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Desiccation in oil protects bacteria in thermal processing

Ren Yang[a], Jie Xu[a,1], Stephen P. Lombardo[b], Girish M. Ganjyal[c], 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
[c] School of Food Science, Washington State University, P.O. Box 646376, Pullman, WA 99164-6376, USA

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ABSTRACT

Edible oils have long been considered to have a protective effect on bacteria from thermal inactivation, but the mechanism for this effect remains unclear. Our recent study suggests that the water activity (a_w) of oil decreases exponentially with increasing temperature. Therefore, in thermal processing, the a_w of the bacteria inside oil may also decrease making the bacteria more resistant to heat. To validate this hypothesis, the equilibrium a_w of bacteria (*Enterococcus faecium* NRRL B2354, or *E. faecium*) in peanut oil samples, with different initial a_w (0.93, 0.75, 0.52 & 0.33) at room temperature, were measured at elevated temperatures up to 80 °C. Meanwhile, the thermal resistances of *E. faecium* in these samples were also tested at 80 °C. Results indicate that the a_w of the bacteria-in-oil systems changed in the same manner as that of pure peanut oil; it decreased exponentially with temperature from 0.93, 0.75, 0.52 & 0.33 (at ~23 °C) to 0.36, 0.30, 0.21 & 0.13 (at 80 °C), respectively. This confirmed that bacterial cells experienced desiccation in oil during the thermal treatments. The thermal death rates of *E. faecium* in peanut oil samples followed first-order kinetics. The D_{80} value (time needed to achieve 1-log reduction at 80 °C) increased exponentially with the reduced a_w at 80 °C, from 87 min at a_w 0.36 to 1539 min at a_w 0.13. A graphical comparison ($\log D_{80}$ vs. high-temperature a_w) showed a similarity between the thermal resistance of *E. faecium* in oil and that in dry air, which supports the hypothesis that oil protects bacteria from thermal treatments through desiccation.

1. Introduction

Outbreaks in recent years indicate that contamination of *Salmonella* spp. is still a risk in high-fat low-moisture products, such as nut butter and spreads (Cavallaro et al., 2011; Nascimento et al., 2018; Sheth et al., 2011; U.S. CDC, 2013, 2016). The U.S. Food and Drug Administration recommends a 5-log reduction of *Salmonella* for peanut-derived ingredients (Food & Administration, 2009). So far, thermal processing is the most effective and commonly used method to achieve that goal.

Proper thermal process design requires knowledge of the thermal death kinetics of the target pathogen in the product (Silva & Gibbs, 2012). In previous studies, *Salmonella* spp. have exhibited very high heat tolerance in peanut butter (He, Guo, Yang, Tortorello, & Zhang, 2011; He, Li, Salazar, Yang, Tortorello, & Zhang, 2013; Kenney & Beuchat, 2004; Li, Huang, & Chen, 2014; Ma, Zhang, Gerner-Smidt, Mantripragada, Ezeoke, & Doyle, 2009; Shachar & Yaron, 2006). The low water activity (a_w) of peanut butter and the protection from the high oil content (about 50% in peanut butter) were believed to be the major causes of the enhanced thermal resistance of *Salmonella*. However, it is not clear how oil enhances the heat tolerance of bacteria, and under what circumstances will the protection becomes stronger or weaker. With these questions unanswered, the protective effect of oil toward bacteria could be a potential risk for the safety control of high-fat low-moisture food products.

The protective effect of oil occurs when bacteria are heated in lipid materials, which leads to an enhanced thermal resistance as compared to bacteria suspended in buffers or high-moisture food systems (Molin & Snygg, 1967; Senhaji & Loncin, 1977). This effect has been observed on a variety of bacterial strains (spores and vegetative cells) within non-aqueous menstruum like edible oils (pure or blends), glycerin, triolein, and others (LaRock, 1975; Molin & Snygg, 1967; Mverance & LaRock, 1973). It was also reported that adding a small amount of water into the oil could reduce this protective effect, but the role of water content is unclear (LaRock, 1975; Molin & Snygg, 1967). Revealing the mechanism of this protective effect of oil could benefit both academia and the food industry in further understanding the thermal death mechanism of microorganisms and better process design for pathogen

* Corresponding author.
E-mail address: jtang@wsu.edu (J. Tang).
[1] Current Address: Depart of Food Science and Technology, The Ohio State University, Columbus, OH 43210, USA.

