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Evaluation of radio-frequency heating in controlling *Salmonella enterica* in raw shelled almonds

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ABSTRACT

This study was conducted to investigate the efficacy of radio-frequency (RF) heating to reduce *Salmonella enterica* serovars Enteritidis, Typhimurium, and Senftenberg in raw shelled almonds compared to conventional convective heating, and the effect of RF heating on quality by measuring changes in the color and degree of lipid oxidation. Agar-grown cells of three pathogens were inoculated onto the surface or inside of raw shelled almonds using surface inoculation or the vacuum perfusion method, respectively, and subjected to RF or conventional heating. RF heating for 40 s achieved 3.7-, 6.0-, and 5.6-log reductions in surface-inoculated *S.* Enteritidis, *S.* Typhimurium, and *S.* Senftenberg, respectively, whereas the reduction of these pathogens following convective heating for 600 s was 1.7, 2.5, and 3.7 log, respectively. RF heating reduced internally inoculated pathogens to below the detection limit (0.7 log CFU/g) after 30 s. However, conventional convective heating did not attain comparable reductions even at the end of treatment (600 s). Color values, peroxide values, and acid values of RF-treated (40-s treatment) almonds were not significantly ($P > 0.05$) different from those of nontreated samples. These results suggest that RF heating can be applied to control internalized pathogens as well as surface-adhering pathogens in raw almonds without affecting product quality.

1. Introduction

Consumption of nut products has continually increased from 2000 to 2011, as consumers have taken greater interest in health and nutrition (Statista, 2014). Almonds are among the most popular nuts, accounting for 28% of the nut market in 2011 (Almond Board of California, 2012). However, outbreaks of salmonellosis have been associated with the consumption of raw almonds in the United States, Canada, and Sweden in 2001, 2004, and 2006, respectively (Centers for Disease Control and Prevention, 2004; Ledet et al., 2007). *Salmonella enterica* serovar Enteritidis phage type 30 (PT 30) was identified as the outbreak strain. No outbreaks involving serovars Typhimurium and Senftenberg on almonds have been reported; however, other nut-associated outbreaks were caused by these foodborne pathogens. Of the 5 nut-associated outbreaks reported to the Centers for Disease Control and Prevention between 1998 and 2008, 20% were caused by *S.* Typhimurium (Jackson et al., 2013). In March 2009, a multistate outbreak of *S.* Senftenberg infections associated with pistachios occurred in the United States (Centers for Disease Control and Preservation, 2009).

As of 2007, in response to these outbreaks, the U.S. Department of Agriculture has mandated that almonds be processed to achieve a minimum 4-log reduction of *Salmonella* using a validated process prior to export. To reduce the microbial load of almonds, several processes including propylene oxide (PPO) fumigation, oil roasting, dry roasting, blanching, and steam heating have been approved by the U.S. Food and Drug Administration (FDA) (U.S. Department of Agriculture, 2007). However, as PPO treatment is controversial due to residues remaining on almonds, the European Union and many other countries have banned its use (Cornucopia Institute, 2007). In addition, with conventional thermal treatments, externally generated heat is only slowly transferred to bulk almonds due to low thermal conductivities, necessitating lengthy treatments, which may result in thermal damage to almonds (Doores, 2002). Several alternative methods, such as high pressure, irradiation, and infrared heating, have been suggested; however, constraints of high installation cost, poor consumer acceptance, and difficulty of scaling up for large volume commercial applications still limit widespread use of these treatments (Goodridge et al., 2006; Prakash et al., 2010; Yang et al., 2010).

For the above reasons, it is necessary to develop new pasteurization

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technologies for almonds. One of these alternatives is radio-frequency (RF) heating which involves the use of electromagnetic energy at frequencies between 1 and 300 MHz. Among these, only selected frequencies (13.56, 27.12, and 40.68 MHz) are permitted for domestic, industrial, scientific and medical applications so as not to interfere with communication systems (Piyasena et al., 2003). RF generates heat rapidly within food materials due to molecular friction and space charge displacement in response to an externally applied alternating electric field. This technology can deliver thermal energy quickly to every part of the bulk food product in which pathogens may reside (Kinn, 1947; Zhao et al., 2000). Thus, RF heating could potentially replace conventional heating for solid and semi-solid foods which have low thermal conductivities. In our previous study, the effectiveness of RF thermal processing for pasteurization of powdered foods was investigated (Jeong and Kang, 2014).

Recently there have been some research efforts to apply RF heating as a new thermal intervention for treatment of almonds (Gao et al., 2010; Gao et al., 2011). However, these studies were limited to disinfestation and product quality following RF treatment, and did not assess microbial inactivation. Furthermore, a comparison between the pasteurization efficacy of RF heating and approved methods including conventional heating has not been reported. Accordingly, microbial inactivation rates between RF heating and conventional heating need to be evaluated in order to be approved for industrial use by the FDA. Also, because of the possibility of pathogen internalization within almonds during growing and distribution (Danyluk et al., 2008), more studies on the inactivation of internalized foodborne pathogens in almonds are required.

In this study, the efficacy of RF treatment and conventional convective heating, especially dry roasting which is somewhat comparable to RF heating in treatment condition were comparable for reducing populations of *S*. Enteritidis, *S*. Typhimurium, and *S*. Senftenberg in raw shelled almonds. Also, the effects of RF heating for controlling internalized pathogens and quality of almonds, including color, peroxide value, and acid value, were investigated through additional experiments utilizing the same treatments.

2. Materials and methods

2.1. Bacterial strains

All bacterial strains, namely, *S*. Enteritidis PT 30 (ATCC BAA-1045), *S*. Typhimurium (ATCC 700408), and *S*. Senftenberg (KVCC 0590) were obtained from the Department of Food and Animal Biotechnology bacterial culture collection of Seoul National University (Seoul, South Korea). These strains were isolated from human or animal. Stock cultures were kept frozen at 80 °C in 0.7 ml of tryptic soy broth (TSB; Difco, Becton, Dickinson, Sparks, MD) containing 0.3 ml of 50% glycerol. For this study, working cultures were prepared by streaking for isolation onto tryptic soy agar (TSA; Difco), incubating at 37 °C for 24 h, and storing at 4 °C.

