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Isothermal inactivation of *Enterococcus faecium* NRRL B-2354 as surrogate for *Salmonella* in oat flour

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ABSTRACT

This study identified the survival kinetic and thermal resistance (*D*- and *z*-values) of *Salmonella* with its potential surrogate, *Enterococcus faecium*, in oat flour during isothermal processing. Oat flour (a_w = 0.40) was uniformly inoculated with *S.* Enteritidis PT30 and *E. faecium* (10^{8-9}CFU/g). Samples (1 ± 0.1 g) were in plastic bags heated at 75, 80, and 85°C to assess bacterial survival. The survival inactivation of *S.* Enteritidis PT30 and *E. faecium* were analyzed using Log-linear and Weibull models. The survival curves followed first-order kinetic. $D_{75°C}$, $D_{80°C}$ and $D_{85°C}$ values of *S.* Enteritidis PT30 and *E. faecium* in oat flour decreased from 16.08 ± 1.36 to 2.27 ± 0.35 min and 23.61 ± 1.39 to 3.87 ± 0.42 min respectively, with corresponding z-values of 13.4 ± 0.37 and 12.1 ± 0.41°C. Results from this research can be applied to validate the thermal process to ensure the low moisture food safety.

Keywords: *E. faecium*, *Salmonella*, oat flour, thermal processing

Yulaf ununda *Salmonella* ve *Enterococcus faecium* NRRL B-2354'ün izotermal inaktivasyon kinetiği

ÖZ

Bu çalışmada *Salmonella*'nın ısıl işlem sırasında yulaf ununda hayatta kalma ve ısıl toleransının (*D*- ve *z*-değerleri) patojen özellik göstermeyen *Enterococcus faecium* ile birlikte belirlenmesi amaçlamıştır. Yulaf unu (a_w=0.40) örnekleri nem kontrollü bir ortamda *S.* Enteritidis PT30 ve *E. faecium* ile eşit şekilde inoküle (10^{8-9} CFU/g) edilmiştir. Un örnekleri (1±0.1 g) daha sonra plastik torbalarda vakumlanarak paketlenmiş ve bakterilerin sıcaklık direncini değerlendirmek üzere 75, 80 ve 85°C'de sıcak su banyosunda ısıl işleme tabi tutulmuştur. *S.* Enteritidis PT30 ve *E. faecium*'un hayatta kalma eğrileri, iki farklı model (Log-lineer ve Weibull) kullanılarak analiz edilmiş ve mikroorganizmaların hayatta kalma eğrilerinin birinci derece kinetik modelini takip ettiği gözlemlenmiştir. *S.* Enteritidis PT30 ve *E. faecium*'un yulaf ununda *D*-değerlerinin sıcaklık artışı ile 16.08±1.36'dan 2.27±0.35 dakikaya ve 23.61±1.39'dan 3.87±0.42 dakikaya düştüğü gözlemlenirken, karşılık gelen *z*-değerleri 13.41±0.37 ve 12.13±0.41°C olarak belirlenmistir. Bu araştırmadan elde edilen *D*- ve *z*-değerleri, düşük nemli gıdaların mikrobiyal güvenliğini sağlamak adına ısıl işlemlerin doğrulanmasında kullanılabilir.

