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Influence of water activity on inactivation of *Escherichia coli* O157:H7, *Salmonella* Typhimurium and *Listeria monocytogenes* in peanut butter by microwave heating

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ABSTRACT

This study evaluated the efficacy of a 915 MHz microwave with 3 different electric power levels to inactivate three pathogens in peanut butter with different a_w. Peanut butter inoculated with *Escherichia coli* O157:H7, *Salmonella enterica* serovar Typhimurium and *Listeria monocytogenes* (0.3, 0.4, and 0.5 a_w) were treated with a 915 MHz microwave with 2, 4, and 6 kW for up to 5 min. Six kW 915 MHz microwave treatment for 5 min reduced these three pathogens by 1.97 to >5.17 log CFU/g. Four kW 915 MHz microwave processing for 5 min reduced these pathogens by 0.41−1.98 log CFU/g. Two kW microwave heating did not inactivate pathogens in peanut butter. Weibull and Log-Linear + Shoulder models were used to describe the survival curves of three pathogens because they exhibited shouldering behavior. T_d and T_{5d} values were calculated based on the Weibull and Log-Linear + Shoulder models. T_d values of the three pathogens were similar to D-values of *Salmonella* subjected to conventional heating at 90 °C but T_{5d} values were much shorter than those of conventional heating at 90 °C. Generally, increased a_w resulted in shorter T_{5d} values of pathogens, but not shorter T_d values. The results of this study can be used to optimize microwave heating pasteurization system of peanut butter.

1. Introduction

Escherichia coli O157:H7 is an increasingly common cause of illness, including bloody diarrhea and hemolytic uremic syndrome (Besser et al., 1999). *Salmonella enterica* serovar Typhimurium is the most commonly isolated *Salmonella* serotype and causes non-typhoidal salmonellosis which has a symptom of self-limiting gastroenteritis (Boyle et al., 2007). *Listeria monocytogenes* is a one of the most widespread gram-positive bacteria which causes abortion, stillbirth, neonatal sepsis, meningitis, sepsis, and gastroenteritis (Salamina et al., 1996). Robertson et al. (2016) reported that there were 163 outbreaks from 2007 to 2012, in the United States, comprising 89 *E. coli*, 56 *Salmonella*, and 11 *L. monocytogenes* cases. The 4132 cases of illnesses were reported due to 163 outbreaks, *Salmonella* comprised 68%, STEC comprised 26% of illnesses, and *L. monocytogenes* showed the highest fatality (38%). Low a_w foods have usually been considered safe regarding foodborne pathogens because the optimum a_w for growth of these pathogens is over 0.95. But, unfortunately, there have been several outbreaks due to low a_w foods contaminated with *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes*. In 1998, there was an outbreak caused by *E. coli* O157:H7 in southern Ontario, Canada. The major source of contamination was dry fermented Genoa salami (Williams et al., 2000). There was a large outbreak in Norway and Finland due to *S.* Typhimurium-contaminated chocolate in 1987. Because of this outbreak 361 people were infected with *S.* Typhimurium and many young children developed acute hemorrhagic diarrhea (Kapperud et al., 1990). Also, a *L. monocytogenes* infection outbreak in Finland was traced to contaminated butter (Lyytikäinen et al., 2000).

Peanut butter has low a_w which disrupts the growth of foodborne pathogens, and generally, is pasteurized by conventional

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heating at 70–75 °C (Ha et al., 2013). But, unfortunately, multistate outbreaks caused by peanut butter have been reported. In 2007 in the USA, there was a large outbreak of salmonellosis due to consumption of *Salmonella enterica* serovar Tennessee-contaminated peanut butter. Because of this outbreak, 425 people were infected with *S.* Tennessee and 71 people were hospitalized (CDC, 2007). In 2008–2009, a multistate outbreak of *S.* Typhimurium infections linked to peanut butter occurred. This outbreak affected 714 people from 46 states of the USA; 24% of whom required hospitalization and resulted in 9 deaths (CDC, 2010). Also, in 2012 in the USA, 42 cases of *Salmonella* Bredeney infections linked to peanut butter consumption were reported (CDC, 2012). And also, there have been several studies which confirmed that conventional heating is not sufficient to inactivate *Salmonella* in peanut butter. Ma et al. (2009) reported that conventional thermal treatment at 71 °C for 50 min reduced *Salmonella* Tennessee by just 2 log CFU/g. He et al. (2011) also reported that thermal treatment at 72 °C for 60 min reduced *Salmonella enterica* by less than 2 log CFU/g in peanut butter with 0.4 a_w.

Microwave heating is a form of dielectric heating which is used industrially for the processing of food and also used domestically for cooking or thawing of food. Microwave irradiation produces efficient volumetric heating by utilizing the ability of microwave which can penetrate the material directly without any need of intermediate heat transfer medium (Zhu et al., 2007). Microwave heating is greatly affected by water in food because of the dipolar nature of water. When an electric field is applied to water, the dipolar water molecules try to realign in the direction of the electric field. This million times per second realignment due to the high frequency of microwaves cause internal friction of water molecules resulting in the volumetric heating of food. Because of this reason, microbial inactivation of food by microwave heating is focused on foods which have moisture contents higher than 50%, such as, milk (Choi et al., 1993), juice (Cañumir et al., 2002), meat (Shamis et al., 2008) and poultry (Pucciarelli and Benassi, 2005).