2.2. Preparation of pathogen inocula

For each experiment, inoculum was prepared individually for each strain using the method described by Danyluk et al. (2005). A loopful (ca. 10 μl) from a single isolated colony of each strain of *S*. Enteritidis, *S*. Typhimurium, and *S*. Senftenberg was cultured in 30 ml of TSB at 37 °C for 24 h, then a loopful was transferred into 30 ml of TSB, and incubated at 37 °C for 18 h. For production of a bacterial lawn, 1 ml of the overnight culture was spread onto each of three TSA plates and followed by incubation at 37 °C for 24 h. The bacterial lawn was dislodged by adding 9 ml of 0.2% peptone water (PW; Difco) to each plate and rubbing with a sterile cotton swab. Cell suspensions were collected from the three plates and pooled, corresponding to approximately 10^9 CFU/ml. These final suspensions of the three pathogenic serovars were used in this study.

2.3. Sample preparation and inoculation

Raw shelled almonds of the variety Nonpareil were purchased from a local grocery store (Seoul, South Korea) and sorted to remove any damaged kernels before being used for experiments. For surface inoculation, 25 ml of prepared inoculum (*S*. Enteritidis, *S*. Typhimurium, and *S*. Senftenberg) was added to 400-g samples inside sterile stomacher bags (Labplas, Inc., Sainte-Julie, Quebec, Canada), and then mixed by hand for 1 min. The inoculated samples were dried for 24 h inside a biosafety hood (24 ± 2 °C) with the fan running until the moisture content and water activity of the samples equaled those of a noninoculated samples (ca. 4.20%, dry basis and 0.42, respectively). The final cell concentration was 10^6 to 10^7 CFU/g.

Internal inoculation with foodborne pathogens was performed according to the procedure reported by Niemira (2007) using a vacuum perfusion method. Twenty-five milliliters of the inoculum was diluted in 500 ml of sterile distilled water. Almonds samples sufficient for one replication were immersed in the combined pathogen suspension and placed in a vacuum oven (OV-11; Jeio tech. Co., Ltd., Daejeon, South Korea). A vacuum was drawn to about 96 kPa in order to pull gas from the intercellular spaces of the almonds. After 4 min, the vacuum was quickly broken, thereby drawing the inoculum into the sample. The vacuum perfusion was repeated three times to fully perfuse the almonds. For the removal of pathogens on exposed surfaces, the fully perfused almonds were agitated in 500 ml of 300 ppm sodium hypochlorite solution for 3 min, washed with sterile distilled water for 3 min. The internally-inoculated almonds were dried for 24 h in order to ensure the properties of the samples were close to the original almonds, resulting in an internalized cell concentration of 10^5 to 10^6 CFU/g. This method was chosen for almonds by comparing dye (Red No. 40 AR6223; Emerald Performance Materials, Cincinnati, OH) internalizations after dipping for 20 min, syringe injection, and the vacuum perfusion method.

2.4. Experimental apparatus

The RF heating and conventional convective heating system (Fig. 2) consisted of a RF heater (Seoul National University, Seoul, South Korea; Dong Young Engineering Co. Ltd., Gyeongnam, South Korea), a convection oven (CK9230; Convex Korea Co. Ltd., Gyeonggi, South Korea), and a temperature signal conditioner (TMI-4; FISO Technologies Inc., Quebec, Canada). The RF electric field with a frequency of 27.12 MHz was generated between two parallel-plate electrodes (30.0 × 35.0 cm; 0.6 cm thick) spaced 5.5 cm apart. The convection oven was composed of four electric resistive emitters arranged horizontally in parallel with the four emitting surfaces facing each other. The heated air in the roasting oven was circulated by fan. The temperature signal conditioner was connected to a computer for control using FISO Commander 2 Control and Analysis Software (FISO Technologies Inc.).

2.5. RF heating and conventional convective heating treatment

For the RF heating treatment, 25 g of inoculated almonds were placed in a polypropylene jar, 4.5 cm in diameter and 4.0 cm deep (NALGENE 2118-0002; Thermo Scientific, Hudson, NH), which was placed on the center of the bottom electrode. For conventional convective heating, almond samples were spread in a single layer on a sterilized stainless rack located 10 cm from the upper and the lower emitters. A roasting temperature of 150 °C, as conventionally used in industry, was selected (Almond Board of California, 2007). RF and convective heating were applied to each prepared sample and heated to about 100 °C and 150 °C, respectively, in order to maximize the efficacy of pasteurization while maintaining product quality.

2.6. Temperature measurement

A fiber optic temperature sensor (FOT-L; FISO Technologies Inc., Quebec, Canada) connected to a temperature signal conditioner was used to measure real-time temperatures in samples during RF and convective heating. The sensor was placed directly on the surface of the non-inoculated almonds or inserted into the center of the kernel located in middle, and the temperature was recorded at 5 s intervals. In the case of convective heating, the temperature was recorded at 10 s intervals after 40 s of treatment. The fiber optic did not interfere with the temperature profile of the treated sample, since it was coated with electric insulating material (Wang et al., 2003). The temperature values of RF and convective heated samples were compared to determine the heating rate.

2.7. Bacterial enumeration

For enumeration of pathogens, 25 g of treated almonds were placed at room temperature for 30 s, transferred immediately into sterile stomacher bags containing 100 ml of 0.2% PW pre-chilled in a 4 °C refrigerator (detection limit, 0.7 log 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 blanks of 0.2% PW, and 0.1 ml of sample or diluent was spread-plated onto a selective medium, xylose lysine desoxycholate agar (XLD; Oxoid, Ogdensburg, NY), for enumeration of *S. enterica*. Where low levels of surviving cells were anticipated, 1 ml of undiluted homogenate was equally divided onto four plates of medium and spread-plated. All agar plates were incubated at 37 °C for 24 h, and typical black colonies were counted. To confirm identity of the pathogens, colonies randomly selected from the enumeration plates were subjected to the *Salmonella* latex agglutination assay (Oxoid).

2.8. Enumeration of heat-injured cells

The overlay (OV) method was used to enumerate heat-injured cells of *S. enterica* using TSA as a nonselective medium for recovery of injured cells (Lee and Kang, 2001). Appropriate dilutions were spread-plated onto TSA medium and incubated at 37 °C for 2 h to allow heat-injured microorganisms to repair, and then 7 to 8 ml of XLD selective medium was overlaid on the plates. After solidification, plates were further incubated for an additional 22 h at 37 °C and black colonies were enumerated. Preliminary experiments verified that the 2-h incubation recovery period on TSA did not result in multiplication of uninjured cells in the control samples (data not shown).

