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Research Paper

Understanding water activity change in oil with temperature

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ARTICLE INFO	ABSTRACT
	Our recent studies and several publications suggest that the low water activity (a_w) of oil in thermal processing might be a major contributing factor towards the increased thermal resistance of bacteria in oils. In this study, we developed a reliable method to measure the water activity of oil by measuring the equilibrium relative humidity in a small headspace. Using this method, water activity of peanut oil was found to decrease exponentially with increasing temperature. A model derived from excess Gibbs free energy was fitted to the observations with an $R^2 = 99.6\%$ and RMSE = 0.01 (a_w). Our results suggest that the sharply reduced water activity of oil resulting from a rise in temperature could cause desiccation of bacteria. This is a possible explanation for the protective effect of oil in thermal processing.

1. Introduction

Outbreaks of *Salmonella* spp. associated with peanut butter have occurred in many countries in recent decades (Cavallaro et al., 2011; Killalea et al., 1996; Sheth et al., 2011; Shohat et al., 1996). *Salmonella* remains a high risk for peanut butter and related food products as thermal treatments are often ineffective. Laboratory studies indicate that bacteria inoculated into oil rich products, such as peanut butter and chocolate, have a notable increased thermal resistance, which has been partially ascribed to the protective effect of oil/fat (Kenney and Beuchat, 2004; Ma et al., 2009; Shachar and Yaron, 2006).

Progress has been made toward understanding the protective effect of lipids in the past century. Earlier reports associated with the protective effect of lipid materials were mostly focused on *Bacillus* spores (Bartlett and Kinne, 1913; Jones and Pearce, 1954; Molin and Snygg, 1967). Thermal death studies of spores in various edible oils showed that the water content and the type of oil are critical to the thermal death time of spores (Ababouch et al., 1987; Ababouch and Busta, 1987; Molin and Snygg, 1967; Senhaji and Loncin, 1977). Later studies on non-spore forming bacteria (vegetative cells) in oil also showed a remarkably increased thermal resistance (LaRock, 1975; Severance and LaRock, 1973; Zaleski et al., 1971). This protective effect for both spore and non-spore forming organisms is not limited to edible oils and fats but also includes other nonaqueous materials such as triolein, octane, paraffin oil, and kerosene (LaRock, 1975; Molin and Snygg, 1967; Severance and LaRock, 1973).

The mechanism of the protective effect of oil remains inconclusive. It has been attributed to several factors including the poor heat conductivity of lipids (Lang, 1935), protective free fatty acids (Ababouch et al., 1987, 1995; Ababouch and Busta, 1987; Molin and Snygg, 1967), and the low moisture content of oil (LaRock, 1975; Molin and Snygg, 1967; Zaleski et al., 1971). The most convincing hypothesis, suggested by Senhaji and Loncin (Senhaji, 1977; Senhaji and Loncin, 1977), suggests that the water activity of oil, which decreases with rising temperature, is the cause of the oil protection phenomenon. Yet Senhaji estimated the water activity of oil at elevated temperatures based upon water solubilities of oil only through mathematic equations with no experimental verification.

Our recent studies on thermal inactivation of *Salmonella* and *E. faecium* suggest that the actual water activity at the treatment temperature is a key factor influencing the heat tolerance of bacteria in low-moisture foods. The thermal death times of these microorganisms in almond flour, wheat flour, whey protein powder, and sands (SiO_2) increase exponentially with decreasing water activities controlled at 80 °C (Liu et al., 2018; Xu et al., 2019). The water activities of different matrices respond differently to temperature changes. For example, water activity increases in wheat flour but decreases in peanut butter when temperature increases (Syamaladevi et al., 2016a). The same study reported that the thermal death time of *Salmonella* is greater in peanut butter than in wheat flour at 80 °C, even though their initial water activities are the same at room temperature (Syamaladevi et al., 2016a). These findings suggest a close connection between the lipid protection phenomenon and the considerably enhanced heat resistance of bacteria due to reduced water activity. To date, however, there has been no study

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that directly links the changes in water activity of oil at high-temperature to the thermal resistance of bacteria in oil and oil-rich food systems during thermal treatments. A major reason that hinders the investigation is the extremely small amount of water in oil which makes the water activity difficult to be accurately measured or controlled in experiments. But more importantly there is a general lack of understanding in the food research community that water solubility in oil changes sharply with temperature, which, in turn, significantly changes water activity in oil. Mineral and vegetable oils are used as insulating and cooling fluids in high-power electric transformers and as dielectric media in high voltage equipment (Rafiq et al., 2015). Moisture in transformer oils has a detrimental effect on the performance of the transformers and other high voltage equipment (Du et al., 2001; Rafiq et al., 2015). A previous study investigated the change of water solubility in transformer oils by measuring the relative humidity (RH) inside oil using a sensor that was immersed in the oil (Du et al., 2001). But their method contaminates the sensors resulting in measurement errors.

The objectives of this study were to: 1) develop a method to accurately measure the water activity of oil at elevated temperatures; and 2) establish a quantitative relationship between the water activity of oil and temperature.

