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Water activity change at elevated temperatures and thermal resistance of *Salmonella* in all purpose wheat flour and peanut butter

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ABSTRACT

Water activity (a_w) is a major factor affecting pathogen heat resistance in low-moisture foods. However, there is a lack of data for a_w at elevated temperatures that occur during actual thermal processing conditions, and its influence on thermal tolerance of pathogens. The objective of this study was to gain an in-depth understanding of the relationship between temperature-induced changes in a_w and thermal resistance of *Salmonella* in all purpose flour and peanut butter at elevated temperatures (80 °C). Equilibrium water sorption isotherms (water content vs. water activity) for all purpose wheat flour and peanut butter over the range of 20 to 80 °C were generated using a vapor sorption analyzer and a newly developed thermal cell. The thermal resistance (D_{80}-values) of *Salmonella* in all purpose wheat flour and peanut butter with initial a_w of 0.45 (measured at room temperature, ~20 °C) was determined via isothermal treatment of small (<1 g) samples. When increasing sample temperature from 20 to 80 °C in sealed cells, the a_w of all purpose flour increased from 0.45 to 0.80, but the a_w of peanut butter decreased from 0.45 to 0.04. The corresponding estimated D_{80}-values of *Salmonella* in all purpose flour and peanut butter with 20 °C a_w of 0.45 were 6.9 ± 0.7 min and 17.0 ± 0.9 min, respectively. The significantly ($P < 0.05$) higher D_{80}-value of *Salmonella* in peanut butter than in all purpose flour may be partially attributed to the reduced a_w in peanut butter in comparison to the increased a_w in all purpose flour at 80 °C. The improved understanding of temperature-induced changes in a_w of low-moisture products of different composition provides a new insight into seemly unpredictable results, when using heat treatments to control *Salmonella* in such food systems.

1. Introduction

There is a common misconception that low-moisture foods (with $a_w < 0.6$) are safe as they will not support the growth of microorganisms (GMA, 2009). But several strains of foodborne pathogens, including *Salmonella* spp., can survive for significant periods of time as evidenced by recent outbreaks of salmonellosis linked to low-moisture foods, such as spices, whole raw almonds, peanut butter, baby formula, wheat flour, puffed cereals, and cookie dough (CDC, 2002; Isaacs et al., 2005; Keller, VanDoren, Grasso, & Halik, 2013; Kirk et al., 2004; Komitopoulou & Penaloza, 2009; Nummer, Shrestha, & Smith, 2012; Park, Oh, & Kang, 2008; Podolak, Enache, Stone, Black, & Elliott, 2010; Uesugi, Danyluk, & Harris, 2006; Zweifel & Stephan, 2012). Thermal treatments can be effective means to control pathogens in intermediate and high-moisture foods ($a_w \geq 0.6$); however, in a low-moisture environment, some pathogens are extremely difficult to control. Published research reported sharp increases in thermal resistance of pathogens like *Salmonella* at lower water activities (Archer, Jervis, Bird, & Gaze, 1998; Bari et al., 2009). For example, it takes around 3 min at 65 °C to achieve a 6 log reduction of *Salmonella* spp. in liquid milk (Jay, 1992) while in a dry product with a low-moisture content, it requires more than 30–100 times longer to achieve a similar level of reduction (Bari et al., 2009; Chang, Han, Reyes-De-Corcuera, Powers, & Kang, 2010; Du, Abd, McCarthy, & Harris, 2010; Villa-Rojas et al., 2013).

Most of the published studies only relate heat resistance of *Salmonella* to a_w of food matrices measured at room temperature (Archer et al., 1998), not the a_w at the treatment temperatures. In dynamic thermal treatments, a_w changes sharply as temperature increases (Bassal, Vasseur, & Lebert, 1993; Iglesias & Chirife, 1977). The degree and direction of changes in a_w depend on the chemical composition of foods at a fixed water content (Labuza, Kaanane, & Chen, 1985). There is no commercial instrument available to measure a_w of foods above 60 °C.

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Consequently, there is little knowledge about how the a_w of low-moisture foods at elevated temperatures might affect microbial inactivation depending on the major components.

However, such considerations can be found in very early literature. For example, Murrell and Scott (1966) used five methods to relate thermal inactivation kinetics with water activity of bacterial spores (*Bacillus megaterium*, *Bacillus stearothermophilus* ATCC 7953, and *Clostridium botulinum* type E ATCC 9564), considering the change in water activity at elevated temperatures. In general, the spores were equilibrated with LiCl, or NaOH or H_2SO_4 solutions and treated at elevated temperatures for specific periods of time and cooled rapidly in ice water (Murrell & Scott, 1966). The thermal resistance of spores increased initially with increasing a_w, reached maximum thermal resistance between 0.2 and 0.4 a_w and then decreased steadily from 0.4 to 1.0 a_w. The thermal resistance observed between 0.2 and 0.4 a_w was up to 10,000 times greater than the highest thermal resistance observed at 1.0 a_w (Murrell & Scott, 1966).