2.9. Quality measurement

To evaluate the effect of RF heating on quality during storage, changes in color, peroxide value, and acid value were measured. The accelerated shelf life tests were conducted in a temperature and humidity chamber (TH-TG-300; Jeio tech. Co., Ltd.) at 35 °C and 30% relative humidity for 10 and 20 days. Storage conditions of 1 and 2 years at 4 °C were chosen based on commercial practices using a Q_{10} value of 3.4 for lipid oxidation and validated by real-time storage experiments (Taoukis et al., 1997; Wang et al., 2006). Kernel skin and core color of RF-treated and untreated uninoculated almonds were measured at random locations using a Minolta colorimeter (CR400; Minolta Co., Osaka, Japan). The values of L*, a*, and b* were used to quantify color attributes and indicate lightness, redness, and yellowness of the sample, respectively.

The peroxide value and acid value were determined by the oil extracted from the almond samples using a solvent recovery extractor (4002842; JP Selecta S.A., Barcelona, Spain) and tested according to AOCS official methods Cd 8–53 and Cd 3a–63, respectively. After titration of the almond oil in acetic acid/chloroform solutions (3:2 [v/v]) with 0.1 N sodium thiosulfate, the peroxide value was calculated by the following Eq. (1):

$$\mathrm{PV} = \frac{(S - B) \times N_1 \times 1000}{W} \quad (1)$$

where PV is the peroxide value (meq/kg), S and B is consumption of 0.1 N sodium thiosulfate (ml) at the end point for the sample and the blank, respectively, N_1 is the normality of sodium thiosulfate, and W is the weight of the almond oil (g).

The acid value was calculated by Eq. (2), based on the titration of the almond oil in ether/ethanol solutions (1:1 [v/v]) with 0.1 N potassium hydroxide.

$$\mathrm{AV} = \frac{V \times N_2 \times 56.11}{W} \quad (2)$$

where AV is the acid value (%), V is consumption of 0.1 N potassium hydroxide (ml) at the end point for the sample, and N_2 is the normality of potassium hydroxide.

2.10. Statistical analysis

All experiments were performed in triplicate. Data were analyzed by the analysis of variance procedure and Duncan's multiple-range test of the Statistical Analysis System (SAS Institute, Cary, NC). A P value of < 0.05 was used to determine significant differences.

3. Results

3.1. Temperature curves of almonds

Average surface and center temperatures of almonds during RF and conventional convective heating are shown in Fig. 3. The rate of RF heating was much higher than that of conventional convective heating on the almond surface, especially the initial heating rate. The surface temperature increased immediately in response to the RF electric field when the almond samples were subjected to RF heating, while it began to rise after approximately 30 s of convective heating. During 40 s of RF heating, almond surface temperature reached 93 °C. For convective heating, the mean time taken to reach 93 °C was ca. 180 s. The overall patterns of temperature increase for almond centers were similar to those for almond surfaces. The center temperature reached 97 °C after ca. 35 s and 320 s of RF and convective heating, respectively. Furthermore, ca. 70 s was required for the initiation of temperature increase during convective heating.

3.2. Survival curves of foodborne pathogens

Populations (log CFU/g) of *S.* Enteritidis, *S.* Typhimurium, and *S.* Senftenberg on almond surfaces during RF and convective heating are shown in Fig. 4. Significant ($P < 0.05$) log reductions of the three pathogens were observed after 20 s of RF heating and 300 s of convective heating. RF heating for 40 s achieved 3.7-, 6.0-, and 5.6-log reductions in *S.* Enteritidis, *S.* Typhimurium, and *S.* Senftenberg, respectively, whereas convective heating did not attain comparable reductions even at the end of treatment. After 600 s of convective heating, levels of these pathogens were reduced by 1.7, 2.5, and 3.7 log CFU/g, respectively. The reduction of *S.* Enteritidis was significantly ($P < 0.05$) smaller than that of *S.* Typhimurium and *S.* Senftenberg during both heating treatments.

The vacuum perfusion method effectively introduced the inoculum into almond kernels (Fig. 1). Fig. 5 shows the different effect of RF and convective heating on inactivation of internally inoculated *S.* Enteritidis, *S.* Typhimurium, and *S.* Senftenberg. The reduction patterns of the three pathogens in almonds were similar to those of the surface-inoculated pathogens. RF heating yielded about a 290 s shorter initiation time of significant ($P < 0.05$) reduction compared to convective

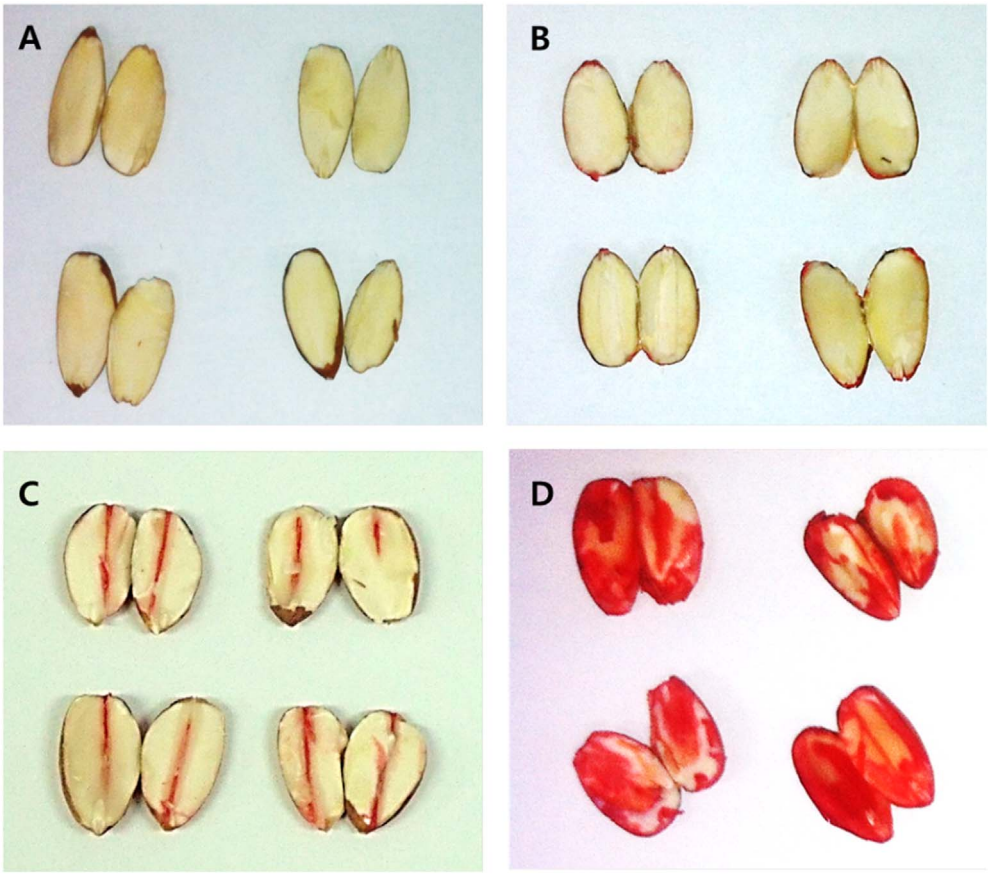

Fig. 1. Dye internalization after dipping, syringe injection, and vacuum perfusion method. (A) control; (B) dipping for 20 min; (C) syringe injection; (D) vacuum perfusion at 96 kPa.

