



# Thermal inactivation of *Salmonella*, *Listeria monocytogenes* and *Enterococcus faecium* NRRL B-2354 in desiccated shredded coconut

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## ABSTRACT

Desiccated shredded coconut (DSC) products have been implicated in multiple *Salmonella* outbreaks and *Listeria monocytogenes* recalls. The objective of this study was to evaluate the thermal resistance of *Salmonella* and *L. monocytogenes* in DSC as impacted by water activity ( $a_w$ ), and the suitability of *Enterococcus faecium* NRRL B-2354 as a surrogate for these foodborne pathogens during thermal processing of DSC. The inactivation kinetics of each strain in DSC fitted the log-linear model well; their thermal tolerances were inversely related to  $a_w$ . The  $D_{80^\circ\text{C}}$ ,  $D_{85^\circ\text{C}}$ , and  $D_{90^\circ\text{C}}$  values of *Salmonella* were 38.7, 15.5, and 6.0 min at  $a_w$  0.45 compared to 53.2, 28.0, and 12.5 min at  $a_w$  0.25 under respective temperatures. For  $a_w$  0.25 and 0.45 DSC, D-values of *E. faecium* ranged from 49.6 to 6.5 min and 85.5 to 24.2 min at 80–90 °C, which were 1.4–1.9 and 1.1–1.3 times of those of *Salmonella* at the tested temperatures, indicating *E. faecium* is an appropriate surrogate of *Salmonella* during thermal processing of DSC. Compared to *Salmonella*, *L. monocytogenes* exhibited less thermal resistance in DSC. This study provides useful information for the food industry to develop thermal inactivation strategies to control *Salmonella* and *L. monocytogenes* during the post-drying process of DSC.

## 1. Introduction

Coconut (*Cocos nucifera*) belongs to the family of Palmae. According to the Food and Agriculture Organization Corporate Statistical Database, the global production of coconuts in 2019 was 62.47 million metric tons (FAOSTAT, 2020). Among a wide range of coconut products, desiccated coconut is the second most common product traded globally with 200,000 million tons produced worldwide (FAO, 1999). It is commonly used as an ingredient in household and processed foods.

The desiccated coconut was ranked in the third category of low  $a_w$  foods ( $L_{a_w}F$ ), under the subcategory of dried fruits and vegetables as the third greatest concern from a microbiological food safety perspective by the Food and Agriculture Organization of the United Nations (FAO, 2014). *Salmonella* is a major pathogen of concern in  $L_{a_w}F$  because it has caused many outbreaks and recalls in recent years such as in infant powdered milk (Jourdan-da Silva et al., 2018), chocolate (Werber et al., 2005), almonds (Isaacs et al., 2005), peanut butter (Medus et al., 2009), and pistachios (FDA, 2016). Desiccated shredded coconut (DSC) and coconut products were previously implicated in multiple *Salmonella* outbreaks in the United States and around the world (Berginski, Pareth, & Brunn, 1998; Galbraith, Hobbs, Smith, & Tomlinson, 1960; Semple,

Parry, & Graham, 1961; Ward, Duckworth, O'Brien, & Brusin, 1999). Dried and frozen shredded coconut were recently linked to two multi-state *Salmonella* outbreaks in the U.S. (CDC, 2018a, 2018b; Luna et al., 2018), and there have been shredded coconut and coconut products recalls due to possible contamination by *Salmonella* (CFIA, 2018; FDA, 2019a). This evidence indicates *Salmonella* is a potential risk to coconut products, requiring additional control measures.

In the U.S., *L. monocytogenes* is a third leading cause of death from foodborne illness, causing ~260 deaths per year with a 20% mortality rate (Buchanan, Gorris, Hayman, Jackson, & Whiting, 2017). *L. monocytogenes* has been identified as a potential hazard in  $L_{a_w}F$  and is associated with an increasing number of voluntary recalls in the U.S. (FDA, 2018b; 2019b, 2020). *L. monocytogenes* survives in various  $L_{a_w}F$  such as nonfat dry milk powder (Ballom, Tsai, Taylor, Tang, & Zhu, 2020), almond kernel and in-shell pistachios (Kimber, Kaur, Wang, Danyluk, & Harris, 2012), peanut kernels and pecan halves (Brar, Proano, Friedrich, Harris, & Danyluk, 2015) during one-year of storage. Recent recalls associated with coconut-containing granola in Canada (CFIA, 2017) and organic coconut butter in the U.S. (FDA, 2019b) due to potential contamination with *L. monocytogenes*, heighten the need to control *L. monocytogenes* in coconut products in addition to *Salmonella*.