Water is one of the key factors which control the effect of microwave heating. And also, the a_w of food affects the heat resistance of microorganisms in food. Goepfert et al. (1970) reported that reduced a_w increased the D-value of salmonellae. But there has been no study investigating the effect of a_w of foods on microwave heating and inactivation of pathogens in foods by microwave heating treatment. Recently, we reported that microwave heating is effective for the pasteurization of peanut butter (Song and Kang, 2016). Therefore, in this study we evaluated the effect of a_w on inactivation of three foodborne pathogens (*E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes*) in peanut butter by microwave heating and obtained the inactivation kinetics of the three pathogens.

2. Materials and methods

2.1. Bacterial strains and cell suspension

Strains of *E. coli* O157:H7 (ATCC 35150, ATCC 43889, ATCC 43890), *S.* Typhimurium (ATCC 19585, ATCC 19115, DT 104), and *L. monocytogenes* (ATCC 15313, ATCC 19111, ATCC 19115) were obtained from the bacteria culture collection of Seoul National University (Seoul, Republic of Korea) for this study. Stock cultures were kept frozen at −80 °C in 0.7 ml of Tryptic Soy Broth (TSB; Difco, BD, Sparks, MD) and 0.3 ml of sterile 50% (V/V) glycerol. Working cultures were streaked onto Tryptic Soy Agar (TSA; Difco, BD), incubated at 37 °C for 24 h and stored at 4 °C. Each strain of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* was cultured in 5 ml TSB at 37 °C for 24 h, harvested by centrifugation at 4000*g* for 20 min at 4 °C and washed three times with sterile distilled water. The final pellets were resuspended in sterile distilled water, corresponding to approximately 10^8–10^9 CFU/ml. Mixed culture cocktails were prepared by blending together equal volumes of each test strain.

2.2. Sample preparation and inoculation

Experiments were performed using commercially processed creamy peanut butter. The peanut butter used for this study was purchased at a local grocery store (Seoul, Republic of Korea) and stored at room temperature (22 ± 1 °C). Twenty-five g of peanut butter samples were aseptically placed in sterile 100 ml Pyrex beakers. For inoculation, 0.2 ml of culture was inoculated into the sample and thoroughly mixed for 1 min with a sterile spoon to ensure even distribution of the pathogens. A_w of inoculated peanut butter was 0.30. To increase peanut butter a_w, a select volume of sterile distilled water was mixed into the inoculated peanut butter samples to adjust the a_w to 0.4 and 0.5. Generally peanut butter has water activity range from 0.20 to 0.33 (Burnett et al., 2000). But some studies used peanut butter with a_w 0.4 to 0.5 (Ha et al., 2013; Ma et al., 2009; Shachar and Yaron, 2006) so we used these a_w (0.3, 0.4, and 0.5). Uniform distribution of inoculum was confirmed by similar log CFU counts (log 5–6 CFU/g) obtained from 1 g subsamples of inoculated peanut butter taken from three randomly selected locations and appropriate tenfold serial dilutions (method described in section 2.4 *Bacterial enumeration*) spread-plated onto Sorbitol MacConkey agar (SMAC; Difco), Xylose Lysine Desoxycholate agar (XLD; Difco), and Oxford Agar Base with antimicrobial supplement (OAB; MB Cell) for enumeration of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes*, respectively. After inoculation, we removed excess peanut butter to obtain 25 g of inoculated sample because total sample weight was increased due to inoculation and adjustment of a_w. Water activity of inoculated peanut butter was measured with an Aqualab model 4TE a_w meter (Decagon Devices, Pullman, WA).

2.3. Microwave heating treatment

Microwave treatment was performed in a previously described apparatus (Sung and Kang, 2014). The beaker containing 25 g of peanut butter sample was located at the center of the turntable. For temperature measurements, the geometric center temperature of a non-inoculated sample adjusted to 0.3, 0.4, and 0.5 a_w in a beaker was measured by a fiber optic sensor (FOT-L; FISO Technologies Inc., Quebec, Canada) connected to a signal conditioner (TMI-4; FISO Technologies Inc., Quebec, Canada). For the inactivation study, 25 g of peanut butter with different a_w was placed in a 100 ml Pyrex beaker. An inoculated sample-filled beaker was subjected to microwave heating at 3 different power levels (2, 4, and 6 kW) for up to 5 min.

2.4. Bacterial enumeration

After microwave heating treatment, 25 g of sample was mixed with 25 ml of 0.2% peptone water (PW). Then, the sample and 0.2% PW mixture was diluted in 200 ml of sterile 0.2% PW and homogenized for 2 min in a stomacher (EASY MIX, AES Chemunex, Rennes, France). After homogenization, 1 ml aliquots of homogenized samples were 10-fold serially diluted in 9 ml of sterile 0.2% PW, and 0.1 ml of sample or diluent was spread-plated onto SMAC, XLD, and OAB for enumeration of the three pathogens. Where low populations of surviving cells were anticipated, 1 ml aliquots of the original homogenate were equally distributed between four plates and spread-plated. All plates were incubated at 37 °C for 24 h and colonies were counted.

2.5. Modeling of survival curves and T_d and T_{5d} values calculation

Survival curves for microwave heating treatments were obtained by plotting the logarithm of the surviving population versus treatment time (min). To fit survival curves and calculate parameters for fitting the model, the Geeraerd and Van Impe Inactivation model Fitting Tool (GInaFiT) was used (Geeraerd et al., 2005). Because our survival curves were obtained under non-isothermal conditions and showed a shouldering effect, the curves were fitted with the Weibull model (equation (1)) (Mafart et al., 2002) and Log-Linear + Shoulder model (equation (2)) (Geeraerd et al., 2000).

$$\log\left(\frac{N}{N_0}\right) = -\left(\frac{t}{\delta}\right)^p \quad \text{Weibull model} \tag{1}$$

where N = the number of survivors at microwave heating time, N_0 = initial population of pathogen, t = treatment time (min), δ = scale parameter (min), and p = shape parameter.

$$\log\left(\frac{N}{N_0}\right) = -\frac{k_{max}*t}{\ln(10)} + \log\left(\frac{Exp(k_{max}*Sl)}{1+(Exp(k_{max}*Sl)-1)*Exp(-k_{max}*t)}\right) \quad \text{Log} - \text{Linear} + \text{Shoulder model} \tag{2}$$

where N = the number of survivors at microwave heating time, N_0 = initial population of pathogen, t = treatment time (min), k_{max} = maximum inactivation rate (1/min), and Sl = shoulder length.

