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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

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ABSTRACT

The influence of moisture content during radio-frequency (RF) heating on heating rate, dielectric properties, and inactivation of foodborne pathogens was investigated. The effect of RF heating on the quality of powdered red and black pepper spices with different moisture ranges was also investigated. Red pepper (12.6%, 15.2%, 19.1%, and 23.3% dry basis, db) and black pepper (10.1%, 17.2%, 23.7%, and 30.5% db) inoculated with *Escherichia coli* O157:H7 and *Salmonella enterica* serovar Typhimurium were treated in a RF heating system with 27.12 MHz. The heating rate of the sample was dependent on moisture content up to 19.1% (db) of red pepper and 17.2% (db) of black pepper, but there was a significant decrease in the heating rate when the moisture content was increased beyond these levels. The dielectric properties of both samples increased with a rise in moisture content. As the moisture content increased, treatment time required to reduce *E. coli* O157:H7 and *S.* Typhimurium by more than 7 log CFU/g (below the detection limit, 1 log CFU/g) decreased and then increased again without affecting product quality when the moisture content exceeded a level corresponding to the peak heating rate. RF treatment significantly ($P < 0.05$) reduced moisture content of both spices. These results suggest that RF heating can be effectively used to not only control pathogens but also reduce moisture levels in spices and that the effect of inactivation is dependent on moisture content.

1. Introduction

Radio-frequency (RF) heating is a highly appealing technology by which internal heating as a result of molecular friction is rapidly generated in response to an applied alternating electric field at frequencies between 1 and 300 MHz (Piyasena et al., 2003). This technology can offer the advantage of more uniform heating than conventional heating because of the direct interaction between electromagnetic waves and foods (Marra et al., 2009). With conventional food heating, externally generated heat is transferred to the food product by conduction, convection, or radiation (Doores, 2002). Conversely, RF generates heat rapidly within foods due to the oscillating movement of polar dielectric molecules and the space charge displacement caused by an externally applied AC electric field (Piyasena et al., 2003; Marra et al., 2009). Therefore, it has potential for thermal processing of solid and semi-solid foods which have low thermal conductivities while affecting few appearance, texture, or organoleptic changes and simultaneously promoting microbiological safety and shortening the processing time (Luechapattanaporn et al., 2005). Commercial RF processing has been developed based on applied research of RF heating (Demeczky, 1974; Houben et al., 1991; Zhao et al., 2008), and it has been successfully used in drying, baking and thawing of frozen meat and in meat processing (Piyasena et al., 2003).

Although considered to be a promising food processing technology, RF sterilization is rather limited due to a lack of in-depth technical information. Dielectric properties have assumed great importance associated with RF heating, since these properties of food affect their heating rate during RF heating (Tang, 2005). These properties are affected by sample moisture content, salt content, density, temperature, frequency of the applied alternating field, and a few other factors, though moisture content is generally considered to be the most critical factor (Orsat and Raghavan, 2005; Tang, 2005). In spice manufacturing and storage, moisture content is also important and is monitored continuously due to frequent fluctuations resulting from changes in relative humidity. This is because moisture content serves as an indicator not only for storage stability but also for microbiological safety of powdered foods including spice products (Schweiggert et al., 2007). Accordingly, moisture content should be taken into account as a factor in RF heating of spices.

Ignorance of dielectric properties relevant to RF sterilization can cause improper heating, leading to cold spots or product burning. To prevent undesirable RF heating, there have been some research efforts

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to clarify the impact of moisture content on dielectric properties of solid or semisolid foods (Sacilik et al., 2007; Gao et al., 2012; Guan et al., 2004; Roebuck and Goldblith, 1972). However, there has been no comprehensive research on the effect of moisture content on heating rate, dielectric properties, and microbial inactivation in foods. Also, thus far, the impact of RF heating on product quality at different moisture levels has not been studied. Therefore, it is necessary to examine the influence of moisture content on the heating rate of food products in order to maximize process effectiveness as well as ensure uniform commercial sterilization without affecting product quality when applying RF heating.