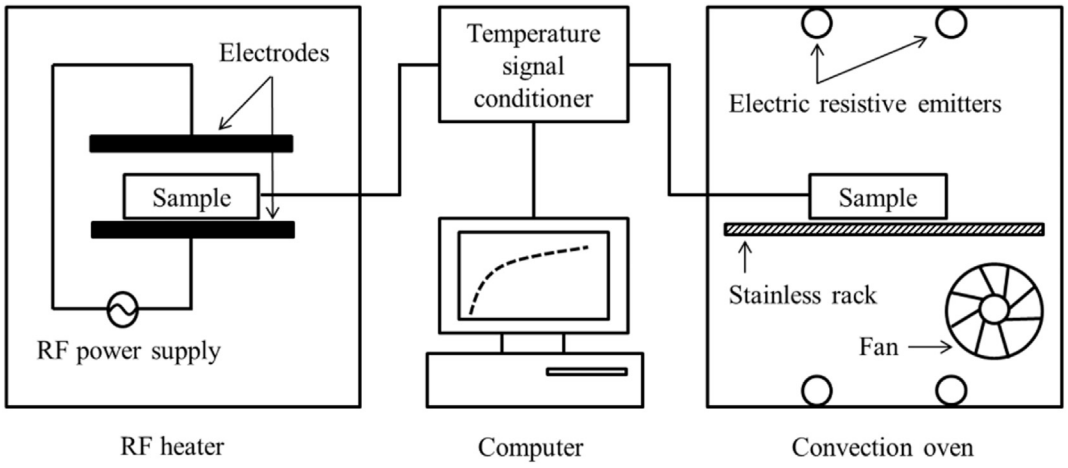

Fig. 2. Schematic diagram of RF and conventional convective heating system at Seoul National University (Seoul, South Korea).

heating. The numbers of *S*. Enteritidis, *S*. Typhimurium, and *S*. Senftenberg were greatly reduced to undetectable levels after RF heating for 30 s. However, convective heating only reduced *S*. Senftenberg to below the detection limit after the maximum treatment of 600 s. Log reductions of 2.5 and 3.0, respectively, were observed in *S*. Enteritidis and *S*. Typhimurium at the same treatment time.

3.3. Recovery of heat-injured cells

Table 1 shows numbers of surviving cells, including heat-injured *S*. Enteritidis, *S*. Typhimurium, and *S*. Senftenberg, on the surfaces and insides of almonds following RF heating. When inoculated almonds were treated with RF heating, slightly higher numbers of these pathogens were detected on the agar for recovery (OV-XLD) than on the selective agar (XLD). Especially, after 20 s corresponding to approximately 50 °C, there were differences in microbial levels between XLD and OV-XLD, resulting in injured populations on surface- and internally-inoculated almonds of 0.3 and 0.2 log CFU/g, respectively, for *S*. Enteritidis; and 0.1 log CFU/g, respectively, for *S*. Typhimurium. Overall, lower reductions of *S*. Enteritidis, *S*. Typhimurium, and *S*. Senftenberg were observed at various stages of RF treatment by the injured cell recovery procedure than by direct plating on selective media. However, at all treatment time intervals, no significant ($P > 0.05$) differences between levels of surviving heat-injured and uninjured cells were observed in raw shelled almonds.

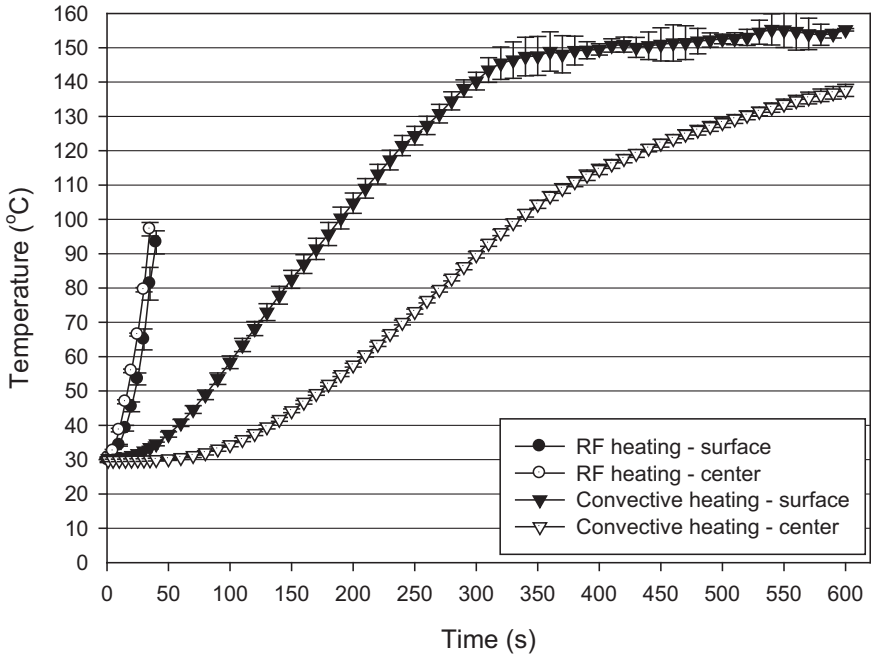

Fig. 3. Temperature curves of surface and center of almonds during RF and conventional convective heating. The results are means from three experiments, and error bars indicate standard deviations.