Anahtar Kelimeler: *E. faecium*, *Salmonella*, Isıl işlem, Yulaf unu

INTRODUCTION

Foodborne pathogens have become a significant global food safety concern. In 2017, 841 foodborne pathogen-related outbreaks were reported, resulting in numerous illnesses, hospitalizations, and tragically, some deaths [1]. Most outbreaks (29%) and illness (34%) are linked to *Salmonella* in various foods [1]. This emphasizes the critical need for thorough preventive measures and efficient control strategies to reduce the risks linked to *Salmonella*. Historically, low-moisture foods (LMF) including flour and dry vegetables have been considered to have minimal risk of microbial contamination. This is primarily because their water activity (below 0.70) acts as a barrier, preventing the growth of microorganisms [2]. Despite their generally perceived safety, LMFs have often been involved in multiple recalls and incidents. From 2006 to 2019, there were 26 salmonellosis outbreaks reported due to the contamination of LMFs such as nuts [3, 4, 5], spices [6], peanut butter [7], pet food [8], and cereals [9]. The presence of *Salmonella* in LMFs (particularly flours) poses a significant hazard, increasing the need for enhanced preventive measures to minimize or eliminate outbreaks [10, 17, 18, 22, 40]. Hence, *Salmonella* presents a growing concern within LMFs, evident from various recalls and outbreaks. Typically, the thermal resistance of microorganisms specific to the product formulation is considered to guide the industrial pasteurization protocols when developing industrial isothermal processes [11]. Although food processing environments generally exclude pathogens, innovative food safety approaches

may still necessitate microbial validation. Therefore, a surrogate has been identified as a non-pathogenic organism used to simulate the behavior of a pathogen under specific conditions. For example, *Enterococcus faecium* NRRL B-2354 (*E. faecium*) is often considered as a surrogate for *Salmonella* in thermal studies due to its similar heat resistance properties in LMFs [12]. This surrogate has been instrumental in validation studies for various food processing technologies used with LMFs, including extrusion [14], moist-air convection heating [15], infrared pasteurization [16], and radio frequency heating [17, 18]. This study examines the isothermal inactivation of *S*. enteritidis PT30 and the use of *E. faecium* as a surrogate in oat flour for process validation.

MATERIAL AND METHOD

Oat flour (OF) samples with a 10.3 % w.b. moisture content, as indicated on the package, were obtained commercially from a local store in Nottingham, UK. Water activity ($a_{w,\ 25°C}$) was measured by using a a_w meter (Decagon Devices, Pullman, WA).

Bacterial inoculation of oat flour

S. Enteritidis PT30 and *E. faecium* were grown in TSB (9 ml) (Tryptic Soy Broth) (BD, Sparks, MD, USA) extracted with 0.6% (w/v) yeast for 24 h at 37°C. Subsequently, the culture (1 ml) was evenly spread on TSA (Tryptic Soy Agar) (BD, USA) plates. The lawn of both microorganisms formed on TSA was then collected into 20 ml (0.1% PW (Peptone water)) (BD, USA) and centrifuged at 2600 g for 30 min as explained in [19]. After removing the supernatant, the resuspended pellet in 4 ml (0.1% PW) was used to inoculate OF sample by following the method described by [19]. The concentrated pellet (1 ml) of *S. Enteritidis* PT30 or *E. faecium* was added to 10 g of OF in a sterile bag and mixed thoroughly until a homogeneous mixture was achieved. Then, 10 g of the inoculated OF were used to uniformly inoculate 90 g of OF which was then homogenized using a mini stomacher (Norfolk, UK) for 5 min at 260 rpm. To avoid the impact of a_w on the thermal tolerance of both microorganisms in OF, samples were spread on the trays and then placed in a humidity controller (SP Industries, PA, USA) for up to 3 days to achieve the target a_w level of 0.4 at 25°C.

Isothermal treatment

The inoculated samples (1 ± 0.1 g), sealed in sterile plastic bags, were arranged in a holder and completely submerged in a heating bath (Fisher Scientific, Newington, USA) set at 75, 80, and 85°C. The duration to achieve the process temperature, approximately 6 s, was not considered as a factor in the reduction of the total bacterial count due to high thermal resistance and log heating time as reported by [20]. Samples were removed from the water bath at various time intervals, up to 120 min based on the specific process conditions. After heating, the samples were quickly placed in an iced bath. Each experiment was performed at least three times on different dates to ensure the reproducibility and accuracy of the results.