The goodness of fit of the two models was evaluated by the coefficient of determination (R^2) and mean squared error (MSE). The R^2 value is the coefficient of determination and a larger R^2 value (as it approaches 1) indicates better the fit of the model to the data. MSE is the average of the squares of the error and a smaller MSE value (as it approaches 0) indicates better fit of the model to the data. And also, T_d and T_{5d} values were calculated based on Weibull (equation (1)) and Log-Linear + Shoulder model (equation (2)) by using Microsoft Excel 2010 software package (Microsoft Coporation).

2.6. Statistical analysis

All data were analyzed with one-way ANOVA using Statistical Analysis System (SAS Institute, Cary, NC, USA) and Duncan's multiple range test to determine if there were significant differences ($P < 0.05$) in mean values of microorganism populations, T_d, and T_{5d} values. Microbial counts were transformed to $\log_{10}$ values for analysis. One log was used for calculations in the case of populations below the detection limit.

3. Results

3.1. Temperature-time histories of peanut butter

Average center temperatures of peanut butter with different a_w are shown in Fig. 1. Differences in temperature between peanut butters of different a_w were detected during 1–5 min of 915 MHz microwave treatment. After 5 min of 6 kW microwave treatment, there was ca. 18 °C difference between 0.3 a_w (94.76 °C) and 0.4 a_w peanut butter (111.8 °C) and ca. 15 °C between 0.4 a_w (111.8 °C) and 0.5 a_w peanut butter (127.35 °C). Following microwave treatment for 5 min at 4 kW, those temperature differences were 14 °C between 0.3 (59.64 °C) and 0.4 a_w (73.52 °C) peanut butter and 10 °C between 0.4 (73.52 °C) and 0.5 a_w (83.38 °C) peanut butter. Two kW microwave treatment for 5 min increased the temperature of peanut butter (0.3, 0.4, and 0.5 a_w) by up to 40.92, 48.97, and 50.93 °C, respectively.

3.2. Inactivation of foodborne pathogens in peanut butter by microwave treatment

Reductions in the viable counts of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* cells in peanut butter with different a_w during microwave heating at different power levels are shown in Tables 1–3. No significant ($P > 0.05$) log reductions of the three pathogens in any of the peanut butter samples were observed after 2 kW microwave heating for 5 min. *E. coli* O157:H7 survival curves following microwave heating at different power levels of peanut butter with different a_w are shown in Table 1. Treatment at 4 kW for 5 min inactivated populations of this pathogen in peanut butter (0.3, 0.4, and 0.5 a_w) by about 1.26, 1.29, and 1.24 log CFU/g, respectively. The 6 kW treatment for 5 min reduced *E. coli* O157:H7 in peanut butter (0.3, 0.4, and 0.5 a_w) by 2.78, 3.03, and 4.03 log CFU/g, respectively. Table 2 shows survival curves of *S.* Typhimurium in peanut butter with different a_w after microwave heating. Microwave heating at 4 kW for 5 min reduced *S.* Typhimurium in peanut butter (0.3, 0.4, and 0.5 a_w) by 1.18, 1.67, and 1.98 log CFU/g, respectively. Six kW microwave heating for 5 min inactivated this pathogen by 2.50, 3.51, and >5.17 log CFU/g (0.3, 0.4, and 0.5 a_w peanut butter), respectively. Reduction of *L. monocytogenes* in peanut butter of varying a_w by different microwave heating power levels is shown in Table 3. Inactivation of *L. monocytogenes* shows a similar trend to that of *S.* Typhimurium. Treatment at 4 and 6 kW for 5 min inactivated this pathogen by 0.41 and 1.97 (0.3 a_w), 1.13 and 2.80 (0.4 a_w), and 1.25 and 3.82 log CFU/g (0.5 a_w), respectively. Generally, *L. monocytogenes* showed more resistance to microwave heating than *E. coli* O157:H7 and *S.* Typhimurium and *S.* Typhimurium showed the least resistance. Especially, *S.* Typhimurium in 0.5 a_w peanut butter was reduced to under the detection limit (1.0 log CFU/g, more than 5 log reduction) when treated with 6 kW microwave heating for 5 min, but *L. monocytogenes* was reduced by 3.82 log CFU/g.

3.3. Suitable model of reduction curves

In this study, we used the Weibull (equation (1)) and Log-Linear + Shoulder (equation (2)) models to describe the experimental data obtained by microwave heating. Table 4 shows R^2 and MSE values of these two models to compare their goodness of fit. The R^2 values with the Weibull model were higher than 0.95, except for 5 out of a total of 18 cases. A similar situation was observed in R^2 values with the Log-Linear + Shoulder model which were higher than 0.95, except in 4 cases. The mean R^2 values of each of the two models were 0.945 (Weibull model) and 0.951 (Log-Linear −Shoulder model). Similar trends were observed in MSE values. The MSE values with the Weibull and Log-Linear + Shoulder models were smaller than 0.1 except in 3 cases (Weibull model) and 2 cases (Log-Linear + Shoulder model). The mean MSE values of each of the two models were 0.044 (Weibull model) and 0.045 (Log-Linear + Shoulder model). Instead, we used Weibull and Log-Linear + Shoulder models to calculate T_d and T_{5d} values of pathogens in peanut butter. The Weibull and Log-Linear + Shoulder model parameters are shown in Table 5.