Powdered red (*Capsicum* spp.) and black (*Piper nigrum*) pepper spices were selected as a model to investigate the effects of RF heating on a solid food. Because of their low moisture content, spices are nonperishable commodities, but they are natural products and may be burdened with high levels of microorganisms (Schweiggert et al., 2007). Since they are utilized in ready-to-eat foods which are not subjected to further cooking, use of contaminated spices can lead to severe foodborne illnesses (Little et al., 2003). A large multistate outbreak of *Salmonella* Montevideo infections associated with salami products occurred in the United States in November 2009. The implicated foods were made with contaminated red and black pepper powders. During this outbreak, a reported 272 persons in 44 states and Washington, DC became ill (Centers for Disease Control and Prevention, 2010). In the United States 14 pepper-associated outbreaks were reported to the Centers for Disease Control and Prevention (CDC) between 1998 and 2009. Few decontamination methods exist for reducing the microbial of spices, such as fumigation with ethylene oxide, irradiation, and steam treatment; however, constraints on unhealthful residues, poor consumer acceptance, and quality deterioration still limit widespread use of conventional technologies (Schweiggert et al., 2007; Waje et al., 2008). No outbreaks involving *Escherichia coli* O157:H7 on red and black pepper spices have been reported. However, *E. coli* O157:H7 could be a potential risk in spice due to its survivability in low a_w food (Park and Beuchat, 2000).

The objectives of this study were to examine the impact of moisture content on the heating rate of powdered red and black pepper spices. The effects of RF heating for inactivating *E. coli* O157:H7 and *S.* Typhimurium in red and black pepper powders of various moisture contents, as well as dielectric properties and quality of spices, including color, volatile flavor components, and post-treatment moisture content, were investigated.

2. Materials and methods

2.1. Bacterial strains

All bacterial strains, namely, *E. coli* O157:H7 (ATCC 35150, ATCC 43889, ATCC 43890), and *S.* Typhimurium (ATCC 19585, ATCC 43971, ATCC 700408) were obtained from the Department of Food and Animal Biotechnology culture collection at Seoul National University (Seoul, South Korea). Stock cultures were stored at −80 °C in 0.7 ml of tryptic soy broth (TSB; Difco, Becton, Dickinson, Sparks, MD, USA) and 0.3 ml of 50% glycerol. For all experiments, working cultures were streaked onto tryptic soy agar (TSA; Difco), incubated at 37 °C for 24 h, and stored at 4 °C.

2.2. Preparation of pathogen inocula

Each strain of *E. coli* O157:H7 and *S.* Typhimurium was cultured individually in 5 ml of 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; Difco). The final pellets were resuspended in 3 ml of 0.2% PW, corresponding to approximately 10^8 to 10^9 CFU/ml. This concentration was confirmed by direct plating method in a preliminary experiment. To inoculate red and black pepper spices, suspended pellets of all strains of both pathogens were combined to produce a mixed culture cocktail (six strains). These cocktails at a final concentration of ca. 10^9 CFU/ml were used in subsequent experiments.

2.3. Sample preparation and inoculation

Commercially dried red and black pepper powders were purchased from a local grocery store (Seoul, South Korea). The red and black pepper spices were dried overnight to an initial moisture level of 10.6 ± 0.1% (dry basis, db) and 6.8 ± 0.1% (db), respectively. Moisture content was determined using a halogen moisture analyzer (HB43-S; Mettler Toledo, Columbus, OH). In order to maintain original quality, samples were stored at an ambient temperature of 22 ± 2 °C in ziplock bags prior to any conditioning. In this study, four moisture levels each of red pepper (12.6%, 15.2%, 19.1%, and 23.3% db) and black pepper (10.1%, 17.2%, 23.7%, and 30.5% db) were selected, covering the possible moisture range where small particles will not become agglomerated. Samples were inoculated in such a way as to simultaneously obtain the desired moisture levels. One ml of the mixed culture cocktail (*E. coli* O157:H7 and *S.* Typhimurium) and predetermined amounts of sterile distilled water were added to 25 g of each sample of powdered red and black pepper spices in polyethylene bags at room temperature (22 ± 2 °C), and then mixed by hand for 10 min to allow uniform moisture distribution throughout the sample. The final cell concentration was 10^7 to 10^8 CFU/g. Inoculated and conditioned spice samples were then immediately subjected to RF heating.