Enumeration of microbial survival

Following the heat treatment, each sample, including both treated and control groups, was aseptically opened and transferred into stomacher bags. Control samples were utilized to verify the inoculation level of each microorganism in OF prior to thermal treatment. They were subsequently diluted using 0.1% PW at a 1:10 ratio. Initially, the heated OF were homogenized using a stomacher for up to 5 min at 260 rpm, as described in [21]. To enumerate the survival of *Salmonella Enteritidis* PT30 and *E. faecium* in OF, the dilutions were transferred on mTSA and eTSA, respectively, as described in [19, 22]. The composition of modified mTSA and eTSA were listed in [18, 19]. The plates were stored aerobically for up to 24-48 h at 37°C h, followed by enumeration of colonies and conversion of populations to log CFU/g. The final concentration of *S.* Enteritidis PT30 and *E. faecium* in OF (control) was determined to be approximately $10^{8\text{-}9}$ CFU/g. No bacterial growth of *Salmonella* and *E. faecium* was observed in un-inoculated OF using the same plating technique used in this study. Hence, the natural microflora was not considered. Log reductions were determined by comparing the initial population to the survivor counts after thermal treatment.

Modeling inactivation kinetics

The isothermal kinetics of both microorganisms were described using following primary models below [23],

The first-order kinetic (Eq. 1):

$$\log\left(\frac{N}{N_0}\right) = -\frac{t}{D} \qquad \text{Eq. 1}$$

Weibull (Eq. 2):

$$\log\left({}^{N}/_{N_0}\right) = -\left[{}^{t}/_{\delta}\right]^{\alpha} \qquad \text{Eq. 2}$$

N and N_0 represent the bacterial count (CFU/g) at times (t) and 0, respectively; t represents the duration of the thermal process (min) after come-up time (CUT), and D is the required time (min) to achieve one log (10-fold) reduction in microbial population at a specific temperature (°C); δ indicates to the steepness of the inactivation curve of bacterial population; α refers the shape of the curve, being linear (α=1) or nonlinear (α≠1) with decreasing (α <1) or increasing (a>1) inactivation rate over time [19].

The survival data were applied to both models to describe the model fitness using the root mean square error (RMSE) (Eq. 3) (log CFU/g) [24].

$$RSME = \sqrt{\frac{\sum_{i=1}^{n}\left(\log\left(N/N_0\right)_{data,i} - \log\left(N/N_0\right)_{model,i}\right)^2}{n-p}}$$

$\log(N/N_0)_{data,i}$ is observed log reductions, and $\log(N/N_0)_{model,i}$ is the estimation of log reductions. n represents the count of observations, and p denotes model parameters. RMSE was calculated by using three independent replicates. The Integrated Pathogen Modeling Program (IPMP) [25] was employed to evaluate model's fitness and directly provide RMSE. The differences in *D*-values in each sample heated at different temperatures were analyzed using ANOVA (Minitab Inc., State College, PA). The log of *D*-values is plotted against heating temperature, which is commonly referred to as the isothermal death time when the log-linear model appropriately shows survival curves. The slope of the obtained curves (-1/z) is where (z) represents the change in process temperature required to shift a one-log cycle in the *D*-value.

RESULTS AND DISCUSSION

D and z- values in oat flour

The natural microbial count in OF samples was determined to be as 2.29 ± 0.32 log CFU/g. This count was sufficiently low to ensure it would not affect the counts obtained during the inactivation process. Figure 1 represents the survival kinetics of both *S*. Enteritidis PT30 (a) and *E. faecium* (b) in OF at $a_{w,25°C}$ = 0.40.