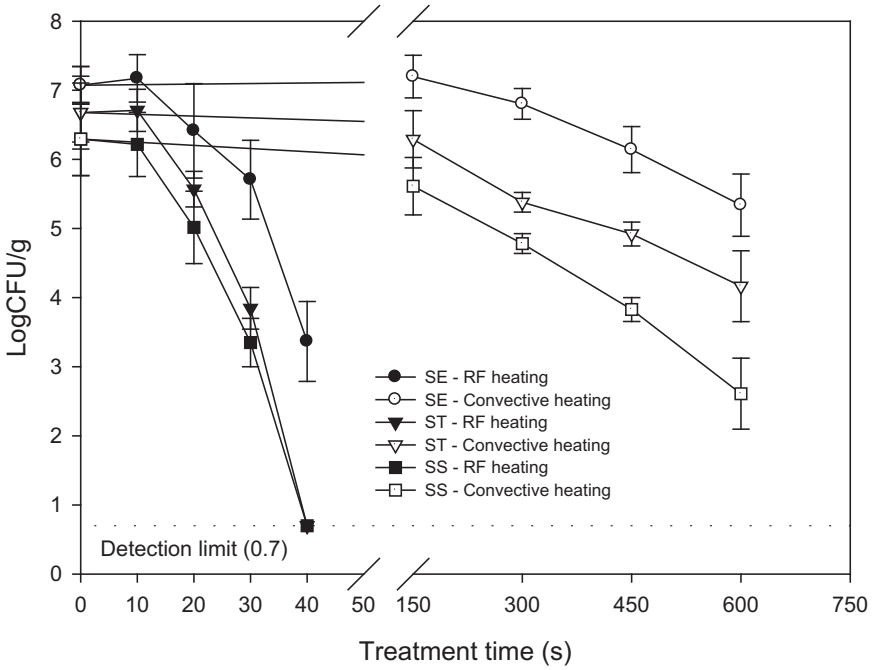

Fig. 4. Survival curves for *Salmonella* Enteritidis, *Salmonella* Typhimurium, and *Salmonella* Senftenberg on almond surfaces treated with RF or conventional convective heating. The results are means from three experiments, and error bars indicate standard deviations.

3.4. Effect of RF heating on product quality

Color values of kernel skin and almond cores after RF heating for the time interval (40 s) required to achieve maximum reduction of *S.* Enteritidis, *S.* Typhimurium, and *S.* Senftenberg are summarized in Table 2. The L*, a*, and b* values of RF-treated samples were not significantly ($P > 0.05$) different from those of untreated samples during the entire storage time. Table 3 shows the lipid oxidation parameters of almonds following RF treatment. There were no significant ($P > 0.05$) differences in PV and AV between untreated and treated almonds. Although they varied slightly in accordance with RF heating at several storage times, no statistically significant differences ($P > 0.05$) were detected between any of the tested samples. Therefore, RF heating for 40 s did not degrade the quality of raw shelled almonds.

4. Discussion

In the present study, RF heating resulted in a much more rapid heating rate compared with conventional convective heating. This result is in agreement with earlier research by Wang et al. (2003) who reported that lethality was achieved in macaroni and cheese with

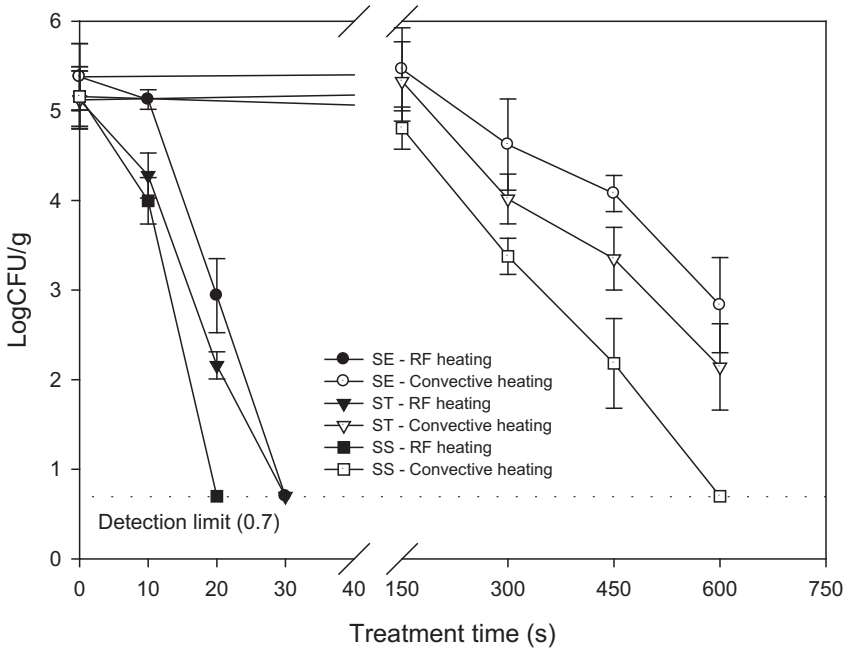

Fig. 5. Survival curves for *Salmonella* Enteritidis, *Salmonella* Typhimurium, and *Salmonella* Senftenberg inside of almonds treated with RF or conventional convective heating. The results are means from three experiments, and error bars indicate standard deviations.

the use of RF processing within shorter times than with conventional retort processing. Similar results were observed in ground beef and ready-to-eat aquatic foods such as caviar heated by RF and a water bath (Al-Holy et al., 2004; Guo et al., 2006). Although it has been reported that there was no non-thermal effect of RF energy on microbial inactivation (Geveke et al., 2002; Ponne et al., 1996), we determined that RF treatment produced significantly greater reduction of all tested pathogens even at the same temperature as convective heating. This is agreement with the effect of heating rate on inactivation of pathogens. The behavior of bacteria in foods that are heated slowly may mimic heat shock response, resulting in increased thermal resistance (Mackey and Derrick, 1987; Stephens et al., 1994; Wesche et al., 2009).

Besides rapid heating, uniformity is one of the advantages of RF heating. Guo et al. (2006) concluded that the temperature variation in RF heating between the surface and center of ground beef was much lower than that achieved in water-bath heating. This result was similar to our data which showed that the gap in treatment time required for the surface and center of almonds to reach the same temperature (93 °C) was only 5 s for RF heating; however, the time gap within samples was 140 s in conventional convective heating, indicating surface overheating of the samples. Also, maximum temperature of RF-treated almonds was much lower than that of conventionally heated almonds. These patterns reflect the means of RF heating and the nature of heat transfer. Unlike other heat transfer modes, RF heating is an internal heating process resulting from the direct interaction between electromagnetic waves and foods. The energy conversion from electrical energy to heat occurs within the food itself and generates relatively uniform heating (Zhao et al., 2000). Conversely, conventional heating requires heat energy that is generated externally, and heat is transferred to the food product by convection, conduction, radiation, or a combination thereof. Because of these heat transfer modes, heating uniformity is decreased which results in cold spots in the food which could permit survival of harmful microorganisms (Doores, 2002).