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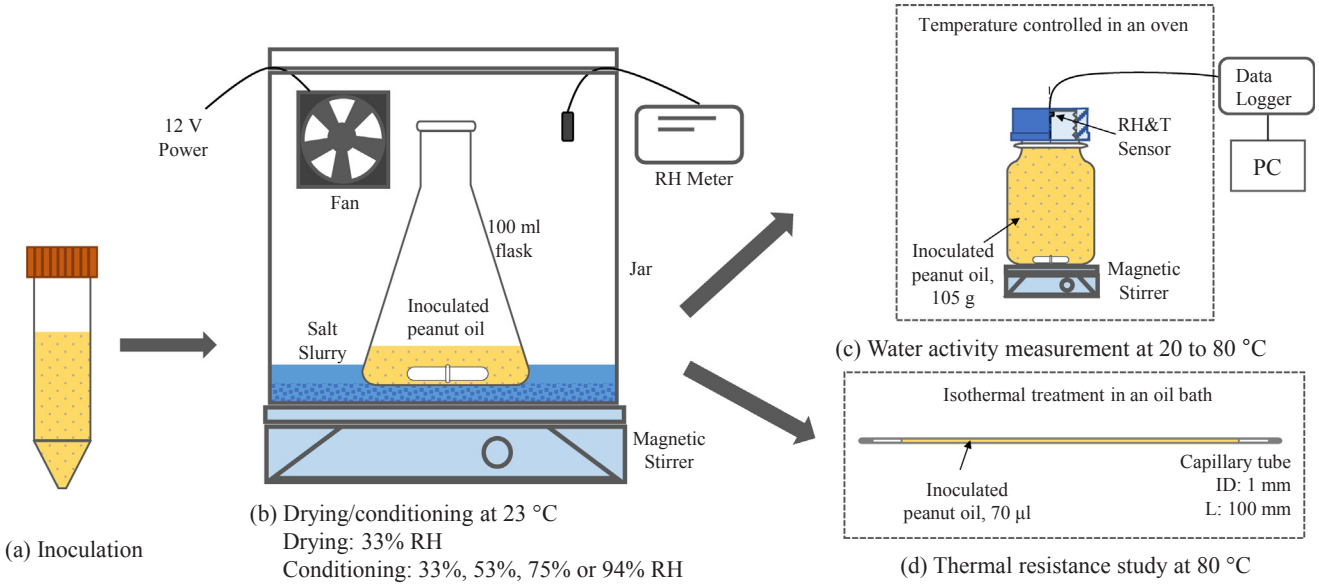

Fig. 1. Experimental flow chart of the study. Including (a) inoculated peanut oil, (b) drying/conditioning apparatus for inoculated peanut oil, (c) device for water activity measurement of sample, and (d) capillary tube used for thermal resistance study of *E. faecium* in peanut oil.

control of low-moisture products containing high-fat content.

Our recent study (Yang, Guan, Sun, Sablani, & Tang, 2020) provides both experimental evidences and theoretical support that the a_w of peanut oil decreases exponentially as temperature increases. It is also well-known that desiccated bacteria are more resistant to heat (Goepfert, Iskander, & Amundson, 1970; Murrell & Scott, 1966). Hence, we hypothesize that the low a_w environment in oil at elevates temperatures causes desiccation of bacteria cells and enhances their resistance to thermal inactivation. Senhaji (1977) proposed a similar hypothesis for *Bacillus* spores in soybean oil associated with canning. However, in that study, the effect of changing a_w in oil samples on the thermal resistance of *Bacillus* spores was not directly measured at a given temperature. Rather, the researcher first extrapolated the effect of a_w on the thermal resistance of bacteria from tests on inoculated oil samples with added water to adjust a_w at high temperatures; and second, tested inoculated oil samples either floating on a bed of water or agitated with water during thermal treatments. No qualitative relationship was established between the a_w of the oil samples and the thermal resistance of the tested bacteria in oil.

It has not been experimentally verified if the a_w of a bacteria-in-oil system would change with temperature equally as the a_w of pure oil. When the temperature is elevated, a_w of the oil decreases sharply, but the a_w of bacterial cells may increase due to the increased water mobility in these cells at elevated temperatures (Syamaladevi, Tang, & Zhong, 2016). Theoreticall, the difference in a_w between oil and bacteria will drive water molecules out of bacteria cells into the oil matrix. This process will stop once thermal dynamic equilibration is achieved, thus is when the a_w of bacteria and oil become equal. The equilibrium a_w depends on the total mass of the bacteria and water holding capacity of oil. Yang et al. (2020) developed a method to measure the water activity of pure oil through the equilibrium relative humidity (RH) in a small headspace, which was used in this study to measure the equilibrium a_w of the bacteria-in-oil system at elevated temperatures. If the equilibrium a_w of inoculated oil samples behaves similarly as pure oil, that is, reduces sharply with temperature, then the desiccation of bacterial cells can be confirmed.