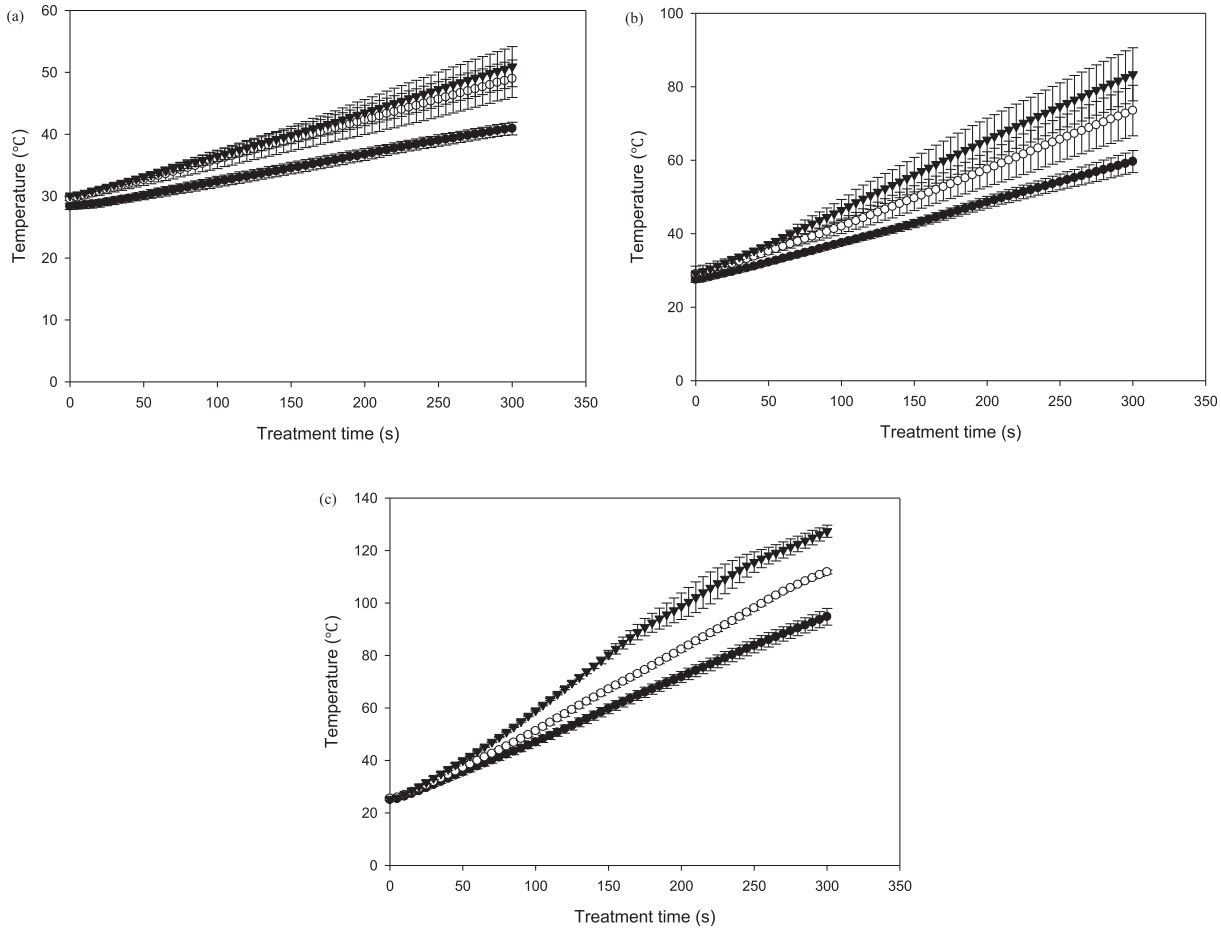

Fig. 1. Temperature histories of peanut butter of different a_w treated with different microwave power levels. (a) 2 kW, (b) 4 kW, (c) 6 kW. ● 0.30 a_w; ○ 0.40 a_w; ▼ 0.50 a_w.

Table 1
Effect of microwave heating at different power levels on the inactivation of *E. coli* O157:H7 in peanut butter with different a_w.

