut butter (CDC, 2009), and black pepper (CDC, 2010), have resulted in several foodborne outbreaks in the United States, indicating that low water activity food can no longer be considered as inherently microbiologically safe.

Milk is a nutrient-rich liquid that is often spray-dried into powder forms for a longer shelf-life. Milk powders are commonly used as ingredients in many food products including ready-to-eat foods, such as confectionery, drink mixes, seasoning, nutritional bars, and dry blend infant formula. However, no pathogen lethality step is applied to eliminate foodborne pathogens in milk powders after spray drying (LiCari and Potter, 1970), which could potentially result in serious food safety issues. For instance, 37 babies in France were known to have fallen sick due to the presence of *Salmonella* Agona in powdered milk in 2018 (Marler, 2018). Several recalls and outbreaks have also been linked to *Salmonella*-contaminated dry milk powder products (Park et al., 2004; Brouard et al., 2007; Rodríguez-Urrego et al., 2010; Jourdan-da Silva et al., 2018).

After spray drying of milk powders, there is a risk of cross-contamination through poor postprocessing

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practices, such as poor environmental sanitation, inappropriate storage conditions, or mishandling during transportation (Podolak et al., 2010). Before further processing of milk powders, especially for ready-to-eat foods, an effective pasteurization process should be integrated to minimize the risk of contamination of final products.

As required by the US FDA (2018), validation studies are required to scientifically demonstrate that processing technologies effectively limit the hazards they are designed to address. Also, due to the difficulty of directly introducing the target pathogen into the processing plant, the use of a surrogate to replace the pathogen is recommended by FDA (2013) to conduct the validation study in the industrial processing plant. Several studies (Bianchini et al., 2014; Liu et al., 2018; Verma et al., 2018; Wei et al., 2018) have previously demonstrated that *Enterococcus faecium* NRRL B-2354 is a suitable surrogate for *Salmonella* during thermal processing of low water activity foods.

In commercial practice, ingredients such as milk powders are usually stored at 23°C for a long period before usage. It has been reported that high storage temperature (45°C) could greatly affect lipid oxidation in whole milk powder (WMP), and the maximum level of free radicals was detected after 47 d of storage at 45°C (Stapelfeldt et al., 1997). According to US Dairy Export Council (2017), the typical storage time for WMP is 6 to 9 mo at room conditions (<27°C, <65% relative humidity). Flavor deterioration of WMP has been reported to occur after 3 mo of storage (Drake et al., 2003). However, the effect of storage time on the thermal inactivation kinetics of *Salmonella* in milk powders is currently unknown. Therefore, it is important to understand the effect of storage time on thermal inactivation kinetics for process validation studies. It is also important to evaluate the suitability of surrogate at different storage times and temperatures.

The overall goal of this study is to evaluate the effect of storage time on thermal inactivation kinetics of *Salmonella* and *E. faecium* NRRL B-2354 in milk powders. The specific objectives were to (1) determine the effect

of storage time (30, 60, and 90 d) on the thermal inactivation kinetics of *Salmonella* and *E. faecium* NRRL B-2354 in milk powders at 85°C and water activity of 0.10, and (2) evaluate *E. faecium* NRRL B-2354 as a surrogate for *Salmonella* at different temperatures (85, 90, and 95°C) after 30 d of storage of milk powders at a water activity of 0.10.

MATERIALS AND METHODS

Milk Powder Products

Three batches of grade A pasteurized WMP and nonfat dry milk (NFD) from different production lots were received from Mars Inc. (McLean, VA). The milk powders were stored inside polyethylene bags and held at ambient temperature (23 ± 2°C) before inoculation. On receiving the samples, the proximate analysis and background microorganism tests were conducted as described in Wei et al. (2020a). A water activity meter (4TE, METER Group, Pullman, WA) was used to measure the water activity of milk powders at 25°C.

Inoculum Preparation

The details of the bacterial serotypes used in this study are described in Table 1. A *Salmonella* cocktail, consisting of 5 serotypes of *Salmonella enterica* ssp. *enterica* that were previously isolated from different food-borne outbreaks, was used to inoculate both powder samples. *Enterococcus faecium* NRRL B-2354 was used to inoculate both powder samples for surrogate tests.

