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Inactivation of *Salmonella* Senftenberg, *Salmonella* Typhimurium and *Salmonella* Tennessee in peanut butter by 915 MHz microwave heating

Won-Jae Song [a, b], Dong-Hyun Kang [a, b, *]

[a] Department of Agricultural Biotechnology, Center for Food and Bioconvergence, and Research Institute for Agricultural and Life Sciences, Seoul National University, Seoul 151-921, Republic of Korea
[b] Institutes of Green Bio Science & Technology, Seoul National University, Pyeongchang-gun, Gangwon-do 232-916, Republic of Korea

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ABSTRACT

This study evaluated the efficacy of a 915 MHz microwave with 3 different levels to inactivate 3 serovars of *Salmonella* in peanut butter. Peanut butter inoculated with *Salmonella enterica* serovar Senftenberg, *S. enterica* serovar Typhimurium and *S. enterica* serovar Tennessee were treated with a 915 MHz microwave with 2, 4 and 6 kW and acid and peroxide values and color changes were determined after 5 min of microwave heating. *Salmonella* populations were reduced with increasing treatment time and treatment power. Six kW 915 MHz microwave treatment for 5 min reduced these three *Salmonella* serovars by 3.24–4.26 log CFU/g. Four and two kW 915 MHz microwave processing for 5 min reduced these *Salmonella* serovars by 1.14–1.48 and 0.15–0.42 log CFU/g, respectively. Microwave treatment did not affect acid, peroxide, or color values of peanut butter. These results demonstrate that 915 MHz microwave processing can be used as a control method for reducing *Salmonella* in peanut butter without producing quality deterioration.

1. Introduction

Non-typhoidal salmonellae are among the most important foodborne pathogens and can cause gastroenteritis, bacteremia and subsequent focal infection (Hohmann, 2001). Scallan et al. (2011) estimated there are over 9 million foodborne illnesses in the United States annually; about a million of these cases are caused by non-typhoidal *Salmonella* spp. Based on this estimate, about twenty thousand people are hospitalized and 378 die annually due to non-typhoidal *Salmonella* spp. People believe that low water activity (a_w) foods are free from contamination with *Salmonella* because the optimum a_w for growth of *Salmonella* is 0.99 (Mattick et al., 2000). However, several incidents of *Salmonella* contamination of low a_w foods have been reported. In 2001, there was an international outbreak caused by multi-resistance *Salmonella enterica* serovar Typhimurium DT 104 in Australia and Sweden. The main source of contamination was traced to halvah (a candy made of sesame seed, sugar and flavoring) imported from Turkey (Brockmann et al., 2004). In 1993, the US Centers for Disease Control and Prevention (CDC) reported three cases of infection with *S. enterica* serovar Tennessee in infants in Canada and the USA. Infection occurred due to contaminated powdered infant formula (CDC, 1993). Also, *S. enterica* serovar Senftenberg isolated from infant formula milk was reported (Rushdy et al., 1998).

Peanut butter, which usually contains over 90% peanuts with optional added sweeteners, salt and stabilizers (Grasso et al., 2010), constitutes the largest segment of peanut consumption. This is due to the increase in the consumption of peanut butter from 1999 to 2008 while peanut candy and snack consumption has not (USDA, 2010). Peanut butter has low a_w (less than 0.35) which precludes the growth of foodborne pathogens (He et al., 2013). But unfortunately, multistate outbreaks caused by peanut butter have been reported. A total of 628 persons from 47 states were infected with *S.* Tennessee traced to contaminated peanut butter in 2007. Symptoms of infection included diarrhea, abdominal cramps, fever and dysuria (CDC, 2007). Between September 2008 and April 2009, there was a large multistate outbreak caused by *Salmonella* Typhimurium. This outbreak resulted in 714 illnesses, 166 hospitalizations and 9 deaths. The major source of these *S.* Typhimurium infections was peanut butter (Cavallaro et al., 2011). Also, in 2012, 41 cases of *S. enterica* serovar Bredeney infections were identified in

* Corresponding author. Department of Food and Animal Biotechnology, Department of Agricultural Biotechnology, Seoul National University, Kwanak-ro 1, Kwanak-gu, Seoul 151-742, Republic of Korea.
E-mail address: kang7820@snu.ac.kr (D.-H. Kang).

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20 states (CDC, 2013).