The thermal resistance of *Salmonella* in low-moisture foods is a function of a_w as well as type of food components such as carbohydrates, proteins, and fats (Senhaji, 1977; Li, Fu, Bima, Koontz, & Megalis, 2014). Understanding the change of thermodynamic properties such as a_w of low-moisture foods at elevated temperatures is imperative in designing protocols for thermal processing methods to control *Salmonella* populations in low-moisture foods. More systematic thermal inactivation experiments at different temperatures and initial water activities of selected low-moisture foods should be conducted and the water activity difference at those temperatures should be considered in order to develop thermal processing protocols to inactivate *Salmonella*. The objective of the current study is to determine a_w values as a function of temperature during adsorption and desorption for all purpose flour and peanut butter; and relate the thermal resistance of *Salmonella* with the changes in water activity of these selected low-moisture foods at elevated temperatures.

2. Materials and methods

All purpose wheat flour (Gold medal brand, General Mills, Inc., Minneapolis, MN, USA) and 100% natural creamy peanut butter (Adams brand, Smucker Foods Canada Corp. Markham, ON, Canada) were purchased from a local store. All purpose flour (wheat flour as main component) and peanut butter were selected for this study based on their connection to recent *Salmonella* outbreaks (CDC, 2007; McCallum et al., 2013). They were also chosen due to differences in their food composition (~70% carbohydrates and ~10% protein in all purpose wheat flour and ~50% fat, 20% protein and 20% carbohydrate in peanut butter).

2.1. Water sorption isotherms methods at selected temperatures

2.1.1. Vapor sorption analyzer

An Aqualab vapor sorption analyzer (VSA) (Decagon Devices, Inc., Pullman, WA) was used to generate adsorption and desorption isotherms of all purpose flour and peanut butter at 20, 40 and 60 °C (at least two replicates) following the dynamic vapor sorption (DVS) method described by Yu, Schmidt, Bello-Perez, and Schmidt (2008). The VSA is capable of generating equilibrium relative humidity conditions between 3 and 90% (corresponding water activities of 0.03–0.90) with an accuracy of ±0.005 at operating temperatures from 15 to 60 °C. During the test, a food sample inside the VSA was exposed to a preselected relative humidity level until a constant sample mass was achieved. Once the sample reached equilibrium, the corresponding water activity and water contents were recorded. The VSA then incrementally set another level of relative humidity to bring the water content of the sample to a different equilibration value. We selected a 10% incremental change in relative humidity to achieve water activity intervals of 0.1 for the water sorption curve. The equilibrium water content values at each water activity step were calculated from the weight change data.

2.1.2. Thermal cell with relative humidity sensor

For a_w measurements above 60 °C, we developed a sealed thermal cell containing a commercial relative humidity sensor (HX15-W, Omega Engineering, Inc.) to measure water activity in collaboration with Decagon Devices (Fig. 1). In this design, a high temperature relative humidity sensor measured the relative humidity of the air headspace above the food sample at elevated temperatures at equilibrium. In principle, the a_w of the food sample is equivalent to the relative humidity of the headspace in thermodynamic equilibrium with the food sample at the same temperature and pressure.

The water vapor pressure (P) can be calculated from water vapor concentration (C) using the following equation:

$$P = \frac{CR(T + 273.15)}{m_r} \tag{1}$$

where T is the temperature in °C, m_r is the molecular weight of water (kg/mol) = 0.018 and R = 8.314 J/mol. K. The a_w of food/relative humidity (RH) of air is related to water vapor pressure:

$$a_w = RH = \frac{P}{P_s} \times 100 \tag{2}$$

where P_s is the saturation vapor pressure. P_s can be determined at a temperature by:

$$P_s = 0.611 \exp\left(\frac{17.502T}{240.97 + T}\right). \tag{3}$$

The reported values of water activities of lithium chloride (13.41 molal) and sodium chloride (6 molal) solutions at selected temperatures were used to calibrate the a_w instruments at elevated temperatures (Table 1) (Gibbard & Scatchard, 1973; Gibbard, Scatchard, Rousseau, & Creek, 1974). The osmotic coefficients of LiCl and NaCl solutions at the above two molalities at various temperatures were used to calculate the water activities by the following equation (Gibbard & Scatchard, 1973; Gibbard et al., 1974):

$$\ln a_w = -\frac{\varphi \nu n_s}{n_w} \tag{4}$$

where a_w is the water activity, φ is the osmotic coefficient, M_w is the molar mass of water in kg/mol, ν is the number of ions formed when one mol of salt is dissolved in water, m_s is the molality (mol/kg H_2O) of salt, n_w is the amount of water and n_s is the amount of salt, both in mol.

Inside the thermal cell, during experiment, the relative humidity of the air in the headspace above the food matrix was monitored. When the temperature and relative humidity of air in the headspace did not change for significant time periods (~30 min), we concluded that an equilibrium condition was reached inside the thermal cell. The equilibrium relative humidity of the air headspace was considered as being equivalent to the water activity of the food matrix.