2. Theoretical considerations for water activity measurement in oil samples

The water activity of a common food sample is often determined by measuring the RH or the vapor pressure of the headspace in a closed container after equilibrium is established between the headspace and the sample (Labuza et al., 1976). Ideally, for an accurate measurement, the water holding capacity of the headspace should be negligible compared to that of the measured sample. This usually is not a problem when a relatively large quantity of hygroscopic sample occupies most of the space in the enclosed test cell. But large errors may occur when using the method to measure water activities of oil samples because of their extremely low water content. Thus, in order to design a reliable method for the water activity measurement for oil, it is necessary to evaluate possible water activity change caused by the equilibration between the sample and the headspace and find a way to reduce the change to an acceptable level.

Based on previous studies (Hilder, 1968, 1971; Parsons and Holmberg, 1937), we may assume that the moisture content of an oil sample is proportional to its water activity. Similarly, the moisture content of air is also proportional to its RH according to the Ideal Gas Law. In a closed system where an oil sample and a headspace reach equilibration, the amount of water molecules that oil gained equals to what the headspace air lost, and vice versa. That is:

$$(a_{w.e} - a_w) \cdot V_{oil} \cdot C_{s.oil} = -\left(a_{w.e} - \frac{RH_i}{100\%}\right) \cdot V_{air} \cdot C_{s.air} \qquad \text{Eq. 1}$$

where, a_w is the original water activity of the sample; $a_{w.e}$ is the water activity of the sample after equilibration with the headspace in a closed system; V is the volume unit in m^3; C_s is the volume-based saturation molar concentration of water molecules in mol/m^3; and RH_i is the initial RH of the headspace air.

The change in water activity upon equilibration, Δa_w, can be derived from Eq. 1 as:

$$\Delta a_w = a_{w.e} - a_w = \frac{\left(\frac{RH_i}{100\%} - a_{w.e}\right) \cdot V_{air} \cdot C_{s.air}}{V_{oil} \cdot C_{s.oil}} = \frac{\left(\frac{RH_i}{100\%} - a_{w.e}\right)}{v \cdot c} \qquad \text{Eq. 2}$$

where, v is the volume ratio between oil and headspace (V_{oil}/V_{air}); and c is the ratio of saturation moisture concentration between oil and air ($C_{s.oil}/C_{s.air}$).

According to Eq. 2, the change in water activity upon equilibration is proportional to the difference between the initial RH and the equilibrium

a_w of the headspace and inversely proportional to the volume ratio and the ratio of moisture concentration ($c = C_{s.oil}/C_{s.air}$).

2.1. Evaluating the saturation moisture concentration of oil and air

In order to evaluate and compare the ability of air and oil to contain water, their moisture concentrations at saturated states were calculated at temperatures from 0 to 100 °C as follows.

At low pressures and temperatures, water vapor follows the Ideal Gas Law. The saturation molar concentration of water in air is:

$$C_{s.air} = \frac{n_s}{V} = \frac{P_s}{RT} \qquad \text{Eq. 3}$$

where n_s is the amount of water molecules (mol) in a saturated air of volume V (m^3); R is the universal gas constant which equals 8.314 J·mol^{-1}·K^{-1}; T is absolute temperature in K; and P_s is the pressure at which water vapor is in thermodynamic equilibrium with its condensed state. Several researchers attempted to predict the relationship between the vapor pressure of water and temperature, among which, Buck's equation (Eq. 4) gave the best accuracy within the range of 0–100 °C (Lide, 2005):

$$P_s = 611.21 exp\left(\left(18.678 - \frac{T - 273.15}{234.5}\right)\left(\frac{T - 273.15}{T - 16.01}\right)\right) \qquad \text{Eq. 4}$$

For a solution of water dissolved in oil, water is a minor component due to its small solubility in oil. Thus, Henry's law applies (Atkins et al., 2018; Hilder, 1968):

$$\frac{P}{P_s} = \gamma \cdot x \qquad \text{Eq. 5}$$

where P is the equilibrium water vapor pressure of the gaseous phase, γ is the activity coefficient of water in oil; and x is the mole fraction of water in oil (the number of moles of water/the total number of moles of oil). The activity coefficient of water in oil, γ, is dependent on temperature, pressure, and composition of the solvent. However, since we only discuss the cases under approximately one standard atmospheric pressure, and for a given type of oil, γ only changes with temperature (Hilder, 1968).

Upon saturation, where $P = P_s$, the mole fraction $x = 1/\gamma$. Assuming that the small amount of water content has no impact on the volume of oil, the saturation molar concentration of water in oil is expressed as:

$$C_{s.oil} = \frac{x}{(1 - x) \cdot M_o/(1000 \times \rho)} = \frac{1000 \times \rho}{(\gamma - 1) \cdot M_o} \qquad \text{Eq. 6}$$

where, x is the mole fraction of water; ρ is the density of oil in kg/m^3; and M_{oil} is the average molar mass of oil in g/mol, which can be determined from the saponification value of the sample (triglycerides) (Thomas et al., 2015):

$$M_{oil} = \frac{3 \times M_{KOH}}{sap} \qquad \text{Eq. 7}$$

where, sap is the saponification value in mg/g; and M_{KOH} is the molar mass of potassium hydroxide which is equal to 56.106 g/mol (Lide, 2005).

The saponification value of peanut oil ranges from 187 to 196 (Thomas et al., 2015). We choose an intermediate value of 191, which gives an average molar mass of peanut oil as 881 g/mol.

