Food Microbiology 34 (2013) 145–150

Contents lists available at SciVerse ScienceDirect

Food Microbiology

journal homepage: www.elsevier.com/locate/fm

Inactivation of *Salmonella enterica* serovar Typhimurium and *Escherichia coli* O157:H7 in peanut butter cracker sandwiches by radio-frequency heating

Jae-Won Ha, Sung-Youn Kim, Sang-Ryeol Ryu, Dong-Hyun Kang*

Department of Food and Animal Biotechnology, Department of Agricultural Biotechnology, Center for food and Bioconvergence, Seoul National University, Seoul 151-921, Republic of Korea

ARTICLE INFO

Article history:
Received 4 October 2011
Received in revised form
29 February 2012
Accepted 27 November 2012
Available online 12 December 2012

Keywords:
Radio-frequency heating
Peanut butter
Inactivation
Foodborne pathogen
Cracker

ABSTRACT

A multistate outbreak of *Salmonella enterica* serovar Typhimurium recently occurred in the USA, which was traced back to various food products made with contaminated peanut butter. This study was conducted to investigate the efficacy of radio-frequency (RF) heating to inactivate *S.* Typhimurium and *Escherichia coli* O157:H7 in peanut butter cracker sandwiches using creamy and chunky commercial peanut butter and to determine the effect on quality by measuring color changes and sensory evaluation. Samples were treated for a maximum time of 90 s in a 27.12 MHz RF heating system. Samples were prepared in the form of peanut butter cracker sandwiches and placed in the middle of two parallel-plate electrodes. After 90 s of RF treatment, the log reductions of *S.* Typhimurium and *E. coli* O157:H7 were 4.29 and 4.39 log CFU/g, respectively, in creamy peanut butter. RF treatment of chunky peanut butter for 90 s also significantly ($P < 0.05$) reduced levels of *S.* Typhimurium and *E. coli* O157:H7 by 4.55 log CFU/g and 5.32 log CFU/g. Color values and sensory characteristics of the RF treated peanut butter and crackers were not significantly ($P > 0.05$) different from the control. These results suggest that RF heating can be applied to control pathogens in peanut butter products without affecting quality.

1. Introduction

Salmonella serotypes are frequently detected in agricultural commodities grown in fields contaminated with animal manures (Islam et al., 2004). *Salmonella enterica* serovar Typhimurium is the most prevalent pathogen among *Salmonella* serotypes. The most common symptoms caused by *S.* Typhimurium are diarrhea, abdominal pain, mild fever, and chills (Baird-Parker, 1990; Rhee et al., 2003a). For young children, the elderly, and immunocompromised individuals, mortality rates may increase by up to 40% (Shimoni et al., 1999). Peanuts, an important agronomic crop and the source of peanut butter, can become contaminated with *Salmonella* strains during all production steps: growth, harvest, transportation and storage (Mattick et al., 2000). Contaminated peanut butter and products like peanut butter cracker sandwiches can lead to serious foodborne illnesses when they do not undergo adequate antimicrobial treatment (Burnett et al., 2000; Park et al., 2008; Dennis and Maki, 2009). In 2008, a multistate outbreak of *S.* Typhimurium infection occurred in the USA, which was traced back to food products made with contaminated peanut butter (CDC, 2012). By October 2012, a reported 714 persons in 44 states and Canada had become ill due to the outbreak (CDC, 2012).

Escherichia coli O157:H7 has not been frequently linked to foodborne illness outbreaks associated with low a_w food (He et al., 2011). However, the survival of *E. coli* O157:H7 is enhanced as a_w decreases (Ryu et al., 1999; Park and Beuchat, 2000) and this factor could be a potential risk in low a_w foods. *E. coli* O157:H7 has emerged as an important foodborne pathogen, causing hemorrhagic colitis which is occasionally complicated by hemolytic uremic syndrome (Doyle, 1991; Wang et al., 1996), and requires a very low dose (fewer than 700 organisms) to become infected (Tuttle et al., 1999).

For this reason, peanut butter usually undergoes pasteurization at temperatures of 70–75 °C before packaging, although raw peanuts have been subjected to a validated pasteurization process. However, Li et al. (2009) reported thermal treatments of peanut butter at 90 °C for less than 30 min are insufficient to kill large populations (5 log CFU/g) of *Salmonella* in highly contaminated peanut butter. Shachar and Yaron (2006) found that thermal treatments (usually less than 20 min) currently used to pasteurize peanut butter are not sufficient for the destruction of salmonellae. Several researchers have suggested that salmonellae have increased

* Corresponding author. Tel.: +82 2 880 4927; fax: +82 2 883 4928.
E-mail address: kang7820@snu.ac.kr (D.-H. Kang).

0740-0020/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.fm.2012.11.018

heat resistance in low-a_w foods or in foods with high lipid content (Juneja and Eblen, 2000, 2001; Mattick et al., 2000). Accordingly, there is a need for the development of alternative technologies as a secondary intervention to reduce pathogens, including *Salmonella*, in peanut butter products by at least 4–5 log in the event that hygienic conditions in the manufacturing facility are inadequate.

