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The effect of water activity and temperature on the inactivation of *Enterococcus faecium* in peanut butter during superheated steam sanitation treatment

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ABSTRACT

The objective of this study was to investigate the inactivation kinetics of *Enterococcus faecium* in peanut butter under different water activities (a_w) and superheated steam temperatures. Peanut butters were prepared at 4 different initial water activities (0.19, 0.40, 0.60, and 0.80) and *E. faecium* was inoculated into the peanut butter (7.4–8.7 log CFU/g). The inoculated peanut butter samples were exposed at 4 different superheated steam temperatures (125 °C, 175 °C, 225 °C, and 250 °C). Survivor data were modelled using Weibull and log-linear models to describe the inactivation kinetics of *E. faecium*. The decimal reduction times (*D*-value), temperature sensitivity (z_T) and a_w sensitivity (z_{aw}) of the *D*-value were determined from a log-linear model, and inactivation parameters from the Weibull model were also evaluated. An increase in a_w of peanut butter and superheated steam temperature decreased the *D*-value of *E. faecium*. The z_{aw}-value and z_T-value were determined to be 0.60 ± 0.09 and 194.66 ± 40.69 °C, respectively (R^2 > 0.89). The inactivation kinetics of *E. faecium* on surfaces contaminated with peanut butter can provide comprehensive information to superheated steam sanitation treatment which may be applied to environmental surfaces for effective microbial inactivation without the introduction of water.

1. Introduction

Sanitation of food processing environmental surfaces is essential in preventing environmental cross-contamination and ensuring the microbial safety of food products (Park & Yoon, 2019). However, sanitation in dry food processing environments represents a unique challenge since conventional wet sanitation approaches utilize water which is typically excluded from otherwise dry processing facilities. Moisture introduced from sanitation regimens can facilitate microbial growth and the formation of harborage sites in niches and microenvironments in dry facilities. Relevant industries include grain products, nut and nut butters, pet food, dairy powders and infant formulas, and spices (Marriott et al., 2018). Therefore, effective dry sanitation approaches are essential for safe food production in dry processing environments (Burnett & Hagberg, 2014).

Various dry sanitation methods have been used to reduce microbial contamination on food plant surfaces, such as alcohol-based sanitizers (Du et al., 2010), dry heat (McKelvey & Bodnaruk, 2013), hot oil (Grasso et al., 2015), gaseous ozone (Kim et al., 2003), gaseous chlorine dioxide (Nam et al., 2014; Trinetta et al., 2012), and UV (Kim et al., 2018). However, compared to wet sanitation methods, the disinfection efficiencies of these dry sanitation methods are limited (Burnett & Hagberg, 2014; Kim et al., 2020). Therefore, it is necessary to develop an effective sanitization technology to inactivate the microbial pathogens in dry processing environments.

It is well established that moist heat (saturated steam) is more efficient in microbial inactivation than dry heat (hot air) because saturated steam has higher thermal conductivity and heat capacity than hot air (Alder & Simpson, 1982). Recently, superheated steam has been evaluated as an emerging dry thermal technology for microbial inactivation. Superheated steam is a form of steam which has been heated to increase its temperature over the saturation point at a given pressure (Van Deventer & Heijmans, 2001). In contrast to saturated steam, a decrease in temperature will not lead to condensation unless the temperature

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decreases to below the saturation point. Due to the higher temperature than saturated steam, superheated steam can effectively inactivate pathogens in dry environmental conditions without introducing moisture. Hence, superheated steam has been used to decontaminate various food processing surfaces (Ban et al., 2014; Hu et al., 2016; Kim et al., 2019; Kohli, 2019; Kondjoyan & Portanguen, 2008; Kwon et al., 2019).

Food plant sanitation is a two-step process that involves cleaning (removal of organic soil, such as peanut butter) and sanitization (inactivation of remaining microbial targets). In commercial food plant environments, it is not uncommon that cleaning is incompletely accomplished and the mitigating effect of the residual food soil on sanitization efficacy is an important problem. Moreover, in many food facilities that handle low moisture foods, legacy equipment is not hygienically designed, often possessing many crevices that are difficult to clean with most physical strategies. One of the advantages of superheated steam is its ability to penetrate such niches where residual food soil is harbored.

Water activity (a_w) has been shown to greatly affect the thermal resistance of pathogens (Syamaladevi et al., 2016). Thus, a_w of food soils on treated surfaces can affect the decontamination efficiency of superheated steam. In addition, superheated steam temperature (ranging from 125 to 300 °C) significantly affects its decontamination efficiency (Ban et al., 2014; Hu et al., 2016; Kwon et al., 2019). However, the interacting effects of a_w and superheated steam temperature on inactivation kinetics are not well understood and are underreported in the literature.

Peanut butter is a common low-moisture food that has been associated with previous outbreaks of salmonellosis. Complete removal of peanut butter residues from environmental surfaces is difficult. Consequently, peanut butter was used in this study as a matrix that potentially mitigates the effect of superheated steam. The non-pathogenic, vegetative bacterium, *Enterococcus faecium*, has been adopted as a surrogate for *Salmonella* in low-moisture foods to investigate inactivation efficiency of thermal processes (Bianchini et al., 2014; Ceylan & Bautista, 2015; Jeong et al., 2011; Yang, Xie, et al., 2020). To our knowledge, there is limited kinetic data on superheated steam inactivation of *E. faecium*. Therefore, the objective of this study was to investigate the effect of peanut butter a_w and different superheated temperatures on the inactivation kinetics of *E. faecium* during superheated steam sanitation treatment.