For the generation of adsorption isotherms above 60 °C, food samples were vacuum dried with 10 kPa pressure inside the vacuum oven at 50 °C overnight, then placed in air tight containers and equilibrated for two weeks with saturated salt solutions at room temperature (~20 °C). The water contents of the samples were adjusted by exposing to specific relative humidities, 11.3%, 22.5%, 32.8%, 43.2%, 52.9%, 65.8%, 75% and 86%, provided by supersaturated solutions of LiCl, CH_3COOK, $MgCl_2$, K_2CO_3, $MgNO_3$, $NaNO_2$, NaCl and KCl (Fisher Scientific, Houston, TX) (Greenspan, 1977). After equilibration, the water activities of those samples were determined at 80 °C using the newly developed thermal cell with a RH sensor. A convection oven (Yamato Scientific America

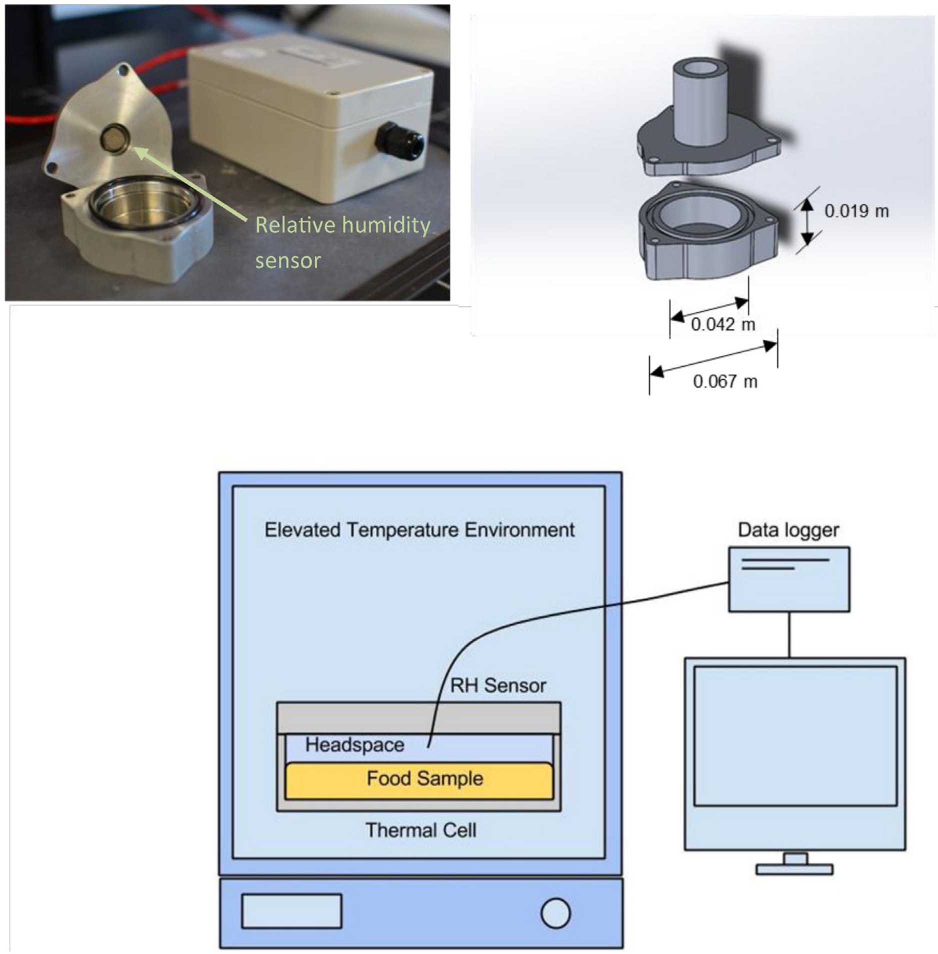

Fig. 1. Thermal cell with RH sensor to determine water activity of foods at elevated temperatures.

Inc., CA, USA) was used to heat the samples in sealed cells. After each water activity measurement at elevated temperatures, the sealed cell was removed from the oven and kept at room temperature for ~30 min to reach ambient temperature. The water content of the equilibrated samples was determined using a vacuum oven (Yamato Scientific America Inc., CA, USA) with 10 kPa pressure inside the chamber at 80 °C for 10 h.

For the generation of desorption isotherms above 60 °C, food samples were conditioned inside a humidity chamber with water to achieve ~100% relative humidity (corresponding to a_w of 1). The samples were then placed in air-tight containers and equilibrated for two weeks with supersaturated salt solutions at room temperature (~20 °C), similar to the generation of adsorption isotherms. After equilibration, the water activities of samples at 80 °C and the corresponding water contents were determined as described in the previous section.

2.1.3. Modeling of water sorption isotherms at selected temperatures

Several empirical and semi-empirical equations have been reported in the literature to quantify the temperature influence on sorption isotherm of foods. In this study, we fit the most frequently used equations including the Modified Henderson Model, Modified Halsey Model, Modified Oswin Model, Chung–Pfost Model, and Guggenheim, Anderson and de Boer (GAB) Model to the sorption data of the tested samples (Kaymak-Ertekin & Gedik, 2004; Quirijns, van Boxtel, van Loon, & van Straten, 2005). The GAB equation based on the assumption of multilayered adsorption with no lateral interactions was selected as the best fit model to describe all purpose flour sorption isotherms based on regression coefficient (R^2), root mean square error (*RMSE*) and mean relative percent error (*P*) values. The GAB Model is expressed as:

Table 1
Water activity values of 13.4 m LiCl and 6 m NaCl at different temperatures.