Radio-frequency (RF) heating involves the use of electromagnetic energy at frequencies between 1 and 300 MHz to generate heat in dielectric material. Dielectric heating can be more uniform than conventional heating because of the direct interaction between food materials and electromagnetic waves (Zhao, 2000). Conventional food heating methods require heat energy to be generated externally and then transferred to the food product by convection, conduction, or radiation (Doores, 2002). Thus, it takes considerable time for sufficient heat to reach the cold spot of a product in order for the particulates to attain a high enough temperature for proper sterilization, which consequently causes thermal damage by surface overheating. By contrast, RF generates heat rapidly within the product, due to the frictional interactions of polar dielectric molecules rotating and the space charge displacement in response to an externally applied AC electric field (Kinn, 1947; Zhao, 2000; Orsat et al., 2004). In addition, compared to microwave heating, RF heating offers the advantages of providing more uniform heating due to deep penetration and simple uniform field patterns (Marra et al., 2009). Therefore, RF heating could potentially replace conventional heating in food processing and has the potential as a novel thermal process to sterilize products that include peanut butter without affecting product quality.

This study was undertaken to evaluate the efficacy of RF heating to inactivate *E. coli* O157:H7 as well as *S.* Typhimurium in peanut butter cracker sandwiches used creamy and chunky commercial peanut butter, and to determine the effect of RF heating on the quality of peanut butter cracker sandwiches by measuring the color change and sensory evaluation.

2. Materials and methods

2.1. Cultures and cell suspension

Three strains each of *S.* Typhimurium (ATCC 19585, ATCC 43971, DT 104) and *E. coli* O157:H7 (ATCC 35150, ATCC 43889, ATCC 43890) were obtained from the bacterial culture collection of Seoul National University (Seoul, Korea). Each strain of *S.* Typhimurium and *E. coli* O157:H7 was cultured in 5 ml of tryptic soy broth (TSB; Difco, Becton Dickinson, Sparks, MD, USA) at 37 °C for 24 h, followed by centrifugation (4000 × *g* for 20 min at 4 °C), and washing three times with buffered peptone water (BPW; Difco). The final pellets were re-suspended in 9 ml of BPW, corresponding to approximately 10^7–10^8 CFU/ml. Subsequently, suspended pellets of each strain of the two pathogen species (six strains total) were combined to produce mixed culture cocktails. These culture cocktails consisting of a final concentration of approximately 10^8 CFU/ml were used in this study.

2.2. Sample preparation and inoculation

Experiments were performed using commercially processed creamy and chunky peanut butter. A peanut butter sample (60 g) was aseptically placed in sterile 250-ml glass beaker. For inoculation, 2.0 ml of culture cocktail was applied to the sample and gently but thoroughly mixed for 1 min with a sterile spoon to ensure even distribution of the pathogens. It was then dried for 1 h inside a biosafety hood (22 ± 2 °C) until the a_w of the sample equaled that of a non-inoculated sample (ca. 0.4). During the drying interval the sample was stirred gently at 10 min intervals to prevent pockets of high moisture. Commercially-produced saltine crackers were purchased at a local grocery store (Seoul, Korea) and were used to simulate commercially-prepared foods that contain peanut butter. Crackers (5.0 cm in diameter) were first sterilized for about 10 min under a germicidal UV lamp before 3 g of inoculated peanut butter was coated between two crackers for minimizing other interruptive bacterial factors. Three peanut butter cracker sandwiches (ca. 25 g) were placed parallel to each other in the middle of a sterile polypropylene bowl (11.0 cm in diameter and 4.5 cm deep) for each RF treatment.

2.3. RF heating system

A RF heating system with a maximum power of 9 kW at a frequency of 27.12 MHz was used in this study (Fig.1). This system was developed and constructed at Seoul National University (Seoul, Korea) and Dong Young Engineering Co., Ltd. (Gyeongnam, Korea). The RF treatment cavity consisted of two parallel-plate electrodes (30.0 × 35.0 cm; 0.6 cm thick) and the distance between the two electrodes was 11.0 cm. A sample in a polypropylene bowl was placed on the center of the bottom electrode. Treatments consisted of 10 s, 30 s, 50 s, 70 s, and 90 s RF exposure of both creamy and chunky peanut butter cracker sandwiches in order to maximize the efficacy of pasteurization while maintaining product quality.

2.4. Temperature measurement

A fiber optic temperature sensor (FOT-L, FISO Technologies Inc., Quebec, Canada) connected to a signal conditioner (TMI-4, FISO Technologies Inc., Quebec, Canada) was used to measure real-time temperatures in samples during RF heating. The sensor was directly inserted into the peanut butter of each three cracker sandwiches (top, middle, bottom) simultaneously and the temperature was manually recorded every 5 s. Since the fiber optic sensors were coated with electrical insulating material, they did not interfere with the temperature profile of the treated sample (Wang et al., 2003).