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Coconut is susceptible to microbial contamination throughout production, harvesting, post-harvest manufacturing process, distribution, and retailing (Strawn, Schneider, & Danyluk, 2011). Pasteurization of raw coconut meat in a water bath at 80 °C for 8–10 min is used in the industry to control microbial contamination before processing dried, shredded, or chipped coconut (Schaffner, Mosbach, Bibit, & Watson, 1967). However, *Salmonella* survived during the manufacturing of the desiccated coconut (Meedeniya, 1969). Different *Salmonella* serovars were isolated from desiccated coconut samples (Meedeniya, 1969; Velaudapillai, Nitiananda, & Meedeniya, 1963). Given that the recent outbreaks and recalls of DSC products due to potential *Salmonella* and *L. monocytogenes* contamination, it is important to understand and determine temperature and time combinations to achieve a 5-log or greater reduction of *Salmonella* and *L. monocytogenes* to ensure microbial safety of the products.

Bacterial resistance to thermal treatment increases dramatically when they adapt to  $a_w$ F (Liu, Rojas, Gray, Zhu, & Tang, 2018; Villa-Rojas et al., 2013). However, no information is available about the thermal resistance of *Salmonella* in DSC, neither is there information about the fate of *L. monocytogenes* in DSC during thermal treatments. The objectives of this study were to evaluate the thermal resistance of *Salmonella* and *L. monocytogenes* in DSC, assess the influences of  $a_w$  on the thermal inactivation of *Salmonella* and *L. monocytogenes* in DSC, and determine the suitability of *E. faecium* as a surrogate strain for controlling *Salmonella* and *L. monocytogenes* in DSC during thermal treatment.

## 2. Materials and methods

### 2.1. Bacterial strains and growth conditions

A 3-strain *Salmonella* cocktail, 3-strain *L. monocytogenes* cocktail, and *E. faecium* NRRL B-2354 were used in this study. Information about strains, serovars, and source are provided in Table 1. All strains were stored at −80 °C in trypticase soy broth supplemented with 0.6% (w/v) yeast extract (TSBYE) (Hardy Diagnostics, Santa Maria, CA) and 20% glycerol. The bacterial cultures were individually activated twice in TSBYE by culturing at 35 ± 2 °C for 24 h. DSC was inoculated with lawn-grown bacterial inoculum. Each bacterial strain was prepared by plating onto sterile tryptic soy agar with 0.6% yeast extract (TSAYE) (Hardy Diagnostics, Santa Maria, CA) at 35 ± 2 °C for 24 h. Each bacterial lawn was collected from TSAYE using a plastic hockey-stick spreader in 5 mL of phosphate-buffered saline (PBS, pH 7.4), and centrifuged at 8000×g, 4 °C for 15 min (Centrifuge 5810 R®, Eppendorf North America, Hauppauge, NY). The resulting pellet was re-suspended in sterile PBS to achieve ~10<sup>10–11</sup> CFU/mL. Equal volumes of the resuspended bacterial suspensions were combined to obtain the 3-strain cocktail of *Salmonella*

**Table 1**  
Bacterial strains used in this study.

Designation	Strain and Serovar	Origin	Source
PT-30	<i>Salmonella</i> Enteritidis	Raw almonds	Dr. Linda Harris, UC Davis
K4643	<i>Salmonella</i> Tennessee	Peanut butter	Dr. Nathan Anderson, FDA
447967	<i>Salmonella</i> Agona	Toasted oat cereal	Dr. Nathan Anderson, FDA
NRRL B-57618	<i>Listeria monocytogenes</i> 1/2a	Human clinical isolate	USDA-ARS
NRRL B-33053	<i>Listeria monocytogenes</i> 4b	Coleslaw outbreak	USDA-ARS
NRRL B-33466	<i>Listeria monocytogenes</i> 1/2b	Environmental isolate	USDA-ARS
NRRL B-2354	<i>Enterococcus faecium</i>	Milk and ice cream mix	USDA-ARS

UC Davis: University of California, Davis; USDA-ARS: United States Department of Agriculture- Agricultural Research Service; FDA: United States Food Drug and Administration.

or *L. monocytogenes* inoculums.