	Temperature (°C)				
	0	25	50	75	100
a_w of 13.4 molal LiCl	0.22	0.25	0.28	0.31	0.34
a_w of 6 molal NaCl	0.76	0.76	0.76	0.77	0.77

$$\frac{X}{X_m} = \frac{CKa_w}{(1-Ka_w)(1-Ka_w+CKa_w)} \tag{5}$$

where X is the dry basis water content of the material, X_m is the monolayer water content (dry basis), C and K are parameters which have physical meaning based on the multilayer adsorption of water. The parameter C is a measure of strength of binding water to the primary binding sites of the food; a higher value for C indicates greater strength of binding of water and larger enthalpy difference exists between the monolayer and multilayer water molecules (Quirijns et al., 2005). The parameter K is a correction factor, which has a more entropic than enthalpic contribution (Quirijns et al., 2005). The parameters C and K are thermodynamic in nature and the temperature dependence of these parameters can be expressed as:

$$C = C_o \exp\left(\frac{\Delta H_C}{RT}\right) \tag{6}$$

$$K = K_o \exp\left(\frac{\Delta H_K}{RT}\right) \tag{7}$$

where ΔH_C is generally positive, which is the difference in enthalpy between monolayer and multilayer sorption. ΔH_K is generally negative, which is the difference between the heat of condensation of water and is the heat of sorption of a multi-molecular layer (Quirijns et al., 2005). In most cases, the monolayer water content (X_m) is considered as a constant but a similar expression to describe the temperature dependence of X_m can be presented.

$$X_m = X_{mo} \exp\left(\frac{\Delta H_X}{RT}\right) \tag{8}$$

Incorporating the temperature dependence of the parameters X_m, C and K to the GAB Model will allow one to predict the sorption isotherms of foods at selected temperatures, where T is the temperature in K.

The Modified Halsey Model was the best fit model to describe the sorption isotherms of peanut butter based on R^2, *RMSE*, and *P* values. The Modified Halsey Model is expressed as:

$$X = \left(-\frac{\exp(A + B \times T)}{\ln a_w}\right)^{\frac{1}{C}} \tag{9}$$

where X is the dry basis water content of the material, a_w is the water activity, T is the temperature in °C, A, B, and C are equation parameters. Nonlinear optimization by Excel® Solver software program (Microsoft corporation, Redmond, WA, USA) was used to obtain the parameters in the GAB and Modified Halsey Models.

The regression coefficient (R^2), root mean square error (*RMSE*), and mean relative percent error (*P*) were determined using the following equations to analyze the fit and prediction quality of the models.

$$R^2 = 1 - \left(\frac{\sum_{i=1}^{n} \left(y_{i,obs} - y_{i,calc}\right)^2}{\sum_{i=1}^{n} \left(y_{i,obs} - \bar{y}\right)^2}\right) \tag{10}$$

$$P(\%) = \frac{100}{N} \sum_{i=1}^{N} \frac{\left|y_{i,obs} - y_{i,calc}\right|}{y_{i,obs}} \tag{11}$$

$$RMSE = \sqrt{\frac{\sum_{i=1}^{n} \left(y_{i,calc} - y_{i,obs}\right)^2}{n}} \tag{12}$$

The best fit model was characterized by a higher R^2, and smaller *P* and *RMSE* values compared to other models.

2.1.4. Thermal resistance of Salmonella in selected food matrices at elevated temperatures

To obtain the three *Salmonella* inoculums, a stock culture of *Salmonella* Enteritidis PT 30 acquired from Dr. Linda Harris at UC-Davis (ATCC® BAA-1045™), kept in 20% glycerol 80% Trypticase Soy Broth with 0.6% Yeast Extract (TSBYE) held at −80 °C, was successively transferred twice into TSBYE (24 h incubation periods at 37 °C). One milliliter of culture was streaked into Trypticase Soy agar (TSA) amended with with 0.6% YE and incubated for 24 h at 37 °C. The cells were harvested by washing the lawn twice with 10 mL of 0.1% peptone water (PW), centrifuged (6000 ×g at 4 °C for 15 min) and resuspended in 3 mL of PW. Three batches of food samples (100 g each) were inoculated with 1 mL of this inoculum. After inoculation, samples were equilibrated for ~5 days to ~0.45 a_w in a controlled humidity chamber. *Salmonella* populations were enumerated as described subsequently to confirm homogeneity (±0.1 log CFU/g) and quantify survival at several different time intervals during equilibration. The inoculated concentration of *Salmonella* in all purpose flour was 8.96 ± 0.05 log CFU/g, while the concentration after equilibration was 7.66 ± 0.10. In peanut butter, the inoculated concentration was 8.74 ± 0.22 log CFU/g, while the concentration after equilibration was 8.21 ± 0.09 log CFU/g. The background mesophilic bacterial populations for all purpose flour and peanut butter were 1.9 ± 0.2 log CFU/g and 2.1 ± 0.2 log CFU/g, respectively. However, considering the high level of inoculated *Salmonella*, this background population level did not cause any interference.