Peanut butter is usually pasteurized by conventional heating at temperatures of 70–75 °C before packaging (Ha et al., 2013). But Ma et al. (2009) reported that thermal treatment at 71 °C for 50 min resulted in 2 log reductions of *Salmonella* in peanut butter ($a_w = 0.45$, pH = 5.12). Shachar and Yaron (2006) also reported that 90 °C treatment for 30 min reduced *Salmonella* by about 3 log CFU/g. Conventional heating of peanut butter is a time and energy intensive intervention which consumes large amounts of water. Moreover, food ingredients are heat sensitive which means that food quality deterioration can occur during conventional heating (Chemat et al., 2011). Heating of peanut butter is primarily conductive in nature, and thus much heat has to be applied before the cold spot located at or near the geometric center of product is raised sufficiently to inactivate pathogens, resulting in over-heating of product near the periphery. Especially, peanut butter has a high oil contents which is susceptible to lipid oxidation resulting in rancidity and off-flavor (Riveros et al., 2010). Due to holding food samples at high temperature for an extended time, lipid oxidation is increased because high temperature decreases the activation energy needed for oxidation (Kanner, 1994). Based on these limitations, there is a need to develop new intervention strategies involving shorter time intervals to reduce *Salmonella* in peanut butter.

Microwave heating has been used for food processing over a period of several decades. Microwave heating can be used in food processing, including drying, thawing, tempering, baking, pasteurization and sterilization of food materials. In contrast to conventional heating, microwave radiation can penetrate the material directly (without any need for an intermediate medium to transfer heat); and also, microwave heating allows for volumetric heating of materials (Zhu et al., 2007). Therefore, microwave treatment can process foods in a shorter time with higher efficiency and with fewer changes in flavor and nutritional qualities compared to conventional heating (Vadivambal and Jayas, 2010). There are two major frequencies for microwave processing of foods: 2450 MHz is usually used in domestic microwave ovens and 915 MHz is mainly utilized in industrial microwave equipment (Datta and Davidson, 2000). However, most industrial microwave systems operate at 915 MHz in the USA because they enable greater penetration depth than those of 2450 MHz (Wang et al., 2003).

There have been many research studies which have confirmed the effect of conventional heat treatment on inactivation of pathogens in peanut butter (He et al., 2011, 2013; Ma et al., 2009; Shachar and Yaron, 2006). However, conventional thermal treatment is not effective for reducing pathogens in peanut butter because of the long treatment time required. Therefore, in this study, we evaluated the effect of a 915 MHz microwave system on the inactivation of *Salmonella* Senftenberg, Typhimurium and Tennessee in creamy peanut butter. Unlike the other two *Salmonella* serovars, there have been no outbreaks traced to *S.* Senftenberg in peanut butter. But *S.* Senftenberg has reportedly been isolated from peanut butter (Burnett et al., 2000); therefore, we also investigated the effect of a 915 MHz microwave system on the inactivation of *S.* Senftenberg. Also, changes of color, acid, and peroxide values of peanut butter were studied.

2. Materials and methods

2.1. Bacterial strains and cell suspension

S. Senftenberg KVCC 0590 and *S.* Tennessee KVCC 0592 were obtained from the Korea Veterinary Culture Collection (KVCC; Anyang, Republic of Korea) and *S.* Typhimurium DT 104 was obtained from the bacteria culture collection of Seoul National University (Seoul, Republic of Korea) for this study. Stock cultures were prepared by mixing 0.7 ml of cultures grown in tryptic soy broth (TSB; Difco, BD, Sparks, MD) for 24 h at 37 °C with 0.3 ml of sterile 50% (v/v) glycerol and kept frozen at −80 °C. 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 *S.* Senftenberg, *S.* Typhimurium, and *S.* Tennessee 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 0.2% peptone water (PW; Bacto, Sparks, MD). The final pellets were resuspended in sterile 0.2% PW, corresponding to approximately 10^8–10^9 CFU/ml.

2.2. Sample preparation and inoculation

Experiments were performed using commercially processed creamy peanut butter purchased at a local grocery store (Seoul, Republic of Korea) and stored at room temperature (22 ± 1 °C). The composition of the peanut butter (in the order listed on the product label) consisted of roasted peanuts, sugar, hydrogenated vegetable oil (cottonseed, soybean and rapeseed oil) and salt. The nutrition facts label indicated 16 g of fat, 7 g of protein, 6 g of total carbohydrate and 150 mg of sodium per each 32 g serving. Thirty g samples of peanut butter were aseptically placed in sterile 100 ml Pyrex glass beakers. For inoculation, 0.3 ml of culture was added to each sample and thoroughly mixed for 2 min with a sterile spoon to ensure even distribution of the pathogen. Uniform distribution of inoculum was confirmed by similar log CFU counts (log 5–6 CFU/g) on xylose lysine desoxycholate agar (XLD; Difco) that were obtained from 1 g sub-samples of inoculated peanut butter taken from three randomly selected locations. After inoculation, approximately 5 g was removed to obtain 25 g of inoculated sample. Water activity of inoculated peanut butter was 0.26 and pH was 5.99. A_w and pH were measured by an Aqualab model 4 TE a_w meter (Decagon Devices, Pullman, WA) and a pH meter (Mettler-Toledo, Switzerland).