2.5. Bacterial enumeration

At selected time intervals, each of three treated peanut butter cracker sandwiches (ca. 25 g) were removed to room temperature for 30 s then immediately transferred into sterile stomacher bags

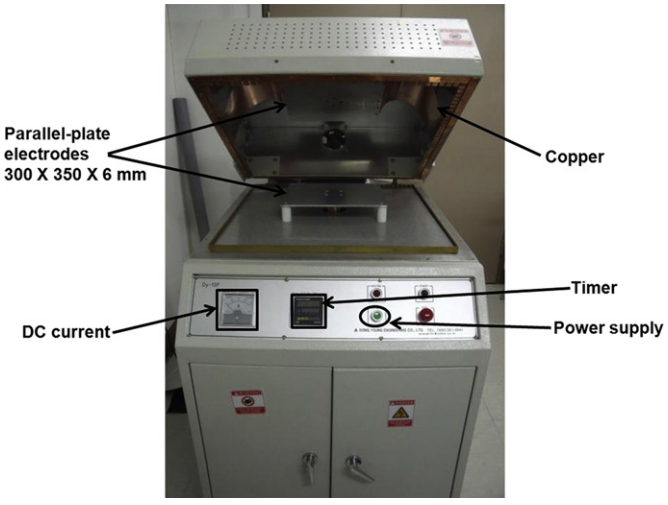

Fig. 1. Radio-frequency (27.12 MHz) heating system used in this study.

(Labplas Inc., Sainte-Julie, Quebec, Canada) containing 225 ml of BPW (detection limit, 10 CFU/g) and homogenized for 2 min with a stomacher (EASY MIX, AES Chemunex, Rennes, France). After homogenization, 1 ml aliquots of sample were serially diluted in 9 ml of BPW, and 0.1 ml of sample or diluent was spread-plated onto each selective medium. Xylose Lysine Desoxycholate agar (XLD, Difco) and Sorbitol MacConkey agar (SMAC, Difco) were used as selective media for the enumeration of *S.* Typhimurium and *E. coli* O157:H7, respectively. Where low levels of surviving cells were anticipated, 1 ml of undiluted stomacher bag contents was equally distributed onto four plates of each medium and spread-plated. All agar media were incubated at 37 °C for 24 h and colonies were counted.

2.6. Enumeration of heat-injured cells

Phenol red agar base with 1% sorbitol (SPRAB; Difco) was used to enumerate heat-injured cells of *E. coli* O157:H7 (Rhee et al., 2003b). After incubation at 37 °C for 24 h, typical white colonies characteristic of *E. coli* O157:H7 were enumerated. Randomly selected isolates from SPRAB plates were subjected to serological confirmation as *E. coli* O157:H7 (RIM, *E. coli* O157:H7 Latex Agglutination Test; Remel, Lenexa, Kan.), because SPRAB is not typically used as a selective agar for enumerating *E. coli* O157:H7. The overlay (OV) method was used to enumerate injured cells of *S.* Typhimurium (Lee and Kang, 2001). TSA was used as a nonselective medium to repair and resuscitate heat injured cells. One hundred microliters of appropriate dilutions were spread onto TSA medium and plates were incubated at 37 °C for 2 h to allow injured microorganisms to repair and resuscitate (Kang and Siragusa, 1999). Then the plates were overlaid with 7–8 ml of selective medium, XLD. After solidification, plates were further incubated for an additional 22 h at 37 °C. Following incubation, presumptive colonies of *S.* Typhimurium with typical black colonies were enumerated.

2.7. Color measurement and sensory evaluation

Color values of L*, a*, and b* were used to measure the color of peanut butter and surface of crackers. Samples were measured at random locations on peanut butter and cracker surfaces using a Minolta colorimeter (model CR300, Minolta Co., Osaka, Japan). L*, a*, and b* values indicate color lightness, redness, and yellowness of the sample, respectively.

Sensory evaluation was carried out to determine how specific attributes (flavor, texture and overall acceptability) varied over five RF-treated samples when compared to a non-treated control. In all sensory tests, the panelists consisted of 13 members from the Department of Food and Animal Biotechnology, and scores were obtained by rating the sensory attributes using the following 9-point hedonic scales: 9 = extremely good, 8 = very good, 7 = good, 6 = below good/above fair, 5 = fair, 4 = below fair/above poor, 3 = poor, 2 = very poor, 1 = extremely poor (Jeong et al., 2012). Samples, labeled with three-digit random numbers, were placed on white paper plates and served after being cooled at room temperature. Presentation order was randomized to avoid psychological error in judgment and the panelists were asked to use water to clean their palate between products.

2.8. Statistical analysis

Triplicate data was analyzed by analysis of variance using the ANOVA procedure with Duncan's multiple range test of SAS (SAS Institute, Cary, NC, USA). Value of $P < 0.05$ was used to indicate significant difference.