2. Materials and methods

2.1. Bacterial cultures and preparation of inoculum

Enterococcus faecium NRRL B-2354, provided from the bacterial culture collection of Michelle Danyluk at the University of Florida (Lake Alfred, FL), was used. The preparation procedure developed by Jeong et al. (2011) was followed with slight modifications. *E. faecium* was grown in tryptic soy broth supplemented with 0.6% (w/v) yeast extract (TSBYE; Thermo Fisher Scientific, Waltham, MA) for 24 h at 37 °C, and then 1 ml of the culture was spread plated on TSAYE agar plates (150 by 15 mm) to obtain uniform lawns. After 24 h of incubation at 37 °C, the bacterial lawns from 6 plates were harvested by flooding with 60 ml of 0.1% sterile peptone water (Thermo Fisher Scientific, Waltham, MA). The bacterial broth was centrifuged at 8,000 g for 10 min at 4 °C and the supernatant was discarded. Then, the bacterial pellet was re-suspended in 6 ml of 0.1% peptone water before use.

2.2. Sample preparation and inoculation

Experiments utilized peanut butter sample as the model food residue to evaluate superheated steam efficacy. Two days before experiment, 49.5 g of peanut butter (100% peanuts, Crazy Richard's, Plain City, OH) samples were inoculated with 500 μl of the *E. faecium* culture and prepared at 4 different a_w levels (0.19, 0.40, 0.60, and 0.80) at 25 °C as shown in Table 1. To adjust the a_w levels, a pre-determined amount of deionized water was mixed with peanut butter. Following homogenization, a water activity meter (Aqua Lab 4 TE, METER®, Pullman, WA) was used to measure the a_w of the inoculated peanut butter in triplicate at room temperature (25 °C). The prepared peanut butter samples were kept at 25 °C for 48 h to condition the *E. faecium* in the low a_w environment. The initial populations of *E. faecium* in the inoculated samples after conditioning ranged from 7.4 to 8.7 log CFU/g.

0.6 mm thickness thin-film layer of peanut butter was chosen based on preliminary experimentation with the aim of minimizing variations in thermal history and moisture distribution within the test matrix during the superheated steam experiments. A custom 3D printed mold was used for casting the peanut butter layer (31.5 mm × 20.0 mm × 0.60 mm, length × width × thickness) (Fig. 1). By sliding a blade over a flat surface, the excess coating of peanut butter was scraped away and the resulting thin-film layer of peanut butter was a uniform. This layer was applied to one side of aluminum foil. The sample-coated aluminum foil was attached to a custom coupon holder.

2.3. Superheated steam system

2.3.1. Temperature measurement in the treatment chamber

The schematic diagram of the superheated steam equipment with a superheated steam generator (HGA-S, MHI Inc., Cincinnati, OH) is shown in Fig. 2. To investigate the effect of water supply flow rate on the temperature distribution within the treatment chamber, the temperatures at different distances (2 cm, 4 cm, 6 cm, 8 cm, 10 cm, and 12 cm) from the steam source were measured at different flow rates of water which ranged from 17.0 ml/min to 26.2 ml/min. To monitor and record the temperature within the superheated chamber, tyke-K thermocouples and a portable data acquisition module (Advantech, Taibei, Taiwan) were used. During superheated steam treatment, the flow rate of water was adjusted to maintain the superheated steam temperature at each target temperature (125 °C, 175 °C, 225 °C, and 250 °C). The uniformity of superheated steam temperature was assessed at each target temperature during superheated steam treatment.

2.3.2. Superheated steam treatment

Prior to experiments, the superheated steam chamber was pre-heated to target temperature (1 h for reaching steady state conditions). The peanut butter (initially at 25 °C) was mounted on a custom coupon holder and introduced into the preheated treatment chamber to a predetermined location, 4 cm distance from the steam source (Fig. 2). A k-type thermocouple monitored temperature of the sample at its geometric center during superheated steam treatment. The samples were treated at various process temperature (125 °C, 175 °C, 225 °C, and 250 °C) for a given time interval, and then quickly removed and immediately transferred to 10 ml of 0.1% peptone water to stop the thermal process.

2.4. Enumeration of Enterococcus faecium

Samples suspended in 10 ml of peptone water were homogenized for 2 min by using a vortex mixer (Mini Vortexer, Thermo Fisher Scientific, Waltham, MA) until no clumps were observed. From the homogenized samples, 100 μl was plated onto TSAYE plates either directly or after serial dilutions. When the samples showed low microbial counts, 1 ml of

Table 1
Water activity of different peanut butter samples at 25 °C.