For testing, samples (~0.7 g) were loaded into aluminum test cells (4 mm thickness) (Chung, Birla, & Tang, 2008). Triplicate samples were placed in an isothermal water bath (80 °C). After reaching ~79.5 °C, test cells were removed at predetermined time intervals and immediately cooled in ice water. Samples were tenfold serially diluted in PW, plated in duplicate on modified TSA (0.6% yeast extract, 0.05% ammonium iron citrate, 0.03% sodium thiosulfate) for peanut butter and XLT4 agar without the selective agent (turgitol) for all purpose flour, and *Salmonella* survivors were enumerated after 48 h incubation at 37 °C. We observed that the two different agar media did not influence the thermal resistance values of *Salmonella*. Even though we used different agar media for peanut butter and all purpose flour, the D-values were computed from the resulting log CFU/g data by linear regression analysis performed using the log-linear model (Villa-Rojas et al., 2013).

$$\log N = \log N_o - \frac{t}{D} \tag{13}$$

where N is the number of bacteria (CFU/g) at time t (min), N_o is the initial number of bacteria (CFU/g) and D is the decimal reduction time (min).

2.2. Statistical analysis

The water activity and thermal resistance of *Salmonella* data at the selected temperatures were analyzed for statistical significance using SAS 9.1 (SAS Institute, Inc., Cary, NC). A value of $P < 0.05$ was selected as statistically significant using proc. GLM by Fisher's Least Square Difference (LSD) method. The experiments involved a completely randomized design.

3. Results and discussion

3.1. Water sorption isotherms at selected temperatures

3.1.1. All purpose flour

The adsorption and desorption isotherms of all purpose wheat flour at tested temperatures are presented in Figures 2&3.The sorption isotherms of all purpose flour at the selected temperatures followed a typical type II behavior, which is common for several foods such as ready-to-eat cereals (Labuza and Altunakar, 2007). Type II isotherms exhibit typically two bending regions, one around a_w of 0.2–0.4 and another at 0.6–0.7, which was more visible in the desorption isotherm of all purpose flour (Labuza and Altunakar, 2007). This behavior was attributed to the building up of sorption multilayers and filling of small pores at low water activities and swelling and filling up of large pores at higher water activities (Labuza and Altunakar, 2007). We observed hysteresis for all the isotherms at the selected temperatures i.e., the water content for the desorption curve was greater than the water content for adsorption for the same water activities (Labuza and Altunakar, 2007).

Water adsorption and desorption isotherms of all purpose flour show a significant increase in water activity with increasing temperature (Figs. 2 & 3). For instance, water activity of all purpose flour with 10% water content (dry basis) increased from 0.16 to above 0.5 when temperature was increased from 20 to 80 °C. The increase in water activity with temperature has been observed in carbohydrate and protein rich foods (Bandyopadhyay, Weisser, & Loncin, 1980). Hydrophilic substances like carbohydrates interact and dissolve in water by the formation of hydrogen bonds with water molecules, resulting in hydration of

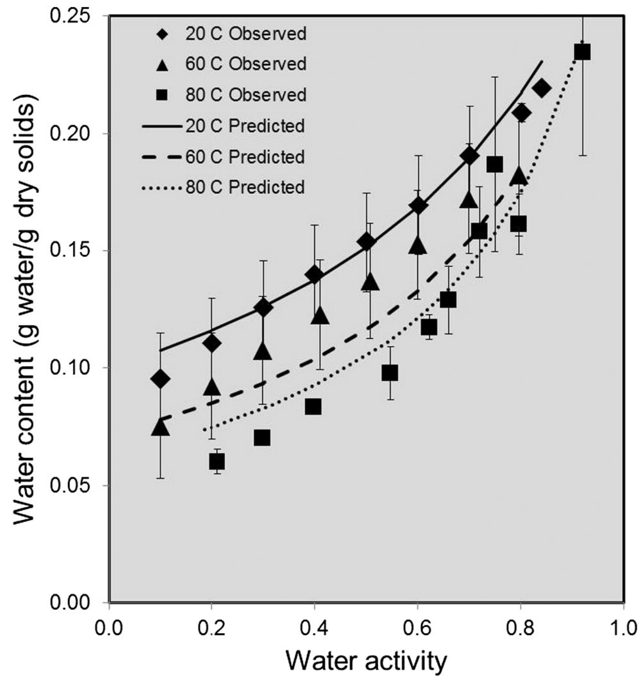

Fig. 2. Adsorption isotherms of all purpose wheat flour at tested temperatures (water content data points are average of at least two independent samples).

macromolecules. In protein rich foods, it is believed that water molecules may interact with hydrophilic sites of protein structure (Iglesias & Chirife, 1977). Elevated temperatures may disrupt the hydrogen bonds between water and hydrophilic molecules, resulting in an increase in the number of free water molecules and consequently, an increase in water activity. Further, structural changes in food macromolecules may happen at elevated temperatures which could affect their interaction with water (Iglesias & Chirife, 1977). For example,