### 2.2. Inoculation of desiccated shredded coconut

For this study, natural, unsweetened 100% DSC was purchased from a local store. To determine the background microbiota, three 10-g portions of DSC were added to 90 mL sterile PBS, homogenized for 2 min with a stomacher (Stomacher® 400 Circulator, U.K.), serially diluted, and plated on TSAYE in duplicate. The counts were enumerated after incubating at 35 ± 2 °C for 48 h. For bacterial inoculation of DSC, inside a biosafety cabinet, 400 µL of a 3-strain *Salmonella* cocktail, 3-strain *L. monocytogenes* cocktail, or *E. faecium* NRRL B-2354 was added to 40 g of DSC in a stomacher bag (Nasco Whirl-Pak™, Fisher Scientific) to achieve approximately 10<sup>8–9</sup> CFU/g of DSC. These inoculated DSC samples in a stomacher bag were tightly sealed, then hand-mixed vigorously for 10 min to ensure consistent distribution of inoculum.

The inoculated DSC samples were divided into two 150 mm Petri dishes (Fisher Scientific, USA) and spread in an even layer, then placed in an  $a_w$ -equilibration chamber (custom designed at Michigan State University). The inoculated samples were equilibrated for a minimum of 4 days at room temperature (RT, 22 ± 0.5 °C) to achieve target  $a_w$  (0.25 and 0.45). These  $a_w$  values present a low and high boundary  $a_w$  of DSC under different environmental relative humidities. The  $a_w$  of the individual samples were monitored and samples were used for thermal inactivation after reaching the target  $a_w$  ± 0.02 as measured by a water activity meter (Aqualab Series 3, Decagon Devices, Inc., Pullman, WA). The bacterial population was determined immediately after inoculation, and after four days of equilibration. Four 1-g inoculated DSC samples were randomly sampled, serially diluted, and enumerated as shown in the 2.4 section. The initial inoculation level of each prepared sample was 10<sup>8–9</sup> CFU/g; this level was maintained during equilibration.

### 2.3. Thermal inactivation

The heat resistance (D- and z-values) of *Salmonella*, *L. monocytogenes*, and *E. faecium* in DSC were determined from the inoculated and equilibrated samples. About 0.50 g of DSC was loaded into thermal death time (TDT) aluminum test cells (18 mm diameter, 4 mm thickness) (Chung, Birla, & Tang, 2008), sealed and then subjected to isothermal treatments in an ethylene glycol oil bath (Isotemp Heat Bath Circulator, Model 5150 H24, Fisher Scientific). The thermal treatments for *Salmonella* and *E. faecium* were at 80, 85, and 90 °C, while those for *L. monocytogenes* were conducted at 75, 80, and 85 °C. The treatment temperatures were selected based on the common pasteurization temperature range and preliminary tests to yield desired levels of thermal inactivation of the target bacteria in DSC. The loaded TDT test cells with T-type thermocouples at the sample geometrical center were used to determine the come-up-time (CUT), the time required for the sample center to reach within 0.5 °C of the target temperature. The CUT measured at 75–95 °C was 1.5 min. The time at CUT was set to 0 min, i.e. the timing of heat treatment was initiated at CUT. For each isothermal treatment, three TDT test cells were taken out at each of five selected time points, then immediately chilled in an ice-water bath for 2 min to stop further inactivation of bacteria. Each thermal inactivation was repeated three times independently.

### 2.4. Bacterial enumeration

The heat-treated DSC sample was transferred from TDT cells to a Whirl-Pak bag (Nasco, Ft. WI) and diluted at 1:10 with sterile 1 × PBS, pH 7.4, hand massaged for 0.5 min and further homogenized for 2 min at 230 rpm in a stomacher (Stomacher® 400 Circulator, U.K.). The resulting bacterial suspensions were 10-fold serially diluted and 100 µL of an appropriate dilution were plated on TSAYE plates in duplicate followed by incubation at 35 ± 2 °C for 24–48 h and counted for microbial populations which were converted to log CFU per gram. The

detection limit was 10 log CFU/g.

## 2.5. D-value and z-value analysis

To analyze and compare the inactivation kinetics of isothermal treatment of *Salmonella*, *L. monocytogenes*, and *E. faecium*, the first-order kinetic/log-linear model was used in this study (Equation (1)):

$$\log(N/N_0) = -t/D \quad (1)$$

where  $N_0$  is the bacterial population (CFU/g) at CUT,  $N$  is the population of survivors (CFU/g) at the time ( $t$ );  $t$  is the isothermal treatment time (min) after the CUT,  $D$  is the time in min required to reduce the microbial population by 90% at a selected temperature ( $^{\circ}\text{C}$ ).  $D$ -value was estimated from the thermal inactivation curve using log-linear regression analysis. The  $z$ -values in  $^{\circ}\text{C}$  were determined from the regression of log  $D$ -value versus temperature and were calculated as  $z = \text{slope}^{-1}$  for the linear trend lines. The survival data of bacteria after isothermal treatment was applied to fit the first-order kinetic model and estimate the model parameter. The goodness-of-fit of the model was quantified to interpret the performance of the model by the root mean square error (RMSE) (log CFU/g) (Motulsky & Christopoulos, 2004) and the data were analyzed through the Integrated Pathogen Modeling Program (IPMP) (Huang, 2014):

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

where  $\log\log(\frac{N}{N_0})_{\text{data},i}$  is the measured log reduction,  $\log\log(\frac{N}{N_0})_{\text{model},i}$  is the predicted log reduction from the model,  $n$  is the total number of observations, and  $p$  is the number of model parameters.