The lawn-based pelletized inoculum method was used to prepare the inoculum based on a standard procedure as described in Hildebrandt et al. (2016), which yields a stable bacterial population before isothermal treatment and consistent *D*-value. The frozen stock of each bacteria was thawed at 37°C for 5 min, then was subjected to 2 sequential transfers (24 h at 37°C) in 10 mL of trypticase soy broth (TSB; Becton, Dickinson and Company, Franklin Lakes, NJ) with 0.6% (wt/vol) yeast extract (YE; Becton, Dickinson and Company). A loop (~10

Table 1. Bacterial serotypes used in this study

Bacterial name	Source ¹	Related outbreak	Reference
<i>Salmonella</i> Agona 447967	FDA, ORA Regional Laboratory (Jefferson, AR)	Oats cereal	CDC, 1998
<i>Salmonella</i> Montevideo 488275	FDA, ORA Regional Laboratory (Jefferson, AR)	Black and red pepper	CDC, 2010
<i>Salmonella</i> Mbandaka 698538	FDA, ORA Regional Laboratory (Jefferson, AR)	Sprouts	Jackson et al., 2013
<i>Salmonella</i> Reading Moff 180418	FDA Culture Collection (Bedford Park, IL)	Alfalfa sprout	CDC, 2016
<i>Salmonella</i> Tennessee K4643	University of Georgia (Athens, GA)	Peanut butter	CDC, 2007
<i>Enterococcus faecium</i> NRRL B-2354	USDA, ARS (Peoria, IL)	—	Almond Board of California, 2014

¹FDA = Food and Drug Administration; ORA = Office of Regulatory Affairs; ARS = Agricultural Research Service.

μL) of the incubated culture was streaked onto tryptic soy agar (TSA; Becton, Dickinson and Company) supplemented with 0.6% (wt/wt) YE and incubated at 37°C for 24 h to prepare the working stock plate. A 10- μL loop was used to harvest one isolated colony from a working stock plate and was then transferred to 10 mL of TSBYE and incubated for 24 h at 37°C. The incubated culture (0.1 mL) was then spread-plated onto a TSAYE plate and incubated for 24 h at 37°C. The grown bacterial lawn was then harvested with 3 mL of 0.1% buffered peptone water (Becton, Dickinson and Company) with an L-shaped spreader. By mixing the equal amount of lawn from each *Salmonella* serotypes, the *Salmonella* cocktail inoculum was prepared with the bacterial population of approximately 1,010 cfu/mL. Similarly, *E. faecium* NRRL B-2354 inoculum was prepared with the bacterial population of approximately 10^9 cfu/mL.

Milk Powder Inoculation and Equilibration

The WMP or NFDM from each batch was weighed (100 g) and transferred to a resealable 1-gallon polyethylene bag. A sterile 15-mL centrifuge tube (Thermo Fisher Scientific, Rochester, NY) with a finger-operated spray head contained a 10-mL aliquot of the prepared *Salmonella* cocktail or *E. faecium* NRRL B-2354 inoculum was used to spray-inoculate both milk powder samples. The inoculated sample was then mixed by hand for 5 min to manually detach powders that were stuck to the inner lining of the bag. Subsequently, the sample was transferred into a sterile Whirl-Pak bag (2.04 L, Nasco, Fort Atkinson, WI) and then placed in a paddle mixer (9000471, Neutec Group Inc., Farmingdale, NY) and mixed for 20 min to homogenize the inoculum distribution and minimize the clumps. The mixed inoculated sample (5 mm thickness) was then placed on a sterile aluminum tray (230 $\times$ 300 $\times$ 15 mm) and transferred into a custom-made relative humidity chamber (Lau and Subbiah, 2020a) set to a target relative humidity of 10% to equilibrate the inoculated sample to a water activity of 0.10 at 25°C. Based on the previous study (Wei et al., 2020a), a low water activity of 0.10 was selected, which could represent the worst-case scenario where *Salmonella* exhibits high thermal resistance. This low water activity (0.10) represents a conservative study, as *Salmonella* resistance is higher at lower water activity. After 5 d of equilibration in the chamber, the inoculated sample was packed into heat-sealable aluminized pouches (7.62 cm $\times$ 7.62 cm $\times$ 0.10 cm, IMPAK Corporation, Los Angeles, CA) in quantities of approximately 2 g and then sealed for storage at

23°C for 30, 60, and 90 d. The whole packing process was conducted inside the relative humidity chamber to ensure a minimal change in the water activity.