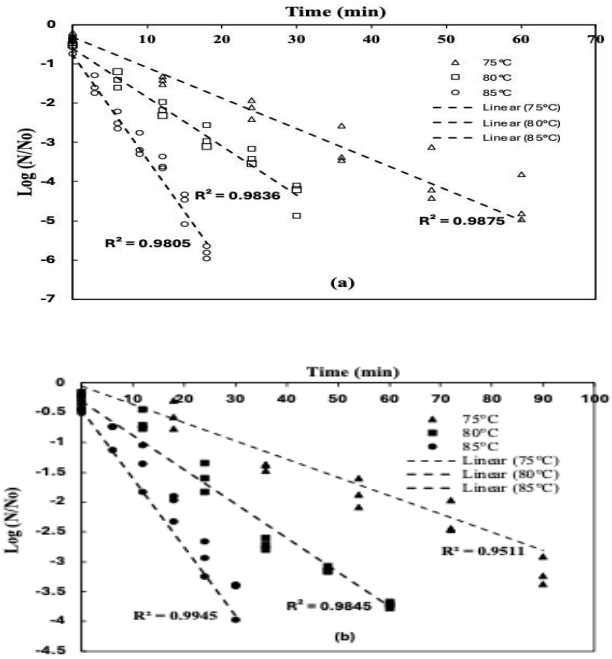

Figure 1 The inactivation curves for *S*. Enteritidis PT30 (a) and *E. faecium* (b) in OF with an a_w of 0.40 at 25ºC were determined at various temperatures. Each experiment was independently repeated three times.

The survival data for both *S. Enteritidis PT30* and *E. faecium* aligned well with the models, showing comparable RMSE values (Table 1).

Table 1 Estimates for the parameters of the primary models, along with RMSE values

		Linear Model		Weibull Model		
	Temperature (ºC)	D-value (min)	RMSE (log CFU/g)	δ(min)	α	RMSE (log CFU/g)
Salmonella	75	17.08 ± 1.36	0.51	17.56 ± 3.42	1.65 ± 0.14	0.72
	80	7.34 ± 0.69	0.27	8.27 ± 0.64	0.86 ± 0.08	0.42
	85	2.27 ± 0.35	0.21	1.92 ± 0.46	0.72 ± 0.07	0.33
E. faecium	75	20.61 ± 1.39	0.32	24.42 ± 4.56	1.13 ± 0.38	0.52
	80	11.56 ± 0.96	0.42	10.12 ± 0.92	0.64 ± 0.26	0.61
	85	3.87 ± 0.42	0.19	2.78 ± 0.62	0.72 ± 0.07	0.27

* RMSE was calculated by IPMP software using experimental observations
* Lower RMSE signify a better model fit.

This study defined the thermal resistance of each microorganism by employing the log-linear model (Figure 2).

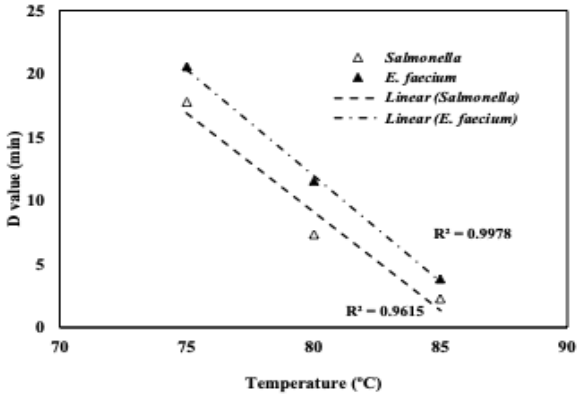

Figure 2 Thermal tolerance of *Salmonella* and *E. faecium* in oat flour.

Given that the Weibull model did not significantly enhance the fitness of inactivation curves compared to the log-linear model. Thus, the thermal tolerance of both *Salmonella* and *E. faecium* was analyzed using the log-linear model. The *D*-values for *S*. Enteritidis PT30 in OF derived from the slopes of the trend lines at 75, 80, and 85°C, were 16.08 ± 1.36, 7.34 ± 0.69, and 2.27 ± 0.35 min, respectively (Figure 1). As the temperature for inactivation increased, the thermal tolerance of *Salmonella* in OF decreased accordingly. The *D*-values obtained for *Salmonella* in this study align with those reported in the previous studies. For example, [26] and