## 2.6. Statistical analyses

Data were analyzed by one-way Analysis of Variance (ANOVA) and mean differences were separated by Tukey's multiple-comparison test using the generalized linear model from Statistical Analysis Systems (SAS, 2000).  $P < 0.05$  were considered significant.

## 3. Results

### 3.1. Thermal inactivation of *Salmonella* and *L. monocytogenes* in desiccated shredded coconut

The populations of *Salmonella*, *L. monocytogenes*, and *E. faecium* were stable during the initial four days of equilibration in the moisture chambers. At  $a_w$  0.25, from initial  $\sim 9$  log CFU/g, there were 0.46, 0.56, and 0.29 CFU/g log reductions of *Salmonella*, *L. monocytogenes*, and *E. faecium*, respectively. The inactivation kinetics of both *Salmonella* and *L. monocytogenes* in DSC fitted the log-linear model well (Fig. 1A and B). At a selected temperature, the  $D$ -values of both *Salmonella* and *L. monocytogenes* in DSC increased as the  $a_w$  of DSC decreased ( $P < 0.05$ ). For the samples that were preconditioned to  $a_w$  0.25, the  $D$ -values calculated from the slope of the trend lines for *Salmonella* at 80, 85, and  $90^{\circ}\text{C}$  were  $53.2 \pm 0.8$ ,  $28.0 \pm 0.5$  and  $12.5 \pm 0.1$  min, respectively (Table 2 and Fig. 1A). The  $D$ -values for *Salmonella* in  $a_w$  0.45 DSC were smaller with  $38.7 \pm 0.9$ ,  $15.5 \pm 0.3$ , and  $6.0 \pm 0.1$  min, respectively, under the same temperatures. Similarly, the thermal tolerance of *L. monocytogenes* in DSC was inversely related to  $a_w$  of DSC. The  $D$ -values for *L. monocytogenes* at 80 and  $85^{\circ}\text{C}$  were  $40.2 \pm 1.2$  and  $17.1 \pm 2.0$  min in  $a_w$  0.25 DSC and  $14.2 \pm 0.9$  and  $6.2 \pm 1.6$  min in  $a_w$  0.45 DSC, respectively (Table 2 and Fig. 1B). Data indicated that the thermal resistance of *Salmonella* in DSC was greater than that of *L. monocytogenes* at each  $a_w$  and temperature combination (Table 2). The  $z$ -values for *Salmonella* and *L. monocytogenes* in  $a_w$  0.25 DSC were 15.9 and  $14.4^{\circ}\text{C}$ , respectively, and  $12.4^{\circ}\text{C}$  and  $13.9^{\circ}\text{C}$  in  $a_w$  0.45 DSC, respectively