Determination of Thermal Resistance at Different Storage Times

Stored inoculated milk samples were sampled on 30, 60, and 90 d to evaluate the effect of storage time on the heat resistance of *Salmonella* and *E. faecium*. Isothermal treatment was conducted at 85°C by using the custom-designed thermal death time (TDT) sandwich (Lau and Subbiah, 2020b) for both WMP and NFDM to obtain the isothermal death curve of *Salmonella* and *E. faecium* at 85°C. The TDT sandwich has 2 resistive heating elements that could apply dry heat to samples. Each packed inoculated sample was placed in between the 2 heating elements in one TDT sandwich unit for isothermal treatment of one time and temperature combination.

Before conducting the isothermal treatment, the come-up time was measured in triplicates by a type T thermocouple (5TC-TT-T-40-36, Omega Engineering Inc., Norwalk, CT) with an accuracy of $\pm 0.5^\circ\text{C}$ inserted to the inside center of an aluminized pouch. The come-up time was the average time required for the sample to be heated to 84.5°C by the TDT sandwiches plus twice the standard deviation, which would ensure that at least 95% of TDT sandwiches would reach at least 84.5°C at time zero in the inactivation curve.

Six equally spaced time points including time zero were used for the isothermal treatment of inoculated samples to obtain thermal death curves for *Salmonella* or *E. faecium* in both WMP and NFDM. Immediately after each treatment, the treated pouch was transferred into an ice-water bath (0°C) for at least 1 min before it was enumerated for bacterial survivors.

Surrogate Evaluation at Different Temperatures

To evaluate the suitability of *E. faecium* as a surrogate for *Salmonella* at different temperatures, isothermal treatments at the higher temperatures (90 and 95°C) were conducted for *Salmonella* or *E. faecium* in inoculated milk powders. Based on the results of the storage study as described in the previous section, a single storage time was used to determine the thermal inactivation kinetics for both bacteria at higher temperatures. Inoculated samples were isothermally treated by the TDT sandwiches as described in the previous section to obtain thermal inactivation curves for *Salmonella* or *E. faecium* in both WMP and NFDM at

90 and 95°C. All isothermal treatments for inoculated WMP and NFDM were performed in 3 independent replicates by using inoculated samples from each batch.

Bacterial Enumeration

Enumeration was performed as previously described (Wei et al., 2019). Briefly, the *Salmonella* or *E. faecium* survivors in the thermally treated sample were enumerated by serially 10-fold diluting samples in 0.1% buffered peptone water and were then spread-plated onto TSAYE supplemented with 0.05% (wt/vol) ammonium iron citrate (Sigma-Aldrich Co., St. Louis, MO), and 0.03% (wt/vol) sodium thiosulfate (mTSA; Fisher Chemical, Fair Lawn, NJ) for *Salmonella* or TSAYE supplemented with 0.05% (wt/vol) ammonium iron citrate, and 0.025% (wt/vol) esculin hydrate (eTSA; Acros Organics, Morris Plains, NJ) for *E. faecium* and incubated for 24 h at 37°C (Smith et al., 2016; Liu et al., 2017; Wei et al., 2020b). Colonies with a black center were counted as *Salmonella* on incubated mTSA plates and black colonies were counted as *E. faecium* on incubated eTSA plates, respectively. The untreated WMP or NFDM samples were enumerated as a control to monitor the bacterial population change during the 90 d of storage.

Curve Fitting of The Survivor Models

The *Salmonella* or *E. faecium* survivors in both milk powders were fitted with 2 primary inactivation models, the log-linear model [1] and the Weibull-type model [2] as described in Peleg and Cole (1998):

$$\log_{10} \left(\frac{N}{N_0} \right) = -\frac{t}{D}, \quad [1]$$

$$\log_{10} \left(\frac{N}{N_0} \right) = -\left(\frac{t}{\delta} \right)^\beta, \quad [2]$$

where N and N_0 (cfu/g) are the *Salmonella* or *E. faecium* survivors at time t and 0; t is the instantaneous time of the thermal treatment (min); D is the decimal reduction time, which is the time required to reduce the microbial population by 10-fold; δ (min) is the scale parameter; and β is the shape parameter of the Weibull inactivation curve, where $\beta > 1$ indicates a concave downward trend, $\beta < 1$ indicates a concave upward trend of the inactivation curve, and $\beta = 1$ indicates a linear trend.