[27] provided $D_{80°C}$ values of *Salmonella* in wheat flour (WF), with equivalent a_w levels, as 6.9 ± 0.7 and 5.51 ± 0.22 min, respectively. Furthermore, the *D*-values obtained for *S*. Enteritidis PT30 in OF also aligned with those reported for organic WF which was reported as 17.65 ± 1.58, 7.17 ± 0.35, and 2.92 ± 0.35 min at 75°C, 80°C, and 85°C, respectively [18]. The survival population of *Salmonella* in LMFs during thermal inactivation decreases as a_w decreases. Previous studies have reported similar findings on the thermal inactivation of *Salmonella* in LMFs such as spices [17], WF [18, 19], corn flour [22], pet foods [28], skim milk powder [29], and peanut paste [30]. [31] reported a similar correlation between water activity and thermal resistance for *Listeria monocytogenes* in WF. However, the specific mechanisms driving the heightened thermal tolerance of *Salmonella* in LMFs have yet to be fully understood. In addition to a_w, the thermal tolerance of bacteria is influenced by several other factors, such as bacterial strain [32], the stage of bacterial growth [33, 34, the physical structure of the food [35], fat content [36], and carbohydrates [37]. The slight variations in observed *D*- values can therefore be attributed to a combination of variations, such as the bacterial strain, the food matrix composition, a_w, and the physical state of the food. In this study, *E. faecium* exhibits notably greater heat resistance at the tested temperatures, showing a z-value comparable to that of *S.* Enteritidis PT30, as indicated by statistical significance ($p < 0.05$). The *D*-values for *E faecium* are 75, 80, and 85 °C were 23.61 ± 1.39, 11.56 ± 0.96, and 3.87 ± 0.42 min, respectively (Figure 1). As seen in Figure 2, the *D*-value of *Salmonella* decreases at a significantly higher rate with increasing the temperature as compared to those for *E. faecium*. The *z*-values of *S.* Enteritidis and *E. faecium* in OF were 13.4 ± 0.4 and 12.1 ± 0.4 °C, respectively. The data slightly exceeded the obtained *z*-value of *S. Enteritidis* (14.8°C), which was derived from *D*-values in WF with an a_w of 0.43 [26]. Additionally, these values were higher than the z-value of 12.8°C for WF with a a_w of 0.45 at 25°C.

DISCUSSION

Despite traditionally being considered safe, LMFs have been linked to several significant foodborne pathogen outbreaks. From 2006 to 2017, CDC reports documented 59 *Salmonella* outbreaks associated with a diverse range of products, including LMFs [1]. Among these outbreaks, approximately one-third were associated with LMFs [1]. Multiple *Salmonella* strains were linked to outbreaks involving flour (wheat and oat) or raw dough [1], highlighting the need to validate the thermal treatment of OF to prevent potential *Salmonella* contamination and outbreaks. Despite the roasting process involved in flour production, which includes grinding roasted grains, pathogenic bacteria such as *Salmonella* can survive. Additionally, these bacteria might be introduced during later stages, such as storage or transportation. In the current industry, after the processes of roasting and grinding, flours do not undergo any additional heat treatment until they are incorporated as ingredients in various products [38]. To successfully create and apply accurate procedures within a risk-based safety protocol, it is essential to comprehend the thermal reduction of *Salmonella* under typical treatment conditions. Furthermore, validating *E. faecium* as a potential surrogate for the thermal treatment of OF is essential.

E. faecium for process validation

FDA [10] requires food companies to establish and validate measures to reduce or manage significant risks, such as *Salmonella* in various LMFs [10]. *E. faecium* is frequently utilized as a substitute for *Salmonella* in thermal validation studies for LMFs because of its non-pathogenic nature and higher tolerance to heat and acid [39]. *E. faecium* exhibits greater heat tolerance than *Salmonella* during the thermal processing of different LMFs. This includes extrusion processing of carbohydrate-protein meals [14], thermal heating of low-a_w pet food [28], radio-frequency processing of WF [18], spices [17, 22], and peanut paste [30]. *E. faecium* consistently exhibited higher *D*-values than *Salmonella* at all tested temperatures in OF. The *D*-value ratios between *E. faecium* and *Salmonella* varied from 1.3 to 1.4, indicating that *E. faecium* is an appropriate to be used instead of *Salmonella* during the thermal treatment of OF at $a_w = 0.40$. However, as indicated in previous studies, *E. faecium* may not be a representative microorganism to validate the thermal process across varying a_w levels or temperature conditions in the same food matrix [19]. [40] observed that increasing the initial a_w to 0.45 resulted in *Salmonella* exhibiting greater heat resistance compared to *E. faecium* at all tested temperatures in cocoa powder, with the *D*-value ratios of *E. faecium* to *S.* Enteritidis PT30 ranging from 0.67 to 0.81. This suggests that *E. faecium* might not be a suitable substitute for *Salmonella* as a_w in cocoa powder exceeds a specific level. In a confectionery formulation with a a_w of 0.57, the $D_{80°C}$ of *Salmonella* were higher than the reported values for *E. faecium* in cocoa powder [41]. In this research, the log10 D-values plotted contrary process temperature revealed that the curves for *S.* Enteritidis PT30 and *E. faecium* intersected at around 75, 80, and 85°C at $a_w = 0.40$, indicating the temperatures at which the appropriateness of *E. faecium* as a surrogate changes. To enhance and confirm the accuracy of thermal processing, additional research is required to verify the feasibility of *E. faecium* as a potential surrogate for *Salmonella* in OF.