In recent years, numerous research investigations on internalization

Table 1
Comparison of pathogen populations between uninjured cells and cells including heat-injured cells on surface- or internally-inoculated almonds following RF heating[a].

Inoculation type and treatment time (s)	Population (log_{10} CFU/g) by organism and selective medium					
	S. Enteritidis		*S.* Typhimurium		*S.* Senftenberg	
	XLD[b]	OV-XLD	XLD	OV-XLD	XLD	OV-XLD
Surface						
0	7.1 ± 0.3 Aa	7.5 ± 0.3 Aa	6.7 ± 0.4 Aa	7.0 ± 0.2 Aa	6.3 ± 0.5 Aa	6.0 ± 0.1 Aa
10	7.2 ± 0.3 Aa	7.6 ± 0.4 Aa	6.7 ± 0.3 Aa	7.1 ± 0.3 Aa	6.2 ± 0.5 Aa	6.0 ± 0.3 Aa
20	6.4 ± 0.7 ABa	6.7 ± 0.9 Aa	5.6 ± 0.3 Ba	5.7 ± 0.6 Ba	5.0 ± 0.5 Ba	5.1 ± 0.4 Ba
30	5.7 ± 0.6 Ba	5.5 ± 0.8 Ba	3.9 ± 0.3 Ca	4.3 ± 0.5 Ca	3.4 ± 0.4 Ca	3.1 ± 0.5 Ca
40	3.4 ± 0.6 Ca	3.3 ± 0.4 Ca	ND[c]	ND	ND	ND
Internal						
0	5.4 ± 0.4 Aa	5.6 ± 0.4 Aa	5.1 ± 0.3 Aa	5.0 ± 0.2 Aa	5.2 ± 0.3 Aa	5.2 ± 0.3 Aa
10	5.1 ± 0.1 Aa	5.4 ± 0.2 Aa	4.3 ± 0.3 Ba	4.4 ± 0.4 Aa	4.0 ± 0.3 Ba	4.2 ± 0.5 Ba
20	3.0 ± 0.4 Ba	3.2 ± 0.5 Ba	2.2 ± 0.2 Ca	2.3 ± 0.5 Ba	ND	ND
30	ND	ND	ND	ND	ND	ND

[a] Means ± standard deviations from three replications. Means with the same uppercase 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.
[c] ND, below detection limit (0.7 log CFU/g).

Table 2
Kernel skin and core color values of RF-treated and untreated almonds stored at 35 °C and 30% relative humidity for 20 days[a].

Parameter and treatment type	Storage time (days) at 35 °C and 30% relative humidity[b]		
	0	10	20
Kernel skin color[c]			
L*			
Control	48.58 ± 0.72 a	48.02 ± 1.27 a	49.29 ± 1.64 a
RF treated	48.25 ± 0.83 a	48.32 ± 0.69 a	48.32 ± 0.87 a
a*			
Control	15.07 ± 0.41 a	14.55 ± 0.32 a	15.47 ± 0.51 a
RF treated	15.58 ± 0.86 a	15.53 ± 1.11 a	15.72 ± 0.23 a
b*			
Control	32.08 ± 0.67 a	32.38 ± 0.80 a	32.01 ± 1.52 a
RF treated	32.20 ± 0.93 a	32.35 ± 0.73 a	32.40 ± 0.42 a
Kernel core color (L*)[d]			
Control	75.43 ± 0.49 a	76.04 ± 1.37 a	75.53 ± 1.45 a
RF treated	75.70 ± 1.05 a	75.40 ± 0.79 a	75.24 ± 0.45 a

[a] Values followed by the same letters within the column per parameter are not significantly different ($P > 0.05$).
[b] 10 and 20 days at 35 °C and 30% relative humidity to simulate 1 and 2 years storage at 4 °C, respectively.
[c] Color parameters are L* (lightness), a* (redness), b* (yellowness).
[d] Accepted L* values for good quality are > 40.

Table 3
Peroxide values and acid values of RF-treated and untreated almonds stored at 35 °C and 30% relative humidity for 20 days[a].

Parameter and treatment type	Storage time (days) at 35 °C and 30% relative humidity[b]		
	0	10	20
Peroxide value (meq/kg)[c]			
Control	0.40 ± 0.00 a	1.46 ± 0.46 a	1.53 ± 0.58 a
RF treated	0.40 ± 0.00 a	1.12 ± 0.12 a	1.56 ± 0.55 a
Acid value (%)[c]			
Control	0.61 ± 0.08 a	0.62 ± 0.05 a	0.67 ± 0.03 a
RF treated	0.63 ± 0.11 a	0.67 ± 0.02 a	0.67 ± 0.03 a

[a] Values followed by the same letters within the column per parameter are not significantly different ($P > 0.05$).
[b] 10 and 20 days at 35 °C and 30% relative humidity to simulate 1 and 2 years storage at 4 °C, respectively.
[c] Accepted PV and AV for good quality are < 5 meq/kg and 1.5%, respectively.

of foodborne pathogens within various forms of fresh produce have been conducted. These studies have reported that pathogenic bacteria can be introduced and possibly infiltrate into fresh fruits and vegetables following pre-harvest and/or post-harvest processes (Barak et al., 2011; Beuchat and Mann, 2010; Danyluk et al., 2008; Franz et al., 2007; Mitra et al., 2009). Internalization of *S.* Enteritidis PT 30 into almond kernels was detected by confocal laser scanning microscopy (Danyluk et al., 2008). In 1999, the FDA declared that conventional surface decontamination treatments are generally ineffective in reducing internalized pathogens. However, no researchers have proposed any decontamination methods in an effort to inactivate internalized *Salmonella* in almonds. In this study, as with the comparison of efficacy of RF and convective heating for controlling surface-adhering pathogens, RF heating significantly decreased the treatment time required to reduce internalized *S.* Enteritidis, *S.* Typhimurium, and *S.* Senftenberg in almonds to below detectable levels. During RF heating, the heating rate was much higher at the center than on the surface of almonds. These results are in agreement with the internal generation of heat within the product caused by molecular friction.