E. faecium has been identified as a valid surrogate of *Salmonella* in several low-moisture foods (Almond Board of California, 2014; Ceylan & Bautista, 2015; Liu, Rojas, Gray, Zhu, & Tang, 2018) and has shown stronger thermal resistance compared to *Salmonella* spp. in peanut butter (Kataoka, Enache, Black, Elliott, Napier, Podolak, & Hayman, 2014). It can be a model bacterium for this study because its thermal death times were measured at 80 °C in hot air at different RH conditions in a recent study (Liu, Tang, Tadapaneni, Yang, & Zhu, 2018). The study shows the effect of desiccation in the air on the heat tolerance of this bacteria. By correlating the thermal resistance of *E. faecium* in peanut oil with the measured a_w of oil (at the treatment temperature), the effect of desiccation in oil can be compared with that in the air to determine if they have the same impact on the bacteria.

The objectives of this research were: 1) to verify the desiccation effect of oil on bacteria at elevated temperatures by measuring the equilibrium a_w of bacteria-in-oil systems, and 2) to directly quantify the influence of the a_w of oil on the thermal resistance of *E. faecium* and determine if desiccation is the major cause for the protective effect of the oil.

2. Materials and methods

2.1. Preparation of bacterial inoculum

The inoculum preparation followed the lawn-based method that was used previously in low-moisture food studies (Hildebrandt, Marks, Ryser, Villa-Rojas, Tang, Garces-Vega, & Buchholz, 2016; Xu, Yang, Jin, Barnett, & Tang, 2020). In brief, *E. faecium* NRRL B2354 (from Dr. Linda Harris, University of California, Davis, CA) was stored as stock cultures on Trypticase soy agar (Difco, BD, Franklin Lakes, NJ) with 0.6% yeast extract (Difco, BD) (TSAYE). For each biological independent set of experiments, a single colony of *E. faecium* was subjected to two consecutive transfers of 9 ml Trypticase soy broth (Difco, BD) with 0.6% yeast extract (TSBYE) and each incubated at 37 °C for 24 h. One milliliter of culture was spread on each of three 150 × 15 mm plates of TSAYE and incubated for 24 h at 37 °C. The bacterial lawn from each plate was harvested with 9 ml of sterile 2% buffered peptone water (Difco, BD) with a sterile plate spreader. Bacteria suspensions from three TSAYE plates were combined and centrifuged at 3000*g* for 15 min to obtain a pellet.

2.2. Inoculation and drying

After removing the supernatant, a small amount of DI water (0.3 ml) was added to the centrifugal tube and vortexed for 2 min using a high shear vortex mixer to separate the bacterial pellet. Then, 30 ml of sterile

(treated for 20 min in autoclave) peanut oil (Ventura Foods, LLC. Brea, CA) was added to the inoculum and homogenized with a 5-min vortex to achieve ~10^9 CFU/ml of *E. faecium* in the oil (Fig. 1a). The oil sample was dehydrated for better preservation by conditioning in an air-tight glass jar containing magnesium chloride salt slurry to provide a constant RH (33%) at room temperature (~23 °C) (Fig. 1b). Post-inoculation drying was commonly used to condition the low-moisture food. However, the mass diffusivity of water molecules in oil is small, which leads to extended time for conditioning as well as sedimentation of bacterial cells (Hilder & van den Tempe, 1971). To accelerate the water vapor equilibration and prevent clotting of cells, the 30 ml of inoculated peanut oil was transferred to a 100 ml sterile flask with a magnetic stir bar (3 cm long) rotating at about 800 rpm. A 5-volt fan was placed in the headspace of the jar to speed-up the mass transfer within the air by creating forced convection. The sample was dried at 33% RH in the glass jar for at least 72 h.

2.3. Sample conditioning

After drying in the first jar, the inoculated oil sample in the flask was taken out. The outside of the flask was washed with DI water to remove residual salt, and the flask was then placed in a second jar for conditioning at 53%, 75%, or 94% RH (± 1%) at 23 °C(Fig. 1b). The RH in the second jar was maintained, respectively, by a saturated solution of magnesium nitrate, sodium chloride, or potassium nitrate (Greenspan, 1977). These RH levels were selected to cover a wide range with similar intervals of a_w for the sample. At least 12 h were required for each sample to reach the equilibrium state of the desired RH condition.

2.4. Water activity measurement at elevated temperatures

The water activities of inoculated peanut oil samples, conditioned at different RHs, were measured at elevated temperatures using the method described in Yang et al. (2020). Briefly, 105 g of a pre-conditioned sample was transferred into a 100 ml glass bottle (Pyrex round media bottle, Corning Inc., NY) to occupy the most space in the bottle and allow the RH to be measured with a sensor in a small headspace (Fig. 1c). The sensor (Honeywell HumidIconTM HIH 8000 Series, Morristown, NJ) read both temperature and RH of the headspace air. Before the measurement, each sensor was calibrated against set temperatures at 30 and 80 °C using an oil bath, and against four standard solutions (METER Group, Pullman WA), with a_w of 0.25, 0.50, 0.76 and 0.92, respectively, at 23 °C (Tadapaneni, Yang, Carter, & Tang, 2017).