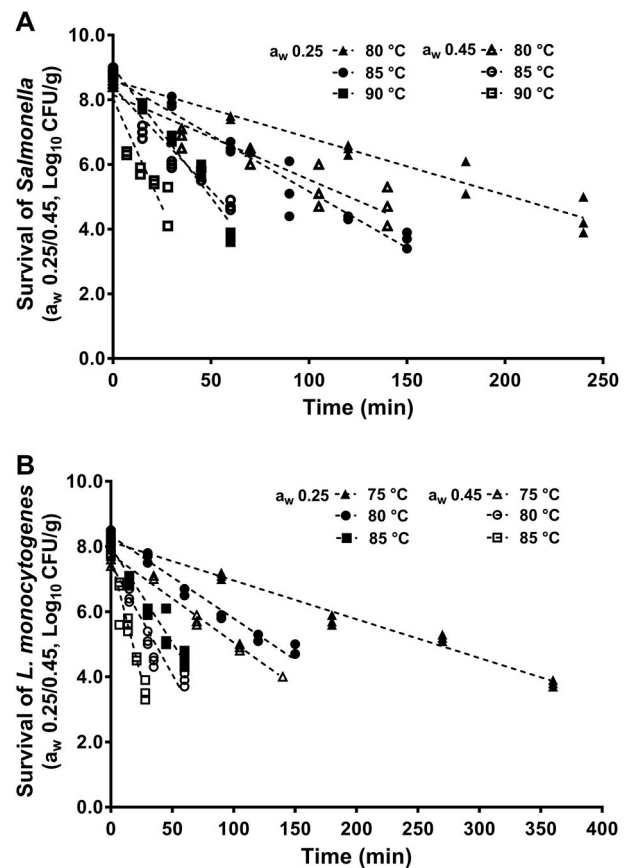


Fig. 1. Representative thermal inactivation kinetic curves of *Salmonella* and *L. monocytogenes* in desiccated shredded coconut at selected temperatures. (A) *Salmonella*. (B) *L. monocytogenes*. The time at CUT was set to 0 min. Experiments were independently repeated thrice.  $a_w$ : water activity measured at  $22^{\circ}\text{C}$ .

(Table 2, Fig. 3A and B).

### 3.2. The suitability of *E. faecium* NRRL B-2354 as a surrogate strain of *Salmonella* in desiccated shredded coconut

The above thermal inactivation indicated that *Salmonella* is more heat resistant than *L. monocytogenes* in DSC, thus *Salmonella* was further selected to be compared with *E. faecium*, a presumable surrogate of *Salmonella* in various  $a_w$  (Kataoka et al., 2014; Perez-Reyes, Jie, Zhu, Tang, & Barbosa-Canovas, 2021). At  $a_w$  0.25 DSC, the  $D$ -values of *E. faecium* were  $85.5 \pm 1.8$ ,  $40.0 \pm 0.6$  and  $24.2 \pm 0.3$  min at 80, 85, and  $90^{\circ}\text{C}$ , respectively, which were 1.4–1.9 times the  $D$ -values of *Salmonella* under the same temperatures (Table 2 and Fig. 2A). In  $a_w$  0.45 DSC, the  $D_{80^{\circ}\text{C}}$ -value of *E. faecium* was 1.3 times that of *Salmonella* ( $49.6 \pm 1.0$  min vs  $38.7 \pm 0.9$  min) (Table 2 and Fig. 2B), and the  $D$ -values of *E. faecium* at  $85^{\circ}\text{C}$  and  $90^{\circ}\text{C}$  were not different ( $P > 0.05$ ) from those of *Salmonella* (Table 2 and Fig. 2B). The  $z$ -values of *E. faecium* at  $a_w$  0.25 and 0.45 were 18.2 and  $11.4^{\circ}\text{C}$ , respectively (Table 2; Fig. 3C). These data indicated that *E. faecium* is a suitable surrogate strain of *Salmonella* in the thermal processing of DSC.

## 4. Discussion

### 4.1. Thermal resistance of *Salmonella* and *L. monocytogenes* in desiccated shredded coconut

The  $D$ -values of *Salmonella* and *L. monocytogenes* in DSC increased significantly as the  $a_w$  decreased, showing increased thermal resistance of *Salmonella* and *L. monocytogenes* under desiccated conditions. This

**Table 2**

Thermal inactivation parameters of *Salmonella*, *L. monocytogenes* and *E. faecium* NRRL B-2354 in desiccated shredded coconut.