Power (kW)	A_w	Microbial populations of *E. coli* O157:H7 (log CFU/g)[a]					
		Control (0 min)	1 min	2 min	3 min	4 min	5 min
2	0.3	6.67 ± 0.30 Aa	6.66 ± 0.05 Aa	6.53 ± 0.21 Aa	6.72 ± 0.21 Aa	6.70 ± 0.11 Aa	6.43 ± 0.22 Aa
	0.4	6.61 ± 0.12 Aa	6.54 ± 0.21 Aa	6.63 ± 0.06 Aa	6.62 ± 0.04 Aa	6.30 ± 0.26 Ab	6.31 ± 0.30 Aa
	0.5	6.59 ± 0.30 Aa	6.55 ± 0.24 Aa	6.78 ± 0.16 Aa	6.65 ± 0.11 Aa	6.48 ± 0.00 Aab	6.67 ± 0.09 Aa
4	0.3	6.76 ± 0.14 Aa	6.61 ± 0.13 Aa	6.50 ± 0.21 Aa	6.46 ± 0.15 Aa	5.90 ± 0.16 Ba	5.50 ± 0.22 Ca
	0.4	6.49 ± 0.16 Aab	6.64 ± 0.08 Aa	6.50 ± 0.10 Aa	6.15 ± 0.16 Ba	5.70 ± 0.09 Cab	5.20 ± 0.27 Da
	0.5	6.25 ± 0.19 Ab	6.42 ± 0.37 Aa	6.36 ± 0.26 Aa	6.06 ± 0.44 Aa	5.48 ± 0.15 Bb	5.01 ± 0.36 Ba
6	0.3	6.80 ± 0.14 Aa	6.45 ± 0.13 ABa	5.97 ± 0.34 Ba	5.30 ± 0.29 Ca	4.54 ± 0.28 Da	4.03 ± 0.39 Ea
	0.4	6.41 ± 0.09 Ab	6.33 ± 0.07 ABa	5.87 ± 0.19 Ba	5.17 ± 0.36 Ca	4.25 ± 0.35 Dab	3.38 ± 0.38 Eab
	0.5	6.69 ± 0.14 Aa	6.56 ± 0.25 Aa	5.76 ± 0.48 Ba	4.80 ± 0.47 Ca	3.93 ± 0.24 Db	2.66 ± 0.32 Eb

Values followed by the same uppercase letters within a row and by the same lowercase letters within a column are not significantly different ($P > 0.05$).
[a] Mean of three replications ± standard deviation.

3.4. Calculation of T_d and T_{5d} values

Calculated T_d and T_{5d} values are shown in Table 6. T_d and T_{5d} values based on the Log-Linear Regression model were not computed because this model could not account for the reduction curves obtained by microwave heating. T_d values calculated based on the Weibull model showed no significant differences between different a_w except for 4 kW microwave heating of *S.* Typhimurium. *S.* Typhimurium in 0.5 a_w peanut butter showed a smaller T_d value than that of this pathogen in 0.3 a_w peanut butter. But significant differences in T_{5d} values computed based on the Weibull model were observed. Especially, T_{5d} values of the three pathogens treated with 6 kW microwave heating showed significant differences in all cases. T_{5d} values of the three pathogens in 0.5 a_w peanut butter were significantly lower than those of the three pathogens in 0.3 a_w peanut butter. And *L. monocytogenes* showed reduced T_{5d} values with increasing a_w when treated with 4 kW microwave heating. T_d values of *E. coli* O157:H7, which were calculated based on the Log-Linear + Shoulder model, were not affected by a_w (no significant differences). *S.* Typhimurium in 0.4 and 0.5 a_w peanut butter showed smaller T_d values than those of *S.* Typhimurium in 0.3 a_w peanut butter and *L. monocytogenes* showed decreased T_d values as a_w increased. T_{5d} values of the three pathogens in 0.5 a_w peanut butter were smaller than those of the three pathogens in 0.3 a_w

Table 2
Effect of microwave heating at different power levels on the inactivation of *S.* Typhimurium in peanut butter with different a_w.

Power (kW)	A_w	Microbial populations of *S.* Typhimurium (log CFU/g)[a]					
		Control (0 min)	1 min	2 min	3 min	4 min	5 min
2	0.3	6.31 ± 0.29 Aa	6.37 ± 0.09 Aa	6.22 ± 0.45 Aa	6.32 ± 0.25 Aa	6.38 ± 0.24 Aa	6.19 ± 0.19 Aa
	0.4	6.33 ± 0.02 Aa	6.28 ± 0.25 Aa	6.37 ± 0.18 Aa	6.33 ± 0.13 Aa	6.22 ± 0.40 Aa	5.98 ± 0.50 Aa
	0.5	6.16 ± 0.12 Aa	6.32 ± 0.21 Aa	6.15 ± 0.41 Aa	6.24 ± 0.25 Aa	6.10 ± 0.35 Aa	5.94 ± 0.41 Aa
4	0.3	6.24 ± 0.15 Aa	6.14 ± 0.13 Aa	6.07 ± 0.25 Aa	6.03 ± 0.21 Aa	5.46 ± 0.22 Ba	5.02 ± 0.24 Ca
	0.4	6.16 ± 0.15 Aa	6.15 ± 0.15 Aa	5.83 ± 0.30 ABa	5.73 ± 0.12 Bab	5.23 ± 0.22 Cab	4.49 ± 0.11 Db
	0.5	5.91 ± 0.19 ABa	6.10 ± 0.18 Aa	5.67 ± 0.23 ABa	5.47 ± 0.33 Bb	4.82 ± 0.36 Cb	3.93 ± 0.33 Dc
6	0.3	6.30 ± 0.18 Aa	6.03 ± 0.28 Aa	5.54 ± 0.26 Ba	5.30 ± 0.45 Ba	4.23 ± 0.17 Ca	3.80 ± 0.34 Ca
	0.4	5.98 ± 0.03 Ab	5.78 ± 0.24 ABa	5.37 ± 0.14 Ba	4.59 ± 0.33 Cab	3.35 ± 0.41 Db	2.47 ± 0.30 Eb
	0.5	6.17 ± 0.13 Aab	5.79 ± 0.10 Aa	5.16 ± 0.26 Ba	4.09 ± 0.50 Cb	2.73 ± 0.37 Db	>1.00 ± 0.00 Ec

Values followed by the same uppercase letters within a row and by the same lowercase letters within a column are not significantly different ($P > 0.05$).
[a] Mean of three replications ± standard deviation.

Table 3
Effect of microwave heating at different power levels on the inactivation of *L. monocytogenes* in peanut butter with different a_w.