During the measurement, a magnetic stirrer, spinning at approx. 400 rpm, created forced convection in the oil to accelerate the thermal dynamic equilibration between the oil and the headspace air (Fig. 1c). The sample was kept at room temperature (~23 °C) for 3 h and then heated to 44, 63, and 82 °C in sequence within a convection oven, each temperature change followed with a 2 h holding session. The RH & temperature of the headspace were recorded every 10 s via computer. The equilibrium RH & temperature were taken as equal to the a_w and temperature of the oil sample.

The tests were conducted in three replicates with freshly conditioned samples and pre-calibrated sensors.

2.5. Isothermal treatment

After conditioning, the oil samples with *E. faecium* of ~10^8 CFU/ml were heated isothermally at 80 °C in capillary tubes to examine the survival populations of *E. faecium* at different lengths of heat treatment (Fig. 1d). The capillary tubes with a 1 mm inner diameter (100 mm long, 0.25–0.4 mm wall thickness; Kimbel Chase, Vineland, NJ) were used for the treatments, for a short come-up time and to minimize the air space which could lower the a_w of the sample according to Yang et al. (2020). In brief, 70 μl of the sample was slowly injected into a sterile glass capillary tube with a pipette and adjusted to the center position by leaning the tube. Both ends of the capillary tube were sealed by holding the tip of the glass tube over a flame until it melted. While applying the glass to the flame, only the tip was exposed, and the rest of the tube was covered in a wet paper towel to prevent heat exposure of the sample. The isothermal treatment was conducted using an oil bath (Neslab GP-400, Newington, NH) filled with ethylene glycol (VWR 264 International, Radnor, PA). Two replicated samples were fully immersed in the ethylene glycol maintained at 80 °C for each of 8 predetermined time lengths after the come-up time (time required for the sample in a capillary tube to reach 79.5 °C). The come-up time was 5 s, measured with a fine (0.5 mm) T-type thermocouple (Omega Engineering, Inc., Stamford, CT) located at the center of a capillary tube filled with non-inoculated peanut oil. To ensure repeatability, each of four a_w levels was tested with three replicated sets of experiments each conducted with independent inoculation, drying, conditioning, and thermal treatment.

2.6. Recovery and enumeration

After the thermal treatment, each sample was immediately chilled by immersing in ice water. The oil in the capillary tube was transferred to a sterile test tube by breaking both ends of the glass with a sterile wire cutter and blowing out the contents with a plastic dropper. 10 ml of sterile BPW plus 0.1% (v/v) of Tween 80 (emulsifier) was added to the test tube and sealed hermetically (0.7:100 dilution). For homogenization, the tube was subjected to a 10 min vortex at 3,200 rpm (Fisherbrand mini vortexer, Waltham, MA). The liquid suspension was subjected to appropriate serial dilutions, then plated in triplicate on differential media for *E. faecium* (TSAYE supplemented with 0.05% ammonium iron (III) citrate and 0.025% esculin) and grown at 37 °C for 48 h. To enumerate colonies of *E. faecium*, all colonies with a black center were counted. The mean colony-forming unit (CFU/ml) of each sample was converted into a logarithmic form for further analysis.

2.7. Data analysis

The measured a_w of inoculated peanut oil samples were compared with the model established for pure peanut oil (Yang et al., 2020):

$$a_w = x \cdot e^{\left(\frac{1714}{T} - 2.7\right)} \tag{1}$$

where x is the mole fraction of water in the water-in-oil solution, its value was determined by fitting Eq. (1) to the experimental data; T is the absolute temperature of the sample in K.

The dry basis moisture content (MC) of each sample was calculated from the mole fraction of water, x, according to its definition using the following equation:

$$MC = \frac{x \cdot M_{water}}{(1 - x) M_{oil}} \tag{2}$$

where M_{water} is the molar mass of water (18.015 g/mol); and M_{oil} is the average molar mass of peanut oil which is about 881 g/mol (Yang et al., 2020).

The thermal resistance of *E. faecium* from each trial was quantified by D-value, the thermal death time in min, which is described as (Peleg, 2006):

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

where N and N_0 are the populations (CFU/ml) at time t and t_0 respectively; t_0 is the come-up time which equals 5 s.

Parameters for the best fits were obtained using Visual Basic (Microsoft Excel 16.0, Seattle, WA). The coefficient of determination (R^2) and root mean squared error (RMSE) were used to quantify the goodness of fit of the model. Levene's test was used to test if the results

have equal variances. Also, One-way ANOVA and Fisher least significant difference (LSD) tests were conducted using Minitab (18.1) to analyse the difference between the means. All differences were considered significant if the probability was less than 0.05.