CONCLUSION

The thermal reduction of *Salmonella* and *E. faecium* in oat flour was defined by employing the log-linear model. The findings from this study indicated that *E. faecium* exhibited significantly higher thermal resistance in OF with $a_{w,25°C}$=0.40 as compared to

Salmonella. This study can further help the LMF industry develop thermal processes to improve the safety of oat flour. Furthermore, the industry might use *E. faecium* as a conservative surrogate for process validation of *Salmonella*, given its greater thermal resistance.

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REFERENCES

[1] Centers for Disease Control (CDC), 2023. Multistate *Salmonella* outbreak linked to flour. https://www.cdc.gov/salmonella/infantis-03-23/index.html

[2] Blessington, T., Theofel, C. G., & Harris, L. J. (2013). A dry-inoculation method for nut kernels. Food Microbiology, 33(2), 292–297. https://doi.org/10.1016/j.fm.2012.09

[3] Centers for Disease Control and Prevention (CDC), 2016. Multistate outbreak of *Salmonella* Montevideo and *Salmonella* Senftenberg infections linked to Wonderful Pistachios (final update). https://www.cdc.gov/salmonella/montevideo-03-16/index.html.

[4] Centers for Disease Control and Prevention (CDC), 2014. Multistate outbreak of human *Salmonella* Enteritidis infections linked to Turkish pine nuts (Final update). https://www.cdc.gov/salmonella/2011/pine-nuts-11-17-2011.html.

[5] Centers for Disease Control and Prevention (CDC), 2009a. Multistate outbreak of *Salmonella* infections linked to pistachio nuts (Final Update). https://www.cdc.gov/ salmonella/2009/pistachio-nuts-4-14-2009.html

[6] Centers for Disease Control and Prevention (CDC), 2010. Multistate outbreak of human *Salmonella* Montevideo infections (final update). https://www.cdc.gov/mmwr/preview/mmwrhtml/mm5950a3.htm

[7] Centers for Disease Control and Prevention (CDC), 2009b. Multistate outbreak of *Salmonella* Typhimurium infections linked to peanut butter, 2008-2009 (final update). https://www.cdc.gov/salmonella/2009/peanut-butter-2008-2009.html.

[8] Centers for Disease Control and Prevention (CDC), 2008a. Multistate outbreak of human *Salmonella* infections caused by contaminated dry dog food–United States, 2006- 2007. MMWR Morbidity and Mortality Weekly Report , 57, 521–524.

[9] Centers for Disease Control and Prevention (CDC), 2008b. Multistate outbreak of *Salmonella* agona infections linked to rice and wheat puff cereal (final update). http s://www.cdc.gov/salmonella/2008/rice-wheat-puff-cereal-5-13-2008.html.

[10] Food and Drug Administration, 2015. Analysis and Evaluation of Preventive Control Measures for the Control and Reduction/elimination of Microbial Hazards on Fresh and Fresh-cut Produce: Chapter VII. The Use of Indicators and Surrogate Microorganisms for the Evaluation of Pathogens in Fresh and Fresh-cut Produce.

[11] Awuah, G. B., Ramaswamy, H. S., & Economides, A. (2007). Thermal processing and quality: Principles and overview. Chemical Engineering and Processing: Process Intensification, 46(6), 584-602.