3. Results

3.1. Average temperature-time histories of peanut butter

The temperatures of creamy peanut butter during treatment with 27.12 MHz RF energy are shown in Fig. 2. Although the temperatures of all samples rapidly increased with increasing treatment time, the heating rates of the middle cracker sandwich and the bottom cracker sandwich were higher than that of the top cracker sandwich. After 90 s of RF heating, the creamy peanut butter of the middle and bottom sandwiches reached ca. 86 °C, and the top one reached 77 °C. In order to raise the creamy peanut butter temperature from room temperature to 60 °C, RF heating for a maximum of 66 s in the top cracker sandwich was required. Fig. 3 shows temperatures of chunky peanut butter during RF treatment. The pattern of temperature increase of the top, middle and bottom cracker sandwiches did not differ from those of creamy peanut butter. The maximum heating time for the top cracker sandwich to reach 60 °C from room temperature was 65 s and the temperature reached after heating for 90 s was a minimum of 78 °C in the top cracker sandwich.

3.2. Inactivation of pathogenic bacteria by RF heating

The survival of *S.* Typhimurium and *E. coli* O157:H7 in creamy peanut butter during 90 s of RF heating are shown in Table 1. The populations of both pathogens decreased with increasing treatment time. Significant ($P > 0.05$) reductions were not observed for 50 s compared to the control. However, treatment for 70 s significantly ($P < 0.05$) reduced levels of *S.* Typhimurium and *E. coli* O157:H7 by 2.77 and 2.93 log CFU/g in creamy peanut butter, respectively. After 90 s of RF treatment, the reductions of *S.* Typhimurium and *E. coli* O157:H7 were 4.29 and 4.39 log CFU/g in creamy peanut butter, respectively. Table 2 shows populations of *S.* Typhimurium and *E. coli* O157:H7 in chunky peanut butter during 90 s of RF heating. There were no significant ($P > 0.05$) reductions in microbial levels until 30 s of treatment had occurred. However, significant reductions of *S.* Typhimurium and *E. coli* O157:H7 were observed after 50 s of treatment. Treatment for 90 s significantly ($P < 0.05$) reduced levels of *S.* Typhimurium by 4.55 log CFU/g and *E. coli* O157:H7 by 5.32 log CFU/g.

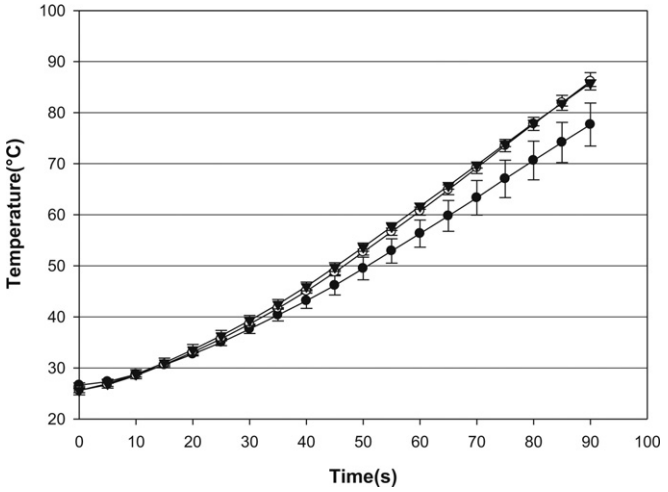

Fig. 2. Time-temperature profile for creamy peanut butter thermally processed by 27.12 MHz RF energy. The error bars indicate standard deviations calculated from triplicates. •, top cracker sandwich; ○, middle cracker sandwich; ▼, bottom cracker sandwich.

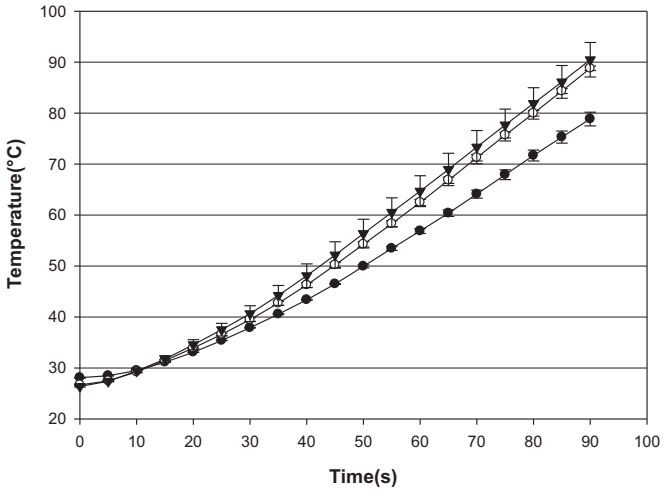

Fig. 3. Time-temperature profile for chunky peanut butter thermally processed by 27.12 MHz RF energy. The error bars indicate standard deviations calculated from triplicates. •, top cracker sandwich; ○, middle cracker sandwich; ▼, bottom cracker sandwich.