The z -value is defined as the increase in temperature required to achieve a 90% reduction of the decimal re-

duction time D and was determined by the following equation:

$$z = \frac{T_2 - T_1}{\log_{10} D_{T_1} - \log_{10} D_{T_2}}, \quad [3]$$

where $\log_{10} D_{T_1} - \log_{10} D_{T_2}$ are the D -values at temperature T_1 and T_2 , respectively.

The bacterial survival data for each isothermal treatment were fitted to Equations [1] and [2] using the Levenberg–Marquardt algorithm for nonlinear least squares regression as implemented in the `curve_fit` function of the Python SciPy library (Newville et al., 2015). The statistical significance of differences between different storage times and different milk powder products were analyzed using Fisher's least significant difference test as implemented in the `LSD_test` function of the R `agricolae` library (de Mendiburu and de Mendiburu, 2019).

Global Models

The log-linear and Weibull models were also globally fitted across all 3 storage times. To evaluate the appropriateness of model fitting, model selection analyses were performed using root mean squared error (RMSE) and corrected Akaike information criteria (AIC_c; Motulsky and Christopoulos, 2003):

$$\text{RMSE} = \sqrt{\frac{\sum_{i=1}^n [\log_{10}(N)_{\text{observed},i} - \log_{10}(N)_{\text{predicted},i}]^2}{n}}, \quad [4]$$

$$\text{AICc} = n \ln \left(\frac{SS}{n} \right) + 2K + \frac{2K(K+1)}{n-K-1}, \quad [5]$$

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, SS is the sum of squares of the residuals, and K is the number of parameters being estimated plus 1. Lower RMSE and AIC_c values indicate that the model prediction is more likely to be correct for the experimental data. According to Motulsky and Christopoulos (2003), the percent likelihood of a given model is more correct, which can be estimated using the following equation:

$$\text{Probability}(\text{model A over B}) = \frac{e^{\left(\frac{\text{AIC}_{c,B} - \text{AIC}_{c,A}}{2} \right)}}{1 + e^{\left(\frac{\text{AIC}_{c,B} - \text{AIC}_{c,A}}{2} \right)}}. \quad [6]$$

RESULTS AND DISCUSSION

Survival of *Salmonella* and *E. faecium* in WMP and NFDM

The bacterial populations of *Salmonella* and *E. faecium* in the prepared inoculum were 10.84 and 9.92 log cfu/mL, respectively. Immediately after inoculation, *Salmonella* and *E. faecium* populations were determined to be 8.13 and 8.86 log cfu/g in WMP and 8.25 and 8.92 log cfu/g in NFDM, respectively. After 24 h equilibration in the relative humidity chamber, the inoculated samples were equilibrated to the water activity level of 0.10 ± 0.01 , and the *Salmonella* and *E. faecium* population were dropped to 7.75 and 8.61 log cfu/g in WMP and 7.86 and 8.78 log cfu/g in NFDM, respectively. The standard deviations of 3 subsamples from inoculated WMP and NFDM were determined to be 0.18 and 0.15 log cfu/g, respectively, which indicated a good homogeneity of the inoculated samples.

The survival results of *Salmonella* and *E. faecium* in WMP and NFDM are shown in Figure 1. During 90 d of storage, the reductions of *Salmonella* in WMP and NFDM were observed to be 0.79 and 0.86 log cfu/g, whereas the reductions of *E. faecium* in WMP and NFDM were observed to be 0.10 and 0.16 log cfu/g (Figure 1). Most of the decrease in the initial population of *Salmonella* was observed in the first 30 d of storage. The results in this study showed that *Salmonella* in milk powder experienced a rapid death rate in the first few weeks of storage followed by a slower death rate are in agreement with several published studies (LiCari and Potter, 1970; Lian et al., 2015). A similar reduction in *Salmonella* population has been observed during the postinoculation process of low water activity foods, such as black pepper (Wei et al., 2018, 2019), cumin seeds (Chen et al., 2019), egg white powder (Wei et al., 2020b), almond (Abd et al., 2012), wheat flour (Forghani et al., 2019), and walnut kernel (Blessington et al., 2012). *Salmonella* cells need time to adapt to a low water activity environment. Thus, a higher death rate was observed during the adaption period and then the death rate leveled off in the remaining storage time.

