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Oil protects bacteria from humid heat in thermal processing

Ren Yang [a], Yucen Xie [a], Stephen P. Lombardo [b], Juming Tang [a,*]

[a] Department of Biological Systems Engineering, Washington State University, P.O. Box 646120, Pullman, WA, 99164-6120, USA
[b] Technical Innovation Center, McCormick & Company, Inc., Hunt Valley, MD, 21031, USA

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ABSTRACT

High-fat low-moisture foods are often implicated in outbreaks of Salmonellosis. Research has suggested that the fat content in peanut butter may play an important role in protecting *Salmonella* from thermal inactivation. Our recent studies indicate that the water activity (a_w) of oil reduces sharply with increasing temperature, which causes desiccation to the bacteria and consequently enhances their thermal resistance. Therefore, a humid heating environment may help accelerate the thermal inactivation rate of bacteria inside oil. In this research, we selected *Enterococcus faecium* NRRL B-2354 (*E. faecium*) as a surrogate of *Salmonella* to study how the bacterium inside oil responds to thermal treatments under different relative humidities (RHs). Specially, we determined the decimal reduction time (*D*-value) of *E. faecium* in peanut oil which was exposed to different RHs (0–100%) at 80 °C. The data revealed that the D_{80} of *E. faecium* in 0.51 mm oil layers reduced exponentially from 6335.8 to 9.6 min with increasing RH (0–61%), then stayed constant (7.7 ± 1.5 min) at higher RH levels (61–100%). Further experiments with reduced sample thicknesses (0.26 & 0.10 mm) revealed significantly lower D_{80} values in thinner oil layers than in thicker oil layers at those high RH levels (61%–100%). The results of this study indicate that the water vapor equilibration was not reached in oil in short-time high-RH thermal treatments. This study suggests that oil protects bacteria from thermal inactivation not only through desiccation of bacterial cells but also by serving as a moisture barrier to hinder environmental water vapor from rehydrating the bacteria during short-time high-RH thermal processing.

1. Introduction

Outbreaks of Salmonellosis from consuming low-moisture foods (LMFs) have become an emerging issue in recent years. According to the reports from the U.S. Centers for Disease Control and Prevention (CDC), the LMFs with high fat-content, including peanut butter, tree-nut butter, and tahini (sesame paste), have caused more outbreaks than the other LMFs over the past 15 years (U.S. CDC, 2020). To reduce the risk of food contamination, the U.S. Food and Drug Administration recommended a 5-log reduction of *Salmonella* spp. in peanut-derived products (U.S. FDA, 2009). Currently, thermal processing is still the most efficient and commonly used method to achieve that goal.

Effective design of thermal processing relies on mathematical models to ensure the safety of the products (Holdsworth, 1985). For the application of the model, thermal resistance studies are required to obtain the thermal death parameters of the pertinent pathogen(s). In previous thermal resistance studies of *Salmonella* in peanut butter, oil was ascribed as the cause of the very high heat tolerance and the nonlinear thermal death behavior of *Salmonella* (Kenney & Beuchat, 2004; Li et al., 2014; Ma et al., 2009; Shachar & Yaron, 2006). Our recent studies suggest that oil protects bacteria from heat through desiccation of the bacterial cells (Yang et al., 2020a, 2020b). Specifically, when oil is heated to a thermal treatment temperature in a closed container, its water activity (a_w) decreases sharply and moisture consequently moves out of the bacteria cells to the surrounding oil matrix through diffusion. This process may take only a few seconds, according to Syamaladevi et al.'s calculation (2016). This desiccation occurs no matter how high the initial a_w of oil is at room temperature. For instance, when a peanut oil sample that has been sufficiently conditioned at 100% relative humidity (RH) is heated from 25 to 80 °C, its a_w will drop from 1.0 to 0.37, leading to a relatively dry heating environment for bacterial cells in oil.

Similarly, bacteria in high-fat LMFs, like peanut butter, can also be influenced by the desiccation effect of the oil. But the oil in peanut butter, likely in the form of tiny droplets, can also absorb moisture from the surrounding environment through moisture diffusion, which may counteract the effect of desiccation. Possibly, the a_w of a tiny oil droplet

* Corresponding author.
E-mail address: jtang@wsu.edu (J. Tang).

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will initially drop as temperature rises and then increase slowly from moisture migration until equilibrium was reached. The time for a_w to come up in an oil droplet to a value very close to the environmental RH (the a_w come-up time) can be critical to the thermal death rate of the bacteria inside the oil. For a process with a relatively long a_w come-up time, most of the bacteria in oil were treated at a desiccated condition. On the other hand, if the a_w of oil quickly reaches an equilibrium with a high RH environment, the bacteria in oil would be treated in oil with a high a_w. It is, however, extremely difficult to monitor the real-time a_w of an oil droplet in a humid heating environment, but the thermal death behavior of the bacteria in oil can be observed through controlled thermal resistance studies.

The first attempt to study the effect of humidity on the thermal resistance of bacteria was reported by Murrell and Scott (1966). In their study, freeze-dried bacteria were treated in a test tube whose headspace was connected to another tube containing a chemical solution. The a_w of the solution, which determined the RH of the headspace, varied with concentration and the type of chemical. Recently, Tadapaneni et al. (2017) designed a more convenient device, referred to as thermal a_w cell (TAC), to study the influence of RH on thermal inactivation of bacteria in low-moisture foods. A TAC has four wells with a shared headspace, one well holds a salt solution, the other three hold inoculated low-moisture samples. The solution controlled the headspace RH during thermal treatments. Tadapaneni et al. (2018) further improved the design to reduce the come-up time in terms of sample temperature and a_w by reducing the number of sample wells from three to one, and enlarging the surface area of the wells for both sample and solution (See improved TAC in Fig. 1). The modified TAC can be an ideal device to study the thermal resistance of bacteria in porous food powders that are fully exposed to a headspace of a certain RH. It is desirable to investigate if this method is appropriate for studying the thermal resistance of bacteria in oils, as oil may act as a barrier to moisture diffusion.