Power (kW)	A_w	Microbial populations of *L. monocytogenes* (log CFU/g)[a]					
		Control (0 min)	1 min	2 min	3 min	4 min	5 min
2	0.3	4.82 ± 0.50 Aa	4.83 ± 0.43 Aa	4.80 ± 0.28 Aa	4.90 ± 0.41 Aa	4.75 ± 0.26 Aa	4.76 ± 0.19 Aa
	0.4	4.89 ± 0.37 Aa	4.75 ± 0.39 Aa	4.83 ± 0.46 Aa	4.75 ± 0.39 Aa	4.69 ± 0.32 Aa	4.68 ± 0.41 Aa
	0.5	5.07 ± 0.12 Aa	4.79 ± 0.43 Aa	4.86 ± 0.49 Aa	4.75 ± 0.35 Aa	4.75 ± 0.45 Aa	4.76 ± 0.33 Aa
4	0.3	5.21 ± 0.33 Aa	5.24 ± 0.23 Aa	5.19 ± 0.16 Aa	5.05 ± 0.27 Aa	4.98 ± 0.32 Aa	4.79 ± 0.36 Aa
	0.4	5.30 ± 0.00 Aa	5.20 ± 0.17 ABa	5.00 ± 0.15 BCa	4.92 ± 0.17 BCa	4.84 ± 0.18 Ca	4.17 ± 0.20 Db
	0.5	5.13 ± 0.16 Aa	5.06 ± 0.10 Aa	5.08 ± 0.04 Aa	4.88 ± 0.16 Aa	4.34 ± 0.17 Bb	3.88 ± 0.22 Cb
6	0.3	5.59 ± 0.26 Aa	5.49 ± 0.14 ABa	5.11 ± 0.22 BCa	4.70 ± 0.41 CDa	4.41 ± 0.24 Da	3.62 ± 0.16 Ea
	0.4	5.52 ± 0.35 Aa	5.33 ± 0.25 Aa	5.16 ± 0.12 Aa	4.43 ± 0.23 Ba	3.56 ± 0.08 Cb	2.72 ± 0.31 Db
	0.5	5.42 ± 0.17 Aa	5.13 ± 0.27 ABa	4.92 ± 0.28 Ba	4.17 ± 0.24 Ca	3.32 ± 0.25 Db	1.60 ± 0.30 Ec

Values followed by the same uppercase letters within a row and by the same lowercase letters within a column are not significantly different ($P > 0.05$).
[a] Mean of three replications ± standard deviation.

Table 4
Comparison of goodness of fit of the Weibull, Log-Linear + Shoulder, and Log-Linear Regression models for the inactivation of *E. coli* O157:H7, *S.* Typhimurium and *L. monocytogenes* in peanut butter treated with microwave heating at different power levels.[a]

Pathogens	Power (kW)	A_w	Weibull model		Log linear + Shoulder model	
			R^2	MSE	R^2	MSE
Escherichia coli O157:H7	4	0.3	0.962	0.0152	0.966	0.0137
		0.4	0.954	0.0237	0.956	0.0228
		0.5	0.842	0.0799	0.855	0.0752
	6	0.3	0.981	0.0358	0.982	0.0324
		0.4	0.994	0.0167	0.997	0.0081
		0.5	0.987	0.0594	0.987	0.0574
Salmonella Typhmiurium	4	0.3	0.949	0.0232	0.955	0.0200
		0.4	0.980	0.0136	0.974	0.0173
		0.5	0.959	0.0485	0.969	0.0324
	6	0.3	0.915	0.1243	0.914	0.1256
		0.4	0.976	0.1044	0.979	0.0904
		0.5	0.984	0.1102	0.981	0.1291
Listeria monocytogenes	4	0.3	0.679	0.0215	0.752	0.0151
		0.4	0.937	0.0179	0.934	0.0189
		0.5	0.975	0.0115	0.982	0.0082
	6	0.3	0.977	0.0209	0.969	0.0288
		0.4	0.977	0.0282	0.989	0.0203
		0.5	0.988	0.0407	0.975	0.0894

[a] R^2, regression coefficient; MSE, mean square error.

peanut butter except for *E. coli* O157:H7 and *L. monocytogenes* when treated with 4 kW microwave heating.

4. Discussion

Peanut butter has recently become an important issue in the area of food safety due to several large outbreaks (CDC, 2007, 2010, 2012) and difficulties of pasteurization of this product. The main cause of peanut butter illness outbreaks is *Salmonella* contamination. Many researchers attempted to pasteurize peanut butter by conventional heating. Shachar and Yaron (2006) reported that conventional heating at 80 and 90 °C for 50 min reduced *Salmonella* Agona, *Salmonella* Enteritidis and *S.* Typhimurium by 3.0 log CFU/g. Ma et al. (2009) and He et al. (2011) also used conventional heating to reduce *Salmonella* Tennessee and *Salmonella enterica* but it was not effective as we mentioned above. The long treatment time required by conventional heat pasteurization is one of the main causes of food quality deterioration. Many food

Table 5
Kinetic parameters of the Weibull and Log-Linear + Shoulder models for *E. coli* O157:H7, *S.* Typhimurium and *L. monocytogenes* in peanut butter of different a_w treated with microwave heating at 4 and 6 kW.