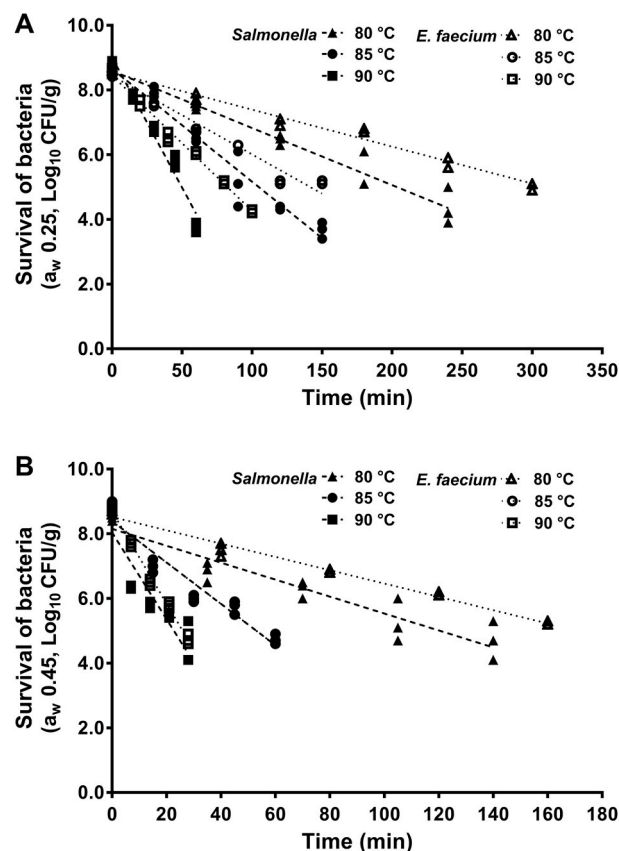
$a_w$	Strains	Temperature (°C)	D-value (min)	95% CI upper limit of D-value	RMSE	z-value (°C)
0.25	<i>Salmonella</i>	80	53.2 ± 0.8 <sup>ab</sup>	57.2	0.16	15.9 ± 0.2
		85	28.0 ± 0.5 <sup>ab</sup>	29.0	0.22	
		90	12.5 ± 0.1 <sup>aA</sup>	14.0	0.36	
	<i>L. mono</i>	75	84.2 ± 1.0 <sup>a</sup>	91.9	0.27	14.4 ± 0.7
		80	40.2 ± 1.2 <sup>aA</sup>	47.2	0.43	
		85	17.1 ± 2.0 <sup>aA</sup>	20.2	0.40	
	<i>E. faecium</i>	80	85.5 ± 1.8 <sup>aC</sup>	93.8	0.28	18.2 ± 0.2
		85	40.0 ± 0.6 <sup>aC</sup>	42.1	0.21	
		90	24.2 ± 0.3 <sup>ab</sup>	25.7	0.18	
0.45	<i>Salmonella</i>	80	38.7 ± 0.9 <sup>bb</sup>	46.8	0.47	12.4 ± 0.2
		85	15.5 ± 0.3 <sup>bb</sup>	17.4	0.39	
		90	6.0 ± 0.1 <sup>bA</sup>	9.4	0.56	
	<i>L. mono</i>	75	37.4 ± 1.0 <sup>b</sup>	34.2	0.29	13.9 ± 0.8
		80	14.2 ± 0.9 <sup>bA</sup>	14.3	0.28	
		85	6.2 ± 1.6 <sup>bA</sup>	7.3	0.35	
	<i>E. faecium</i>	80	49.6 ± 1.0 <sup>bC</sup>	55.2	0.21	11.4 ± 0.3
		85	17.0 ± 0.7 <sup>bb</sup>	18.8	0.18	
		90	6.5 ± 0.3 <sup>bA</sup>	6.9	0.19	

The D-values are the means of three independent trials, expressed as Mean ± SEM. RMSE: the root means square error, log<sub>10</sub> CFU/g which is the goodness-of-fit of the model.  $a_w$ : water activity measured at 22 °C; *L. mono*: *L. monocytogenes*. <sup>a-b</sup>Means of each strain at the selected temperature without a common letter differ significantly ( $P < 0.05$ ) between different  $a_w$ . <sup>A-C</sup>Means of D-values at the selected temperature and  $a_w$  without a common letter differ significantly ( $P < 0.05$ ) among different strains.

inverse relationship between  $a_w$  and D-values has been established for *Salmonella* in several La<sub>w</sub>F including wheat flour (Liu et al., 2018), cocoa powder (Tsai, Ballom, et al., 2019), almond kernels (Villa-Rojas et al., 2013), and peanut butter (He et al., 2013), and *L. monocytogenes* in wheat flour (Taylor, Tsai, Rasco, Tang, & Zhu, 2018), cocoa powder (Tsai, Taylor, et al., 2019), nonfat dry milk powder (Ballom et al., 2020), and almond meal (Zhu, Song, Shen, & Tang, 2020).

Mechanisms responsible for the increased resistance within the La<sub>w</sub>F are not well understood. Limited studies on *Salmonella* indicated that the observed greater heat resistance in low-moisture environments might be due to an increase in potassium influx by the *kdp* transporter (Gruzdev et al., 2012), expression of heat and cold shock proteins, Fe-S clusters, sigma factors (*rpoE* and *rpoS*) (Deng, Li, & Zhang, 2012), osmoprotectant transport (*proPU* and *osmU*) (Finn et al., 2013), trehalose synthesis (Li, Bhaskara, Megalis, & Tortorello, 2012), and others, which together confer protection to heat treatments.