According to the principle of mass transfer, the difference in RH between oil and the headspace, sample thickness, and the binary mass diffusivity of water vapor in the oil are important factors that determine the come-up time for a_w of an oil layer in TAC. For an oil sample at a fixed moisture content, the RH difference between the sample and the headspace is determined by the selection of the RH controlling agent. The come-up time can be reduced by decreasing the sample thickness of the oil layer. The mass diffusivity of water in oil is temperature-dependent thus will be the same for those oil samples treated isothermally at the same temperature.

Therefore, the main objective of this research was to understand the thermal death behavior of bacteria in peanut oil in response to different environmental RHs and sample thicknesses.

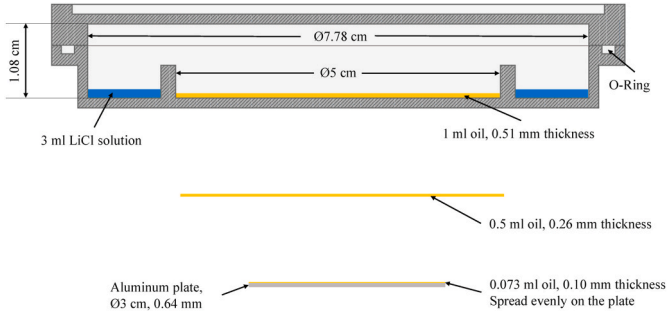

Fig. 1. Schematic representation of TAC (used in this study) containing oil sample and LiCl solution. The oil layers with three different depths are displayed for comparison.

2. Material and methods

2.1. Experimental design

The thermal resistance of *Enterococcus faecium* NRRL B-2354 in peanut oil layers was evaluated corresponding to different sample thicknesses and RHs (with 0.51 mm oil layer at RHs 0–100%, 0.26 mm oil layer at RH 100%, and 0.10 mm oil layer at RHs 61–100%). All experiments were conducted at 80 °C which allowed us to obtain the *D*-values across the whole RH range. The selection of 80 °C also allowed the comparison of the thermal inactivation data for *Enterococcus faecium* NRRL B-2354 from this study with extensively published data for the same bacterial strain in oil and other food matrices to validate our hypothesis. A single bacterial strain of *E. faecium* (NRRL B-2354) was used as inoculum instead of a cocktail of *Salmonella* spp. considering that *E. faecium* is a proven surrogate for industrial process validation of low-moisture food (Ceylan & Bautista, 2015), and a cocktail may show a non-first-order thermal death behavior (Jin & Tang, 2019) which could hinder observations on the bacterial thermal death kinetics.

2.2. Preparation of bacterial inoculum

The inoculation method followed what was described in a previous study (Yang, Xu, et al., 2020). In brief, for each biological independent experiment, a single colony of *E. faecium* NRRL B-2354 (a surrogate of *Salmonella*) (bacterial strain received from Dr. Linda Harris, University of California, Davis), stored on trypticase soy agar (Difco, BD, Franklin Lakes, NJ) with 0.6% yeast extract (Difco, BD) (TSAYE), was subject to two consecutive transfers of 9 ml of trypticase soy broth (Difco, BD) (TSB) with 0.6% yeast extract (TSBYE) each followed by 24 h incubation at 37 °C. Then each 1 ml of culture was spread on a 150 mm × 15 mm plate of TSAYE and incubated for 24 h at 37 °C to form a lawn of bacteria. The lawn was then harvested with 9 ml of sterile 2% buffered peptone water (Difco, BD) with a sterile L-spreader. A bacteria pellet was obtained from three lawns following a 15-min centrifugation at 3000×*g*.

2.3. Inoculation of oil and sample conditioning

The pellet of *E. faecium* was suspended with 0.3 ml of sterile deionized (DI) water and then homogenized with 30 ml of sterile peanut oil using a vortexer (spinning at 3000 rpm for 5 min). To maintain a consistent a_w of the sample, the inoculated oil was conditioned at 33% RH at room temperature (~22 °C) for at least 4 days. This was achieved using a method described in a previous study (Yang, Xu, et al., 2020) by stirring the oil sample inside a sealed container whose inner RH was controlled by saturated magnesium chloride solution.

2.4. RH controlling during thermal treatment

Lithium chloride (LiCl) solutions were used as the RH controlling agent in TACs due to their ability to provide stable RH over a wide temperature range. To obtain desired RHs at 80 °C, an empirical mathematical model was derived based on Gibbard & Scatchart's modeled data (1973) for the a_w estimation of LiCl solutions at temperatures between 0 and 100 °C and molalities between 1 and 18 mol/kg:

$$a_{w.T} = k_m\left(T - T_{ref}\right) + a_{w.Tref} \quad (1)$$

where, $a_{w.T}$ is the a_w of LiCl solution of a certain molality (m, mol/kg) and temperature T (°C); k_m is a constant of a LiCl solution in a certain molality; T_{ref} is the reference temperature in °C, which was 25 °C in this study; and $a_{w.Tref}$ is the a_w at the reference temperature, $T_{ref} = 25$ °C. Two constants, $a_{w.25,}$ and k_m, varies with the molality of the LiCl solution and they were calculated from the molality of the solution with the following empirical equations (fitted from Gibbard & Scatchart (1973)):

$$a_{w.25} = -0.00001419m^4 + 0.00071773m^3 - 0.01024974m^2 - 0.01216982m + 0.98325492$$

$$k_m = 0.00003447m^4 - 0.00203028m^4 + 0.03209317m^4 - 0.06266982m + 0.06807085$$

The goodness of fit of this model was validated with the measured a_w of LiCl solutions reported by Gibbard & Scatchart (1973), which showed a root mean squared error equals 0.002 and the maximum error equals 0.005.