Strains of *Salmonella* may survive high temperatures within low-moisture foods such as nuts, powdered milk, chocolate, peanut butter, and cereal (Doyle and Mazzotta, 2000). Among them, *S.* Enteritidis PT 30 implicated in the outbreak was found to be quite resistant to dry heat compared to other strains evaluated on almonds. Although the cause of its high resistance is still not fully understood, Parker et al. (2010) determined that *S.* Enteritidis PT 30 metabolized L-aspartic acid, L-glutamic acid, L-proline, L-alanine, and D-alanine amino acids efficiently. Their metabolites are required for protein synthesis which may be involved in enhancing cellular resistance (Wesche et al., 2009; Wu, 2009). Some typical industry roasting processes did not achieve a minimum 4-log reduction of *S.* Enteritidis PT 30 (Danyluk et al., 2006). Therefore, the Almond Board of California (ABC) identified *S.* Enteritidis PT 30 as the target pathogen of almonds for the validation test of dry roasting processes (Almond Board of California, 2007). In the present study, although *S.* Enteritidis PT 30 showed more resistance to RF heating than did *S.* Typhimurium and *S.* Senftenberg, a > 4-log reduction was confirmed in raw shelled almonds after RF heating. For conventional convective heating, *S.* Enteritidis PT 30 populations decreased approximately 2-logs.

Even though RF heating was highly effective in reducing foodborne pathogens in almonds, the occurrence of sub-lethally injured cells in RF-treated samples should be considered. Sub-lethal thermal injury may occur during thermal treatments that require extended come-up time (Bunning et al., 1990). Heat-injured cells are potentially as dangerous as their uninjured counterparts because they can undergo resuscitation and become functionally normal under favorable environmental conditions (Lee and Kang, 2001; McCleery and Rowe, 1995). Therefore, sub-lethally injured pathogens in almonds were assessed by plating on selective agar with and without a recovery step. There were no significant ($P > 0.05$) differences between injured cells and uninjured cells in surface- and internally-inoculated almonds after the maximum RF treatment of 40 s and 30 s, respectively. This suggests that RF heating effectively inactivated *S.* Enteritidis, *S.* Typhimurium, and *S.* Senftenberg in raw almonds without generating heat-injured cells during the short come-up time.

It is necessary to investigate the quality changes occurring during RF heating for commercial practical application of this highly appealing technology. Because of the potential of almonds to be affected by elevated temperature during RF treatment, the quality parameters in this study included color values (L*, a*, and b*) of kernel skin and core, PV, and AV used for indicators of possible lipid oxidation. After the maximum treatment applied for inactivation of foodborne pathogens, all tested parameters were not significantly ($P > 0.05$) different from those of the control. These values slightly varied in both untreated and RF-treated almonds during the storage period. However, the quality of almonds stored at 35 °C for 20 days, which simulated two-year storage at 4 °C, remained within the acceptable range (L* > 40, PV < 5 meq/kg, and FA < 1.5%) according to the ABC's standard. These results indicate that RF heating can be applied to control pathogens in raw shelled almonds without affecting product quality during storage.

Industrial scale RF heating for reducing foodborne pathogens in almonds should be based on commercial validation. Pathogen inactivation during RF heating is dependent on sample moisture content, salt content, density, temperature, and certain other factors (Jeong and Kang, 2014; Orsat and Raghavan, 2005). Further studies to enhance the effect of inactivation and shorten the RF treatment time for minimization of quality changes are required. In conclusion, our results indicate that RF heating leads to effective inactivation of *S.* Enteritidis, *S.* Typhimurium, and *S.* Senftenberg in raw shelled almonds without degrading quality. RF heating could be applied to control microbiological contamination in almonds over conventional pasteurization methods.

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References

Al-Holy, M., Ruiter, J., Lin, M., Kang, D.H., Rasco, B., 2004. Inactivation of *Listeria innocua* in nisin-treated salmon (*Oncorhynchus keta*) and sturgeon (*Acipenser transmontanus*) caviar heated by radio frequency. J. Food Prot. 67, 1848–1854.

Almond Board of California, 2007. Guidelines for validation of dry roasting processes. Available at: http://www.almondboard.com/Handlers/Documents/Dry-Roast-Validation-Guidelines.pdf.

Almond Board of California, 2012. 2012 Almond almanac. Available at: http://www.almondboard.com/AboutTheAlmondBoard/Documents/2012%20Almond%20Almanac_FINAL.pdf.

Barak, J.D., Kramer, L.C., Hao, L., 2011. Colonization of tomato plants by *Salmonella enterica* is cultivar dependent, and type 1 trichomes are preferred colonization sites. Appl. Environ. Microbiol. 77, 498–504.

Beuchat, L.R., Mann, D.A., 2010. Factors affecting infiltration and survival of *Salmonella* on in-shell pecans and pecan nutmeats. J. Food Prot. 73, 1257–1268.

Bunning, V.K., Crawford, R.G., Tierney, J.T., Peeler, J.T., 1990. Thermotolerance of *Listeria monocytogenes* and *Salmonella* Typhimurium after sublethal heat shock. Appl. Environ. Microbiol. 56, 3216–3219.

Centers for Disease Control and Preservation, 2009. *Salmonella* in pistachio nuts, 2009. Available at: http://www.cdc.gov/salmonella/pistachios/update.html.

Centers for Disease Control and Prevention, 2004. Outbreak of *Salmonella* serotype Enteritidis infections associated with raw almonds - United States and Canada, 2003–2004. Morb. Mortal. Wkly Rep. 53, 484–487.

Cornucopia Institute, 2007. Fact sheet mandatory sterilization of raw almonds. Available at: http://www.cornucopia.org/almond/Almond_Fact_Sheet.pdf.

Danyluk, M.D., Uesugi, A.R., Harris, L.J., 2005. Survival of *Salmonella* Enteritidis PT 30 on inoculated almonds after commercial fumigation with propylene oxide. J. Food Prot. 68, 1613–1622.

Danyluk, M.D., Harris, L.J., Schaffner, D.W., 2006. Monte Carlo simulations assessing the risk of salmonellosis from consumption of almonds. J. Food Prot. 69, 1594–1599.

Danyluk, M.D., Brandl, M.T., Harris, L.J., 2008. Migration of *Salmonella* Enteritidis phage type 30 through almond hulls and shells. J. Food Prot. 71, 397–401.

Doores, S., 2002. Microwave inactivation of pathogens. In: Juneja, V.K., Sofos, J.N. (Eds.), Control of Foodborne Microorganisms. Marcel Dekker, Inc., New York, pp. 105–164.

Doyle, M.E., Mazzotta, A.S., 2000. Review of studies on the thermal resistance of *Salmonellae*. J. Food Prot. 63, 779–795.