2.4. Experimental apparatus

The RF heating and dielectric measurement system (Fig. 1) consisted of a RF heater (Seoul National University, Seoul, South Korea; Dong Young Engineering Co. Ltd., Gyeongnam, Korea), a temperature signal conditioner (TMI-4; FISO Technologies Inc., Quebec, Canada), a liquid test fixture (16452A; Agilent Technologies, Palo Alto, CA), and a precision impedance analyzer (4294A; Agilent Technologies). The RF heater generated a RF electric field at a frequency of 27.12 MHz and a maximum power of 9 kW. Its cavity was composed 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. The liquid test fixture consisted of two parallel-plate electrodes in contact with the liquid or powder sample to produce a capacitor charge. The distance between the parallel plates was 1.0 mm and the test fixture was connected to the precision impedance analyzer by a four-terminal Bayonet Neill-Concelman (BNC) cable (16452-61601; Agilent Technologies). A precision impedance analyzer measured sample capacitance and equivalent parallel resistance for a frequency range from 20 Hz to 30 MHz which was used to calculate dielectric properties of the sample. The temperature signal conditioner and the precision impedance analyzer were connected to a computer for control using FISOCommander 2 Control and Analysis Software (FISO Technologies Inc.) and IO Libraries Suite (Agilent), respectively.

2.5. RF heating treatment

For the RF heating treatment, 25 g of inoculated red and black pepper powders placed in a polypropylene jar, 6.4 cm in diameter and 10.8 cm deep, (NALGENE 2118-0008; Thermo Scientific, Hudson, NH) was placed on the center of the bottom electrode. In all experiments, the alternating electric field was fixed at 27.12 MHz. RF heating was applied to each prepared sample and heated to 90 °C 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 heating.

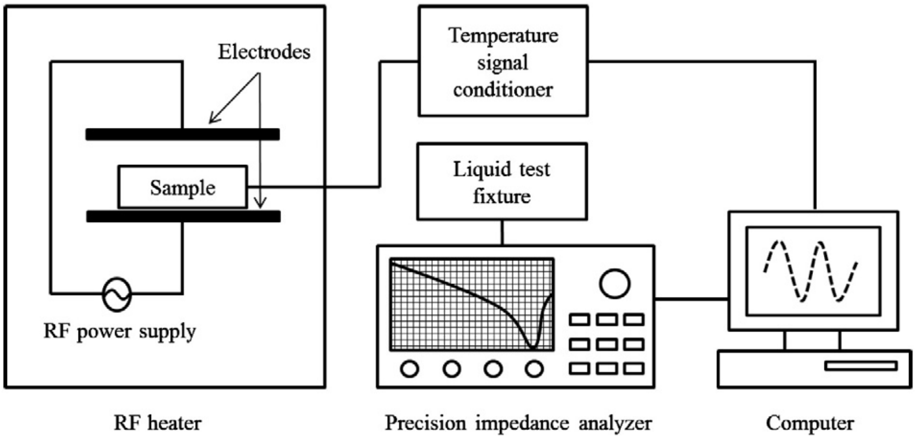

Fig. 1. Schematic diagram of RF heating and dielectric measurement system at Seoul National University (Seoul, South Korea).

The sensor was directly inserted at the center of the treated red and black pepper powders and the temperature was recorded at 1 s intervals. Since the fiber optic sensor was coated with electric insulating material, it did not interfere with the temperature profile of the treated sample (Wang et al., 2003). All experiments were repeated three times, and averages and standard deviations of RF treated sample temperatures were compared to determine the heating rate of samples. The rate of temperature increase was calculated by dividing the difference in temperature between the beginning and the end by the treatment time.

2.7. Dielectric properties measurement

Dielectric properties of samples were determined by the parallel plate method in ASTM D150. Dielectric measurements of spices were taken at 27.12 MHz. The procedure is as follows: the precision impedance analyzer was manually calibrated using a BNC cable to connect it with the liquid test fixture, and then air capacitance of the test fixture was measured. About 2 g of sample was placed into the test fixture and the electrical data of the samples were measured automatically at room temperature (22 ± 2 °C). The dielectric properties of red and black pepper spices were calculated as follows (Eqs. (1) and (2)):

$$\varepsilon' = \frac{C_p}{C_0} \tag{1}$$

where ε' is the dielectric constant, C_p is the spice capacitance (pF), and C_0 is the air capacitance (pF).

$$\varepsilon'' = \frac{1}{C_0 R_p \omega} \tag{2}$$

where ε'' is the dielectric loss factor, C_0 is the air capacitance (pF), R_p is the equivalent parallel resistance (Ω), and ω is the angular frequency (2πf).