Besides  $a_w$ , the rate of bacterial thermal inactivation in La<sub>w</sub>F was impacted by various factors such as the structure of the food matrices (Steinbrunner et al., 2019), the composition of the food (Limcharoenchat et al., 2018; Rachon, Penalzoza, & Gibbs, 2016), source of contamination or local microenvironment (Li et al., 2014), and

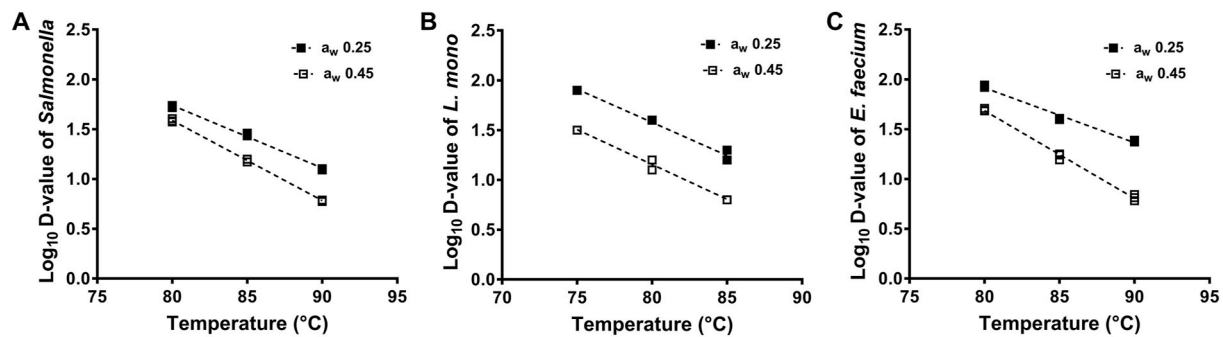


**Fig. 2.** Representative thermal inactivation kinetic curves of the *E. faecium* NRRL B-2354 and *Salmonella* in desiccated shredded coconut at selected temperatures. (A)  $a_w$  0.25. (B)  $a_w$  0.45. The time at CUT was set to 0 min. Experiments were independently repeated thrice.  $a_w$ : water activity measured at 22 °C.

inoculation method (Limcharoenchat et al., 2018). The D-values of *Salmonella* and *L. monocytogenes* in DSC obtained in this study regardless of  $a_w$  were higher than those observed in other food matrices. For instance, the D<sub>80°C</sub>-value of *Salmonella* in  $a_w$  0.45 DSC was 5.4–5.5 times that of wheat flour (Liu et al., 2018) and cocoa powder (Tsai, Ballom, et al., 2019). The D<sub>80°C</sub>-value of *L. monocytogenes* in  $a_w$  0.45 DSC was 3–8 times those in  $a_w$  0.45 non-fat dry milk (Ballom et al., 2020), wheat flour (Taylor et al., 2018), and cocoa powder (Tsai, Taylor, et al., 2019). The D<sub>75°C</sub>- and D<sub>80°C</sub>-values of *L. monocytogenes* in  $a_w$  0.25 DSC were 2.5 and 2.8 times those in  $a_w$  0.25 non-fat dry milk (Ballom et al., 2020).

According to the manufacturer, the DSC contains ~60% fat. *Salmonella* showed greater thermal resistance in fat-rich peanut butter and peanut paste (He et al., 2013; Shachar & Yaron, 2006). The observed greater thermal resistance in desiccated shredded coconut could be attributed to its high-fat content. D<sub>80°C</sub>-values of *Salmonella* in  $a_w$  0.45 and  $a_w$  0.25 DSC in this study were 1.9 and 1.8 times that of *S. Enteritidis* in blanched almond flour containing 48.8% fat at respective  $a_w$  (Xu et al., 2019). Similarly, D<sub>80°C</sub>-values of *L. monocytogenes* in  $a_w$  0.45 and  $a_w$  0.25 DSC was 1.8 and 1.3 times that in  $a_w$  0.45 and  $a_w$  0.25 in almond meal with ~51.6% fat content (Zhu et al., 2020). However, fat might not be solely responsible for higher heat resistance as *Salmonella* in low fat and high carbohydrate peanut butter showed a greater heat resistance than that in high fat and low carbohydrate peanut butter (He et al., 2013). Further studies are warranted to examine bacterial thermal resistance in DSC with differing fat contents.





**Fig. 3.** Log D (decimal reduction time to achieve 90% population reduction at the selected temperature) values of the *Salmonella*, *L. monocytogenes*, and *E. faecium* NRRL B-2354 at  $a_w$  0.25 and 0.45 desiccated shredded coconut at different temperatures. (A) *Salmonella*. (B) *L. monocytogenes*. (C) *E. faecium* NRRL B-2354.  $a_w$ : water activity measured at 22 °C.