Pathogens	Power (kW)	A_w	Weibull model		Log linear + Shoulder model	
			δ (min)	p	Sl (min)	k_{max} (1/min)
Escherichia coli O157:H7	4	0.3	4.59 ± 0.18	2.40 ± 0.52	2.80 ± 0.42	1.27 ± 0.25
		0.4	4.32 ± 0.31	2.36 ± 0.31	2.63 ± 0.28	1.37 ± 0.20
		0.5	4.32 ± 0.73	2.83 ± 0.98	2.80 ± 0.95	1.56 ± 0.33
	6	0.3	2.17 ± 0.54	1.27 ± 0.27	0.72 ± 0.67	1.55 ± 0.32
		0.4	2.62 ± 0.46	1.77 ± 0.25	1.64 ± 0.41	2.09 ± 0.23
		0.5	2.04 ± 0.72	1.70 ± 0.73	1.30 ± 0.88	2.61 ± 0.68
Salmonella Typhmiurium	4	0.3	4.75 ± 0.21	2.86 ± 0.76	3.12 ± 0.27	1.49 ± 0.53
		0.4	4.09 ± 0.25	2.58 ± 0.87	2.61 ± 0.58	1.60 ± 0.45
		0.5	3.70 ± 0.57	2.58 ± 0.31	2.59 ± 0.45	1.90 ± 0.06
	6	0.3	2.56 ± 0.43	1.31 ± 0.05	0.83 ± 0.19	1.38 ± 0.41
		0.4	2.39 ± 0.46	1.79 ± 0.56	1.59 ± 0.75	2.47 ± 0.76
		0.5	2.02 ± 0.39	1.83 ± 0.40	1.60 ± 0.65	3.39 ± 0.58
Listeria monocytogenes	4	0.3	17.28 ± 15.04	1.10 ± 0.34	2.45 ± 2.97	0.87 ± 0.71
		0.4	5.03 ± 0.22	3.34 ± 2.10	2.95 ± 1.57	1.50 ± 0.99
		0.5	4.55 ± 0.21	2.72 ± 0.68	2.95 ± 0.41	1.45 ± 0.35
	6	0.3	3.34 ± 0.83	1.92 ± 0.86	2.41 ± 0.10	1.65 ± 0.21
		0.4	2.90 ± 0.53	1.93 ± 0.29	1.89 ± 0.47	2.05 ± 0.10
		0.5	2.97 ± 0.25	2.50 ± 0.34	2.28 ± 0.21	2.94 ± 0.33

Table 6
Calculated T_d and T_{5d} values for *E. coli* O157:H7, *S.* Typhimurium and *L. monocytogenes* in peanut butter with different a_w based on the Weibull and Log-Linear + Shoulder models.

Pathogens	Power (kW)	A_w	Weibull model		Log linear + Shoulder model	
			T_d	T_{5d}	T_d	T_{5d}
Escherichia coli O157:H7	4	0.3	4.59 ± 0.18 A	9.21 ± 1.09 A	4.57 ± 0.17 A	12.07 ± 1.30 A
		0.4	4.32 ± 0.31 A	8.65 ± 1.00 A	4.27 ± 0.32 A	11.20 ± 1.30 A
		0.5	4.32 ± 0.73 A	8.10 ± 1.08 A	4.26 ± 0.73 A	10.41 ± 1.08 A
	6	0.3	2.17 ± 0.54 A	7.97 ± 1.35 A	2.22 ± 0.52 A	8.39 ± 1.30 A
		0.4	2.62 ± 0.46 A	6.52 ± 0.47 AB	2.70 ± 0.43 A	7.20 ± 0.78 AB
		0.5	2.04 ± 0.72 A	5.65 ± 0.29 B	2.18 ± 0.69 A	5.89 ± 0.28 B
Salmonella Typhmiurium	4	0.3	4.75 ± 0.21 A	8.64 ± 1.56 A	4.72 ± 0.22 A	11.46 ± 1.35 A
		0.4	4.09 ± 0.25 AB	7.98 ± 1.02 A	4.05 ± 0.25 B	10.15 ± 1.29 AB
		0.5	3.70 ± 0.57 B	6.91 ± 0.56 A	3.75 ± 0.47 B	8.66 ± 0.57 B
	6	0.3	2.56 ± 0.43 A	8.79 ± 1.83 A	2.54 ± 0.41 A	9.64 ± 2.17 A
		0.4	2.39 ± 0.46 A	6.24 ± 1.21 B	2.56 ± 0.44 A	6.63 ± 1.24 B
		0.5	2.02 ± 0.39 A	4.92 ± 0.09 B	2.26 ± 0.53 A	5.07 ± 0.11 B
Listeria monocytogenes	4	0.3	17.28 ± 15.04 A	31.17 ± 1.79 A	6.37 ± 0.21 A	22.33 ± 9.36 A
		0.4	5.03 ± 0.22 A	11.43 ± 0.92 B	5.05 ± 0.26 B	13.80 ± 6.21 A
		0.5	4.55 ± 0.21 A	8.49 ± 1.14 C	4.52 ± 0.22 C	11.15 ± 1.46 A
	6	0.3	3.34 ± 0.83 A	9.44 ± 1.89 A	3.75 ± 0.05 A	9.44 ± 0.55 A
		0.4	2.90 ± 0.53 A	6.69 ± 0.43 B	2.96 ± 0.51 B	7.52 ± 0.71 B
		0.5	2.97 ± 0.25 A	5.68 ± 0.22 B	3.03 ± 0.18 B	6.23 ± 0.34 C

Mean values ± standard deviations. Means with the same upper case letter in the same column per pathogen and microwave heating level are not significantly different ($P > 0.05$).

ingredients are heat sensitive, so conventional heating induces chemical and physical changes in foods (Chemat et al., 2011). For these reasons new intervention strategies for pasteurization of peanut butter are needed. Recently, we reported that microwave heating can be used as a pasteurization method for peanut butter (Song and Kang, 2016). In this study, we evaluated the effect of a_w of peanut butter on inactivation of pathogens in peanut butter. *E. coli* O157:H7 has not been frequently linked to peanut butter outbreaks but *E. coli* O157:H7 has been implicated in several outbreaks involving low a_w foods (CDC, 2011; Williams et al., 2000). Kenney and Beuchat (2004) reported that *L. monocytogenes* can survive in 0.33 a_w peanut butter for 24 weeks. Based on these studies, we also confirmed the effect of a_w of peanut butter on inactivation on *E. coli* O157:H7 and *L. monocytogenes* by microwave heating.