2.8. Bacterial enumeration

At selected time intervals, treated 25 g samples were immediately transferred into sterile stomacher bags (Labphas, Inc., Sainte-Juilie, Quebec, Canada) containing 225 ml of 0.2% PW, which were prechilled in a 4 °C refrigerator. After homogenization for 2 min with a stomacher (Easy Mix; AES Chemunex, Rennes, France), 1-ml aliquots of sample were 10-fold serially diluted in 9-ml blanks of 0.2% PW, and 0.1 ml of sample or diluent was spread-plated onto each selective medium. Sorbitol MacConkey agar (SMAC; Difco) and xylose lysine desoxycholate agar (XLD; Difco) were used as selective media for the enumeration of *E. coli* O157:H7 and *S.* Typhimurium, respectively. When low levels of surviving cells were anticipated, 1 ml of undiluted stomacher bag contents was equally divided onto four plates of each medium and spread-plated (detection limit, 1 log CFU/g). All agar media were incubated at 37 °C for 24 h and typical colonies were counted. To confirm identity of the pathogens, colonies randomly selected from the enumeration plates were subjected to serological tests. These tests consisted of the *E. coli* O157:H7 latex agglutination assay (Oxoid, Ogdensberg, NY) and *Salmonella* latex agglutination assay (Oxoid) for *E. coli* O157:H7 and *S.* Typhimurium, respectively.

2.9. Enumeration of heat-injured cells

To enumerate heat-injured cells of *E. coli* O157:H7, RF-treated samples were serially diluted and spread-plated onto phenol red agar base with 1% sorbitol (SPRAB; Difco) at time intervals causing the large temperature change (Rocelle et al., 1995). After incubation at 37 °C for 24 h, typical white colonies were enumerated. Random colonies were selected from SPRAB plates and subjected to serological confirmation as *E. coli* O157:H7 (*E. coli* O157:H7 latex agglutination assay; Oxoid), since SPRAB is not a selective media for enumerating *E. coli* O157:H7. The overlay (OV) method was used to enumerate heat-injured cells of *S.* Typhimurium using TSA as a nonselective agar and XLD as the selective agar (Lee and Kang, 2001). Appropriate dilutions were spread-plated onto TSA medium and incubated at 37 °C for 2 h to allow injured cells to recover, and then 7 to 8 ml of XLD was overlaid on the plates. After solidification, plates were incubated for an additional 22 h at 37 °C, and typical black colonies were enumerated.

2.10. Color and post-treatment moisture content measurement

To measure the effect of RF heating on the color of powdered red and black pepper spices of various moisture contents, a Minolta colorimeter (CR400; Minolta Co., Osaka, Japan) was used to measure the color changes of RF-treated samples. The values of L^*, a^*, and b^* were used to quantify color attributes and measurements were taken from treated and untreated uninoculated red and black pepper taken at random locations. L^*, a^*, and b^* values indicate color lightness, redness, and yellowness of the sample, respectively. After RF heating treatment, the post-treatment moisture content was measured immediately with the halogen moisture analyzer described previously.

2.11. Volatile flavor component measurement

Capsaicinoids and piperine were measured as volatile flavor components in this study for red and black pepper powders, respectively. The total capsaicinoid content was tested according to the method described by Vincent and Ken (1987). This method involves capsaicinoid extraction, cleanup, and separation. Red pepper spice samples (4 g) were mixed with 20 ml of acetonitrile for 2 min with a vortex mixer (WiseMix VM-10; Daihan Wisd., Gangwon, South Korea). The capsaicinoids were eluted by passing the sample extract into a conditioned Sep-pak (WAT054945; Waters, Milford, MA) column. The eluent was passed through a Teknokroma 0.45-mm-pore-size membrane and subjected to the following procedure. For capsaicinoid separation, a high-performance liquid chromatography apparatus (HPLC; Waters 2695; Waters) equipped with an autosampler and a photodiode array detector (Waters 996; Waters) was used. The wavelength was set at 280 nm, and a reversed-phase C_{18} column (5-mm particle size, 4.6-mm diameter, 250-mm length; Young Jin Biochrom Co. Ltd., Gyeonggi, South Korea) where temperature was controlled at 35 °C was used with these conditions of the mobile phase: methanol and triple-distilled water (70:30 [v/v]) at a flow rate of 1 ml/min. A standard calibration curve was obtained by using capsaicin (Sigma Chemical Co., St. Louis, MO) and dihydrocapsaicin (Sigma Chemical Co.) prepared in acetonitrile.