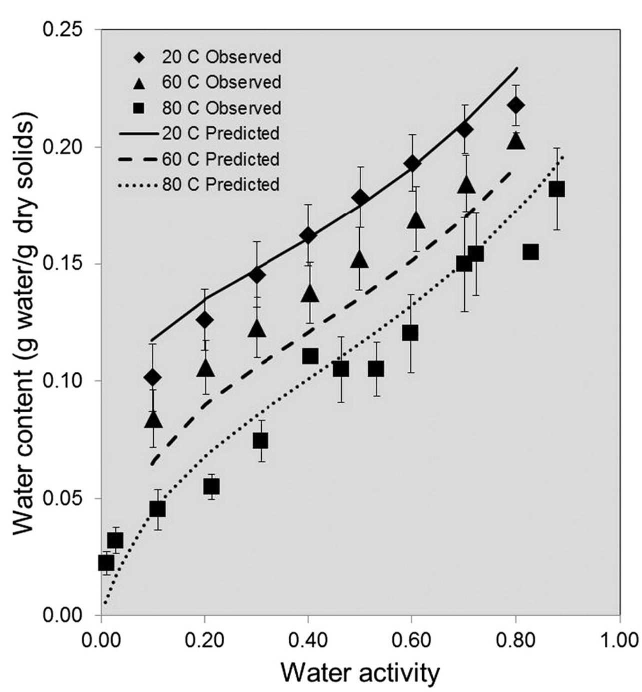

Fig. 3. Desorption isotherms of all purpose wheat flour at tested temperatures (water content data points are average of at least two independent samples).

at elevated temperatures, the binding energy between molecules decreases, which results in an increased distance and reduced attractive forces between the molecules (Quirijns et al., 2005). More water has sufficient energy to escape into the vapor phase resulting in an increase in the a_w of foods at elevated temperatures (Palipane & Driscoll, 1993).

We evaluated the Modified Henderson Model, Modified Halsey Model, Modified Oswin Model, Chung–Pfost Model, and Guggenheim, Anderson and de Boer (GAB) Model to determine the best fit model ($R^2 = 0.80$ for adsorption data and 0.87 for desorption data) to describe the sorption isotherm of all purpose flour, where water content was determined as a function of water activity and temperature. We determined the R^2, *RMSE* and *P* values when the selected models were fitted with the experimental sorption data of all purpose flour at the selected temperatures. The GAB Model (Table 2) was selected to predict the water sorption behavior in all purpose flour as it performed better in fitting the adsorption and desorption data with higher R^2, lower *RMSE* and lower *P* values compared to other selected models.

3.1.2. Peanut butter

The sorption isotherms of peanut butter at the tested temperatures followed a typical type III behavior, which is common for many foods with crystalline components (Labuza and Altunakar, 2007). Small increases in water content sharply increased the a_w of peanut butter during adsorption or desorption at 20 °C (Figs. 4 & 5). This could be attributed to the significant amount of hydrophobic components such as fat in peanut butter. Nonpolar compounds such as fats will not interact or form hydrogen bonds with water molecules. In fat rich foods, water molecules may aggregate together while fat molecules are combined at the center forming a cage like structure, leading to less interaction between water and fat molecules but greater interaction between water molecules themselves as previously reported (Khuwijitjaru, Adachi, & Matsuno, 2002). The arrangement of water molecules around nonpolar fats may be more ordered. The fat molecules will also aggregate together to reduce the interfacial surface with water resulting in a more thermodynamically favorable hydrophobic interaction (Fennema, 1999), these interactions have little impact on the energy of water. Even in products with low water content such as peanut butter, high water vapor pressure may be exerted by free water molecules. Materials with type III isotherm behavior exhibit small water gain up to a point (0.7–0.8 a_w) where the crystalline components begin to dissolve in adsorbed water as observed in peanut butter where fat molecules may be partially crystalline (Labuza and Altunakar, 2007). We observed hysteresis for peanut butter isotherms at the tested temperatures indicating that the water content for the desorption curve was greater than the water content for adsorption.

When temperature was increased, we observed a significant reduction in peanut butter water activity at same water content compared to that observed in all purpose flour (Figs. 4 & 5). Similar observations were reported for other fat rich products such as peanut oil and oleic acid (Loncin, Bimbenet, & Lenges, 1968; Senhaji, 1977). The decrease in water activity of peanut butter at 80 °C compared to that at 20 °C may be attributed to the increase in solubility of nonpolar solids such as fat in water at elevated temperatures, resulting in lower water vapor pressure due to greater interaction between water and fat molecules (Khuwijitjaru et al., 2002). However, more studies are required to understand the interaction between water and food macromolecules such as proteins, carbohydrates, and fats.

We evaluated selected models (as mentioned in the Materials and methods section) to determine the best fit model ($R^2 = 0.87$ for adsorption data and 0.83 for desorption data) to describe the sorption isotherm

Table 2
GAB Model parameters for all purpose flour sorption isotherms.

	X_{mo}	ΔH_X	C_o	ΔH_c	K_o	ΔH_k
Adsorption	0.006	6695	101,109	1332	1.90	−2532
Desorption	0.025	3935	0.0002	32,244	0.784	−765.3