3.3. The effect of RF heating on product quality

Color values of the peanut butter and cracker surfaces after RF treatment are summarized in Tables 3 and 4. L*, a*, and b* values of RF treated creamy peanut butter were measured at a random location on the bottom cracker sandwich which had the highest temperature growth pattern and statistically significant ($P > 0.05$) differences were not detected during the entire heating interval (Table 3). There were also no significant differences of color change on chunky peanut butter (data not shown). L*, a*, and b* values of RF treated (90 s) cracker surfaces were not significantly ($P > 0.05$) different to those of non-treated samples (Table 4). As shown in Table 5, there were no significant ($P > 0.05$) differences among the six samples scored by the hedonic scale for flavor, texture and overall acceptability, indicating that RF treatment for 90 s does not affect the quality of peanut butter cracker sandwiches.

4. Discussion

Snack products made from peanut butter, which comprise a large proportion of low-a_w foods on the market, are a regular part of the modern diet of young children. More frequent outbreaks could occur due to increased consumption of these food types unless appropriate control steps are taken. A past international outbreak (Killalea et al., 1996) caused by *Salmonella* strains in peanut butter coated snacks (in that instance peanut butter was heat-treated to 75 °C before being used for coating) led us to examine the lethal effect of RF treatment on pathogens in peanut butter of snacks. Furthermore, the type of cracker sandwich used in this study as test subjects experienced uniform RF heating due to uniformity of the peanut butter spread between the crackers.

Strains of *Salmonella* have increased heat resistance and are able to survive at high temperatures within a high fat and low water activity environment (Juneja et al., 2001). Peanut butter is a highly concentrated colloidal suspension of lipid and water in a peanut meal phase and provides such an environment as this for *Salmonella*. Mattick et al. (2001) reported that the combination of both high fat and low water activity in foods such as peanut butter or chocolate might have a synergistic effect which could protect the bacterial cells at high temperatures. The two type of products used in this study contained about 40% fats, 6% sodium, and had a a_w of approximately 0.4 as measured with the AquaLab model 4TE water activity meter (Decagon Devices, Pullman, WA, USA), and there was no significant difference between the a_w of creamy and chunky peanut butter.

Shachar and Yaron (2006) reported that *S.* Typhimurium was rapidly killed at 70 °C in saline buffer, and a greater than 7-log reduction was observed within less than 5 min in a heated water bath. However, in a matrix of peanut butter only a 1.4-log reduction was observed at 70 °C, a 2.2-log reduction was observed at 80 °C, and a 2.5-log reduction was observed at 90 °C after 5 min in a heated water bath. Two sequential treatments also were not effective for achieving a 7-log reduction. Based on their calculated parameters of the Weibull model, more than 260 min are needed to reduce *Salmonella* by 7 log units at 70 °C, and more than 60 min is required at 90 °C when using a conventional hot water immersion

Table 1
Populations[a] of *Salmonella* Typhimurium and *Escherichia coli* O157:H7 in creamy peanut butter following RF treatment.

Creamy type	Population (log_{10} CFU/g)			
Treatment time (s)	*S.* Typhimurium		*E. coli* O157:H7	
	XLD[b]	OV-XLD	SMAC	SPRAB
0	6.16 ± 0.20 Aa	6.34 ± 0.27 Aa	6.43 ± 0.46 Aa	6.44 ± 0.27 Aa
10	6.14 ± 0.10 Aa	6.14 ± 0.09 ABa	6.22 ± 0.33 Aa	6.38 ± 0.35 Aa
30	6.07 ± 0.18 Aa	6.21 ± 0.15 ABa	6.38 ± 0.24 Aa	6.40 ± 0.30 Aa
50	5.12 ± 0.60 Aa	5.38 ± 0.70 Ba	5.60 ± 0.60 Aa	5.84 ± 0.50 Aa
70	3.39 ± 0.57 Ba	3.68 ± 0.46 Ca	3.50 ± 0.84 Ba	4.22 ± 0.55 Ba
90	1.87 ± 1.04 Ca	2.38 ± 0.64 Da	2.04 ± 0.91 Ca	2.01 ± 1.14 Ca

[a] Mean of three replications ± standard deviation. Means with the same capital letter in the same column are not significantly different ($P > 0.05$). Means with the same lowercase letter in the same row are not significantly different ($P > 0.05$).
[b] XLD, Xylose Lysine Desoxycholate; OV XLD, overlay XLD agar on TSA; SMAC, Sorbitol MacConkey agar; SPRAB, Phenol red agar base with 1% sorbitol.

Table 2
Populations[a] of *Salmonella* Typhimurium and *Escherichia coli* O157:H7 in chunky peanut butter following RF treatment.