According to Lian et al., (2015), the population of *Salmonella* in skim milk powder dropped by 4.23, 4.69, and 5.58 log cfu/g after 2 mo of storage at the water activity of 0.33, 0.53, and 0.81, respectively. As the water activity of milk powder in this study was much lower at 0.10, a much lower reduction of 0.86 log cfu/g of the *Salmonella* population in NFDM after 90 d of storage was observed. Enhanced survivability of *Salmonella* was observed at lower water activity level during the storage of low water activity foods, as desiccated conditions have been reported to increase survivability

(Jung and Beuchat, 1999; Kataoka et al., 2014; Lian et al., 2015). Differences in reductions of *Salmonella* found in this study when compared with Lian et al. (2015) may also be due to differences in inoculum protocols (Hildebrandt et al., 2016).

Similar to *Salmonella*, several other foodborne pathogens, such as *Enterobacter sakazakii* (Gurtler and Beuchat, 2007) and *Listeria monocytogenes* (Ballom et al., 2020) have been shown to survive well in milk powders at a reduced water activity level. Therefore, the long-term survival of foodborne pathogens in milk powders should be of concern to the dairy industry and it is important to have a kill step to enhance the safety of milk powders.

Effect of Storage Time on the Thermal Resistance of *Salmonella* and *E. faecium*

The effect of storage time on the thermal resistance of *Salmonella* and *E. faecium* was investigated at 85°C and at the water activity of 0.1. The low water activity of 0.1 was selected in this study to represent the worst-case scenario, as increased thermal resistance of *Salmonella* has been reported at reduced water activity levels in both WMP and NFDM (Wei et al., 2020a). The isothermal treatment temperature of 85°C was chosen because there was a minimum quality impact on sensory properties of milk powders at this temperature.

Figure 1. Viability of *Salmonella* and *Enterococcus faecium* population in whole milk powder (WMP) and nonfat dry milk (NFDM) during storage period (23°C). Error bars indicate 1 SD of 3 subsamples.

Table 2. Effect of storage time on model parameter estimates for the log-linear and Weibull models at 85°C¹

Sample	Bacteria	Storage time (d)	D (min)	δ (min)	β
WMP	<i>Salmonella</i>	30	7.98 ± 0.89^f	7.24 ± 0.33^e	0.88 ± 0.10^f
		60	8.00 ± 0.07^f	7.45 ± 1.19^e	1.04 ± 0.19^{def}
		90	7.89 ± 0.34^f	7.67 ± 0.81^e	0.97 ± 0.10^{ef}
	<i>Enterococcus faecium</i>	30	16.96 ± 0.70^{cd}	23.27 ± 1.75^b	1.34 ± 0.14^{abc}
		60	18.38 ± 1.47^{bc}	26.10 ± 0.80^a	1.42 ± 0.07^a
		90	16.36 ± 0.62^d	20.03 ± 0.83^c	1.19 ± 0.05^{bcd}
NFDM	<i>Salmonella</i>	30	10.98 ± 0.52^e	11.48 ± 0.97^d	0.92 ± 0.06^f
		60	11.41 ± 0.92^e	12.90 ± 2.97^d	1.12 ± 0.17^{de}
		90	10.71 ± 0.18^e	13.35 ± 0.70^d	1.20 ± 0.04^{bcd}
	<i>E. faecium</i>	30	19.81 ± 1.75^{ab}	26.11 ± 2.94^a	1.34 ± 0.08^{abc}
		60	19.50 ± 0.27^{ab}	23.12 ± 0.57^b	1.18 ± 0.03^{cd}
		90	20.71 ± 0.76^a	27.49 ± 1.83^a	1.37 ± 0.12^{ab}

^{a-f}Values with differing superscript letters are significantly different within a column ($P < 0.05$).