[12] Food and Drug Administration, 2015b. Food Safety Modernization Act (FSMA) Final Rule for Preventive Controls for Human Food.

[13] ABC, 2014. Guidelines for Using *Enterococcus Faecium* NRRL B-2354 as a Surrogate Microorganism in Almond Process Validation.

[14] Bianchini, A., Stratton, J., Weier, S., Hartter, T., Plattner, B., Rokey, G., et al. (2012). Validation of extrusion as a killing step for *Enterococcus faecium* in a balanced carbohydrate-protein meal by using a response surface design. Journal of Food Protection, 75(9), 1646–1653. https://doi.org/10.4315/0362-028x.jfp-12-085.

[15] Jeong, S., Marks, B. P., & Ryser, E. T. (2011). Quantifying the performance of *Pediococcus* sp.(NRRL B-2354: *Enterococcus faecium*) as a nonpathogenic surrogate for *Salmonella* Enteritidis PT30 during moist-air convection heating of almonds. Journal of Food protection, 74(4), 603-609.

[16] Bingol, G., Yang, J. H., Brandl, M. T., Pan, Z. L., Wang, H., & McHugh, T. H. (2011). Infrared pasteurization of raw almonds. Journal of Food Engineering, 104(3), 387–393. https://doi.org/10.1016/j.jfoodeng.2010.12.034

[17] Ozturk, S., Kong, F., & Singh, R. K. (2020). Evaluation of *Enterococcus faecium* NRRL B-2354 as a potential surrogate of Salmonella in packaged paprika, white pepper and cumin powder during radio frequency heating. Food Control, 108, 106833.

[18] Liu, S., Rojas, R.V., Gray, P., Yang, R., Zhu, M.J., Tang, J., 2018a. *Enterococcus faecium* as a *Salmonella* surrogate in the thermal processing of wheat flour: influence of water activity at high temperatures. Food Microbiology. 74, 92–99.

[19] Liu, S.X., Ozturk, S., Xu, J., Kong, F.B., Gray, P., Zhu, M.J., Sablani, S.S., Tang, J.M., 2018b. Microbial validation of radio frequency pasteurization of wheat flour by inoculated pack studies. Journal of Food Engineering, 217, 68–74.

[20] Li, C., Huang, L., & Chen, J. (2014). Comparative study of thermal inactivation kinetics of 403 *Salmonella* spp. in peanut butter and peanut butter spread. Food Control, 45, 143-149. https://doi.org/10.1016/j.foodcont.2014.04.028

[21] Harris, L. J., Uesugi, A. R., Abd, S. J., & McCarthy, K. L. (2012). Survival of *Salmonella* Enteritidis PT 30 on inoculated almond kernels in hot water treatments. Food Research International, 45(2), 1093–1098. https://doi.org/10.1016/j.foodres.2011. 03.048.

[22] Ozturk, S., Liu, S., Xu, J., Tang, J., Chen, J., Singh, R. K., & Kong, F. (2019). Inactivation of *Salmonella* Enteritidis and *Enterococcus faecium* NRRL B-2354 in corn flour by radio frequency heating with subsequent freezing. Lwt, 111, 782-789.

[23] Peleg, M., 2006. Advanced Quantitative Microbiology for Foods and Biosystems: Models for Predicting Growth and Inactivation. CRC Press.

[24] Motulsky, H., & Cristopoulos, A. (2004). Fitting models to biological data using linear and nonlinear regression: A practical guide to curve fitting. Oxford University Press.