Chunk type	Population (log_{10} CFU/g)			
Treatment time (s)	*S.* Typhimurium		*E. coli* O157:H7	
	XLD[b]	OV-XLD	SMAC	SPRAB
0	6.34 ± 0.29 Aa	6.56 ± 0.20 Aa	6.45 ± 0.25 Aa	6.51 ± 0.11 Aa
10	6.34 ± 0.30 Aa	6.67 ± 0.22 Aa	6.46 ± 0.27 Aa	6.46 ± 0.12 Aa
30	5.94 ± 0.09 Aa	6.33 ± 0.35 Aa	5.90 ± 0.29 Aa	6.16 ± 0.28 Aa
50	4.36 ± 0.25 Ba	5.31 ± 0.22 Bb	4.57 ± 0.19 Ba	5.19 ± 0.18 Bb
70	2.69 ± 0.26 Ca	3.28 ± 0.55 Ca	2.61 ± 0.27 Ca	3.44 ± 0.46 Ca
90	1.79 ± 0.25 Da	1.97 ± 0.35 Da	1.13 ± 0.75 Da	1.87 ± 0.50 Da

[a] Mean of three replications ± standard deviation. Means with the same capital letter in the same column are not significantly different ($P > 0.05$). Means with the same lowercase letter in the same row are not significantly different ($P > 0.05$).
[b] XLD, Xylose Lysine Desoxycholate; OV XLD, overlay XLD agar on TSA; SMAC, Sorbitol MacConkey agar; SPRAB, Phenol red agar base with 1% sorbitol.

Table 3
Color values[a] of RF treated peanut butter.

Treatment time (s)	Parameter[b]		
	L*	a*	b*
0	56.66 ± 0.38 a	10.41 ± 0.70 a	32.51 ± 0.94 a
10	56.80 ± 0.32 a	10.72 ± 0.48 a	33.17 ± 0.58 a
30	56.97 ± 0.94 a	10.85 ± 0.39 a	33.92 ± 0.69 a
50	56.10 ± 0.36 a	10.60 ± 0.44 a	33.55 ± 0.47 a
70	56.19 ± 0.53 a	10.78 ± 0.27 a	33.70 ± 0.33 a
90	56.23 ± 0.34 a	10.35 ± 0.27 a	33.59 ± 0.17 a

[a] Mean of three replications ± standard deviation. Values followed by the same letters within the column per parameter are not significantly different ($P > 0.05$).
[b] Color parameters are L* (lightness), a* (redness), and b* (yellowness).

Table 4
Color values[a] of cracker surfaces treated with RF.

Parameter[b]	Treatment time (s)	Cracker surface
L*	0	62.91 ± 1.08 a
	90	62.39 ± 1.56 a
a*	0	6.72 ± 0.75 a
	90	6.10 ± 0.36 a
b*	0	27.75 ± 0.83 a
	90	28.02 ± 0.34 a

[a] Mean of three replications ± standard deviation. Values followed by the same letters within the column per parameter are not significantly different ($P > 0.05$).
[b] Color parameters are L* (lightness), a* (redness), and b* (yellowness).

method. However, these treatment times are not practical for use by industry and could adversely affect the organoleptic qualities of the products by increasing denaturation or browning. Li et al. (2009) had also reported that a hot water immersion of 71–77 °C for 20 min was not sufficient to inactivate *Salmonella* Tennessee in peanut butter. About a 3 log reduction was observed in peanut butter exposed to 90 °C for 20 min. Therefore, conventional thermal treatments commonly used by industry are not adequate to inactivate *Salmonella* in peanut butter. In the present study, the average bacterial concentrations of *S.* Typhimurium and *E. coli* O157:H7 inoculated into one batch of sample was 6–7 log CFU/g. These inoculation levels were much higher than would normally be encountered in peanut butter since such high levels of pathogens would not occur as post-processing contamination unless GMPs and sanitation were extremely poor. A high inoculum load was used to make enumeration of surviving bacteria easier.

Following treatment with RF, sub-lethally injured *S.* Typhimurium and *E. coli* O157:H7 could assume added significance because they are potentially as dangerous as their uninjured counterparts (McCleer and Rowe, 1995; Lee and Kang, 2001). Tables 1 and 2 show surviving cells or cells including heat-injured *S.* Typhimurium and *E. coli* O157:H7 from creamy and chunky peanut butter treated with RF, respectively. When inoculated creamy peanut butter was treated with RF (Table 1), levels of *S.* Typhimurium were reduced by 1.04, 2.77 and 4.29 log units after 50 s, 70 s and 90 s treatments, respectively. At the same time intervals, 0.26, 0.29 and 0.51 log units of heat-injured cells were detected. In the case of *E. coli* O157:H7, 0.83–2.93 log reductions and 0.24–0.72 log CFU/g of heat-injured cells were observed after 50–70 s treatment, respectively. However, no significant ($P > 0.05$) differences in levels of cells enumerated on XLD and OV-XLD, SMAC and SPRAB were observed during whole treatment time in creamy peanut butter (Table 1). This suggests that RF heating effectively inactivated *S.* Typhimurium and *E. coli* O157:H7 in peanut butter without causing apparent injury to bacterial cells. Statistical differences between levels of surviving cells and cells including those of sub-lethally injured pathogens in chunky peanut butter were similar to those of creamy peanut butter. Only one significant difference was observed at 50 s treatment (Table 2). We postulate that this difference was due to the large temperature range of 48 °C–57 °C among three chunky peanut butter cracker sandwiches (top, middle, bottom) in the 50 s treatment. On the other hand, the temperature range of creamy peanut butter cracker sandwiches was somewhat smaller (46 °C–53 °C). Significant log reductions of pathogens greatly increased at 55–70 s of treatment for each peanut butter. The temperature at this time range was about 60 °C. Accordingly, more sub-lethally injured cells were produced in chunky peanut butter as it approached to 60 °C than in creamy peanut butter.