Culture (ml)	Water (g)	Peanut butter (g)	a_w
0.5	4.1	45.4	0.80
0.5	2.1	47.4	0.60
0.5	1.2	48.3	0.40
0.5	0.0	49.5	0.19

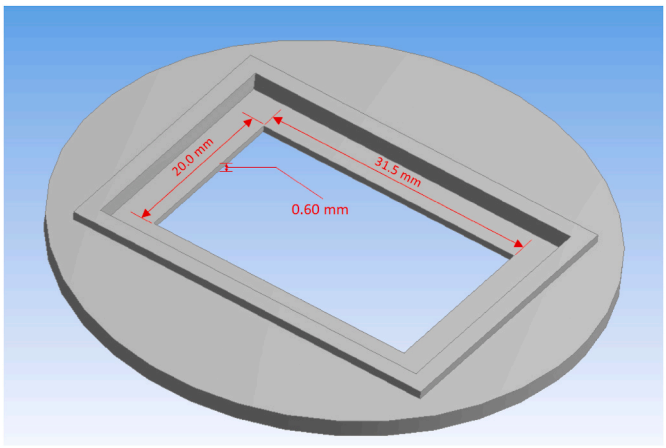

Fig. 1. The custom mold for the thin-film layer of peanut butter.

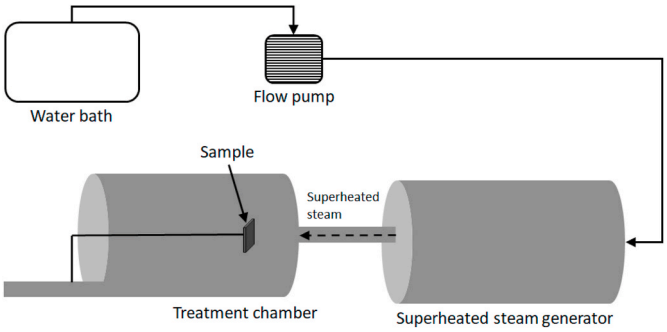

Fig. 2. The schematic diagram of the superheated steam equipment.

the lowest dilution was plated onto 3 TSAYE plates and the resulting colonies were summed. The plates were incubated at 37 °C for 48 h for the enumeration of *E. faecium*. Since 0.48 g (±0.02) of the coated samples were homogenized in 10 ml of peptone water before plating on TSAYE plates, the minimum detection limit in this study was 20 CFU/g.

2.5. Modeling inactivation kinetics

To describe the inactivation kinetics of *E. faecium* at different superheated steam temperatures and a_w levels, the log-linear model and the Weibull model were used to fit the experimental data. Under isothermal conditions, it has generally been assumed that thermal inactivation of microorganisms is exponential with time i.e., log-linear kinetics (McKellar & Lu, 2003).

2.5.1. Log-linear model

The log-linear model can be described by the following equation:

$$log\frac{N}{N_0} = -\frac{t}{D} \quad (1)$$

where N is the number of survivors after a treatment time t (CFU/g), N_0 is initial population (CFU/g), t is the treatment time (sec), and D is the decimal reduction time (sec). To investigate the effect of temperature and a_w on the thermal inactivation kinetics of *E. faecium*, temperature sensitivity (z_T) and a_w sensitivity (z_{aw}) of the D-value can be represented in terms of z-value (McKellar & Lu, 2003). The z-value is defined as the increase in temperature or a_w causing a 90% reduction in D-value. The z-value can be expressed as follows:

$$z = \frac{C_2 - C_1}{\log D_1 - \log D_2} \quad (2)$$

where z is temperature (z_T) or a_w (z_{aw}) sensitivity of the bacterial culture, and C is temperature (°C) or a_w.

2.5.2. Non-linear model

The Weibull model has been widely used in describing the non-linear inactivation of various microorganisms in different experimental conditions (McKellar & Lu, 2003). The Weibull model is expressed by Eq. (3):

$$log\frac{N}{N_0} = -bt^n \quad (3)$$

where b and n are the scale and shape parameters, respectively.

In this study, MATLAB (Mathworks Inc., Natick, MA) was used to fit the inactivation models. The suitability of the two inactivation models described above was estimated by comparison to the experimental data. Generally, the goodness of fit was estimated by the root mean square error (RMSE) (Li et al., 2018). RMSE was calculated using the following equations:

$$\mathrm{RMSE} = \sqrt{\frac{1}{n-p}\cdot\sum_{i=1}^{n}\left[\log\left(N/N_p\right)\right]^2} \quad (4)$$

where N_p is the predicted microbial count (CFU/g), n is the number of data points, and p is the number of parameters in the model.

2.6. Change in moisture content after superheated steam treatment

The moisture contents of peanut butter samples before treatment and after superheated steam treatment achieving 5-log reduction were compared at each a_w. The moisture content was measured according to the AOAC method (1990).

2.7. Statistical analysis

All experiments were carried out in triplicate using independent biological replicates and the standard deviations and mean values were determined. One-way ANOVA and Tukey's multiple comparison tests at the significance level of 0.05 were performed by using SPSS software (SPSS Inc, Chicago, IL).