3. Results and discussion

3.1. Inoculation and conditioning

The mean (± standard deviation) initial population of *E. faecium* across all inoculated samples was 8.6 ± 0.1 log CFU/ml before drying. The mean *E. faecium* population after drying and RH conditioning dropped to 7.7 ± 0.8 log CFU/ml. A similar level of decrease in the population was also observed in other studies on low-moisture foods (Liu, Tang, Tadapaneni, Yang, & Zhu, 2018; Xu, Tang, Jin, Song, Yang, Sablani, & Zhu, 2019), it could be the result of dehydration of bacteria during the conditioning. Overall, the inoculation method developed in this study provided a stable inoculation of *E. faecium* in oil which is easier to apply as compared to the method using freeze-dried inoculum.

3.2. Desiccation of bacteria in peanut oil

The measured a_w of *E. faecium* inoculated peanut oil samples are shown in Fig. 2. At room temperature (22.0–24.8 °C), the measured a_w of these samples were 0.93 ± 0.03, 0.75 ± 0.01, 0.52 ± 0.01 & 0.33 ± 0.01, which were equal to the a_w of the respective salt slurries used for conditioning. As the temperature increased from 23 to 80 °C, the a_w of these samples dropped exponentially to 0.36, 0.31, 0.21, and 0.13, respectively. These data points overlap (RMSE ranged from 0.008 to 0.021) with the predicted curves using Equation (1) that describes changes of the a_w in pure peanut oil with temperature. This indicates that the a_w in inoculated oil samples changed in a similar manner as that in pure peanut oil. According to Syamaladevi, Tang, et al. (2016), the water vapor equilibration between a single bacterium and the environment may happen within seconds. Therefore, the a_w of bacterial cells in oil can be considered equivalent to the a_w of the oil matrix, and it changes simultaneously with temperature. This confirms that the desiccation of bacteria in oil happens in thermal treatments.

The fitted mole fractions of water, *x*, for these four groups of samples with initial a_w of 0.93, 0.75, 0.52, and 0.33 were 0.041, 0.035, 0.024 & 0.015, respectively, and their corresponding moisture contents (dry basis), *MC,* were calculated (using Equation (2)) as 0.088%, 0.075%, 0.050%, & 0.032%. These values indicate that a small change in the moisture content of oil may result in a large variation in the a_w of the oil.

3.3. First-order thermal death kinetics of E. faecium

In Fig. 3, the mean survival populations (log CFU/ml) of *E. faecium* in peanut oils with different a_w are plotted against the lengths of the heat treatments at 80 °C. Each group includes data from three biologically independent experimental sets. A significant linear relationship ($P < 0.05$) was observed between the log-reduction and the thermal treatment time for each data set where the coefficient of determination (R^2) for the linear model ranges from 77 to 96%, and the RMSE (log CFU/ml) ranges from 0.20 to 0.63. A semi-log linear thermal death curve is typical in the isothermal treatment of a single bacterial strain, and it normally indicates consistency in the thermal resistance of bacteria and a homogenous heating environment (Moats, 1971).

The *D*-value of each data set was calculated as the negative inverse slope of the linear regression curve using Equation (3) (Table 1). One-way ANOVA test was used to analyze the log *D*-value under the impact of a_w. The log *D*-values were found normally distributed with equal variances (standard error: 0.10–0.14), and a significant effect of the a_w of the oil was observed ($P < 0.05$).