Based on Hilder's study (Hilder, 1971), the activity coefficient of general edible oils can be approximated using the following equation:

$$ln\gamma = 7.118 + \frac{1222}{T} - 1.459 \, lnT \qquad \text{Eq. 8}$$

where, T is the absolute temperature in K.
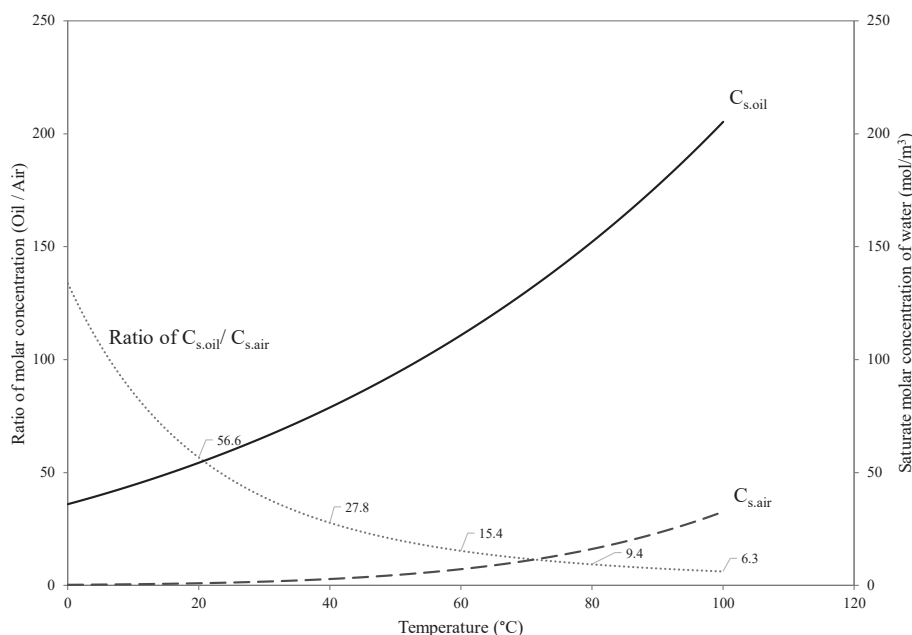
Fig. 1. Estimated volume-specific water capacity (saturation molar concentration of water) in oil and air from 0 to 100 °C. The ratio was simply calculated as their quotient.

Both temperature and moisture content influence the density of oil. However, it is reasonable to postulate that the effect of moisture content is negligible because of the small amount of water in oil. For example, the saturation moisture content of peanut oil (at 80 °C) is calculated to be 0.27% using Eq. 5 and Eq. 8. The temperature-dependent density of several types of edible oil was measured up to 200 °C by Sahasrabudhe, Rodriguez-Martinez, O'Meara & Farkas (Sahasrabudhe et al., 2017). The density of peanut oil can be approximated using an empirical equation linearly regressed from their reported results (RMSE = 0.65 kg/m³):

$$\rho = -0.6153 \cdot T + 1092.43 \qquad \text{Eq. 9}$$

where ρ is the density of oil in kg/m³, and T is the absolute temperature in K.

The moisture concentration of oil as a function of temperature can be derived by substituting Eq. 8 and Eq. 9 into Eq. 6. The temperature-dependent saturation molar concentration of air and oil calculated from Eq. 3 and Eq. 6 are presented in Fig. 1 as well as their ratio, $C_{s.oil}/C_{s.air}$.

3. Materials and methods

3.1. Determining the volume ratio between oil and the headspace

Based on Eq. 2, change in water activity upon equilibration can be reduced by increasing the volume ratio between oil and the headspace (*v*). For accurate measurements, it is important to find the least required volume ratio between a sample and the headspace in the closed container.

According to Fig. 1, the volume-based saturation moisture concentration of oil is 133.7 times that of air at 0 °C. This ratio, *c*, decreases with increasing temperature to 6.3 at 100 °C. According to Eq. 2, for a given volume ratio between the oil sample and the headspace, the maximum water activity change upon equilibration would occur at the highest measurement temperature.

In this study, the maximum measurement temperature was 80 °C. Because the normal ambient RH is about 25–60% at room temperature, the air RH decreases sharply with temperature and will fall to less than 4% at 80 °C. Assuming a worst-case scenario where the initial RH of the

headspace is 0% and the equilibrium water activity of oil is 1.0, the water activity change upon equilibration for the water activity of oil can be estimated from Eq. 2 as:

$$\Delta a_w < \left| \frac{1}{v \cdot c} \right| \qquad \text{Eq. 10}$$

At 80 °C, the ratio of saturation moisture concentration, *c*, is equal to 9.4. In order to keep a small water activity change upon equilibration (saying less than 1%), the volume ratio, *v*, should be larger than 10.6. Therefore, we selected 100 ml glass bottles (Pyrex round media bottle, Corning Inc., NY), with a total volume of about 130 ml, to contain about 105 g of oil sample for this experiment (Fig. 2). As temperature increases from 20 to 80 °C, the volume of 105 g of peanut oil will increase from 115.1 ml to 121.8 ml according to the density of peanut oil (Eq. 9). The volume of the headspace would thus decrease from 14.9 ml to 8.2 ml. The change in water activity upon equilibration for the measurement should be no bigger than 0.007 at 80 °C.