Based on the above model, the LiCl solutions were made gravimetrically by dissolving 12, 18, 32, 40, 48, 58, and 80 g of LiCl powder (Fisher Scientific, Co., Fair Lawn, NJ) into 100 g of DI water in a sealed bottle. The respective a_w of those solutions at 80 °C were calculated from Equation (1) to be 0.89, 0.81, 0.71, 0.61, 0.50, 0.40, 0.31, and 0.19, which reflected RHs of 89, 81, 71, 61, 50, 40, 31, and 19%, respectively. After the powder was completely dissolved and naturally cooled overnight (to ~23 °C), the corresponding a_w of those solutions were measured at 25 °C with a dew-point a_w meter (Aqualab, Meter Group, Inc., Pullman, WA) which were 0.88, 0.80, 0.69, 0.57, 0.45, 0.34, 0.24, and 0.13. To obtain RH of 100% and 0%, pure DI water and phosphorus pentoxide powder (98%, Acros Organics, Thermo Fisher Scientific, NJ) were used instead of LiCl solutions.

2.5. Control of sample thickness

Oil layers of three different thicknesses (0.51, 0.26, and 0.10 mm) were formed in the center wells of TACs to study the effect of sample thickness on the thermal resistance of *E. faecium* in oil. Fig. 1 shows the cross-section of a TAC and oil layers used in this study. A flat 0.51 mm thick oil layer was formed at the bottom of the center well (5 cm diameter) of a TAC by spreading 1 ml of oil sample evenly with a pipette tip. The 0.26 mm oil layers were created by adding half the amount of sample (0.5 ml). To further reduce the sample thickness and meanwhile allow the treated sample to be collected for enumeration, 73 μl of inoculated oil was evenly spread on a sterile aluminum plate (3 cm in diameter × 0.64 mm in thickness) with a pipet tip to make a 0.1 mm thin film, then the plate was placed in the center well without touching the surrounding wall.

2.6. Isothermal treatment

Inoculated and preconditioned peanut oil samples were treated in TACs at 80 °C for different lengths of time to determine the thermal death kinetics of *E. faecium* corresponding to different RHs and sample thicknesses. An oil bath (Neslab GP-400, Newington, NH) filled with circulating ethylene glycol (VWR 264, International, Radnor, PA) was used to elevate and maintain the temperature of TACs. A rack specially designed for TACs (Xu et al., 2019) was used to maintain the horizontal position of the test cells during treatment, and to allow two replicated samples treated simultaneously. At each desired heating interval, TACs were removed from the oil bath and immersed in ice water to terminate the treatment. The treatment time intervals for each experimental condition (RH & sample thickness) were determined from preliminary tests to achieve a minimum of 3-log reductions at the endpoint. The exception was for 0% RH, where less than 2-log reductions in *E. faecium* was achieved after a 6-day thermal treatment at 80 °C.

2.7. Recovery and enumeration

The treated samples were collected to enumerate the survival population of *E. faecium*. For the sampling of oil layers with a thickness of 0.51 or 0.26 mm, the TACs were opened and inclined at an angle of about 45° to allow the oil to pool to one side, then the oil was stirred with a pipet tip and 0.1 ml was collected for serial dilution. For the oil layers of 0.1 mm depth, the whole 73 μl of oil was sampled together with the metal plate. Each sample (with/without metal plate) was transferred into a sterile Whirl-Pak bag (4 oz) with 10 ml of sterile buffered peptone water (BPW) plus 0.1% (v/v) of Tween 80 (Fisher Scientific, Co.), to have a 1:100 or 0.73:100 dilution. For homogenization, the bag was treated in a stomacher (Stomacher 400, Seward Laboratory Systems Inc., Bohemia, NY) at 300 rpm for 10 min. The homogenized suspension was subject to appropriate serial dilutions, then plated in triplicates on a differential media (nonselective), TSAYE supplemented with 0.05% ammonium iron (III) citrate (Sigma-Aldrich, St. Louis, MO) and 0.025% esculin hydrate (97%, Acros Organics), for the growth of *E. faecium*. After 48 h of incubation at 37 °C, all colonies with a black center were counted as one colony-forming unit (CFU) of *E. faecium*.

2.8. Data analyses

The log reduction in colony-forming unit per milliliter of the sample (CFU/ml) was plotted against the treatment time. Two models for the bacterial inactivation in food matrices were tested:

1) The first-order kinetic, or the log-linear model (Peleg, 2006):

$$\log\left(\frac{N}{N_0}\right) = -\frac{t}{D} \tag{2}$$

where N and N_0 are the populations (CFU/ml) at time t and t_0, respectively. The come-up time, t_0, for the oil in a TAC was measured as 50 s, which was rounded up to 1 min.