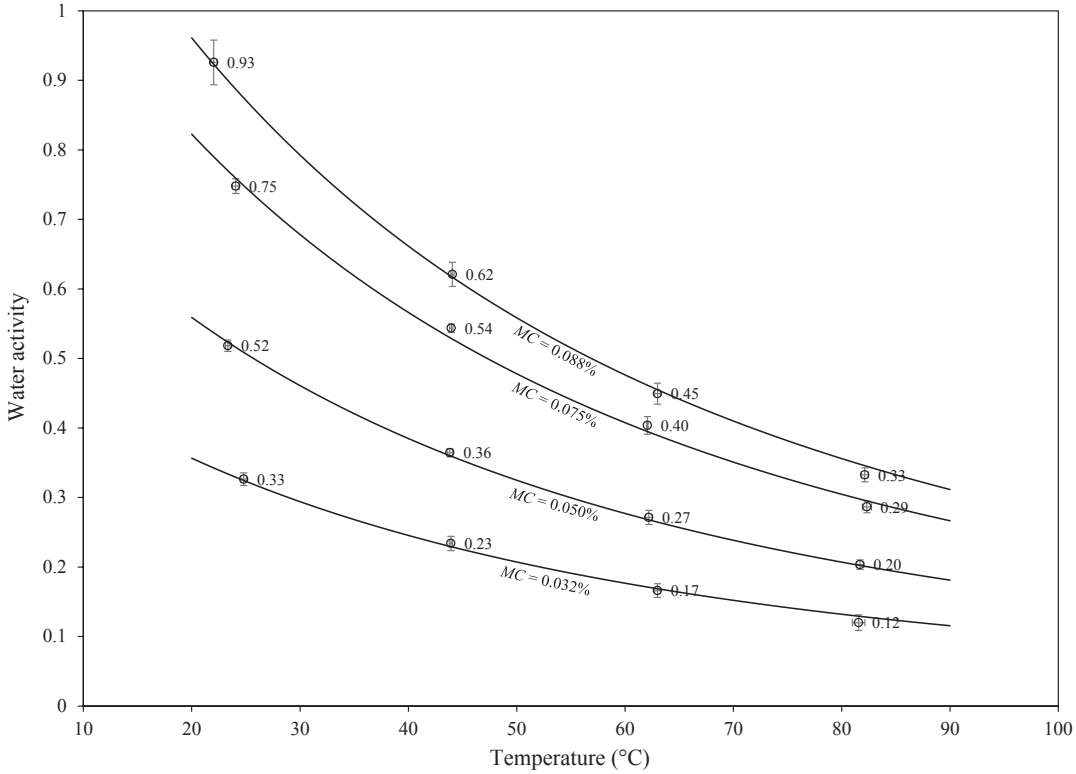

Fig. 2. Measured a_w of *E. faecium*-in-peanut oil samples (circles ± SD) and predicted a_w of pure peanut oil (continuous curves) (calculated from Equation (1)) (n = 3). The dry basis moisture content (*MC*) of each group of samples were labeled under each curve.

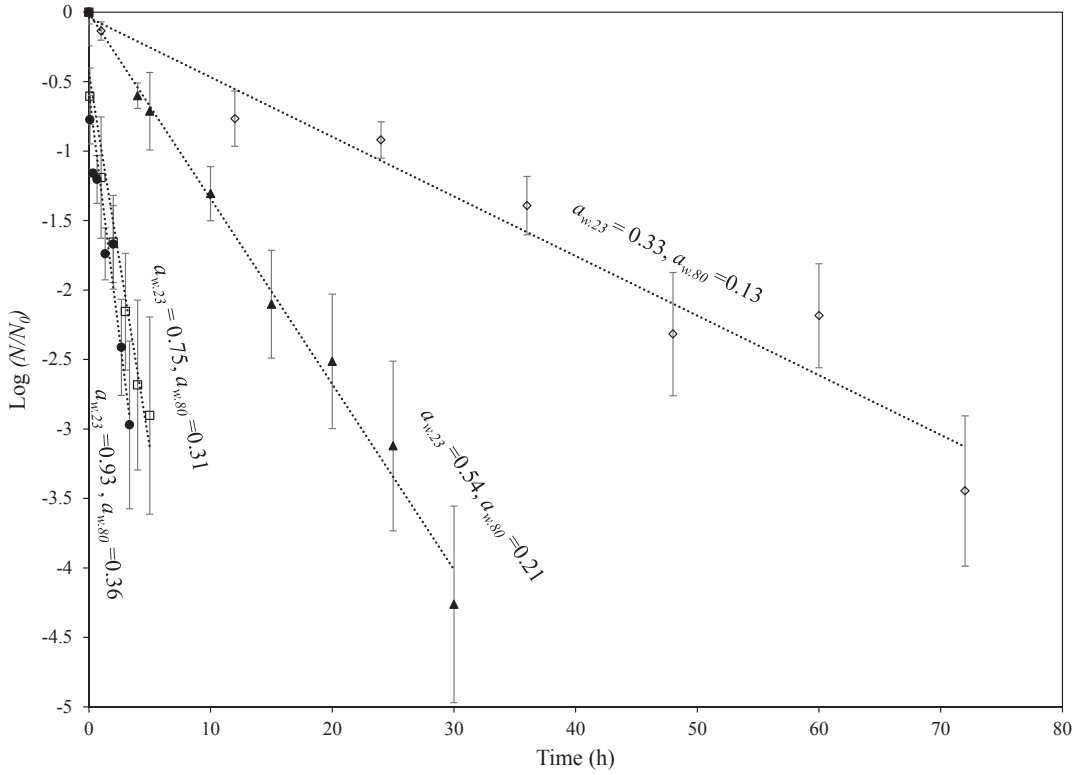

Fig. 3. Survival populations (log CFU/ml, mean ± SE) of *E. faecium* in peanut oil with different water activities after isothermal treatments, at 80 °C, for different amount of time (n = 6). The equilibrium water activities of the bacteria-in-oil system, at 23 ($a_{w.23}$) and 80 °C ($a_{w.80}$), are labeled beside each curve.