3. Results and discussion

3.1. Temperature and moisture distribution within the treatment chamber and test matrix

The temperature of superheated steam within the treatment chamber varied as a function of both the flow rate of water used for generating the steam as well as the distance between the sample coupon and the superheated steam nozzle (Table 2). During operation, the superheated steam generator consumed 1 kW of electrical power to generate superheated steam from water. Temperature in the treatment chamber significantly decreased as the flow rate of water increased since the superheated steam generator needs to heat greater amounts of water at higher flow rates. The superheated steam temperature also decreased as the distance from the steam nozzle increased because of the heat lost to the environment. Thus, superheated steam temperature was significantly affected by both the flow rate of water and the distance from the steam source ($p < 0.05$). Based on these results, the testing coupon was mounted at 4 cm from the steam nozzle source for the microbial inactivation studies. The flow rate of water was adjusted to control the superheated steam temperature at each target temperature. Additionally, the uniformity of superheated steam temperature within the test surface was estimated.

The test sample reached desired target temperature in about 3–4 s after introduction into the superheated steam chamber. Relatively

Table 2
Temperature distributions in the superheated steam treatment chamber at different flow rates of water.

Flow rate of water (ml/min)	Distance from steam nozzle					
	2 cm	4 cm	6 cm	8 cm	10 cm	12 cm
17.0	296.43 °C ± 2.09	266.00 °C ± 0.55	260.22 °C ± 0.53	248.55 °C ± 0.52	246.43 °C ± 0.50	236.56 °C ± 0.52
19.7	251.13 °C ± 1.14	228.41 °C ± 0.33	221.27 °C ± 0.77	221.75 °C ± 0.29	218.55 °C ± 0.52	216.21 °C ± 0.35
22.2	200.91 °C ± 0.75	189.90 °C ± 0.28	184.55 °C ± 0.20	180.34 °C ± 0.25	178.55 °C ± 0.34	169.27 °C ± 0.38
24.3	164.70 °C ± 0.51	156.64 °C ± 0.16	156.25 °C ± 0.15	155.89 °C ± 0.19	154.85 °C ± 0.20	153.63 °C ± 0.24
25.5	135.14 °C ± 0.72	129.15 °C ± 0.14	126.85 °C ± 0.13	126.79 °C ± 0.14	126.05 °C ± 0.12	124.14 °C ± 0.13
26.2	121.24 °C ± 0.11	117.55 °C ± 0.11	116.55 °C ± 0.14	115.02 °C ± 0.14	113.55 °C ± 0.12	112.55 °C ± 0.13

smaller volume of peanut butter sample (0.38 cm^3) within larger treatment chamber (464.94 cm^3) helped to minimize the come-up time. At each target temperature (125 °C, 175 °C, 225 °C, and 250 °C), the temperatures at the geometric center of the test surface were 125.14 °C (±0.12), 175.27 °C (±0.15), 224.97 °C (±0.31), and 250.33 °C (±0.54), respectively. One-dimensional transient heat conduction analytical solution verified that temperature gradient within 0.6 mm sample was negligible and reached steady state within 4 s.

During the treatment, water vapor exchange can occur between superheated steam and the test sample. The relative pressure of superheated steam vapor may explain this phenomena. The ratio of partial vapor pressure of superheated steam (p_v) to saturation vapor pressure of water (p_{sat}) at the same temperature is defined as the relative pressure (Pronyk et al., 2010). In this study, the partial vapor pressure of the superheated steam system is equal to the operating pressure (i.e., atmospheric pressure) because the superheated chamber is primarily composed of water vapor. Thus, the relative pressure superheated steam vapor at 125 °C, 175 °C, 225 °C, 250 °C were estimated as 43.6%, 11.3%, 4.0% and 2.6%, respectively. Thus, when the target process temperature was closer to saturated steam conditions (i.e., T ≤ 125 °C), when the sample at ambient temperature was introduced into the treatment chamber, condensation of vapor on the peanut butter surface was observed (ie. Sample gained moisture from the environment). When the temperature increased beyond 150 °C, at thee beginning stages of treatment, the superheated steam can cool (lose internal energy), resulting in a lowering of its temperature without changing state from a gas. Subsequently the product gains heat from the superheated steam, give up moisture to the environment and became desiccated.

Researchers have reported increases in water activity with increasing temperature during thermal processing of low moisture foods (such as