2.3. Microwave heating treatment

Microwave treatment was performed in a previously described apparatus (Sung and Kang, 2014). For treatment, 25 g of peanut butter (adjusted to $a_w = 0.26$ with addition of sterile distilled water) was placed in a 100 ml Pyrex beaker. For the inactivation study, an inoculated sample-filled beaker was placed at the center of the turntable inside the cavity and subjected to microwave heating at 3 different power levels (2, 4, and 6 kW) for up to 5 min. For temperature measurements, the geometric center temperature of a non-inoculated sample in a beaker was measured with a fiber optic sensor (FOT-L; FISO Technologies Inc., Quebec, Canada) connected to a signal conditioner (TMI-4; FISO Technologies Inc., Quebec, Canada). To assess quality changes during microwave heating, non-inoculated sample-filled beakers were treated with microwave heating at 3 different power levels (2, 4, and 6 kW) for 5 min.

2.4. Bacterial enumeration

After microwave heating treatment, 25 g of sample was mixed with 50 ml of 0.2% PW pre-chilled in ice-water to rapidly cool the sample, thus reducing the effect of residual heat. Then, the sample and 0.2% PW mixture was diluted with 175 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 ten-fold serially diluted in 9 ml of sterile 0.2% PW, and 0.1 ml of sample or diluent was spread-plated onto XLD for the enumeration of *S.* Senftenberg, Typhimurium, and Tennessee. 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. Experiments were conducted three times.

2.5. Acid value, peroxide value and color measurement

After 5 min of microwave heating treatment, the acid and peroxide values of peanut butter were measured as indicators of lipid oxidation. Acid value titrations were determined according to the American Oil Chemists' Society (AOCS) Cd 3d-63 (1998). Peroxide values were determined by iodometric titration according to AOCS Ja 8–87 (2009). Acid value is milligrams of potassium hydroxide necessary to neutralize the free acid in 1 g of sample and peroxide value is the amount of peroxide oxygen per 1 kg of sample. Also, Hunter's color values (*L*, *a* and *b*) were measured using a Minolta colorimeter (model CR300, Minolta Co., Osaka, Japan) after 5 min of microwave heating. Untreated samples were used as controls. *L*, *a*, and *b* values indicate color lightness, redness, and yellowness of the sample, respectively. Experiments were conducted three times.

2.6. Statistical analysis

All data were analyzed by one-way ANOVA using the 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. Microbial counts were transformed to $\log_{10}$ values prior to analysis.

3. Results and discussion

3.1. Time-temperature profiles of peanut butter during 915 MHz microwave heating treatment

Microwaves are part of the electromagnetic spectrum and fall within a frequency range of 300 MHz–300 GHz. The most important mechanism of microwave heating is its interaction with water molecules which can generate heat inside of foods (Papadopoulou et al., 1995), whereas conventional heating depends on conduction and convection to transport heat from the heating source through an intermediate medium (e.g., water or steam) to the interior of the product, which requires a relatively longer period of time (Mullin, 1995).

The time–temperature profiles of peanut butter during microwave heating at 3 power levels (2, 4, and 6 kW) are shown in Fig. 1. After 5 min of microwave heating, the center temperatures of peanut butter increased to 22, 49, and 101 °C at 2, 4, and 6 kW, respectively. Two kW of microwave heating did not generate heat in peanut butter; however, 6 kW increased the temperature of peanut butter to nearly 100 °C. Differences in microwave power greatly affected temperature increase in peanut butter.