Table 5
Sensory attributes of peanut butter cracker sandwiches following RF treatment (at day zero).

Treatment time(s)	Sensory attributes[a]		
	Flavor	Texture	Overall
0	6.6 ± 1.43 a	7.0 ± 1.15 a	6.5 ± 1.58 a
10	6.0 ± 1.63 a	5.7 ± 1.64 a	5.6 ± 1.35 a
30	6.0 ± 1.70 a	6.6 ± 1.26 a	6.0 ± 1.25 a
50	6.5 ± 1.58 a	6.5 ± 1.27 a	6.0 ± 1.33 a
70	6.6 ± 1.71 a	6.1 ± 1.73 a	6.6 ± 1.43 a
90	5.9 ± 1.45 a	6.0 ± 1.25 a	6.0 ± 1.41 a

Means with the same letters within the column for each attribute did not differ significantly ($P > 0.05$).
[a] Results from panelist scorecard analysis, 9 point hedonic scale, 9 = extremely good, $n = 13$.

This study clearly shows that RF heating is very effective for reducing levels of *S.* Typhimurium and *E. coli* O157:H7 in peanut butter cracker sandwiches regardless of the peanut butter type used. Furthermore, RF heating offers a considerable speed advantage over conventional heating as an alternative control technique for a final process prior to packaging by industry. However, the heating rate of samples during RF treatment is known to be affected by the chemical composition (i.e., fat and salt content), its temperature, moisture content, structure of the material, density, and a few other factors (Piyasena and Dussault, 2003; Orsat et al., 2004). In addition, positioning between the two electrodes influenced the heating rate of cracker sandwiches in this study. The bottom and middle cracker sandwiches, being closer to the electrode, were more affected by electromagnetic waves generated than was the top cracker sandwiches; this was true for both two types of peanut butter. Therefore, before commercial application of this method, further research will needed to fully understand the influence of various factors on the heating rate in peanut butter to maximize the effectiveness of RF heating.

Acknowledgments

This research was supported by the WCU (World Class University) program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (R32-2008-000-10183-0). This research was also supported by R&D Convergence Center Support Program, Ministry for Food, Agriculture, Forestry and Fisheries, Republic of Korea.

References

Baird-Parker, A.C., 1990. Foodborne salmonellosis. Lancet 336, 1231–1235.
Burnett, S.L., Gehm, E.R., Weissinger, W.R., Beuchat, L.R., 2000. Survival of Salmonella in peanut butter and peanut butter spread. J. Appl. Microbiol. 89, 472–477.
CDC, 2012. Investigation Update: Multistate Outbreak of Human *Salmonella* Typhimurium Infections. Available at: http://www.cdc.gov/salmonella/typhimurium/index.html (last access on October 2012).
Dennis, G., Maki, M.D., 2009. Coming to grips with foodborne infection – peanut butter, peppers, and nationwide *Salmonella* outbreaks. N. Engl. J. Med. 10, 1–5.
Doores, S., 2002. Microwave inactivation of pathogens. In: Juneja, V.K., Sofos, J.N. (Eds.), Control of Foodborne Microorganisms. Marcel Dekker, New York, pp. 105–164.
Doyle, M.P., 1991. Escherichia coli O157:H7 and its significance in foods. Int. J. Food Microbiol. 12, 289–302.
He, Y.S., Guo, D.J., Yang, J.Y., Tortorello, M.L., Zhang, W., 2011. Survival and heat resistance of *Salmonella enterica* and *Escherichia coli* O157:H7 in peanut butter. Appl. Environ. Microbiol. 77, 8434–8438.
Islam, M., Morgan, J., Doyle, M.P., Phatak, S.C., Millner, P., Jiang, X., 2004. Fate of *Salmonella enterica* serovar Typhimurium on carrots and radishes grown in fields treated with contaminated manure composts or irrigation water. Appl. Environ. Microbiol. 70, 2497–2502.
Jeong, S.H., Bradley, P.M., Elliot, T.R., Janice, B.H., 2012. The effect of X-ray irradiation on *Salmonella* inactivation and sensory quality of almonds and walnuts as a function of water activity. Int. J. Food Microbiol. 153, 365–371.
Juneja, V.K., Eblen, B.S., 2000. Heat inactivation of *Salmonella* Typhimurium DT104 in beef as affected by fat content. Lett. Appl. Microbiol. 30, 461–467.
Juneja, V.K., Eblen, B.S., Marks, H.M., 2001. Modeling nonlinear survival curves to calculate thermal inactivation of *Salmonella* in poultry of different fat levels. Int. J. Food Microbiol. 70, 37–51.