Franz, E., Visser, A.A., Van Diepeningen, A.D., Klerks, M.M., Termorshuizen, A.J., van Bruggen, A.H., 2007. Quantification of contamination of lettuce by GFP-expressing *Escherichia coli* O157:H7 and *Salmonella enterica* serovar Typhimurium. Food Microbiol. 24, 106–112.

Gao, M., Tang, J., Wang, Y., Powers, J., Wang, S., 2010. Almond quality as influenced by radio frequency heat treatments for disinfestation. Postharvest Biol. Technol. 58, 225–231.

Gao, M., Tang, J., Villa-Rojas, R., Wang, Y., Wang, S., 2011. Pasteurization process development for controlling *Salmonella* in in-shell almonds using radio frequency energy. J. Food Eng. 104, 299–306.

Geveke, D.J., Kozempel, M., Scullen, O.J., Brunkhorst, C., 2002. Radio frequency energy effects on microorganisms in foods. Innovative Food Sci. Emerg. Technol. 3, 133–138.

Goodridge, L.D., Willford, J., Kalchayanand, N., 2006. Destruction of *Salmonella* Enteriditis inoculated onto raw almonds by high hydrostatic pressure. Food Res. Int. 39, 408–412.

Guo, Q., Piyasena, P., Mittal, G., Si, W., Gong, J., 2006. Efficacy of radio frequency cooking in the reduction of *Escherichia coli* and shelf stability of ground beef. Food Microbiol. 23, 112–118.

Jackson, B.R., Griffin, P.M., Cole, D., Walsh, K.A., Chai, S.J., 2013. Outbreak-associated *Salmonella enterica* serotypes and food commodities, United States, 1998–2008. Emerg. Infect. Dis. 19, 1239–1244.

Jeong, S.G., Kang, D.H., 2014. Influence of moisture content on inactivation of *Escherichia coli* O157:H7 and *Salmonella enterica* serovar Typhimurium in powdered red and black pepper spices by radio-frequency heating. Int. J. Food Microbiol. 176, 15–22.

Kinn, T.P., 1947. Basic theory and limitations of high frequency heating equipment. Food Technol. 1, 161–173.

Ledet, M.L., Hjertqvist, M., Payne, L., Pettersson, H., Olsson, A., Plym, F.L., Andersson, Y., 2007. Cluster of *Salmonella* Enteritidis in Sweden 2005–2006-suspected source: almonds. Eur. Secur. 12, E9–10.

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.

Mackey, B.M., Derrick, C.M., 1987. Changes in the heat resistance of *Salmonella* Typhimurium during heating at rising temperatures. Lett. Appl. Microbiol. 4, 13–16.

McCleery, 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.

Mitra, R., Cuesta-Alonso, E., Wayadande, A., Talley, J., Gilliland, S., Fletcher, J., 2009. Effect of route of introduction and host cultivar on the colonization, internalization, and movement of the human pathogen *Escherichia coli* O157:H7 in spinach. J. Food Prot. 72, 1521–1530.

Niemira, B.A., 2007. Relative efficacy of sodium hypochlorite wash versus irradiation to inactivate *Escherichia coli* O157:H7 internalized in leaves of romaine lettuce and baby spinach. J. Food Prot. 70, 2526–2532.

Orsat, V., Raghavan, G.S.V., 2005. Radio-frequency processing. In: Sun, D.W. (Ed.), Emerging Technologies for Food Processing. Elsevier Academic Press, Inc., San Diego, pp. 445–468.

Parker, C.T., Huynh, S., Quiñones, B., Harris, L.J., Mandrell, R.E., 2010. Comparison of genotypes of *Salmonella enterica* serovar Enteritidis phage type 30 and 9c strains isolated during three outbreaks associated with raw almonds. Appl. Environ. Microbiol. 76, 3723–3731.

Piyasena, P., Dussault, C., Koutchma, T., Ramaswamy, H.S., Awuah, G.B., 2003. Radio frequency heating of foods: principles, applications and related properties—a review. Crit. Rev. Food Sci. Nutr. 43, 587–606.

Ponne, C.T., Balk, M., Hancioglu, Ö., Gorris, L.G., 1996. Effect of radio frequency energy on biological membranes and microorganisms. LWT Food Sci. Technol. 29, 41–48.

Prakash, A., Lim, F.T., Duong, C., Caporaso, F., Foley, D., 2010. The effects of ionizing irradiation on *Salmonella* inoculated on almonds and changes in sensory properties. Radiat. Phys. Chem. 79, 502–506.

Statista, 2014. Per capita consumption of tree nuts (shelled) in the United States from 2000 to 2011 (in pounds). Available at: http://www.statista.com/statistics/184216/per-capita-consumption-of-tree-nuts-in-the-us-since-2000/.

Stephens, P.J., Cole, M.B., Jones, M.V., 1994. Effect of heating rate on the thermal inactivation of *Listeria monocytogenes*. J. Appl. Bacteriol. 77, 702–708.

Taoukis, P.S., Labuza, T.P., Saguy, I.S., 1997. Kinetics of food deterioration and shelf-life prediction. In: Valentas, K.J., Rotstein, E., Singh, R.P. (Eds.), Handbook of Food Engineering Practice. CRC Press, Boca Raton, pp. 374.

U.S. Department of Agriculture, 2007. Almonds grown in California; outgoing quality control requirements. Fed. Regist. 72, 15021–15036.

Wang, Y., Wig, T.D., Tang, J., Hallberg, L.M., 2003. Sterilization of foodstuffs using radio frequency heating. J. Food Sci. 68, 539–544.

Wang, S., Tang, J., Sun, T., Mitcham, E.J., Koral, T., Birla, S.L., 2006. Considerations in design of commercial radio frequency treatments for postharvest pest control in in-shell walnuts. J. Food Eng. 77, 304–312.

Wesche, A.M., Gurtler, J.B., Marks, B.P., Ryser, E.T., 2009. Stress, sublethal injury, resuscitation, and virulence of bacterial foodborne pathogens. J. Food Prot. 72, 1121–1138.

Wu, G., 2009. Amino acids: metabolism, functions, and nutrition. Amino Acids 37, 1–17.

Yang, J., Bingol, G., Pan, Z., Brandl, M.T., McHugh, T.H., Wang, H., 2010. Infrared heating for dry-roasting and pasteurization of almonds. J. Food Eng. 101, 273–280.

Zhao, Y., Flugstad, B., Kolbe, E., Park, J.W., Wells, J.H., 2000. Using capacitive (radio frequency) dielectric heating in food processing and preservation–a review. J. Food Process Eng. 23, 25–55.