2) The Weibull model (Peleg, 2006):

$$\log\left(\frac{N}{N_0}\right) = -\left(\frac{t}{\delta}\right)^n \tag{3}$$

where δ (in min) is the scale parameter; and n is the shape parameter. The shape parameter, n, describes if the curve is linear ($n = 1$), or with a changing inactivation rate (increasing when $n > 1$, decreasing when $n < 1$). Since the Weibull model is empirical, it was only used to indicate whether an additional degree of freedom (n, the shape factor) would help fitting the data and how the inactivation rate changes with the treatment time.

For each set of data, the goodness of fit was quantified for both models by the root mean squared error (RMSE) (Motulsky & Christopoulos, 2004):

$$\mathrm{RMSE} = \sqrt{\frac{\sum_{i=1}^{n}\left[log\left(\frac{N}{N_0}\right)_{\mathrm{data,i}} - log\left(\frac{N}{N_0}\right)_{\mathrm{model,i}}\right]^2}{n-p}} \tag{4}$$

where $\log (N/N_0)_{\mathrm{data,i}}$ is the measured log reduction from experiment, $\log (N/N_0)_{\mathrm{model,i}}$ is the calculated log reduction from the model, n is the number of data points, and p is the number of degrees of freedom.

The corrected Akaike information criterion ($\mathrm{AIC_c}$) (Equation (5)) was calculated to show which model is more likely to be correct for the data:

$$\mathrm{AIC_c} = n \cdot ln\left(\frac{\mathrm{SS}}{n}\right) + 2K + \frac{2K(K+1)}{N-K-1} \tag{5}$$

where n is the number of data points, SS is the sum of squares of the residuals, and K is the number of parameters plus one. $\mathrm{AIC_c}$ is more accurate than AIC when the number of data points is small compared to the number of model parameters (Motulsky & Christopoulos, 2004). The $\mathrm{AIC_c}$ evaluates if a decrease in SS is justified by the addition of parameters. A lower $\mathrm{AIC_c}$ indicates that the model is more likely to be correct.

One-way ANOVA and Fisher least significant difference (LSD) tests were used to compare the difference between the means of $\log D_{80}$. All differences were considered significant if the probability was less than 0.05 ($P < 0.05$). The statistical analysis was done using computer software, Minitab 18.1 and Microsoft Excel 16.0.

3. Results and discussion

3.1. Model comparison

The log-linear model (Equation (2)) and the Weibull model (Equation (3)) were fit to each of the 16 data sets (depth & RH) (Table 1). For most cases in the 0.51 mm sample thickness group, the Weibull model had a better fit than the log-linear model except for two data sets, judging from the lower AICc values. But the log-linear model was better in four out of five data sets for the 0.10 mm sample thickness group. The Weibull model indicated a slightly accelerating inactivation rate in most situations. Both models provided a good prediction of the results as the RMSEs were relatively low (<0.67), considering that the RMSE of a primary model (log-linear or Weibull model) generally ranges from 0.3 to 1.1 in LMF studies (Limcharoenchat et al., 2018; Smith et al., 2016).

3.2. Effect of RH in D values of E. faecium in oil

Fig. 2 shows the thermal death curves (at 80 °C) of *E. faecium* in 0.51 mm oil layers when exposed to RHs from 0 to 100%. It is evident that *E. faecium* was more resistant to heat when treated under a lower RH. The variations in thermal death curves were less apparent at those higher RHs (61–100%), as the thermal death curves overlapped with each other (Fig. 2(a)). Within RHs 0-61%, the mean D_{80}-values (Table 1) decreased sharply with the increasing RH from 6335.8 min to 9.6 min; the mean logD_{80} (Fig. 3) exhibited a linear relationship with the RH of the heating environment. However, at RH levels higher than 61%, the mean D_{80}-values remained relatively constant, ranging from 6.3 to 8.2 min. The results from ANOVA tests (grouping letters, Table 1) using transformed D_{80}-values (logD_{80}) suggested a significant ($P < 0.05$) effect of RH in the range between 0 and 61%, but no significant difference was found among log*D*-values at those higher RHs (i.e., 71%, 81%, 89% & 100%).

3.3. Effect of sample thickness

A possible reason for the similar *D* values for *E. faecium* in the 0.51 mm oil samples at the RH levels between 61 and 100% is that the total thermal treatment time (30 min) was too short for the a_w of oil to reach a constant value (the equilibrium a_w) throughout the entire oil layer. To prove this hypothesis, thinner oil layers (0.26 & 0.10 mm) were tested to study the effect of sample thickness at 100%RH. Fig. 4 shows the thermal death curves of *E. faecium* in different oil thicknesseses. The population of *E. faecium* reduced faster in a thinner oil layer. The inactivation curves for *E. faecium* in the 0.10 & 0.51 mm oil samples had better linearity ($n = 1.2$) than that for the 0.26 mm oil samples ($n = 1.6$), as their shape factors (for Weibull model), *n*, were closer to 1 (Table 1). The survival curve for the 0.26 mm thick oil sample seems to overlap with the curve for the 0.51 mm sample within the first 5 min of thermal treatment, and then two curves deviated with different inactivation rates. The mean D_{80}-values of *E. faecium* in these oil layers were 7.2, 3.7 & 0.9 min, respectively (Table 1). This comparison clearly showed the effect of oil sample thickness on the thermal death of *E. faecium* in a high-humidity environment.