Kang, D.H., Siragusa, G.R., 1999. Agar underlay method for recovery of sublethally heat-injured bacteria. Appl. Environ. Microbiol. 65, 5334–5337.
Killalea, D., Ward, L.R., Roberts, D., de Louvois, J., Sufi, F., Stuart, J.M., Wall, P.G., Susman, M., Schwieger, M., Sanderson, P.J., Fisher, I.S., Mead, P.S., Gill, O.N., Bartlett, C.L., Rowe, B., 1996. International epidemiological and microbiological study of outbreak of *Salmonella agona* infection from a ready to eat savoury snack – I: England and Wales and the United States. Br. Med. J. 313, 1105–1107.
Kinn, T.P., 1947. Basic theory and limitations of high frequency heating equipment. Food Technol. 1, 161–173.
Lee, S.Y., Kang, D.H., 2001. Suitability of overlay method for recovery of heat-injured *Listeria monocytogenes* and *Salmonella* Typhimurium. Food Sci. Biotechnol. 10, 323–326.
Li, M., zhang, G., Peter, G., Vijaya, M., Ifeoma, E., Doyle, M.P., 2009. Thermal inactivation of *Salmonella* in peanut butter. J. Food Prot. 8, 1596–1601.
Marra, F., Zhang, L., Lyng, J.G., 2009. Radio frequency treatment of foods: review of recent advances. J. Food Eng. 91, 497–508.
Mattick, K.L., Jorgensen, F., Legan, J.D., Lappin-Scott, H.M., Humphrey, T.J., 2000. Habituation of *Salmonella* spp. at reduced water activity and its effect on heat tolerance. Appl. Environ. Microbiol. 66, 4921–4925.
Mattick, K.L., Jorgensen, F., Wang, P., Pound, J., Vandeven, M.H., Ward, L.R., Legan, J.D., Lappin-Scott, H.M., Humphrey, T.J., 2001. Effect of challenge temperature and solute type on heat tolerance of *Salmonella* serovars at low water activity. Appl. Environ. Microbiol. 67, 4128–4136.
McCleer, D.R., Rowe, M.T., 1995. Development of a selective plating technique for the recovery of *Escherichia coli* O157:H7 after heat stress. Lett. Appl. Microbiol. 21, 252–256.
Orsat, V., Bai, L., Raghavan, G.S.V., 2004. Radio-frequency heating of ham to enhance shelf-life in vacuum packaging. J. Food Eng. 27, 267–283.
Park, C.M., Beuchat, L.R., 2000. Survival of Escherichia coli O157:H7 in potato starch as affected by water activity, pH and temperature. Lett. Appl. Microbiol. 31, 364–367.
Park, E.J., Oh, S.W., Kang, D.H., 2008. Fate of Salmonella Tennessee in peanut butter at 4 and 22°C. Food Microbiol. Saf. 73, 82–86.
Piyasena, P., Dussault, C., 2003. Continuous radio-frequency heating of a model viscous solution: influence of active current, flow rate, and salt content on temperature rise. Can. Biosyst. Eng. 45, 327–334.
Rhee, M.S., Lee, S.Y., Dougherty, R.H., Kang, D.H., 2003a. Antimicrobial effects of mustard flour and acetic acid against *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella enterica* serovar Typhimurium. Appl. Environ. Microbiol. 69, 2959–2963.
Rhee, M.S., Lee, S.Y., Hillers, V.N., McCurdy, S.M., Kang, D.H., 2003b. Evaluation of consumer-style cooking methods for reduction of *Escherichia coli* O157:H7 in ground beef. J. Food Prot. 66, 1030–1034.
Ryu, J.H., Deng, Y., Beuchat, L.R., 1999. Survival of *Escherichia coli* O157:H7 in dried beef powder as affected by water activity, sodium chloride content and temperature. Food Microbiol. 16, 309–316.
Shachar, D., Yaron, S., 2006. Heat tolerance of *Salmonella enterica* serovars Agona, Enteritidis, and Typhimurium in peanut butter. J. Food Prot. 11, 2687–2691.
Shimoni, Z., Pitlik, S., Leibovici, L., Samra, Z., Konigsberger, H., Drucker, M., Agmon, V., Ashkenazi, S., Weinberger, M., 1999. Nontyphoid *Salmonella* bacteremia: age-related differences in clinical presentation, bacteriology, and outcome. Clin. Infect. Dis. 28, 822–827.
Tuttle, J., Gomez, T., Doyle, M.P., Wells, J.G., Zhao, T., Tauxe, R.V., Griffin, P.M., 1999. Lessons from a large outbreak of *Escherichia coli* O157:H7 infections: insights into the infectious dose and method of widespread contamination of hamburger patties. Epidemiol. Infect. 122, 185–192.
Wang, G., Zhao, T., Doyle, M.P., 1996. Fate of enterohemorrhagic *Escherichia coli* O157: H7 in bovine feces. Appl. Environ. Microbiol. 62, 2567–2570.
Wang, Y., Wig, T.D., Tang, J., Hallberg, L.M., 2003. Sterilization of foodstuffs using radio frequency heating. J. Food Sci. 68, 539–543.
Zhao, Y., 2000. Using capacitive (radio frequency) dielectric heating in food processing and preservation – a review. J. Food Proc. Eng. 23, 25–55.