3.2. Experimental set-up

Due to the slow binary mass transfer of water in oil (Hilder and van den Tempe, 1971), it could take several weeks for a deep and static oil sample to reach the thermal dynamic equilibrium state with its headspace air in a closed system. We utilized a magnetic stir bar to create forced convection in oil, thus improving the mass transfer of water molecules within the oil and accelerating the equilibration process. The speed was initially set to stir at about 800 rpm at room temperature and then decreased to about 400 rpm for temperatures higher than 30 °C. This was to avoid possible contamination caused by the vortex of low viscous oil samples at those high temperatures to the sensor installed on the lid of the container.

For RH measurement, we used the same type of sensor (Honeywell HumidIcon™ HIH 8000 Series, Morristown, NJ) as the one in a previous study (Tadapaneni et al., 2017). It consisted of a temperature sensor and a thermoset-polymer electrical capacitive hygrosensor. The accuracy was ±0.5 °C for temperature measurement, and ±2.0% for RH measurement (HIH8000 Series Datasheet, Honeywell).

A hole was drilled through a 2-inch plastic screw cap (the universal cap found on a Pyrex media bottle) to insert a four-pin socket that was
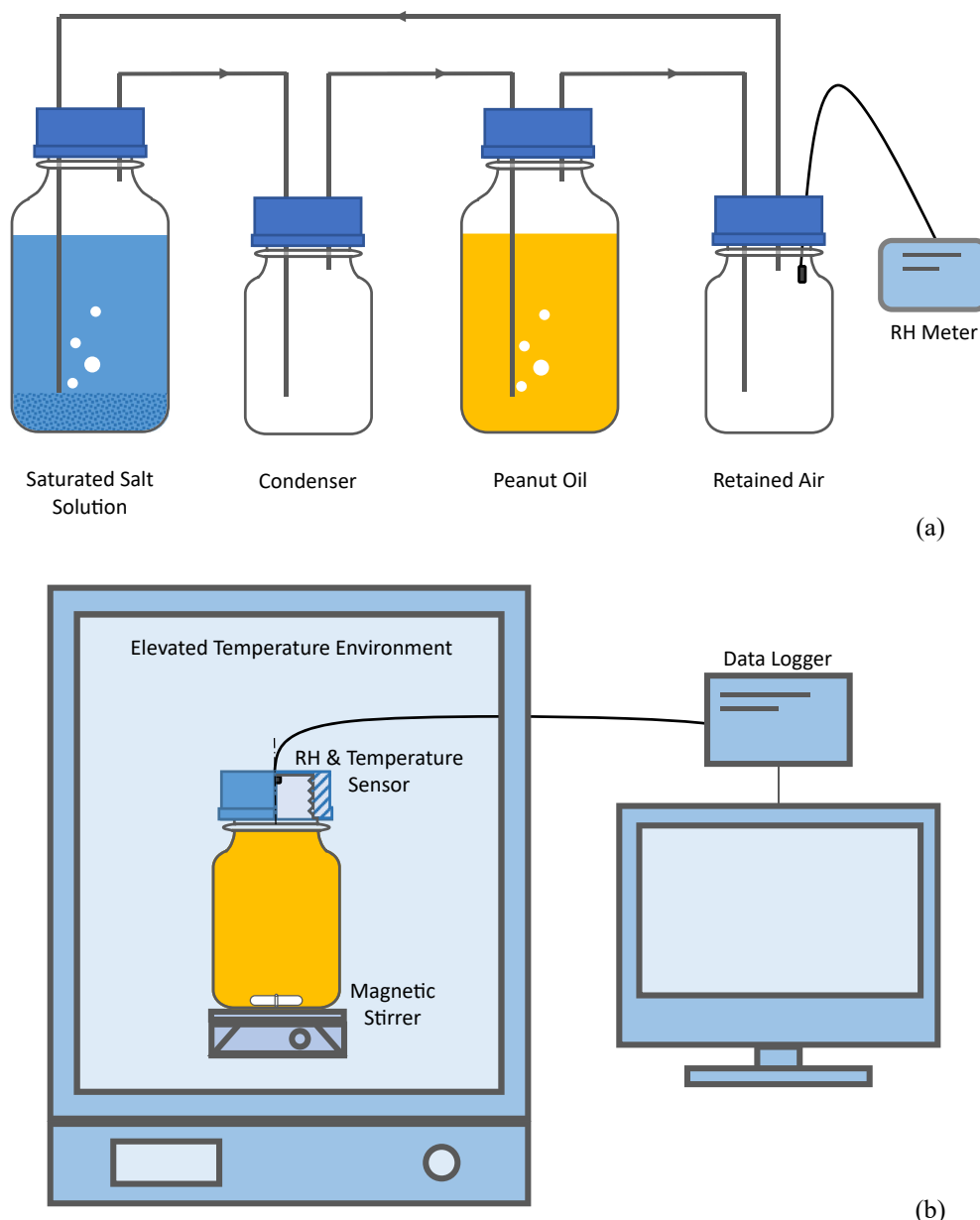
Fig. 2. Experimental apparatus for a) rapid water activity conditioning of a large quantity of oil; and b) water activity measurement of oil by analyzing its headspace air with an electrical capacitive sensor.

used to connect the sensor to a data logger (METER Group, Inc., Pullman, WA). Dielectric epoxy sealing compound was applied from both sides of the cap to fix the socket to the cap and ensure hermetic sealing. The sensor was inserted into the socket from the inner side of the cap. This set-up allows the sensor to be easily replaced if it became contaminated or malfunctioned (Fig. 2).