There are many factors which affected temperature increase during microwave heating. One of them is product size. In this study, we used 25 g of peanut butter which is not a suitable size for industrial processing. Other research studies which confirmed the effect of conventional heating on peanut butter pasteurization reported that less than 1 min of come-up time was required for samples to reach 90 °C. But these studies used small amounts of peanut butter (Li et al., 2014; Ma et al., 2009) and flattened peanut butter samples to increase heat transfer (He et al., 2011, 2013; Li et al., 2014; Ma et al., 2009). We used a 915 MHz microwave which provided much greater penetration depth than a 2450 MHz microwave. Microwave heating produces wavelengths of 0.33 (915 MHz) and 0.12 (2450 MHz) m in free space, respectively (Tanaka et al., 1999). The penetration depth of a wave is proportional to its wavelength, thus, a 915 MHz microwave shows deeper penetration depth than a 2450 MHz microwave. Because of these reasons, a 915 MHz microwave can reduce the differences between pilot scale and industrial scale processing.

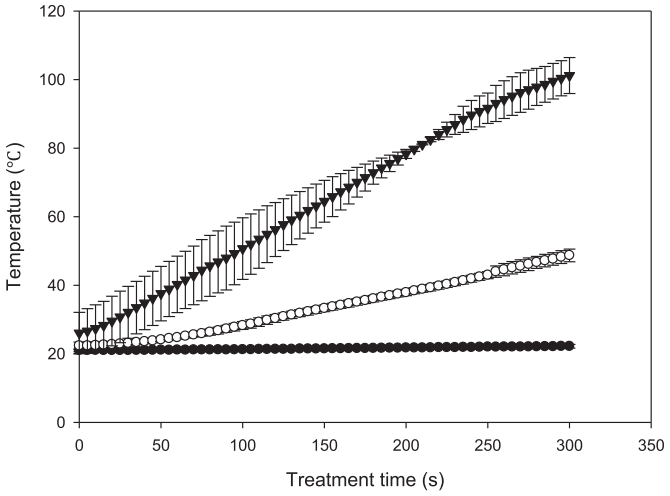

Fig. 1. Time-temperature profiles of the geometric center of peanut butter during microwave heating at 2 kW (●), 4 kW (○) and 6 kW (▼).

3.2. Effect of 915 MHz microwave heating at different power levels on inactivation of Salmonella serovars in peanut butter

Reductions of *Salmonella* in peanut butter are shown in Figs. 2–4. Microwave heating at 2 kW did not significantly reduce *S.* Senftenberg, *S.* Typhimurium and *S.* Tennessee. Microwave heating at 4 kW for 1, 3, and 5 min reduced *S.* Senftenberg by 0.11, 0.59, and 1.14 log CFU/g, respectively. Six kW microwave heating for 1, 3, and 5 min inactivated this pathogen by 0.41, 1.60, and 3.28 log CFU/g, respectively (Fig. 2). The reduction trend of *S.* Typhimurium was similar to that of *S.* Senftenberg. Four kW microwave heating reduced *S.* Typhimurium by 0.16, 0.44, and 1.18 log CFU/g after 1, 3, and 5 min, respectively. This pathogen was reduced by 0.49, 1.85, and 3.24 log CFU/g when treated with microwave heating at 6 kW for 1, 3, and 5 min, respectively (Fig. 3). The reduction trend of *S.* Tennessee in peanut butter by microwave heating was similar to those of *S.* Senftenberg and *S.* Typhimurium. Microwave heating at 4 kW for 1, 3, and 5 min reduced this pathogen by 0.11, 0.68, and

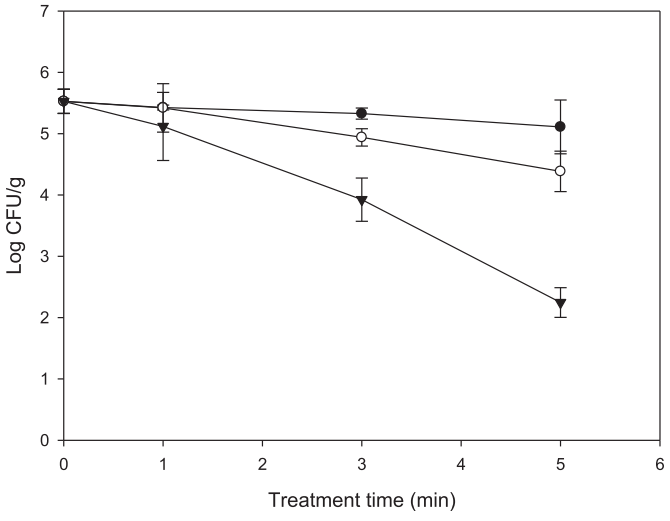

Fig. 2. Survival curves for *S.* Senftenberg KVCC 0590 in peanut butter treated with microwave heating at 2 kW (●), 4 kW (○) and 6 kW (▼).