3.4. Effect of water activities and D-values of E. faecium

The *D*-value of *E. faecium* in oil increased sharply with the reducing a_w at 80 °C (Table 1). Specifically, when treated at 80 °C in the peanut oil with high-temperature a_w of 0.36, 0.31, 0.21, and 0.13, the D_{80}-values of *E. faecium* were 87, 137, 525 and 1539 min, respectively. The corresponding times required to achieve a 5-log reduction of *E. faecium* in these oil samples were calculated and are included in Table 1. Based on Table 1, it would take about 7.2 (± 2.0) hours for a thermal treatment at 80 °C to achieve a 5-log reduction of *E. faecium* in peanut oil preconditioned to 93% RH at 23 °C. If the oil was exposed to 33% RH before the thermal treatment, it would take 128.2 (± 29.0) hours (approx. 18 times more) to achieve the same level of inactivation of *E. faecium*. We must clarify that these calculations are not for recommendations to process a specific food product, but rather to show the high thermal resistance of *E. faecium* (a surrogate of *Salmonella* spp.) in oil sample and the large variations in the thermal resistance corresponding to different a_w.

The *D*-value of *E. faecium* was found to increase exponentially with the decrease in a_w of oil measured at 80 °C (Fig. 4). A linear relationship was observed between log *D*-value and a_w with z_{aw} = 0.17, that is a 0.17 increase in a_w would cause a 1-log decrease in *D*-value of *E. faecium*. The D_{80}-values of *E. faecium* were several orders of magnitude higher in peanut oil (observed in this study) than in hot water or buffer, which was between 0.16 and 2.45 min (data extrapolated from Sörqvist, 2003; Harris, Uesugi, Abd, & McCarthy, 2012, assuming z = 9.6 °C). Such large differences between the *D*-value of *E. faecium* in oil and buffer can be ascribed to the vast differences in a_w of those two media. A similar observation was also noted in a recent study on the thermal resistance of *Salmonella* in three powdered foods (Xu, Tang, Jin, Song, Yang, Sablani, & Zhu, 2019). The researchers also reported an exponential relationship between the *D*-value of bacteria and the high-temperature a_w of the food matrix within the range of a_w from 0.32 to 0.89.

In a recent study (Liu, Tang, Tadapaneni, Yang, & Zhu, 2018), *E. faecium* was heated at 80 °C at various controlled RHs between 0.18 and 0.72. A semi-log relationship was observed between the *D*-value of *E. faecium* at 80 °C and the RH at the same temperature. In Fig. 4, the log*D* values of *E. faecium* from our observations were plotted against the a_w of peanut oil measured at both room temperature and 80 °C to compare with data from Liu, Tang, Tadapaneni, Yang, and Zhu (2018). When using room temperature a_w as the indicator, the thermal resistance of *E. faecium* was much larger in oil compared to that in the air of different RHs. But the data points moved to Liu et al.'s curve after plotting against the high-temperature-a_w and overlap at two points (a_w 0.36 and 0.31). The similarity in the thermal resistance of *E. faecium* in peanut oil and when treated at controlled RHs indicates a similarity in the protective mechanism- desiccation of bacteria.

Table 1
Thermal death parameters (mean ± SE) of *E. faecium* in peanut oil at 80 °C, corresponding to the equilibrium water activities of peanut oil (n = 3).

Conditioning RH at 23 °C	equilibrium a_w at 80 °C	Mean D_{80}-value (min)	Mean Log D_{80}	Time to achieve a 5-log reduction of *E. faecium* (h)
33%	0.13	1539 ± 347	3.17 ± 0.10^{C}	128.2 ± 29.0
53%	0.21	547 ± 118	2.71 ± 0.11^{B}	45.6 ± 9.9
75%	0.31	140 ± 43	2.11 ± 0.14^{A}	11.7 ± 3.6
94%	0.36	87 ± 24	1.91 ± 0.11^{A}	7.2 ± 2.0

Variations in grouping letters indicate a significant difference (P < 0.05).