¹ D = decimal reduction time; δ = scale parameter for Weibull model; β = shape parameter for Weibull model; WMP = whole milk powder; NFDM = nonfat dry milk. Values are mean $\pm$ SD of triplicates.

Figure 2. Experimental and predicted survival of *Salmonella* and *Enterococcus faecium* in whole milk powder stored for 30 d (●, dashed lines), 60 d (+, dotted lines), and 90 d (◆, solid lines) at 85°C. N and N_0 (cfu/g) are the *Salmonella* or *E. faecium* survivors at time t and 0.

The model parameters of the log-linear model and the Weibull model for both WMP and NFDM were compared across the 3 storage times in Table 2. For both WMP and NFDM, the D -values of *Salmonella* were not significantly ($P < 0.05$) different among the storage times. Similarly, there were no significant ($P < 0.05$) differences of D -values among the storage times for the Weibull model parameters of both WMP and NFDM. The fitted models were plotted against the inactivation data in Figure 2 for WMP and Figure 3 for NFDM. The proximity (closeness) and similar curvatures (slope and shape) of the curves for different storage times also reveal the similarity in model parameters.

These results suggest that the thermal resistance of *Salmonella* is not affected by storage time. Abd et al. (2012) and Limcharoenchat et al. (2019) also reported that the storage time had no effect on the heat resistance of *Salmonella* in almonds, which is in agreement

with this study. Similarly, the thermal resistance of *L. monocytogenes* adapted to NFDM (Ballom et al., 2020), chicken meat powder, and pet food (Rachon et al., 2016) was shown to remain constant during prolonged storage.

Therefore, a conclusion can be drawn that when viable cells of *Salmonella* reach stable status in the low water activity environment, their heat resistance would not significantly change with increasing storage time up to 90 d. Although *Salmonella* can survive in low water activity foods for extended periods, this study suggests that their resistance to thermal treatment does not change significantly during the storage of 90 d at 85°C. While the effect of storage period on thermal resistance at other pasteurization temperatures needs to be evaluated, this result implies that the pasteurization protocol does not need to consider the prior storage period up to 90 d at 85°C. This may increase the flexibility

Figure 3. Experimental and predicted survival of *Salmonella* and *Enterococcus faecium* in nonfat dry milk stored for 30 d (●, dashed lines), 60 d (+, dotted lines), and 90 d (◆, solid lines) at 85°C. N and N_0 (cfu/g) are the *Salmonella* or *E. faecium* survivors at time t and 0.

Figure 4. Log-linear and Weibull models fitted to *Salmonella* and *Enterococcus faecium* inactivation data of 90 and 95°C. *Salmonella*-90°C (●, blue lines), *Salmonella*-95°C (★, green lines), *E. faecium*-90°C (+, red dotted lines), and *E. faecium*-95°C (◆, black lines). N and N_0 (cfu/g) are the *Salmonella* or *E. faecium* survivors at time t and 0.

Table 3. Effect of temperature on model parameter estimates for the log-linear and Weibull models after 1 mo of storage¹

Sample	Bacteria	Temperature (°C)	D (min)	δ (min)	β
WMP	<i>Salmonella</i>	85	7.98 ± 0.89 ^e	7.24 ± 0.33 ^e	0.88 ± 0.10 ^e
		90	3.35 ± 0.23 ^g	3.47 ± 0.28 ^{gh}	1.04 ± 0.02 ^{cd}
		95	1.68 ± 0.14 ^h	1.40 ± 0.23 ⁱ	0.83 ± 0.07 ^e
	<i>Enterococcus faecium</i>	85	16.96 ± 0.70 ^b	23.27 ± 1.75 ^b	1.34 ± 0.14 ^b
		90	7.90 ± 0.24 ^e	12.23 ± 0.81 ^d	1.46 ± 0.09 ^{ab}
		95	4.16 ± 0.07 ^{fg}	3.67 ± 0.43 ^g	0.92 ± 0.05 ^{de}
NFDM	<i>Salmonella</i>	85	10.98 ± 0.52 ^c	11.48 ± 0.97 ^d	0.92 ± 0.06 ^{de}
		90	4.80 ± 0.52 ^f	5.33 ± 0.81 ^{fg}	1.14 ± 0.16 ^c
		95	1.82 ± 0.24 ^h	1.66 ± 0.37 ^{hi}	0.90 ± 0.11 ^{de}
	<i>E. faecium</i>	85	19.81 ± 1.75 ^a	26.11 ± 2.94 ^a	1.34 ± 0.08 ^b
		90	9.72 ± 0.17 ^d	14.65 ± 0.56 ^c	1.52 ± 0.06 ^a
		95	4.94 ± 0.02 ^f	6.94 ± 0.18 ^{ef}	1.41 ± 0.04 ^{ab}