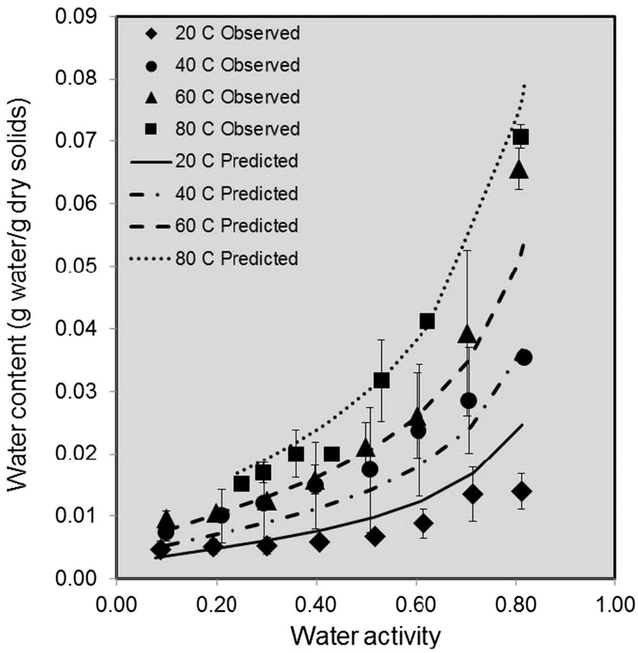

Fig. 4. Adsorption isotherms of peanut butter at tested temperatures (water content data points are average of at least two independent samples).

Table 3
Modified Halsey Model parameters for peanut butter sorption isotherms.

	A	B	C
Adsorption	−6.71	0.024	1.26
Desorption	−10.9	0.055	2.24

of peanut butter. The Modified Halsey Model (Eq. (9)) was the best in predicting the water sorption behavior in peanut butter based on the higher R^2, lower *RMSE* and lower *P* values (Table 3). The predicted water activities at various elevated temperatures can be related to thermal resistance of pathogens to develop thermal processing protocols for the selected low-moisture foods.

3.1.3. Water activity change and thermal resistance of Salmonella at elevated temperatures

The time required to reach ~79.5 °C at the center of the samples inside the aluminum test cell (come-up-time), when the set temperature of the water bath was 80 °C, was considered as the starting point of the inactivation curve (Fig. 6). The come-up-times for all purpose flour and peanut butter were 3.8 and 3 min, respectively. The logarithmic reduction in *Salmonella* populations in all purpose flour and peanut butter during these come-up-time intervals was 1.41 and 0.38, respectively. The logarithmic reduction of *Salmonella* population in all purpose flour and peanut butter was 5.07 and 2.75 after treatment for 24 and 37 min, respectively, excluding the come-up-time. We determined the D_{80}-values of *Salmonella* in all purpose wheat flour and peanut butter with the same initial water activity of 0.45 ± 0.02 at room temperature (~20 °C) from the thermal inactivation data (Fig. 6). The D_{80}-values of *Salmonella* in all purpose flour and peanut butter with initial a_w of 0.45 were 6.9 ± 0.7 min (R^2 = 0.95 ± 0.04) and 17.0 ± 0.9 min (R^2 = 0.92 ± 0.03), respectively. The D_{80}-value of *Salmonella* in all purpose flour was less than half that of peanut butter at a same initial water activity ($P < 0.05$), indicating a greater thermal resistance of *Salmonella* in peanut butter. Considering the desorption isotherm of all purpose flour, the a_w increased from its initial a_w of 0.45 to 0.80, when the temperature increased from 20 to 80 °C (Table 4 & Fig. 7). However, in peanut butter, the water activity decreased from its initial a_w of 0.45 to 0.04, when the temperature increased from 20 to 80 °C (Fig. 7). Microorganisms achieve the same water activity of the surrounding microenvironment (food) during equilibration and heating. With temperature increase, a greater amount of water vapor is formed in a food system, which is attributed to the increased evaporation rate at elevated temperatures. Water vapor diffusion is much faster at elevated temperatures, as a result the food system achieves water activity equilibrium (initial water activity changes to an equilibrium final value depending

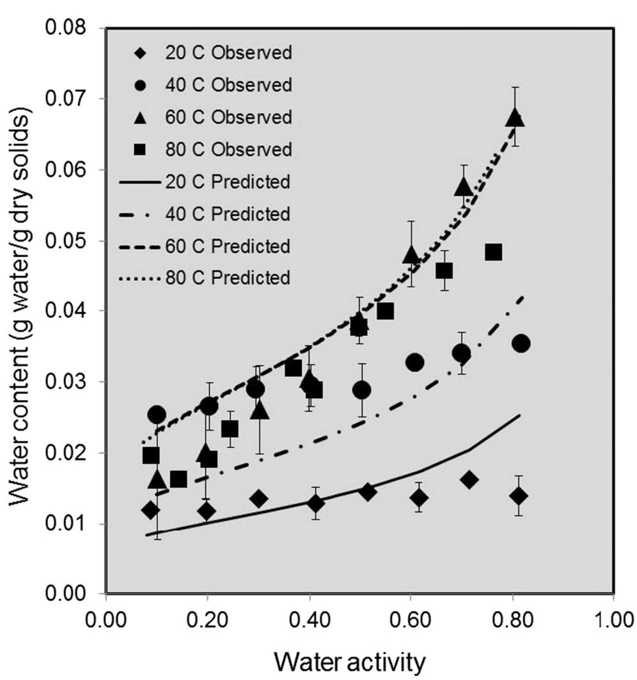

Fig. 5. Desorption isotherms of peanut butter at tested temperatures (water content data points are average of at least two independent samples).