[25] Huang, L. (2017). IPMP Global Fit - A one-step direct data analysis tool for predictive 392 microbiology. International Journal of Food Microbiology, 262, 38–48. 393 https://doi.org/10.1016/j.ijfoodmicro.2017.09.010

[26] Smith, D.F., 2014. Modeling the Effect of Water Activity on Thermal Resistance of *Salmonella* in Wheat Flour. Michigan State University.
[27] Syamaladevi, R.M., et al., 2016. Water activity change at elevated temperatures and thermal resistance of Salmonella in all-purpose wheat flour and peanut butter. Food Research International, 81, 163e170.
[28] Ceylan, E., Bautista, D.A., 2015. Evaluating *Pediococcus acidilactici* and *Enterococcus faecium* NRRL B-2354 as thermal surrogate microorganisms for *Salmonella* for in-plant validation studies of low-moisture pet food products. Journal of Food Protection, 78, 934–939.
[29] Laroche, C., Fine, F., Gervais, P., 2005. Water activity affects heat resistance of microorganisms in food powders. International Journal of Food Microbiology. 97, 307–315.
[30] Kataoka, A., Enache, E., Black, D.G., Elliott, P.H., Napier, C.D., Podolak, R., Hayman, M.M., 2014. Survival of *Salmonella* Tennessee, *Salmonella* Typhimurium DT104, and Enterococcus faecium in peanut paste formulations at two different levels of water activity and fat. Journal of Food Protection, 77, 1252–1259.
[31] Taylor, M. H., Tsai, H. C., Rasco, B., Tang, J., & Zhu, M. J. (2018). Stability of *Listeria monocytogenes* in wheat flour during extended storage and isothermal treatment. Food Control, 91, 434-439.
[32] Goepfert, J.M., Biggie, R.A., 1968. Heat resistance of *Salmonella* Typhimurium and *Salmonella* Senftenberg 775W in milk chocolate. Applied Microbiology, 16, 1939–1940.
[33] Ng, H., Bayne, H.G., Garibaldi, J.A., 1969. Heat resistance of *Salmonella*: the uniqueness of Salmonella senftenberg 775W. Applied Microbiology, 17, 78–82.
[34] Rolfe, M.D., Rice, C.J., Lucchini, S., Pin, C., Thompson, A., Cameron, A.D.S., Alston, M., Stringer, M.F., Betts, R.P., Baranyi, J., Peck, M.W., Hinton, J.C.D., 2012. Lag phase is a distinct growth phase that prepares bacteria for exponential growth and involves transient metal accumulation. International Journal of Bacteriology, 194, 686–701.
[35] Mogollon, M.A., Marks, B.P., Booren, A.M., Orta-Ramirez, A., Ryser, E.T., 2009. Effect of beef product physical structure on *Salmonella* thermal inactivation. Journal of Food Science, 74, M347–M351.
[36] Juneja, V.K., Eblen, B.S., 2000. Heat inactivation of *Salmonella* Typhimurium DT104 in beef as affected by fat content. Letters in Applied Microbiology, 30, 461–467.
[37] He, Y., Li, Y., Salazar, J.K., Yang, J., Tortorello, M.L., Zhang, W., 2013. Increased water activity reduces the thermal resistance of *Salmonella* enterica in peanut butter. Applied and Environmental Microbiology, 79, 4763–4767.
[38] Lima, L.J., van der Velpen, V., Wolkers-Rooijackers, J., Kamphuis, H.J., Zwietering, M.H., Nout, M.J., 2012. Microbiota dynamics and diversity at different stages of industrial processing of cocoa beans into cocoa powder. Applied and Environmental Microbiology, 78, 2904–2913.
[39] Kopit, L.M., Kim, E.B., Siezen, R.J., Harris, L.J., Marco, M.L., 2014. Safety of the surrogate microorganism *Enterococcus faecium* NRRL B-2354 for Use in thermal process validation. Journal of Applied & Environmental Microbiology, 80, 1899–1909.
[40] Tsai, H. C., Ballom, K. F., Xia, S., Tang, J., Marks, B. P., & Zhu, M. J. (2019). Evaluation of Enterococcus faecium NRRL B-2354 as a surrogate for *Salmonella* during cocoa powder thermal processing. Food Microbiology, 82, 135-141.
[41] Rachon, G., Penaloza, W., Gibbs, P.A., 2016. Inactivation of *Salmonella, Listeria monocytogenes* and *Enterococcus faecium* NRRL B-2354 in a selection of low moisture foods. International Journal of Food Microbiology, 231, 16–25.