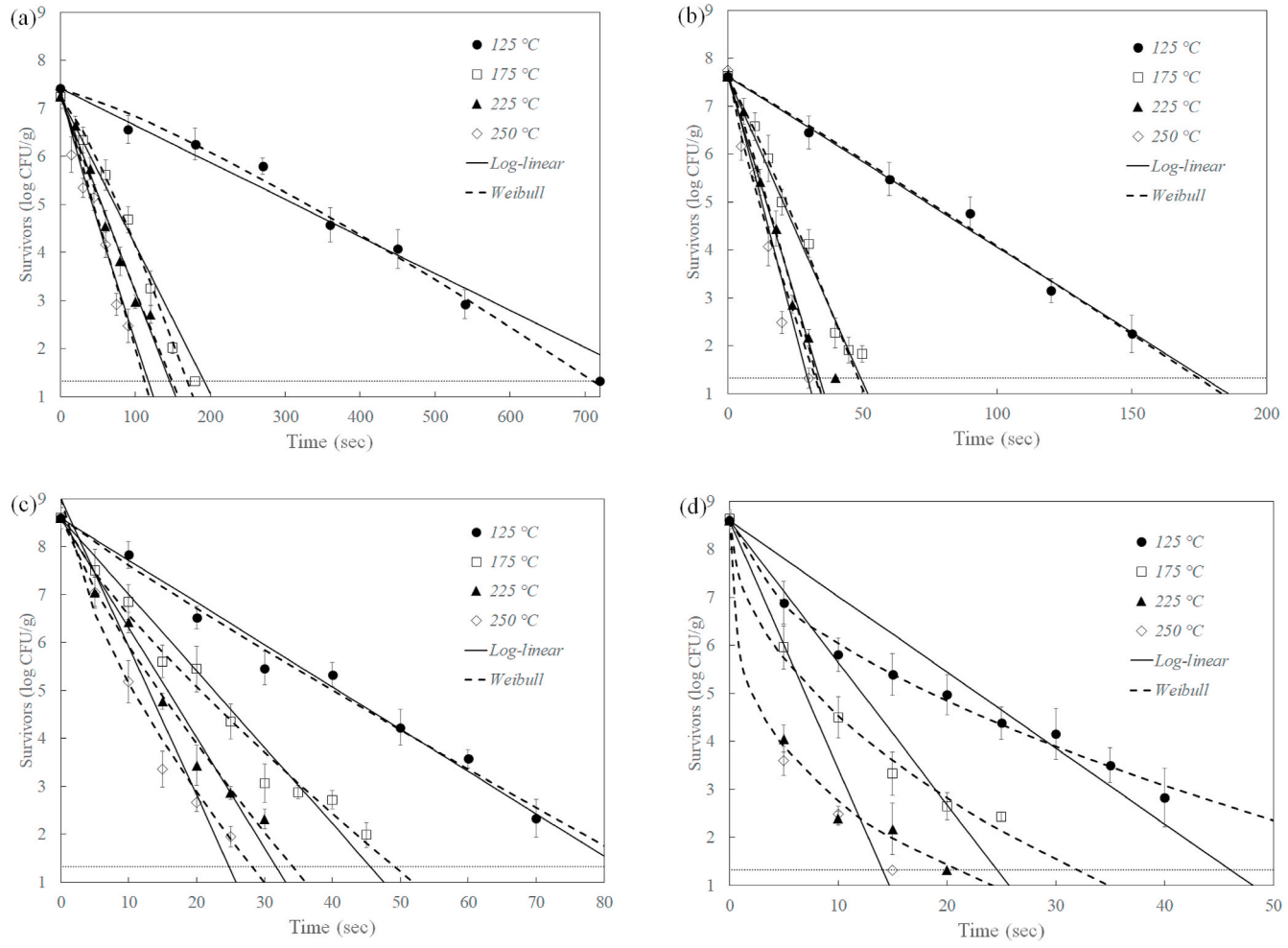

Fig. 3. Inactivation kinetics of *Enterococcus faecium* in peanut butter at (a) 0.19 a_w, (b) 0.40 a_w, (c) 0.60 a_w, and (d) 0.80 a_w during superheated steam treatment at different temperatures. Dotted line indicates the detection limit (1.3 log CFU/g).

cheese powder, corn starch, non-fat milk powder, soy protein powder, and wheat flour) (Jin et al., 2019, 2020; Tadapaneni et al., 2017). On the other hand, the water activity of peanut oil sharply decreased with increasing temperature during thermal processing (Yang, Guan, et al., 2020). Such studies employed closed sample system where changes in water activity can be monitored. On the contrary, during superheating of peanut butter sample, dynamic exchang of moisture occurred, particularly during the earlier stages of heating. The influence of this dynamic moisture transfer between the sample and environment (and changes in product water activity) during the treatment was not determined. Such effort would merit future investigation.

3.2. Effect of peanut butter water activity and superheated steam temperature on the inactivation of Enterococcus faecium

Fig. 3 shows the inactivation curves for *E. faecium* during superheated steam treatment in peanut butter at different temperatures (125 °C, 175 °C, 225 °C, and 250 °C). Regardless of the a_w of peanut butter, the superheated steam temperature greatly affected the inactivation of *E. faecium*. The treatment times required to achieve 5-log reduction at 125 °C were more than 3 times longer than those at 250 °C for different a_w levels investigated. While a >5-log reduction of *E. faecium* was achieved at 250 °C across different a_w levels of peanut butter, only <2-log reduction were achieved at 125 °C. Increasing superheated steam temperature accelerated the inactivation of *E. faecium* and reduced the treatment time, which is consistent with other superheated steam studies reported by Ban et al. (2014), Ban et al. (2018), and Cenkowski et al. (2007). In previous reports, increasing superheated steam temperature accelerated the inactivation of *Geobacillus stearothermophilus* spore in sand (Cenkowski et al., 2007), biofilms on polyvinyl chloride and stainless steel (Ban et al., 2014), and *Salmonella enterica* serovars Typhimurium and Enteritidis on spices (Ban et al., 2018). The results of previous and current studies suggest that the efficacy of superheated steam inactivation of bacteria was highly dependent on superheated steam temperature.