To further support our explanation about the similar *D*-values of *E. faecium* in the 0.51 mm oil samples at high RHs, the thermal resistance of *E. faecium* in 0.10 mm oil layers were tested at RH of 61%, 71%, 81%, & 89% at 80°C. In Fig. 3, the mean logD_{80} of each experimental condition was plotted against the RH for comparison. Different from 0.51 mm oil layers, the logD_{80} obtained from 0.10 mm thick oil samples were similar only at RH of 61 and 71%; it decreased sharply as the RH increased from 71% to 100%. in in oilof The logD_{80} of *E. faecium* in an aqueous buffer ($a_w = 1$) was also plotted in Fig. 3, which was extrapolated from the reported *D*-values of Sörqvist (2003). This data point fell close to the intersection between the extended semi-log linear line (regression from the data at RHs, 0–61%) and the 100% RH axis. The respective D_{80}-values of *E. faecium* in 0.51, 0.26, and 0.10 mm oil layers were about 45, 23, and 6 times of the D_{80}-value of *E. faecium* in the buffer (0.16 min). This indicated that the protective effect of oil from humid heat was weakened with decreasing oil thickness; the *D*-value of bacteria in oil would approach the *D*-value when there was no protection from the oil.

3.4. Moisture diffusion in oil

The effect of oil sample thickness on the *logD*-value of *E. faecium* at high RHs (shown in Fig. 3) can be explained by dynamic moisture diffusion within the oil layers when heated in high humidity environments. When a sample was treated in an environment where the water vapor pressure was different from the water vapor pressure of the sample, moisture diffusion would occur. The rate of moisture transfer in a food sample is generally smaller than that of heat transfer (Muramatsu et al., 2017; Panagiotou et al., 2004). Thus, the moisture equilibration between the oil layer and the humid environment may take a much longer time than the temperature equilibration thus influencing the *D*-value of bacteria. A previous study (Tadapaneni et al., 2018) noted that it took about 4 min for 0.7 g of wheat flour (0.5 mm thick) to reach moisture equilibration in TAC at 80 °C and 50% RH which was much

Table 1
Thermal death parameters (±standard deviations) of *E. faecium* in peanut oil of different sample thicknesses at 80 °C and different relative humidities (RH).

		Log-linear Model				Weibull Model			
Sample thickness (mm)	RH	D_{80} (min)	Log D_{80}[a]	RMSE (log CFU/ml)	AIC$_c$[b]	δ (min)	*n*	RMSE (log CFU/ml)	AIC$_c$[b]
0.10	100%	0.9 ± 0.2	−0.05 ± 0.10^{A}	0.67	−23.1	1.3 ± 0.2	1.3 ± 0.2	0.65	−22.9
	89%	1.5 ± 0.5	0.15 ± 0.13^{B}	0.64	−19.3	1.4 ± 0.9	1.0 ± 0.6	0.64	−16.8
	81%	2.7 ± 0.3	0.42 ± 0.05^{C}	0.36	−57.4	3.7 ± 0.6	1.6 ± 0.5	0.28	−69.8
	71%	6.4 ± 0.4	0.80 ± 0.02^{E}	0.32	−72.2	5.5 ± 0.6	0.9 ± 0.0	0.32	−68.0
	61%	6.2 ± 0.3	0.79 ± 0.02^{E}	0.30	−73.6	6.1 ± 1.4	1.0 ± 0.2	0.29	−72.6
0.26	100%	3.7 ± 0.7	0.56 ± 0.08^{D}	0.63	−27.2	7.9 ± 2.4	1.8 ± 0.6	0.60	−31.5
0.51	100%	7.2 ± 0.8	0.85 ± 0.05^{E}	0.38	−73.4	9.8 ± 1.1	1.4 ± 0.4	0.32	−85.4
	89%	6.3 ± 0.6	0.80 ± 0.04^{E}	0.47	−55.0	8.7 ± 0.7	1.3 ± 0.1	0.37	−70.9
	81%	8.2 ± 1.7	0.91 ± 0.08^{FE}	0.40	−72.8	10.2 ± 3.7	1.3 ± 0.6	0.36	−80.0
	71%	7.5 ± 0.8	0.87 ± 0.04^{FE}	0.54	−47.9	10.7 ± 3.8	1.4 ± 0.5	0.51	−51.2
	61%	9.6 ± 1.6	0.98 ± 0.07^{F}	0.41	−72.0	12.8 ± 2.3	1.3 ± 0.5	0.38	−74.8
	50%	17.9 ± 1.7	1.25 ± 0.04^{G}	0.58	−40.7	25.9 ± 8.2	1.3 ± 0.3	0.49	−51.8
	40%	76.5 ± 11.9	1.88 ± 0.07^{H}	0.27	−105.6	81.8 ± 12.9	1.1 ± 0.3	0.26	−105.1
	31%	261.9 ± 62.4	2.41 ± 0.11^{I}	0.46	−62.5	290.0 ± 38.6	1.1 ± 0.4	0.35	−83.8
	19%	995.5 ± 174.7	2.99 ± 0.08^{J}	0.25	−112.9	973.1 ± 231.3	1.0 ± 0.2	0.24	−114.5
	0%	6335.8 ± 1551.8	3.80 ± 0.10^{K}	0.45	−49.1	4764.0 ± 2896.3	1.0 ± 0.0	0.45	−47.0

[a] Variation in grouping letters indicates a significant difference ($P < 0.05$).
[b] Within a row, a lower AIC$_C$ value indicates that the model is more likely to be correct.