Prior to the tests, RH sensors were calibrated according to Tadapaneni et al. (2017). The procedure included measuring four standard solutions (METER Group, Pullman WA), with water activity of 0.25, 0.50, 0.76 and 0.92 at room temperature and developing a linear calibration equation for each sensor. The calibration equations were used to correct all the measured results.

3.3. Sample preparation

Purified peanut oil (Ventura Foods, LLC. Brea, CA) was purchased from a local grocery store. The initial water activities of peanut oil samples (about 700 ml each) were adjusted by bubbling air of selected

RHs through the samples (Fig. 2a). Three saturated salt solutions (magnesium nitrate, sodium chloride, and potassium nitrate) were used to set the RH of the air to 52%, 75%, and 93%, respectively, at room temperature (22–24 °C) (Greenspan, 1977). The equilibration state of the system was verified after the RH of the retained air (Fig. 2a) reached the water activity of the saturated salt solution and remained constant for more than 8 h. The total equilibration time depended on the volume of sample, pump speed, bubble size, etc. The pump used was an aquarium air pump (Fusion 600, JW Pet Co., Teterboro, NJ) customized by adding return tubing to its air inlet.

3.4. Measurement of moisture content and water activity

The moisture content of each conditioned oil sample was measured using a Coulometric Karl Fisher Titrator (C20SX, Mettler-Toledo International, Inc., Columbus, OH) in three replicates.

For the measurement of water activity, three 100 ml glass bottles each containing a magnetic stir bar (25.5 mm long) were carefully washed,
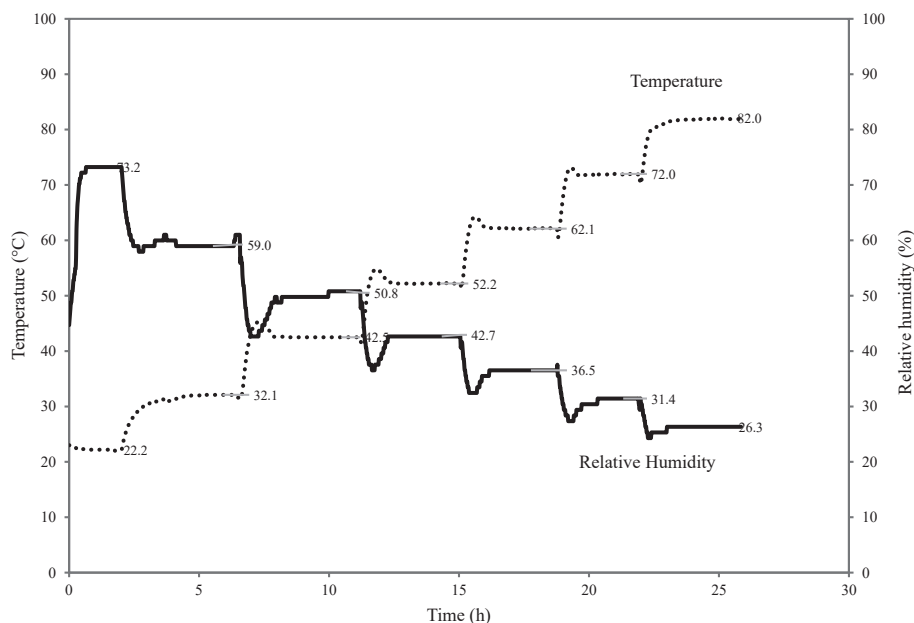
Fig. 3. Example of the temperature and relative humidity profile in the headspace over peanut oil sample during water activity measurement.

rinsed three times with DI water, and dried in an oven for 2 h at 100 °C, then allowed to cool. 105 g of conditioned peanut oil was rapidly poured into each bottle along the inner wall and sealed with a cap embedded with a sensor. The bottles were placed in an oven to raise their temperatures to the desired levels from 30° to 80 °C at 10 °C intervals (see Fig. 2). Temperature and RH in the headspace of the bottles were read and recorded at 1 min intervals via a computer. The equilibrium state at each temperature was confirmed after the RH readings remained constant for more than half an hour.

Due to the thermal expansion of oil and air, before each temperature setting adjustment, the sample bottle cap was unscrewed slightly to vent the air pressure in the bottle and then immediately resealed.

3.5. Modeling and statistical analysis

Water activity (a_w) is defined as the ratio of partial water vapor pressure in a sample vs. partial water vapor pressure of pure water at a given temperature (Barbosa-Canovas et al., 2007), i.e., $a_{w}=p/p_{s}$, therefore can be calculated from Eq. 5.

The activity coefficient of a water in oil solution can be obtained via its excess Gibbs free energy (Atkins et al., 2018):

$$ln\gamma = \frac{\Delta g_e}{RT} = \frac{\Delta h_e}{RT} - \frac{\Delta s_e}{R} \qquad \text{Eq. 11}$$

where Δg_e (J·mol^{-1}), Δh_e (J·mol^{-1}), and ΔS_e (J·mol^{-1}·K^{-1}) are the excess Gibbs free energy, enthalpy, and entropy per mole of water dissolved in oil; R is the ideal gas constant (8.314 J·mol^{-1}·K^{-1}); T is the absolute temperature in K.