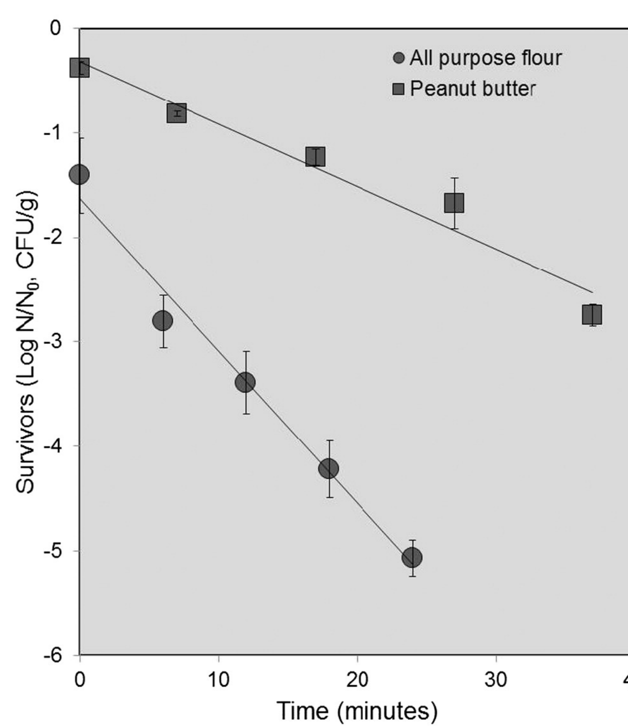

Fig. 6. Thermal inactivation curves of *Salmonella* in all purpose flour and peanut butter.

Table 4
Water activity values of all purpose flour and peanut butter at 20 and 80 °C and D_{80}-values of *S.* Enteritidis PT 30 in those food systems.

Product	a_w at 20 °C	a_w at 80 °C using GAB and Modified Halsey Models	D_{80}-value
All purpose flour	0.45	0.80	6.9 ± 0.7 min
Peanut butter	0.45	0.04	17.0 ± 0.9 min

on the food system) much quicker than at lower temperatures. This water activity equilibration time may be in the similar order of the heat treatment time although more research is needed to confirm this argument. The increased thermal resistance in peanut butter may be partially attributed to the decrease in water activity of peanut butter at elevated temperatures, in comparison to that in all purpose flour (point C to D, Fig. 7). However, further studies to determine the a_w values of low-moisture food matrices with different components over a range of temperature (between 70 and 140 °C) should be conducted to improve our understanding of the relationship between the thermal resistance of pathogens and a_w. The thermal resistance of microorganisms of controlled water activities at treatment temperatures should be determined to confirm the influence of water activity.

4. Conclusion

Water activities of peanut butter and all purpose wheat flour were determined at 80 °C. The thermal resistance values of *S.* Enteritidis PT30 in those two samples at the same room temperature (20 °C) water activity (0.45) were compared. When temperature was increased from 20 to 80 °C, water activity decreased in peanut butter but increased in all purpose flour. The D_{80}-value of *Salmonella* in peanut butter was significantly greater ($P < 0.05$) than that in all purpose flour, partly attributed to the decrease in water activity in peanut butter compared to the increase in water activity in all purpose flour at 80 °C. This study presents the importance of water activity determination at elevated temperatures in order to design and develop thermal treatments to inactivate pathogens in low-moisture foods.

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In all purpose flour with constant water content during desorption, water activity increased from 0.45 (Point A) to 0.80 (Point B) when heated from 20 to 80°C inside a sealed thermal cell until equilibrium. In peanut butter with constant water content during desorption, water activity decreased from 0.45 (Point C) to 0.04 (Point D) when heated from 20 to 80°C inside a sealed thermal cell until equilibrium. The thermal resistance of *Salmonella* should be related with water activity values of all purpose flour and peanut butter at 80°C, not 20°C. It is interesting to note that the thermal resistance of *Salmonella* in peanut butter (D_{80}) was significantly ($P < 0.05$) greater than that in all purpose flour (D_{80}), may be attributed to the reduced water activity at 80°C (Point D) of peanut butter in comparison to the increased water activity at 80°C (Point B) of all purpose flour.

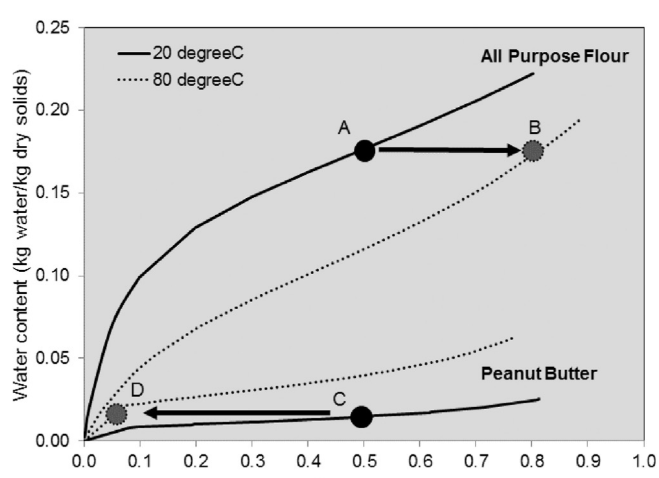

Fig. 7. Desorption isotherms of all purpose flour and peanut butter at 20 and 80 °C, presenting the possible change in water activity during heating and its relationship to thermal resistance of *Salmonella*.

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