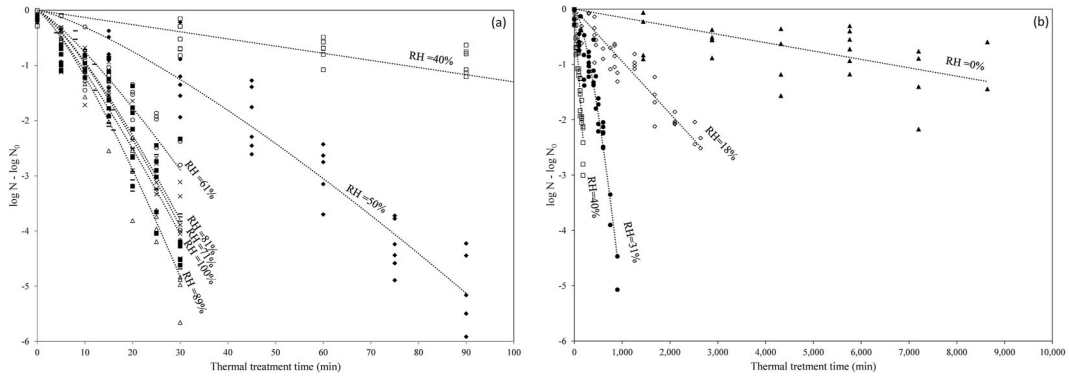

Fig. 2. Thermal death curves of *E. faecium* in 0.51 mm peanut oil layers isothermally (80 °C) treated at controlled relative humidities (RHs) at: (a) 40%–100%, and (b) 0%–40%.

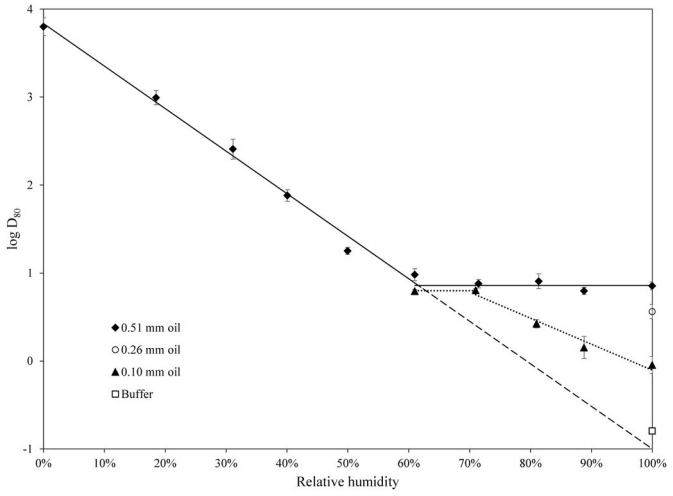

Fig. 3. Logarithmic thermal death time (logD_{80}) of *E. faecium* in peanut oil of different sample thicknesses and at different relative humidities (n = 3). Filled diamonds- 0.51 mm; empty circle- 0.26 mm; filled triangle- 0.51 mm; empty square-in aqueous buffer (Sörqvist, 2003).

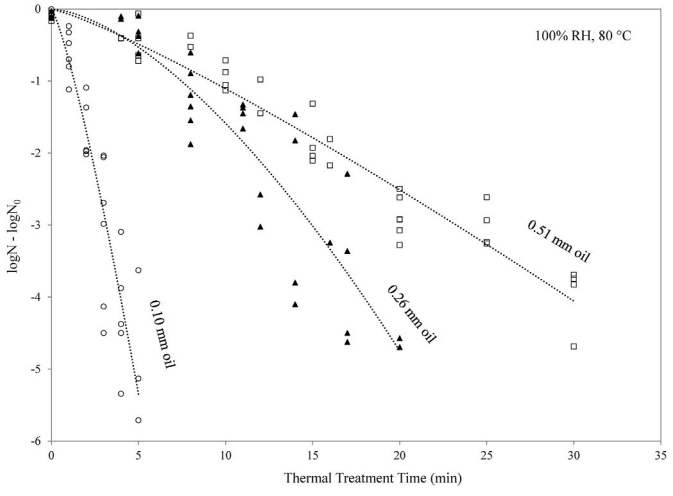

Fig. 4. Thermal death curves of *E. faecium* in peanut oil of different sample thicknesses, treated at 100% relative humidity (RH) and 80 °C.

longer than the temperature come-up time (50 s). When an oil layer was treated in TAC, the a_w come-up time could be longer than 4 min due to the vastly smaller binary mass diffusivity of water in oil than that in the air pores of wheat flour (Cussler, 2009; Hilder & van den Tempe, 1971).

Fig. 5 illustrates a_w distribution within a 0.5 mm oil layer (the top lines and the dashed lines mark the 0.1 and 0.26 mm oil layers) at three different time intervals when heated at 80°C and exposed to the head space RH of 80%. The initial a_w of the peanut oil (inoculated) used in this study was 0.33 at 23 °C. When immersed in the heating bath, the temperature of the oil layer in the TAC increased to 80 °C within 50 s (t_0). The a_w of oil dropped to about 0.13 (Fig. 5(a)). According to Yang, Guan, et al. (2020), the a_w of bacterial cells would also drop to 0.13 due to rapid moisture exchange between the bacteria cells and the oil surrounding them (Syamaladevi et al., 2016). Yet the headspace RH in the test cell remained at high (e.g. 80%), it wascontrolled by the LiCl solution. The difference in water vapor pressure between the headspace and the oil drove moisture to diffuse from the headspace into the oil layer. At a certain time, t_1, a middle point of the diffusion process, a gradient of a_w was established across the depth of the oil layer (Fig. 5 (b)). Consequently, bacterial cells in the oil layer would be would b rehydrated to a different extent—the bacteria within the top 0.10 mm oil layer would absorb more water than the bacteria in the bottom portion of the 0.26 or 0.51 mm oil layer. The variations in a_w within the oil layers remained until the end of a_w come-up time, t_c, when a_w became uniform for the oil layer and the bacterial cells (Fig. 5(c)).