A model for the water activity of oil as a function of temperature and mole fraction of water can be given by combining Eq. 5 and Eq. 11:

$$a_w = x \cdot e^{\left(\frac{\Delta h_e}{RT} - \frac{\Delta s_e}{R} \right)} \qquad \text{Eq. 12}$$

The mole fraction of water in oil, x, can be obtained through the moisture content measurement of the sample. The relationship between the mole fraction of water in oil and the wet-basis moisture content of oil follows:

Table 1
Measured moisture content (*MC*), mole fraction of water (*x*), and temperature-dependent water activity (mean with standard deviation) of peanut oil (n = 3).

Saturated salt solution for sample conditioning	MC (ppm)	x	Temperature (°C)	Water activity (a_w)
Magnesium nitrate Mg(NO$_3$)$_2$	522 ± 8	0.0249	22.2	0.52 ± 0.03
			31.6	0.44 ± 0.04
			42.8	0.38 ± 0.03
			54.1	0.32 ± 0.03
			64.7	0.27 ± 0.03
			75.4	0.22 ± 0.02
			86.2	0.18 ± 0.01
Sodium chloride NaCl	722 ± 5	0.0341	21.9	0.75 ± 0.02
			31.6	0.64 ± 0.01
			42.3	0.53 ± 0.00
			52.2	0.45 ± 0.01
			61.3	0.38 ± 0.01
			71.2	0.32 ± 0.00
			81.1	0.27 ± 0.00
Potassium nitrate KNO$_3$	923 ± 3	0.0432	21.8	0.93 ± 0.02
			31.7	0.79 ± 0.01
			41.9	0.65 ± 0.01
			51.7	0.55 ± 0.02
			61.1	0.47 ± 0.02
			71.0	0.40 ± 0.02
			81.0	0.34 ± 0.02

$$x = \frac{n_w}{n_{tot}} = \frac{MC}{M_w} \div \left(\frac{100\% - MC}{M_{oil}} + \frac{MC}{M_w} \right) \qquad \text{Eq. 13}$$

where, n_w is the mole number of water molecules; n_{tot} is the total amount of molecules including water and oil; *MC* is the moisture content of oil (%, wet basis); M_w is the molar mass of water molecule which is equal to 18.015 g/mol; and M_{oil} is the average molar mass of the oil sample in g/mol which can be calculated from the saponification number of the oil using Eq. 7.

The coefficient of determination (R^2) and root mean square error (RMSE) were used to quantify the goodness of fit of the model.
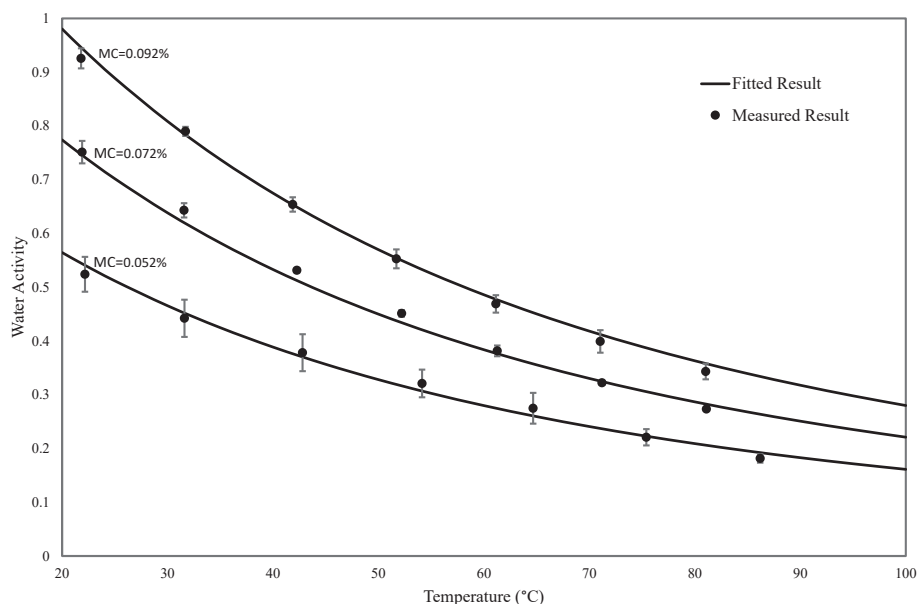
Fig. 4. Mean water activity (± standard deviation) of peanut oil at elevated temperatures measured through the headspace with capacitive sensors and modeled curves from two equations (Eq. 8 & Eq. 14). Wet-basis moisture contents (MC) of three samples can be found beside the first dots (n=3).

4. Results and discussion

4.1. Water activity measurement of peanut oil

A typical time-temperature-RH profile of the headspace over an oil sample is shown in Fig. 3. With the oil sample sealed in the bottle and the magnetic stirrer turned on, the RH reading at the headspace increased rapidly and reached equilibrium within 40 min. Every time when temperature was adjusted, the water activity reading changed with the temperature increase until both became stable. The rapid equilibration of water vapor within the closed bottle at elevated temperatures can be attributed to the forced convection in oil and the increased diffusivity of water molecules in oil (Hilder and van den Tempe, 1971), which helped overcome the major hurdle of mass transfer between oil and headspace. The forced convection is a key element for measuring the water activity of oil, and the total time requirement of this study is mainly dependent on the temperature controlling speed of the oven.