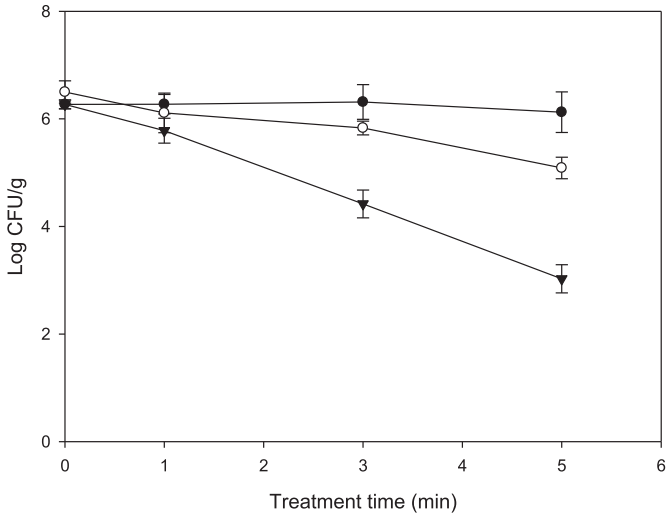

Fig. 3. Survival curves for *S.* Typhimurium DT 104 in peanut butter treated with microwave heating at 2 kW (●), 4 kW (○) and 6 kW (▼).

1.48 log CFU/g, respectively, and 6 kW microwave heating for 1, 3, and 5 min inactivated this pathogen by 0.94, 2.27, and 4.26 log CFU/g, respectively (Fig. 4). Pasteurization of peanut butter by conventional heating has been widely investigated many times, but this method has not been effective for reducing *Salmonella* in peanut butter. Shachar and Yaron (2006) reported that conventional heating treatment at 90 °C for 30 min resulted in about 3 log reduction of *Salmonella* Agona, *Salmonella* Enteritidis, and *Salmonella* Typhimurium in peanut butter. Conventional pasteurization of peanut butter is not an attractive control measure because of the long treatment time. Because conventional heating is not an appealing option, many researchers have focused on non-thermal inactivation methods to control *Salmonella* in peanut butter. Hvizdzak et al. (2010) used electron beam treatment for reduction of *Salmonella* in peanut butter. Three kGy of electron beam radiation reduced *S.* Tennessee and *S.* Typhimurium by 6.75 and 4.86 log CFU/g, respectively, when plated on a selective medium (XLD) for enumeration. Ban and Kang (2014) reported that 3 kGy of gamma irradiation reduced *S.* Typhimurium in three types of peanut butter by 3.5–4.0 log CFU/g. D'Souza et al. (2012) reported that high hydrostatic pressure under

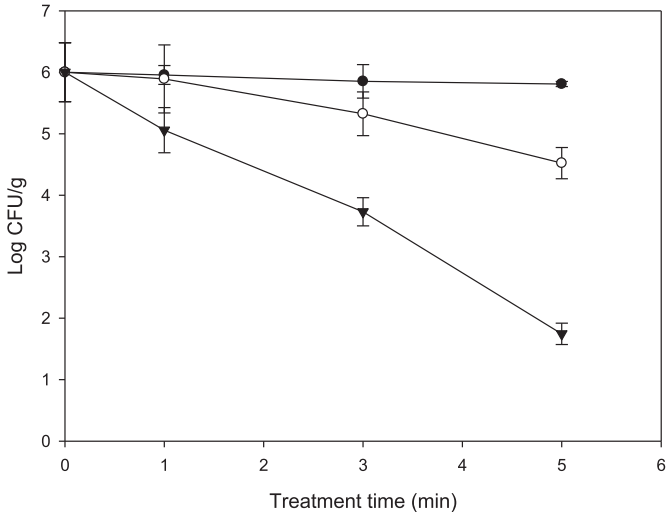

Fig. 4. Survival curves for *S.* Tennessee KVCC 0592 in peanut butter treated with microwave heating at 2 kW (●), 4 kW (○) and 6 kW (▼).

various conditions (400–600 MPa for 4–18 min) reduced cell numbers of a *Salmonella* enterica serovar cocktail in creamy peanut butter by 1.6–1.9 log CFU/g. Ha et al. (2013) used RF for inactivation of *S.* Typhimurium and *Escherichia coli* O157:H7 in peanut butter cracker sandwiches. RF treatment for 90 s reduced these pathogens by 4.55 and 5.32 log CFU/g, respectively.