Piperine concentration in black pepper was also determined using HPLC set at 340 nm. The column which was the same with the one we used to separate the capsaicinoid in red pepper was utilized. The mobile phase was prepared according to the method reported by Chiang (1986) and passed through the column at 1.5 ml/min. Standard piperine was purchased from Sigma Chemical Co. and prepared in methanol. The samples (0.1 g) were homogenized with 5 ml of methanol by vortexing for 2 min in a 10 ml volumetric tube, and methanol was added to the mark. After the solids settled, the supernatant was filtered through a Teknokroma filter and a 20-μl portion of filtrate was injected into the column using the HPLC autosampler.

2.12. Statistical analysis

All experiments were repeated three times with duplicate samples. Data were analyzed by the analysis of variance procedure of Statistical Analysis System (SAS Institute, Cary, NC), and mean values were separated using Duncan's multiple-range test. $P < 0.05$ was used to determine significant differences in the processing treatment.

3. Results

3.1. Temperature curves of powdered red and black pepper spices with different moisture contents

Average temperatures of red and black pepper powders of various moisture contents, respectively, from 12.6% to 23.3% (db), and 10.1% to 30.5% (db) during RF heating at a constant frequency of 27.12 MHz are shown in Fig. 2. At the same moisture level, the temperature increased with increasing treatment times. Higher heating rates resulting from higher moisture content caused heating times to decrease. The heating rate of both spices was dependent on moisture content up to 19.1% (dry basis, db) for red pepper and 17.2% (db) for black pepper, but there was a significant decrease ($P < 0.05$) when moisture content exceeded the above levels. Red pepper with 19.1% (db) and black pepper with 17.2% (db) moisture content increased from 22.2 °C to 90.2 °C when exposed to RF energy for 34 s and 42 s, respectively. For the same treatment time, the temperature of both spices did not exceed 60 °C at the lowest moisture content.

3.2. Influence of moisture content on dielectric properties of powdered red and black pepper spices

The dielectric properties of conditioned powdered red and black pepper spices at each of the four moisture levels and at a fixed frequency of 27.12 MHz are listed in Table 1. Both dielectric constants and dielectric loss factors of red pepper significantly ($P < 0.05$) increased with increasing moisture content from 12.6% to 23.3% (db). Similar patterns of the effect of moisture content were obtained for black pepper in the range of 10.1% to 30.5% (db), however, higher dependence on moisture content was shown in not only dielectric constants but also dielectric loss factors of the samples compared to those of red pepper. In all of the experiments, dielectric properties of the sample varied slightly at the beginning of moisture-conditioning, while those greatly increased as moisture content increased.