The measured water activities of preconditioned peanut oil samples are summarized in Table 1, along with the corresponding moisture contents of the oil samples. The measured water activity of each sample at room temperature equals the reported water activity of the corresponding saturated salt solution that was used to condition the sample. The reported water activities of magnesium nitrate, sodium chloride, and potassium nitrate are 0.53, 0.75 and 0.94, respectively, at 25 °C (Greenspan, 1977), and the respective mean water activity of peanut oil after equilibration with these solutions were measured as 0.52, 0.75 and 0.93 at room temperature. The corresponding measured moisture contents for the samples were 0.052, 0.072 and 0.092%, or 522, 722 and

923 ppm, respectively. This is in agreement with reported moisture contents of 300–1000 ppm in a wide range of vegetable oils in the literature (Woo et al., 2019; Kim et al., 2018). The moisture loss of sample at each temperature from pressure releasing was estimated by multiply the volume of the vented air (about 1.3 ml) with the moisture concentration in air. The sum of moisture loss from pressure releasing throughout the measurement was about 0.3% of the total moisture content of the sample, and is, therefore, negligible. As the oil temperature increased to 80 °C, the water activity of these three samples dropped exponentially from 0.53, 0.75 and 0.94 at 25 °C to 0.21, 0.29 and 0.36, respectively (Fig. 4).

The fitted equation, using model Eq. 12, for the water activity of peanut oil, is:

$$a_w = x \cdot e^{\left(\frac{1714}{T} - 2.7\right)} \qquad \text{Eq. 14}$$

The fitted curves for three peanut oil samples (with x of 0.0249, 0.0341 and 0.0432) calculated using Eq. 14 are presented in Fig. 4 (solid lines). They are in good agreement with the measured results ($R^2 = 99.6\%$, RMSE = 0.01).

4.2. Validation

There are no published research articles covering how water activity of oil changes with temperature elevation. But previous studies on the water solubility of oil have documented activity coefficients of several types of edible oils at various temperatures. The results of those studies were used for the validation of this study.

Table 2
Equations for the prediction of the water activity of peanut oil and their goodness of fit to experimental observations. The first equation was fitted in this study (Eq. 14), the second (Eq. 15) and third (Eq. 8) equations were derived based on Hilder's works (Hilder, 1968, 1971).

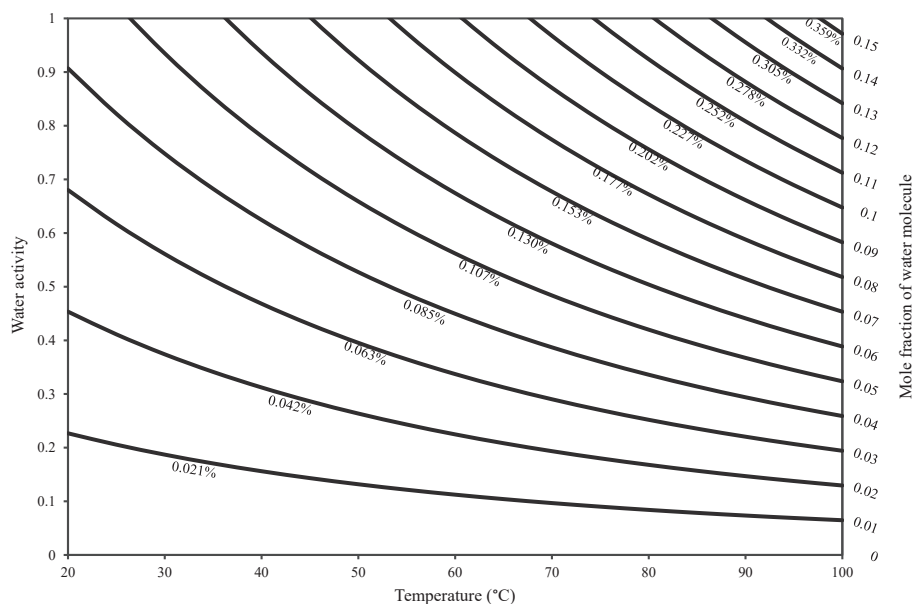| Equation	Δhe (kJ·mol^{-1})	ΔSe (kJ·mol^{-1}·K^{-1})	R^2	RSME (a_w)
Current study (Eq. 14): $a_w = x \cdot \exp\left(\frac{1714}{T} - 2.7\right)$	14.25	0.023	99.6%	0.01
Literature (Hilder, 1968) (Eq. 15): $a_w = x \cdot exp\left(\frac{1600 \pm 40}{T} - 2.5 \pm 0.5\right)$	13.30 ± 0.33	0.021 ± 0.004	87.4%	0.07
Literature (Hilder, 1971) (Eq. 8): $a_w = x \cdot \exp\left(7.118 + \frac{1222}{T} - 1.459 lnT\right)$	NA	NA	91.4%	0.06

Fig. 5. Chart of temperature-dependent water activity of peanut oil at given moisture content (%, wet-basis)/mole fractions of water (Made from Eqs. 13 and 14).

Hilder's investigations on the water solubility of edible oils resulted in two equations to predict the activity coefficients of oil (not type-specific). The first equation was obtained in 1968 by fitting the parameters in Eq. 11 to the published results from different researchers for a group of oil samples measured from 0 to 100 °C:

$$ln\gamma = \frac{1600 \pm 40}{T} - 2.5 \pm 0.5 \qquad \text{Eq. 15}$$

The second equation, Eq. 8 (Hilder, 1971), described an empirical relationship between activity coefficient of oil and temperature. It has one more degree of freedom compared to Eq. 15, and was claimed to have better accuracy at a wider temperature range between 0 and 265 °C.