The moisture content of peanut butter samples were measured to investigate the changes after superheated steam treatment. The moisture contents of peanut butter samples before treatment and immediately after treatment at 125 °C and 250 °C are summarized in Table 3. There was no significant change in the moisture content after superheated steam treatment, except for a decrease of the peanut butter moisture content after superheated steam treatment at 250 °C with an initial a_w of 0.19 ($p < 0.05$). The relatively short treatment time of superheated steam at 250 °C may be why it does not introduce appreciable moisture migration inside peanut butter samples that contain high oil content (>40%) and low moisture content (<14%). It has been well established that the hydrophobic character of oil interferes the moisture diffusion inside foods (Alzamora & Chirife, 1980; Jason, 1965).

Moisture diffusivity (1×10^{-8} m^2/s to 1×10^{-11} m^2/s) in foods is generally lower than thermal diffusivity of foods (9×10^{-8} m^2/s to 1.5×10^{-7} m^2/s) (Panagiotou et al., 2004; Shitzer et al., 2006). Thus, the heat transfer in foods is generally faster than the moisture transfer in foods, especially in oil (Panagiotou et al., 2004; Yang, Xie, et al., 2020).

Table 3
Changes in moisture content of peanut butter before and immediately after superheated steam treatment.

a_w °at 25C	Moisture content (% d.b.)		
	Initial	250 °C	125 °C
0.19	1.70 ± 0.34^{a}	0.76 ± 0.18^{b}	2.22 ± 0.29^{a}
0.40	4.54 ± 0.29^{ab}	4.18 ± 0.14^{b}	4.94 ± 0.15^{a}
0.60	6.63 ± 0.15^{ab}	6.17 ± 0.15^{b}	6.95 ± 0.26^{a}
0.80	13.56 ± 0.20^{ab}	13.00 ± 0.27^{b}	14.07 ± 0.39^{a}

*Values in the same row having the different letters are statistically different ($p < 0.05$).

Our result also showed a similar trend. The time to reach temperature equilibration was within seconds while the noticeable drying effect was observed when the treatment time was longer than 1 min at 250 °C and 0.19 a_w level (Table 3). However, there could be changes in the moisture content at the surface of peanut butter samples. At 125 °C, condensation transiently occured at the beginning of treatment (during come-up time) when the surface temperature was lower than 100 °C. Subsequently as the surface temperature increases, moisture evaporation begins to occur. Hence, minimal changes in the moisture content of peanut butter may be expected during superheated steam treatment.

E. faecium inactivation was greatly affected by initial a_w of peanut butter. When subjected to superheated steam treatment for 30 s at 125 °C, the log reduction of *E. faecium* was 4.45 log CFU/g at 0.80 a_w, whereas it achieved a reduction of 0.51 log CFU/g at 0.19 a_w (Fig. 3a and 3d). Similarly, at 250 °C, the *E. faecium* survivor counts were under the detection limit (i.e., the log reduction > 7.28 log CFU/g) at 0.80 a_w after 15 s treatment, while only 1.21 log CFU/g reduction was achieved when inoculum was subjected to superheated steam treatment for 15 s at 0.19 a_w (Fig. 3a and 3d). The exposure time required to achieve 5-log reduction at 0.19 a_w was more than 9 times longer than that required at 0.80 a_w, regardless of treatment temperature. It has been well known that reducing a_w can protect microorganisms from environmental stress, such as heat (Gould, 1985; Syamaladevi et al., 2016; Xu et al., 2019; Yang, Xie, et al., 2020), pulsed electric fields (Aronsson & Rönner, 2001) electron beam (Black & Jaczynski, 2006), radio-frequency treatment (Xu et al., 2019), and high pressure processing (Daryaei & Balasubramaniam, 2012). The higher thermal resistance of *E. faecium* at lower a_w environments can be explained by water loss from bacterial cells, which leads to more stable protein structures of bacterial cells, which could impedes thermal denaturation of heat-sensitve proteins (Liu et al., 2018).

3.3. Inactivation kinetics of Enterococcus faecium

The log-linear regression model and the Weibull model were used to fit the inactivation data of *E. faecium* at different temperatures and a_w levels during superheated steam treatment (Fig. 3). The estimated model parameters and RMSE values of the log-linear regression model and Weibull model are summarized in Tables 4 and 5, respectively. The *D*-values of *E. faecium* in peanut butter were affected by both superheated steam temperature and a_w of peanut butter (Table 4). By increasing temperature (from 125 °C to 250 °C) and increasing a_w of peanut butter (from 0.19 to 0.80), the *D*-value of 129.70 s at 125 °C and 0.80 a_w greatly reduced to 18.49 s and 6.33 s, respectively. Similar changes in the *D*-value with superheated steam temperature were reported for the inactivation of *Geobacillus stearothermophilus* spores in sand (Cenkowski et al., 2007), *Bacillus cereus* spores on garlic (Jo et al., 2019), and *Salmonella enterica* serovars Typhimurium and Enteritidis on black peppercorns, pecans, and almonds (Ban et al., 2018). However, the effect of a_w on the heat resistance of bacteria during superheated steam treatment has not been reported yet. Similar trends in the effect of a_w on the