The different levels of hydration of bacterial cells in difference depths of the oil samples from the high RH headspace were responsible for the different D values observed in Fig. 3. Specifically, in determining the *D*-value of *E. faecium* in oil under each experimental condition, isothermal treatments were conducted over different lengths of time (with a uniform interval) (Figs. 2 and 4). The total treatment time for each RH depended upon the *D*-value of *E. faecium;* it was normally greater than 3 × *D*. When t_c was smaller than the *D*-value (e.g. *D*/2), the heterogeneous moisture distribution within the oil sample only lasted for a relatively short time (e.g. 1/6 of the total treatment time), so the *D*-value can be considered as obtained from an equilibrium a_w condition. This was the case for the *E. faecium* in 0.51 mm oil layer treated at lower RHs (0%, 19%, 31%, 40%, & 50%) in which t_c was relatively small compared to the D_{80}-values (6335.8, 995.5, 261.9, 76.5, & 17.9 min). On the other hand, when t_c was larger than *D*, the heterogeneous a_w distribution in oil persisted over a relatively large portion of the treatments (e.g. more than 1/3 of the total treatment time). Consequently, the *D*-value obtained from a linear regression would be greater than the *D*-value corresponding to the equilibrium a_w. This was the case for the *E. faecium* in 0.51 mm oil layers treated at higher RHs (61%, 72%, 81%, 88%, & 100%) whose D_{80}-values were between 6.3 and 9.6 min. The a_w come-up time, t_c, decreased with the reduction of sample thickness, thus, the *D*-values of *E. faecium* in a thinner oil layer (e.g. 0.1 mm) were were closer to the *D*-values when there were no protection from oil (dashed line in Fig. 3).

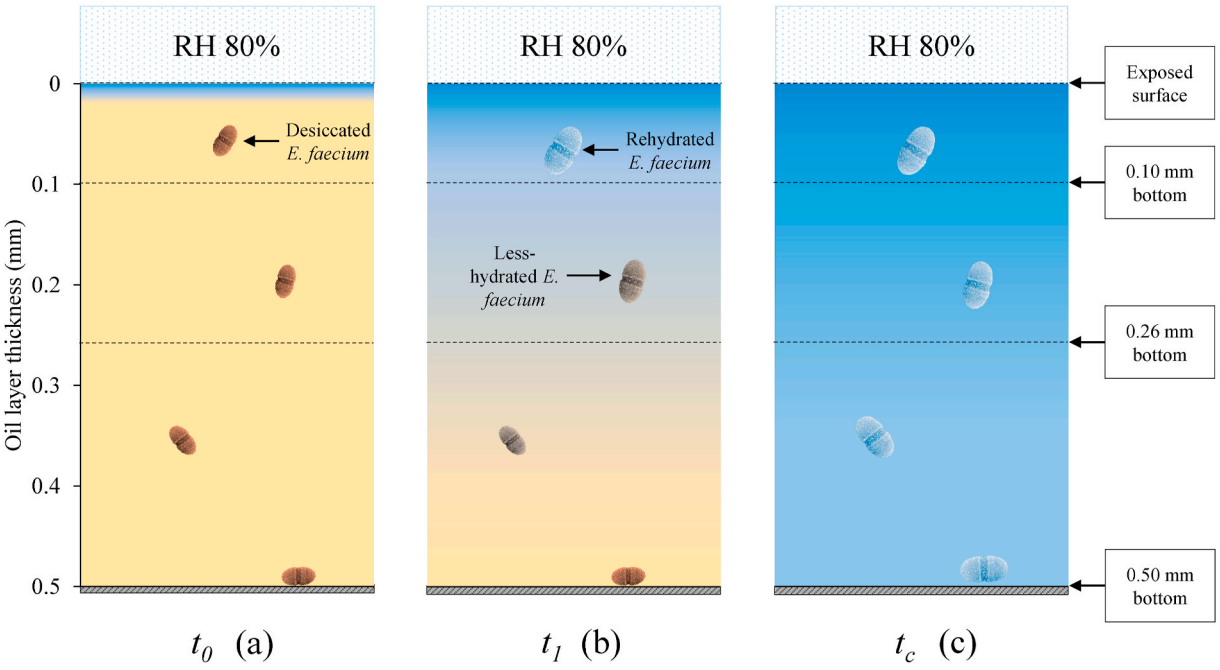

Fig. 5. Graphic illustration of the aw distribution within oil layers exposed to relative humidity (RH) of 80% at 80 °C and three time-points: (a) time t_0 when temperature reaches equilibrium, (b) t_1 when moisture equilibration is in progress, and (c) t_c, the a_w come-up time. Amplified images of *E. faecium* were used to indicate the hydration status of the bacteria cells at different depths in the oil layer. Blue color indicates a high a_w in oil. Yellow color indicates a low a_w in oil. Brown *E. faecium* cells (not to scale) are desiccated by oil to a very low a_w (0.13); larger light blue cells are rehydrated to high a_w levels (close to 0.8); the grey cells are less-hydrated with a_w lower than ~0.7. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