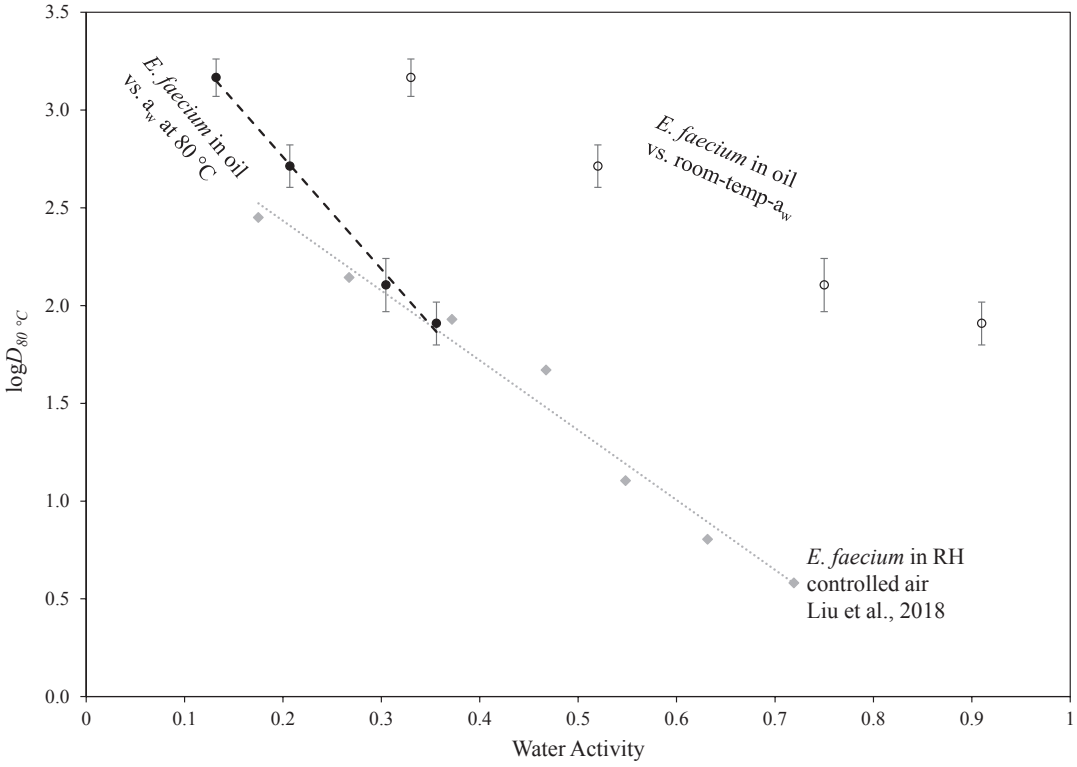

Fig. 4. Thermal resistance (log $D_{80°C}$, mean ± SE) of *E. faecium* in 1) peanut oil vs. room-temperature-a_w of oil (empty circles) (n = 3); 2) peanut oil vs. high-temperature-a_w of oil (80 °C) (solid dots) (n = 3); 3) hot air with controlled relative humidity (grey diamonds) (Liu, Tang, Tadapaneni, Yang, & Zhu, 2018).

Overall, the above results support Senhaji's hypothesis (1997) that desiccation in oil, due to the decrease in a_w of oil with temperature, is the main cause for the protective effect of oil on the thermal resistance of bacteria in oil.

The theory of oil protection can be a powerful tool explaining some phenomenon noted by previous researchers. For example, adding a small amount of water to oil was found to sharply reduce the thermal resistance of the bacteria in oil (Ababouch & Busta, 1987; Molin & Snygg, 1967; Senhaji & Loncin, 1977). This can be explained by the increased a_w of oil at the treatment temperature, as indicated in Figs. 2 and 4. In fact, a very small amount of water added to the oil could easily exceed the water solubility of oil at room temperature. Over-saturated water molecules will not dissolve but may suspend as unstable small globules (Du, Mamishev, Lesieutre, Zahn, & Kang, 2001). These globules could be dissolved as the water solubility of oil raises in thermal treatments, contributing to a higher a_w of oil which would result in lower resistance of bacteria in oil.

In a complex food matric, like peanut butter, the oil may be bound to proteins or starch, or as emulsified tiny oil globules and larger free oil globules/layers. The a_w of the oil globules is not simply dependent on the oil itself but is also influenced by the surrounding components, like proteins or carbohydrates, through moisture diffusion. Thus, the role oil content plays in peanut butter for the protection of bacteria from thermal treatment can be complicated. Further understanding in the a_w of peanut butter and how it changes with temperature may help to establish a valid thermal inactivation model for safety assurance.

4. Conclusion

In summary, we observed a sharp decrease in the a_w of bacteria (*E. faecium*) inside peanut oil at elevated temperatures. The equilibrium a_w of the bacteria-in-oil system was found to be a key factor in the thermal resistance of the bacteria in oil. This effect of a_w on the bacterial thermal resistance was quantitatively similar to the effect of the relative humidity of hot air, indicating that desiccation is the main cause of the protective effect of the oil. This finding improves the fundamental understanding of the thermal death mechanism of bacteria in oil and helps design reliable killing steps of pathogens in oil-rich low-moisture foods.

CRediT authorship contribution statement

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

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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