Table 4
Estimated parameters of the log-linear regression model for the inactivation kinetics of *Enterococcus faecium* in peanut butter at different water activities and different superheated steam treatment temperatures.

a_w °at 25C		Temperature (°C)			
		125	175	225	250
0.19	*D-value* (sec)	129.70	32.41	24.62	18.49
	RMSE	0.29	0.24	0.25	0.30
0.40	*D-value* (sec)	28.03	7.83	5.40	4.61
	RMSE	0.19	0.22	0.24	0.46
0.60	*D-value* (sec)	11.34	6.26	4.35	3.22
	RMSE	0.27	0.38	0.41	0.77
0.80	*D-value* (sec)	6.33	3.38	1.93	–
	RMSE	0.75	0.98	1.48	

Table 5
Estimated parameters of the Weibull model for the inactivation kinetics of Enterococcus faecium in peanut butter at different water activities and at different superheated steam treatment temperatures.

a_w at 25C		Temperature (°C)			
		125	175	225	250
0.19	*B*	0.002	0.01	0.05	0.08
	n	1.20	1.22	0.96	0.92
	RMSE	0.26	0.20	0.25	0.29
0.40	*B*	0.03	0.09	0.14	0.37
	n	1.03	1.09	1.08	0.82
	RMSE	0.20	0.18	0.24	0.44
0.60	*B*	0.12	0.32	0.40	0.83
	n	0.93	0.81	0.82	0.67
	RMSE	0.27	0.32	0.33	0.46
0.80	*B*	0.71	1.28	2.86	–
	n	0.55	0.50	0.31	
	RMSE	0.17	0.25	0.31	

thermal inactivation of bacteria were reported by several researchers (Jin et al., 2020; Liu et al., 2018; Xu et al., 2019). *D*-values of *Salmonella* Enteritidis PT30 in powdered products and *Salmonella* Agona in soy protein powder increased log-linearly with decreasing a_w. Similarly, $D_{80°C}$-value of *E. faecium* in wheat flour increased from 3.81 min to 281.78 min as a_w decreased from 0.70 to 0.11 (Liu et al., 2018). Compared to the $D_{80°C}$-values of *E. faecium* in wheat flour, the *D-values* of *E. faecium* in this study were much smaller possibly due to the higher temperature of superheated steam (Table 4).

3.3.1. Sensitivity of temperature and water activity on decimal reduction time

To investigate the temperature sensitivity and a_w sensitivity of the *D*-value, z_T-value and the z_{aw}-value were determined based on the *D*-values estimated at the different temperatures and a_w levels (Fig. 4). The z_{aw}-value and z_T-value were estimated to be 0.60 ± 0.09 and 194.66 ± 40.69 °C, respectively ($R^2 > 0.89$). The z_T-value in this study was much greater than the z_T-values of *Salmonella* in soy protein powder, which is ranged from 6.7 °C to 13.2 °C in the range of a_w from 0.13 to 0.82 (Jin et al., 2020). Similarly, compared to the z_{aw}-value of *E. faecium* in wheat flour at 80 °C (0.28) (Liu et al., 2018), the z_{aw}-value of *E. faecium* in our study was greater. Thermal processing studies of Jin et al. (2020) and Li et al. (2018) employed sealed test cells which limited moisture exchange. On the other hand, in the current study there was an exchange of water vapor between the treatment environment and sample. This might have resulted in a shift in the a_w of food samples and altered the inactivation of bacteria under these conditions. Additionally, in the current study, due to shorter thermal come-up time of the product, no come-up time correction was considered in the estimation of relevant kinetic parameters.

A very limited number of studies have reported the inactivation kinetic parameters for microorganisms under superheated steam treatment. The inactivation curves of *Salmonella* (Ban et al., 2018) and *G. stearothermophilus* spores (Cenkowski et al., 2007) when subjected to superheated steam treatment showed linearity or a slight concave upward shape, which is similar to the results obtained in our study.

At high a_w levels (0.6 and 0.8), the shape of the inactivation curves were concave upward ($n < 1$ in Weibull model). Previous researchers noted that a_w of peanut products inside packaged container during thermal processing can change by increasing process temperatures. Yang, Guan, et al. (2020) reported that the a_w of peanut oil changed as the temperature increased. As the temperature of peanut oil increased from 25 °C to 80 °C, the a_w of peanut oil, originally conditioned at 0.94 and 0.53, dropped to 0.36 and 0.21, respectively. This could possibly explain the concave upward curves observed in this study.

Kinetic studies using a surrogate with similar or higher thermal resistance than the target pathogens is critical for validation of thermal sanitation processes in dry processing environments, since pathogens, such as *Salmonella*, cannot be tested in commercial process facilities (Niebuhr et al., 2008). Several studies have reported that *E. faceium* is more resistant to heat than *Salmonella* in low-moisture environments (Ceylan & Bautista, 2015; Enache et al., 2015; Liu et al., 2018; Rachon et al., 2016; Xu et al., 2019). However, this study was conducted under superheated steam temperatures (125 °C–250 °C) which are much higher than those temperatures used in earlier studies (<90 °C).