^{a-i}Values with differing superscript letters are significantly different within a column ($P < 0.05$).

¹ D = decimal reduction time; δ = scale parameter for Weibull model; β = shape parameter for Weibull model; WMP = whole milk powder; NFDM = nonfat dry milk. Values are mean ± SD of triplicates.

Table 4. Model parameter estimates for the log-linear and Weibull models globally fitted across all storage times at 85°C¹

Sample	Bacteria	Model	<i>D</i> or δ (min)	β	RMSE (log ₁₀ cfu/g)	AIC _c	Probability
WMP	<i>Salmonella</i>	Log-linear	7.93 (0.32)	—	0.21	-165.80	0.04
		Weibull	7.07 (0.33)	0.89 (0.04)	0.20	-172.15	0.96
	<i>Enterococcus faecium</i>	Log-linear	17.16 (0.78)	—	0.33	-116.79	~0
		Weibull	23.05 (0.62)	1.31 (0.04)	0.23	-158.52	~1
NFDM	<i>Salmonella</i>	Log-linear	11.00 (0.30)	—	0.32	-119.55	0.28
		Weibull	11.9 (0.34)	1.07 (0.03)	0.32	-121.42	0.72
	<i>E. faecium</i>	Log-linear	19.95 (1.42)	—	0.28	-133.69	~0
		Weibull	25.53 (0.83)	1.29 (0.05)	0.21	-166.09	~1

¹*D* = decimal reduction time; δ = scale parameter for Weibull model; β = shape parameter for Weibull model; RMSE = root mean square error; AIC_c = corrected Akaike information criteria; WMP = whole milk powder; NFDM = nonfat dry milk. Microbial populations from 3 storage periods and triplicates were simultaneously used to fit model to estimate model parameters along with the SE of estimate reported in parentheses.

of the application of process control if the results from the effect of storage period hold at other pasteurization temperatures.

In Table 2, it has been shown that *D* and δ values of *E. faecium* were significantly higher ($P < 0.05$) than *Salmonella* at the same storage condition in both WMP and NFDM. This demonstrated that *E. faecium* consistently exhibited greater thermal resistance ($P < 0.05$) than *Salmonella* during the storage of WMP and NFDM.

Thermal Inactivation Kinetics of *Salmonella* and *E. faecium* in WMP and NFDM

Because storage time (90 d) had no effect on the heat resistance of *Salmonella*, the isothermal treatments at

90 and 95°C were conducted by using the inoculated samples at d 30 of storage time to acquire thermal inactivation kinetics of *Salmonella* and *E. faecium*. The bacterial inactivation data were fitted to both log-linear and Weibull models in Figure 4. The model parameters for both WMP and NFDM are summarized in Table 3. For both *Salmonella* and *E. faecium*, *D* and δ values were found to significantly ($P < 0.05$) decrease with increased temperature, which indicated that a higher microbial reduction could be achieved at a higher temperature for the same treatment time. Significantly higher ($P < 0.05$) *D* and δ values of *E. faecium* were also observed at 85, 90, and 95°C than *Salmonella*. Similarly, Liu et al. (2019) conducted isothermal treatments for NFDM with storage time of 48 h after inoculation and found that the decline of *Salmonella* exceeded that of *E. faecium* during isothermal treatment of NFDM at all 3 temperatures (85, 90, and 95°C). These results suggest that *E. faecium* is a suitable nonpathogenic surrogate for *Salmonella* in the thermal processing of milk powder at different process temperatures with 30 d of storage.