Table 2 compares goodness of fitting of Eqs. 8, 14, and 15 to the observations from this study. Eq. 14 was generated through curve fitting and has the best fit to the data. The coefficient of determination of Hilder's two equations (Eqs. 8 and 15) were 87.4% and 91.4%, which were acceptable, but the RMSE's were much larger than that of Eq. 14 (0.06 & 0.07 vs. 0.01) and can hardly be used for the water activity prediction of oil. The second constant ($\Delta S_e/R$) calculated from this work (Eq. 14) falls into the range of Hilder's estimation (2.5 $\pm$ 0.5) (Hilder, 1968), but the first constant ($\Delta h_e/R$) was higher possibly due to the difference in the measurement methods or the difference in the oil samples. Thus, for a more accurate estimation of water activity in a specific oil, separate experimental measurement may be needed .

4.3. Excess enthalpy & isosteric heat

For future studies on water activity of oils, Eq. 12 was simplified by dividing a_w with the specific water activity at a certain reference temperature:

$$a_w = a_{w,r} \cdot e^{\frac{\Delta h_e}{R}\left(\frac{1}{T}-\frac{1}{T_r}\right)} \qquad \text{Eq. 16}$$

where, $a_{w,r}$ is the water activity of the sample at a reference temperature, and T_r is the reference temperature (for example, 298.15 K or 25 °C).

It is evident that Eq. 16 is very similar to the general form of the Clausius-Clapeyron equation (Tadapaneni et al., 2017). Based on these two equations, the excess enthalpy per mole water dissolved in oil, Δh_e, equals to the negative isosteric heat of water absorbed in oil, $-q_{st,n}$. The net isosteric heat is interpreted as the difference between the heat of evaporation for the moisture in oil and the latent heat of evaporation of

pure water (Giraldo et al., 2019). In this study, the calculated net isosteric heat of peanut oil is equal to −14.25 kJ/mol. A negative isosteric heat indicates a weaker bond between water and oil molecules compared to the hydrogen bonds in liquid water.

The parameter Δh_e can be obtained by using this equation to fit the water activity of a specific oil over a wide range of initial water activity and temperatures. Measurement of the moisture contents of an oil sample is no longer necessary. Eq. 16 provides an explicit description of water activity change in oil samples with temperature.

4.4. Significance of this work

A chart (Fig. 5) was generated using Eq. 13 and Eq. 14 to demonstrate the relationship between the water activity, temperature, and moisture content (or mole fraction of water) of peanut oil. This chart shows how water activity decreases with temperature from different initial temperature and water activity combinations. It could be a useful tool in the design of thermal treatments for control of bacterial pathogens in oil-rich low-moisture foods, in which the high-temperature water activity of oil dominates the thermal resistance of pathogens of concern. As previously illustrated (Liu et al., 2018; Xu et al., 2019), it is necessary to elevate the water activity of a product to above 0.8 at 80 °C to achieve a 5-log reduction of *Salmonella* within 12 min thermal treatments at 80 °C. According to Fig. 5, this would not be possible with oil even when it is conditioned at 100% RH (or 1.0 water activity) at room temperature. This can only be achieved by over-saturating the oil with added water to a water content of above 0.202% at room temperature or injecting steam or water to the oil at a higher temperature.

With the method developed in this study, the temperature-dependent water activity of different types of oil, and potentially other nonaqueous materials, can be measured more conveniently compared to the previous method (Hilder, 1968, 1971) which requires the measurement of the moisture content of oil samples equilibrated at selected temperatures and RHs.

The results from this study provide useful insight to explain the protective effect of oil. For an oil sample with no saturated water, its water activity will decrease exponentially with increasing temperature. Due to the difference in water vapor pressure, the water molecules inside bacteria cells will diffuse through the cell membrane into the oil until a thermal dynamic equilibration is achieved. Therefore, it may take less than a second for the bacterial cells to desiccate inside the oil

([Syamaladevi et al., 2016b](#)), leading to enhanced thermal resistance. But further studies are needed to explicitly correlate water activity changes in oils with thermal resistance of bacterial cells in oil samples.

5. Conclusion

We experimentally verified that the water activity of oil decreases exponentially with increasing temperature. This result suggests that microorganisms in oil may dehydrate upon heating due to the thermal dynamic equilibrium of vapor pressure with oil, and the bacterial cells subsequently become more resistant to heat. The temperature-dependent water activity of peanut oil was measured through the equilibrium relative humidity of the headspace above a sufficient amount of oil sample using a capacitive sensor. This method has proven valid and can be a potential tool for future studies on other oils and nonaqueous materials. A simplified model with only two parameters was developed to allow a general description of the water activity change in oil in relationship to temperature.

CRediT author statement

Ren Yang: Methodology, Conceptualization, Investigation, Data Analyses, Writing- original draft, Project administration. **Jiewen Guan**: Investigation, Data Analyses, Writing- review & editing. **Sicheng Sun**: Investigation, Data Analyses. **Shyam S. Sablani**: Validation, Writing- review & editing. **Juming Tang**: Conceptualization, Resources, Validation, Supervision, Writing- review & editing, Funding acquisition.

Declaration of Competing Interest

The authors declare there are no conflicts of interest.

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