3.3.2. Role of inversion temperature in microbial inactivation

In the drying literature, inversion temperature is defined as the temperature at which the drying rates by hot air and superheated steam are equal. Above the inversion temperature, superheated steam drying is faster than hot-air drying. Below the inversion temperature, a thin water film can be formed by condensation of superheated steam, which can act as a barrier against heat transfer between superheated steam and treated surfaces. This phenomenon is commonly observed in superheated steam drying (Ramachandran et al., 2017; Sa-Adchom et al., 2011; Speckhahn et al., 2010). Results of this study suggest that this phenomena also contributed to the inactivation of microorganisms during superheated steam treatment. *D*-values between 125 °C and 175 °C decreased significantly compared to the changes in the *D*-values at the temperatures higher than 175 °C (Table 4). When the z_T-values were estimated in the temperature range from 125 °C to 250 °C, the z_T-values of *E. faecium* at 0.19 and 0.40 a_w levels were 156.25 °C and 163.93 °C, respectively

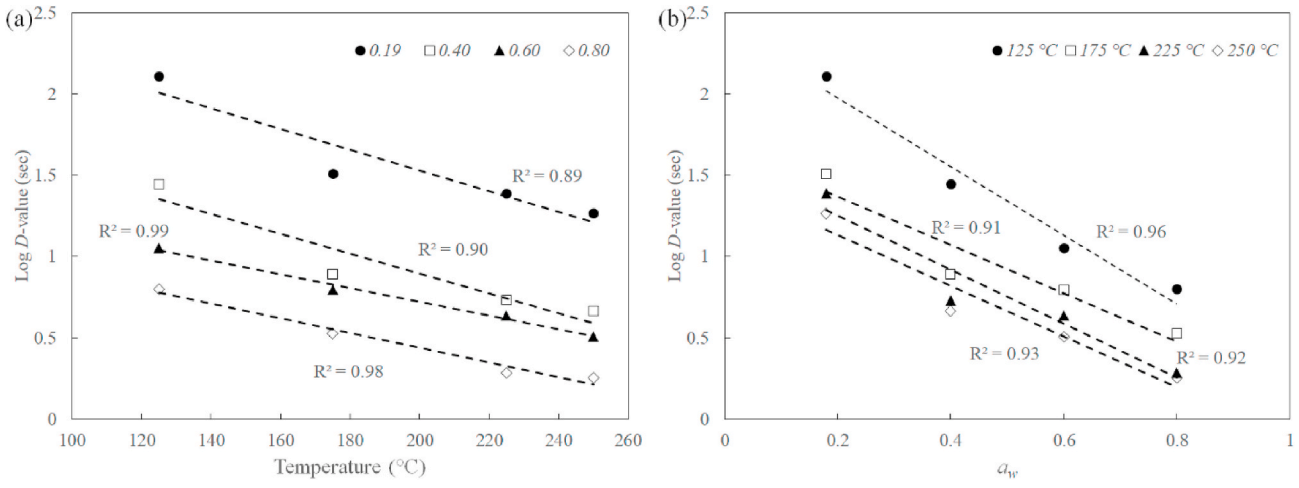

Fig. 4. Effect of (a) temperature and (b) water activity on changes in log D-value of *Enterococcus faecium* in peanut butter during superheated steam treatment.

($R^2 > 0.89$). However, in the range of temperature from 175 °C to 250 °C, the z_T-values were increased to 319.49 °C and 323.62 °C at 0.19 and 0.40 a_w levels, respectively ($R^2 > 0.96$), which indicates that the inactivation of *E. faecium* was highly sensitive to temperature change between 125 °C and 175 °C. This trend was not clearly observed at 0.60 and 0.80 a_w levels, which might be due to the relatively short treatment time (<70 s) at these a_w levels (Fig. 3). More research is needed to gain better understanding on how inversion temperature of superheated steam influences the microbial inactivation.

4. Conclusions

This study investigated the inactivation kinetics of *E. faecium* in peanut butter under different a_w and superheated steam temperatures during superheated steam sanitation treatment. Within the range of superheated steam temperature studied (125 °C–250 °C), increasing superheated steam temperature accelerated the inactivation of *E. faecium*. The results of this study showed that the a_w of peanut butter significantly affected the thermal resistance of *E. faecium* during superheated steam treatment. Log-linear and Weibull models were used to model the inactivation kinetics of *E. faecium* in the range of a_w (0.19–0.80) and superheated steam temperatures (125 °C–250 °C). The *D*-value of *E. faecium* increased as a_w and superheated steam temperature decreased. The inactivation kinetics of *E. faecium* in peanut butter investigated in this study can provide comprehensive information to optimize superheated steam sanitation treatment which may be applied to environmental surfaces for effective microbial inactivation without the introduction of water.

CRediT authorship contribution statement

Hyeon Woo Park: Conceptualization, Data curation, Formal analysis, Methodology, Writing - original draft. **Jie Xu:** Conceptualization, Data curation, Formal analysis, Methodology, Writing - original draft. **V. M. Balasubramaniam:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Methodology, Project administration, Resources, Supervision, Writing - review & editing. **Abigail B. Snyder:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Methodology, Project administration, Resources, Supervision, Writing - review & editing.

Declarations competing of interest

None.

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