#### 4.2. Suitability of *E. faecium* as a surrogate strain for controlling *Salmonella* and *L. monocytogenes*

In our study, *Salmonella* thermal resistance was 1.3–2.7 times of *L. monocytogenes* at the selected  $a_w$  and treatment temperatures, thus, *Salmonella* was selected as a target pathogen of concern during thermal processing of DSC in this study. Previous studies on cocoa powder (Tsai, Ballom, et al., 2019; Tsai, Taylor, et al., 2019) and wheat flour (Liu et al., 2018; Smith, Hildebrandt, Casulli, Dolan, & Marks, 2016; Taylor et al., 2018) also showed that *L. monocytogenes* was less or similarly heat resistant than *Salmonella*.

The Preventive Controls for Human Food Rule (PCHF) under Food Safety Modernization Act (FSMA) (FDA, 2018a) requires the food industry to implement and validate intervention processes for the prevention and control identified hazards, including *Salmonella* in La<sub>w</sub>F to achieve 4 or 5 log reduction via specific processing techniques. To comply with the PCHF requirement, the low-moisture food industry needs to know the actual microbial reduction of a process to document their process controls, which requires a reliable verified surrogate strain that can be used to predict the fate of the target foodborne pathogen, *Salmonella*, during the thermal process of DSC. Ideally, a surrogate strain is a non-pathogenic microorganism that exhibits similar or greater resistance than the target pathogen under the same processing conditions (FDA, 2000). *E. faecium* NRRL-B 2354 was identified as a surrogate of *Salmonella* during almond thermal processing by the Almond Board of California (ABC, 2014). The genomic and functional analyses indicated that *E. faecium* NRRL-B 2354 does not contain virulence genes (Kopit, Kim, Siezen, Harris, & Marco, 2014). Our data indicated that the D-values of *E. faecium* at  $a_w$  0.25 and  $a_w$  0.45 DSC were similar or higher than those of *Salmonella* under the same temperatures, indicating its suitability as a surrogate of *Salmonella* for DSC thermal processing. Consistently, *E. faecium* was shown to be a valid surrogate for *Salmonella* in other La<sub>w</sub>F, where D-values for *E. faecium* were significantly greater in wheat flour (Liu et al., 2018), corn flour (Ozturk et al., 2019), egg powders (Perez-Reyes et al., 2021) and pet foods (Rachon et al., 2016) as compared to *Salmonella*, or similar for those bacteria in peanut kernels, in-shell pistachios, pecans and sunflower kernels (Arias-Rios et al., 2019; Brar & Danyluk, 2019; Moussavi, Frelka, Hildebrandt, Marks, & Harris, 2020). However, *E. faecium* is not always a suitable surrogate for *Salmonella*. For example, the D<sub>80°C</sub>-value of *E. faecium* in the confectionery ( $a_w$  0.57) was smaller than that of *Salmonella* (Rachon et al., 2016). In  $a_w$  0.45 brown rice flour, the suitability of *E. faecium* as a surrogate of *Salmonella* was a function of temperature; the D-values of *E. faecium* at 80 and 85 °C smaller than those of *Salmonella*, though the D-values of *E. faecium* at 70 and 75 °C were 1.3–1.5 times of *Salmonella* (Jin & Tang, 2019). Given that *Salmonella* is more thermal tolerant in DSC compared to *L. monocytogenes*, *E. faecium* can be considered as a suitable surrogate strain for controlling *Salmonella* and *L. monocytogenes* during DSC thermal processing.

#### 5. Conclusion

The thermal resistance as indicated by D-values of *Salmonella* and *L. monocytogenes* in DSC increased as the  $a_w$  of DSC decreased from 0.45 to 0.25. *Salmonella* was found to be more heat resistant than *L. monocytogenes* in DSC at the selected  $a_w$  and treatment temperature. *E. faecium* was a suitable surrogate strain for controlling *Salmonella* and *L. monocytogenes* during thermal processing of DSC. A 32.5 min heating at 90 °C caused a 5.0 and 5.4 log CFU/g reduction of *E. faecium* and *Salmonella*, respectively, in  $a_w$  0.45 DSC, while a 31 min heat treatment at 85 °C can result in a 5.0 log CFU/g reduction of *L. monocytogenes* in  $a_w$  0.45 DSC. Data from this study provide important information to the food industry in developing thermal pasteurization processes to effectively control *Salmonella* and *L. monocytogenes* in DSC.

#### CRedit authorship contribution statement

**Nitin Dhowlaghar:** Conceptualization, Investigation, Formal analysis, Writing – original draft. **Juming Tang:** Writing – review & editing. **Mei-Jun Zhu:** Conceptualization, Supervision, Visualization, Writing – review & editing.

#### Declaration of competing interest

There were no conflict of interest.

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