3.5. A general logD vs. a_w relationship

The above discussion and the results from our previous study (Yang, Xu, et al., 2020) both implied that the a_w of oil is the governing factor in the thermal resistance of bacteria inside oil. To support this concept, the logD_{80} values that were obtained from homogeneous isothermal treatments at RHs 0%, 19%, 31%, 40%, 50%, and 61% (from 0.51 mm oil layers) were plotted against the a_w of the matrix in Fig. 6 with the data reported from the literature (Liu et al., 2018; Sörqvist, 2003; Yang, Xu, et al., 2020). The logD_{80} from Yang, Xu, et al. (2020) was obtained from treating *E. faecium* in peanut oil in closed systems where the a_w was homogeneous and constant upon temperature equilibration. The logD_{80} from Liu et al., 2018 was also obtained with TACs but with *E. faecium* inoculated on sand (silicon dioxide). As previously mentioned, the point at a_w 1.0 was extrapolated from the reported *D* & *z* values of *E. faecium* in aqueous buffer (Sörqvist, 2003). A model was made from linear regression using all this data. The z_{a_w} value, a_w increment for one decimal reduction in D_{80}, was calculated as 0.23. Fig. 6 suggests a universal exponential relationship between the *D*-value and the a_w of *E. faecium* regardless of the matrix the bacteria were located in.

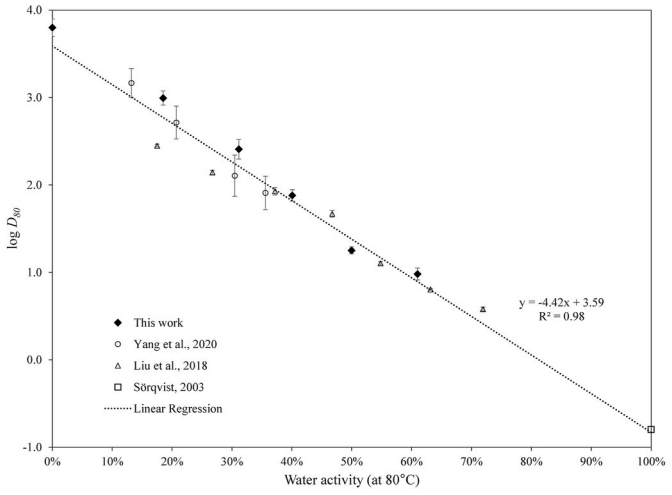

Fig. 6. Logarithmic thermal death time (80 °C) of *E. faecium* corresponding to the a_w (measured at 80 °C) of the food matrix. Filled diamonds-heated in peanut oil exposed to humid air; Empty circle-in peanut oil treated in sealed capillary tubes (Yang, Xu, et al., 2020); Empty triangle-on the surface of silicon dioxide granules exposed to controlled RH (Liu et al., 2018); Filled square-in aqueous buffer (Sörqvist, 2003).

3.6. The protective effect of oil

Based on previous studies (Yang, Xu, et al., 2020) and this work, it can be concluded that moisture in bacterial cells may play a critical role in affecting the thermal resistance of *E. faecium* inside oil. When oil is heated in a closed system, its a_w would decrease sharply with temperature causing desiccation of the bacterial cells and making them highly resistant to thermal inactivation (Yang, Guan, et al., 2020). When oil is heated with a headspace, the changes in oil a_w with increasing temperature would depend upon the rate of moisture exchange between the oil and its ambiance and how rapid moisture migrates within the oil. The slow water vapor diffusion in oil may cause a dynamic heterogeneous a_w condition in oil during thermal treatments resulting in the observed non-first-order thermal death behaviors.

This research was limited to one bacterial strain (*E. faecium*). But the results may provide useful insight into how oils in general protect bacteria in thermal processing. This is because the fundamental principles that govern a_w change and moisture diffusion in oil and the fact that thermal resistance of bacteria sharply increases with reducing a_w do not change with oil types or bacterial species. For example, previous studies (Hilder, 1968; Yang, Guan, et al., 2020) observed that the a_w of different edible oils changed similarly with temperature which would cause desiccation of any bacteria in the oil. The effect of desiccation, whether inside oil or not, on bacterial thermal resistance has also been observed

on other bacterial species in addition to *Enterococcus* and *Salmonella*, like *Listeria, Bacillus,* and *Clostridium* (Murrell & Scott, 1966; Podolak et al., 2017; Taylor et al., 2019; Tsai et al., 2019). Thus, the general conclusion from this study should not be limited to the selected specific type of oil or bacterium.

4. Conclusion

In summary, this study observed the effect of relative humidity and sample thickness on the thermal resistance of *E. faecium* in peanut oil. The result suggests that the thermal resistance of *E. faecium* reduces exponentially with increasing a_w of oil. But due to the slow mass diffusion of water in oil, the a_w come-up time for thicker oil layers exposing to high RHs in thermal treatments can be relatively long, thus compromising the thermal inactivation efficiency in high RH environments. In addition to the desiccation effect of oil in thermal processing, this study suggests that oil as a barrier to moisture diffusion is another mechanism that causes the protective effect of oil on bacteria from thermal inactivation.

CRediT authorship contribution statement

Ren Yang: Methodology, Conceptualization, Investigation, Formal analysis, Writing - original draft, Project administration. **Yucen Xie:** Investigation, Validation, Writing - review & editing. **Stephen P. Lombardo:** Methodology, Writing - review & editing. **Juming Tang:** Conceptualization, Resources, Supervision, Writing - review & editing, Funding acquisition.

Declaration of competing interest

Each named author has substantially contributed to conducting the underlying research and drafting this manuscript. Additionally, to the best of our knowledge, the named authors have no conflict of interest, financial or otherwise.

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