Figure 5 shows the effect of temperature on the *D*-values of *Salmonella* and *E. faecium* in dairy powders. The *z*-values of *Salmonella* and *E. faecium* were determined to be 14.77 and 16.39°C for WMP and 12.82 and 16.58°C for NFDM, respectively. The *z*-values of *Salmonella* and *E. faecium* found in this study were very close to the *z*-values of *Salmonella* and *E. faecium* (16.4 and 16.5°C) reported by Liu et al. (2019) and *z*-values of *L. monocytogenes* (14.6 to 16.0°C) in NFDM at different water activities as reported by Ballom et al. (2020). The determined microbial inactivation kinetics of *Salmonella* and *E. faecium* could be a practical tool for the food industry to identify appropriate thermal processing conditions for pasteurization of milk powders.

Tables 2 and 3 show that *D*-values of both microorganisms in NFDM were slightly higher than WMP. Although the initial water activity of both milk powders was equilibrated 0.10 at 25°C, the water activity

Figure 5. Thermal resistance constants (*z*-values) of *Salmonella* and *Enterococcus faecium* in whole milk powder (WMP) and nonfat dry milk (NFDM).

Figure 6. Global log-linear and Weibull models fitted to *Salmonella* and *Enterococcus faecium* inactivation data combined across all storage times at 85°C at whole milk powder (WMP)-*Salmonella* (★, dash-dot line), WMP-*E. faecium* (●, dashed lines), nonfat dry milk (NFDM)-*Salmonella* (+, dotted lines), and NFDM-*E. faecium* (◆, solid line). N and N_0 (cfu/g) are the *Salmonella* or *E. faecium* survivors at time t and 0.

at 85°C could be different because of differences in the food matrix. As water activity at high temperature plays an important role in thermal inactivation of low-moisture foods, the water activity at 85°C for both milk powders may need to be determined to explain the slight differences in *D*-values. Fat has been shown to have a protective effect on high-moisture foods; however, the protective effect of fat in low-moisture foods is not known and need to be investigated in future.

Bacterial Inactivation Model Comparison

Storage time (90 d) did not have an effect on the thermal resistance of bacteria in WMP and NFDM. Therefore, the thermal inactivation data at different storage times were combined and globally fitted to both log-linear and Weibull models for model comparison. The AIC_c and RMSE analysis (Table 4) indicated that the Weibull model was more likely correct (probability >0.72 for *Salmonella* and >0.99 for *E. faecium*) for all cases. The visualization of the models fitting on the combined data set was shown in Figure 6.

Several studies have reported that the Weibull model is a better fit than the log-linear model in low water activity food, such as *Salmonella* in whey protein powder (Farakos et al., 2013), *Salmonella* in almond kernels (Villa-Rojas et al., 2013), and *Listeria monocytogenes* and *E. faecium* in low water activity food powders (Rachon et al., 2016). However, Smith et al. (2016) reported that the log-linear model provided a better prediction than the Weibull model for *Salmonella* inactivation in wheat flour with minimal difference in AIC_c values for both models at most water activity levels. In terms of model fitting, the Weibull model was shown to be more suitable than the log-linear model for describing the thermal inactivation of *Salmonella* and *E. faecium* in WMP and NFDM.

CONCLUSIONS

In this study, both *Salmonella* and *E. faecium* showed good survivability in WMP and NFDM at low water activity with <0.9 log cfu/g reduction of *Salmonella* and <0.2 log cfu/g reduction of *E. faecium* after 90 d of storage. The thermal resistance of *Salmonella* and *E. faecium* did not change significantly during the 90 d storage of WMP and NFDM. This finding simplifies the process validation study in the industry. *Enterococcus faecium* was a suitable surrogate for *Salmonella* at different temperatures (85, 90, and 95°C). The TDT comparison between *Salmonella* and *E. faecium* in milk powders provides an important foundation for the dairy industry for evaluating different thermal treatment technologies that can achieve a suitable reduction of

target pathogens. Use of *E. faecium*, which is a conservative surrogate for process validation, may result in over-processing beyond what is needed for *Salmonella* reduction, which could affect product quality. Therefore, identifying the kill ratio between *Salmonella* and *E. faecium* could contribute to efficient process validations. Based on microbial inactivation kinetics of *Salmonella* and *E. faecium*, the food industry could establish an appropriate thermal process validation for pasteurization of milk powders.

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