In this study, we confirmed that *S.* Tennessee experienced greater reduction than the other two *Salmonella* serovars which indicates that *S.* Tennessee shows less heat resistance than the other two serovars. There are some studies which correlate with the results of the present study. Goepfert et al. (1970) reported that mean D-values of *S.* Tennessee in aqueous sucrose of different a_w were smaller than those of *S.* Typhimurium. Álvarez-Ordóñez et al. (2009) reported that D-values of *S.* Typhimurium and *S.* Senftenberg in apple and orange juice were not significantly different. Ma et al. (2009) reported that D-values based on first-order kinetics of three strains of *S.* Tennessee isolated from patients associated with a 2006–2007 peanut butter outbreak, 2 isolates of *S.* Tennessee from sporadic cases unrelated to this outbreak, and 5 *Salmonella* strains of other serotypes (*S.* Enteritidis, *S.* Typhimurium and *S.* Heidelberg) were 13.4, 8.6 and 9.4 min at 90 °C, respectively. In this study, we reduced *Salmonella* by about 3–4 log after 5 min treatment with a 6 kW, 915 MHz microwave. Pasteurization of peanut butter by microwave heating can dramatically reduce the processing time.

3.3. Effect of 915 MHz microwave heating at different power levels on physico-chemical properties of peanut butter

Acid and peroxide values are indicators of lipid oxidation of foods. Lipid oxidation is a major cause of quality deterioration of foods containing high levels of lipids and results from exposure to high temperature. But, lipid oxidation can be induced by some non-thermal treatments. Zheng et al. (2010) reported that pulsed electric field-treated peanut oil showed higher peroxide values than that of the control. Another study confirming that peanut butter treated by electron beams showed increased peroxide values was also reported (El-Rawas et al., 2012). Gao et al. (2011) reported that peroxide values of almonds increased after RF heating. But in this study, acid and peroxide values were not affected by microwave heating (Table 1). Acid and peroxide values of microwave heat-treated samples were not significantly different from those of non-treated samples. Both acid and peroxide values slightly increased after microwave heating but these differences were not significant ($P > 0.05$).

3.4. Effect of 915 MHz microwave heating at different power levels on color of peanut butter

Color changes in peanut butter resulting from electron beam and gamma irradiation were reported. Ban and Kang (2014) reported that gamma irradiation in excess of 3 kGy decreased the L^* value of peanut butter. And El-Rawas et al. (2012) reported that during electron beam radiation color changes of peanut butter occurred. *L*, *a*, and *b* values of microwave-treated samples were not significantly different from those of non-treated samples (Table 2). *L* values decreased as

Table 1
Acid and peroxide values[a] of 915 MHz microwave heat-treated peanut butter.

Microwave power (kW)	Acid value (mg KOH/g)	Peroxide value (mEq/kg)
0 (non-treated)	1.04 ± 0.03 A	10.85 ± 0.76 A
2	1.08 ± 0.04 A	11.07 ± 0.56 A
4	1.09 ± 0.08 A	11.07 ± 0.94 A
6	1.12 ± 0.02 A	11.50 ± 0.78 A

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

Table 2
Hunter's color *L* (lightness), *a* (redness), and *b* (yellowness) values[a] of 915 MHz microwave heat-treated peanut butter.

Treatment	Parameter		
	L	*a*	*b*
Control	60.93 ± 1.37 A	5.92 ± 1.87 A	34.30 ± 2.09 A
2 kW	60.58 ± 1.23 A	6.23 ± 1.80 A	34.66 ± 2.03 A
4 kW	59.22 ± 1.83 A	6.70 ± 2.20 A	35.93 ± 2.61 A
6 kW	58.81 ± 1.45 A	7.15 ± 2.18 A	36.02 ± 2.44 A

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

microwave power increased from 0 (non-treated sample) to 6 kW, but these differences were not significant ($P > 0.05$). Ha et al. (2013) reported that there were no significant color changes during pasteurization of peanut butter crackers by RF treatment.

4. Conclusion

In conclusion, this study evaluated the feasibility of 915 MHz microwave heating as a pasteurization technology for peanut butter. In this research, 6 kW microwave heating for 5 min reduced *Salmonella* in peanut butter by 3.24–4.26 log CFU/g without affecting acid value, peroxide value, nor color of peanut butter. But 2 kW microwave heating did not reduce pathogens or increase temperature. Nine hundred fifteen MHz microwave heating at 6 kW could be utilized by the peanut butter industry. However, further investigations need to be performed to ascertain what effect various factors can have on microwave heating of peanut butter, such as frequency, a_w and product size.

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