Both temperature and microbial inactivation of microwave heat-treated peanut butter of different a_w increased as power, treatment time and a_w increased. A_w and water content of foods are two key factors of thermal treatment which greatly affect temperature rise and microbial inactivation. Jeong and Kang (2014) reported that the rate of temperature increased by radio-frequency heating of 12.6% moisture content red pepper was lower than that of 19.1% moisture content red pepper. He et al. (2013) reported that increased a_w of peanut butter resulted in reduced thermal resistance of *Salmonella enterica*. The results from this study show a similar trend. Increasing a_w of peanut butter resulted in higher temperature increase and greater reduction of pathogens.

Microwave heating experiences different starting and ending treatment temperatures and thus usually can-not maintain an isothermal condition. In the case of non-isothermal conditions, inactivation of pathogens in foods can-not be characterized by the Log-Linear Regression model. From this reason, several non-log-linear models have been proposed to explain the survival curves of pathogens, such as the Weibull model (Mafart et al., 2002), Log-Linear + Shoulder or Tail model (Geeraerd et al., 2000), Log-Logistic model (Chen and Hoover, 2003), and Biphasic model (Cerf, 1977). In this study, the Weibull and Log-Linear + Shoulder models were

selected to explain the survival curves of the three pathogens because their survival curves did not describe a straight line. These two models achieved better statistical results than did other non-log-linear models in our preliminary studies. The Log-Linear + Shoulder model showed a higher mean R^2 value (0.951) than that of the Weibull model (0.945) and MSE values of these two models were similar (0.045 and 0.044); therefore the Log-Linear + Shoulder model provided the best fit for microbial inactivation by microwave heating and was especially applicable because inactivation curves of the pathogens in peanut butter by microwave heating showed a shouldering effect. Several studies have confirmed that a shouldering effect occurs during microwave heating (Goldblith and Wang, 1967; Papadopoulou et al., 1995). Valero et al. (2014) also used the Log-Linear + Shoulder model to explain microbial inactivation by microwave heating.

Usually, under non-isothermal conditions, T_d value is used to the resistance of a pathogen to thermal treatment. In this study, we calculated T_d values of the three pathogens in peanut butter of different a_w based on the Weibull and Log-Linear + Shoulder models. Several studies have measured the D-values of pathogens in peanut butter subjected to conventional heating. Ma et al. (2009) reported that the D-value of a cocktail of 5 *Salmonella* strains of non-Tennessee serotypes in 0.45 a_w peanut butter was 9.4 min at 90 °C. He et al. (2011) reported that D-values of 5 strains of *Salmonella enterica* in different peanut butters (0.4 a_w) ranged from 2.33 to 3.55 min at 90 °C. Also, D-values of *S.* Typhimurium in low-fat and regular formulation 0.4 a_w peanut butter ranged from 2.43 to 1.35 min at 90 °C (He et al., 2013). In this study, T_d values of the three foodborne pathogens treated with 6 kW microwave heating were 2.02–3.34 min based on the Weibull model. In the case of the Log-Linear + Shoulder model, T_d values of pathogens treated with 6 kW microwave heating ranged from 2.18 to 3.75 min. Decimal reduction times (D-value and T_d value) of 6 kW microwave heat-treated pathogens were similar to those of 90 °C conventional heating. But T_{5d} values were different from those of conventional heating. He et al. (2013) reported that calculated minimum times to achieve 5 log reduction of *S.* Typhimurium in 0.4 a_w regular formulation peanut butter based on the Weibull model were 23.83 min by conventional heating at 90 °C. Six kW microwave heating of 0.4 a_w peanut butter resulted in T_{5d} values of the three pathogens ranging from 4.92 to 9.44 min. Especially 6 kW microwave heating can reduce *S.* Typhimurium by 5 log after about 5 min He et al. (2013) also reported that increased a_w reduced the heat resistance of *Salmonella*. But, a_w of peanut butter treated with microwave heating generally did not affect T_d values of pathogens. Four out of a total of 12 cases showed a significant difference in T_d values. However, increased a_w generally affected T_{5d} values of pathogens (in 8 of 12 cases). In the case of 6 kW microwave heating, increased a_w resulted in less heat resistance of pathogens when we calculated T_{5d} values.

Our results indicate that microwave heating can be used as a pasteurization intervention for peanut butter. Inactivation curves of the three pathogens fit well to the Weibull and Log-Linear + Shoulder models and we found that the Log-Linear + Shoulder model gave the best fit for peanut butter pasteurization by microwave heating. T_d values of the three pathogens were similar to D-values for conventional heating but T_{5d} values were dramatically lower than those of conventional heating. Generally, a_w of peanut butter affected T_{5d} values of microwave heating but did not influence T_d and *L. monocytogenes* showed the most resistance to microwave heating, whereas *S.* Typhimurium showed less resistance than the other two pathogens. Results of the present study can be used to optimize microwave heating as a pasteurization intervention for peanut butter.

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