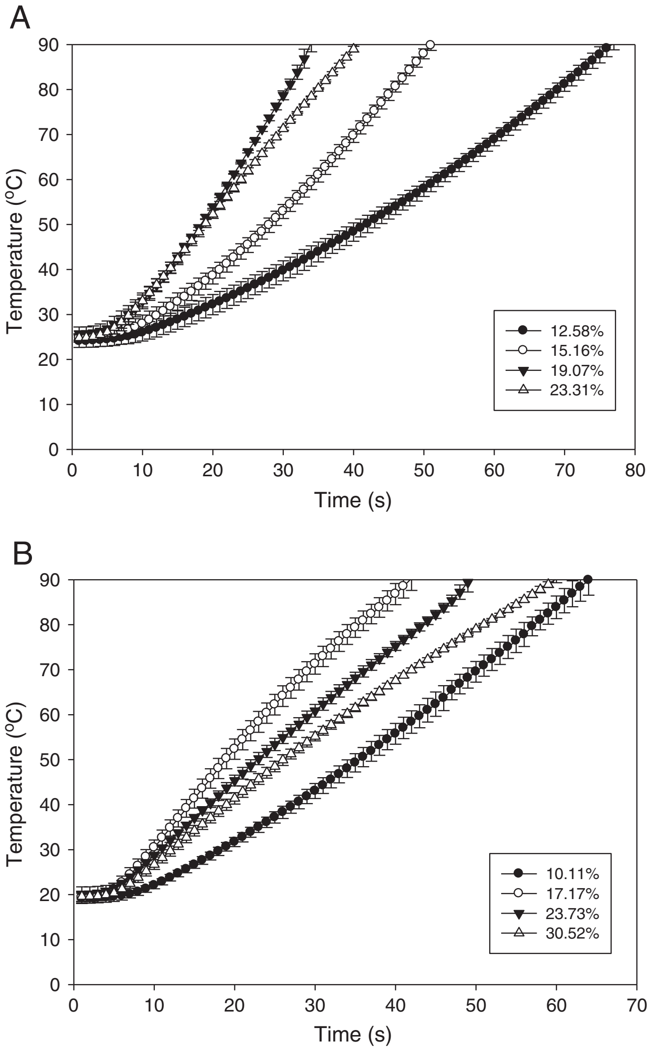

Fig. 2. Temperature curves of red (A) and black (B) pepper powders at controlled moisture levels during RF heating. All moisture contents are expressed on a dry basis. The results are means from three experiments, and error bars indicate standard deviations.

3.3. Relationships between rate of temperature increase, dielectric loss factor, and moisture content of powdered red and black pepper spices

The results of the rate of temperature increase, dielectric loss factor, and moisture contents of powdered red and black pepper were arranged for analysis of tripartite relationships (Fig. 3). In both samples, the dielectric loss factor was proportional to moisture content as listed in Table 1. The rate of temperature increase was significantly ($P < 0.05$) dependent on moisture content up to ca. 19.1% (db) for red pepper and 17.2% (db) for black pepper as shown in Fig. 2. The patterns of relationship between the rate of temperature increase and the dielectric loss factor were similar to those between the rate of temperature increase and moisture content. The rate of temperature increase diminished above ca. 3.23 and 1.53 of dielectric loss factor in red and black pepper powders, respectively.

Table 1
Dielectric properties of red and black pepper powders of varying moisture content at 27.12 MHz.[a]

Moisture content (%, db)	Red pepper		Moisture content (%, db)	Black pepper	
	ε'[b]	ε''		ε'	ε''
12.6	3.17 ± 0.14 d	0.03 ± 0.02 d	10.1	1.72 ± 0.03 d	0.02 ± 0.00 d
15.2	4.64 ± 0.30 c	0.63 ± 0.09 c	17.2	4.45 ± 0.32 c	1.53 ± 0.22 c
19.1	8.78 ± 0.28 b	3.23 ± 0.20 b	23.7	11.11 ± 0.21 b	10.58 ± 0.56 b
23.3	13.80 ± 0.28 a	8.69 ± 0.29 a	30.5	22.62 ± 0.50 a	40.01 ± 0.68 a

[a] Means ± standard deviations from three replications. Values followed by different letters within the column are significantly different ($P < 0.05$).
[b] ε' is the dielectric constant and ε'' is the dielectric loss factor.

3.4. Influence of moisture content on inactivation of foodborne pathogens in powdered red and black pepper spices

Populations (log CFU/g) of *E. coli* O157:H7 and *S.* Typhimurium in red pepper during RF heating are depicted in Fig. 4. As the moisture content increased from 12.6% to 19.1% (db), surviving populations of both pathogens decreased more effectively. However, treatment time required to reduce *E. coli* O157:H7 and *S.* Typhimurium to below the detection limit (1 log CFU/g) increased again when the moisture content exceeded 19.1% (db). The levels of surviving cells of both pathogens were reduced to below the detection limit within 35 s at a moisture content of 19.1% (db). At 23.3% (db), levels of *E. coli* O157:H7 experienced a significant reduction of 4.4 log CFU/g after 35 s and a >5.3-log reduction to below the detection limit after 40 s of treatment. Cell numbers of *S.* Typhimurium were reduced by 2.3 log CFU/g after 35 s and to below the detection limit after 40 s of treatment. At 15.2% (db), levels of both foodborne pathogens were reduced to below the detection limit after 50 s of treatment. The numbers of *E. coli* O157:H7 and *S.* Typhimurium in red pepper were greatly reduced to undetectable levels after 80 s at 12.6% (db).

Fig. 5 shows the survival of *E. coli* O157:H7 and *S.* Typhimurium of black pepper treated with RF heating. The overall reduction patterns of both pathogens in black pepper were similar to those in red pepper.

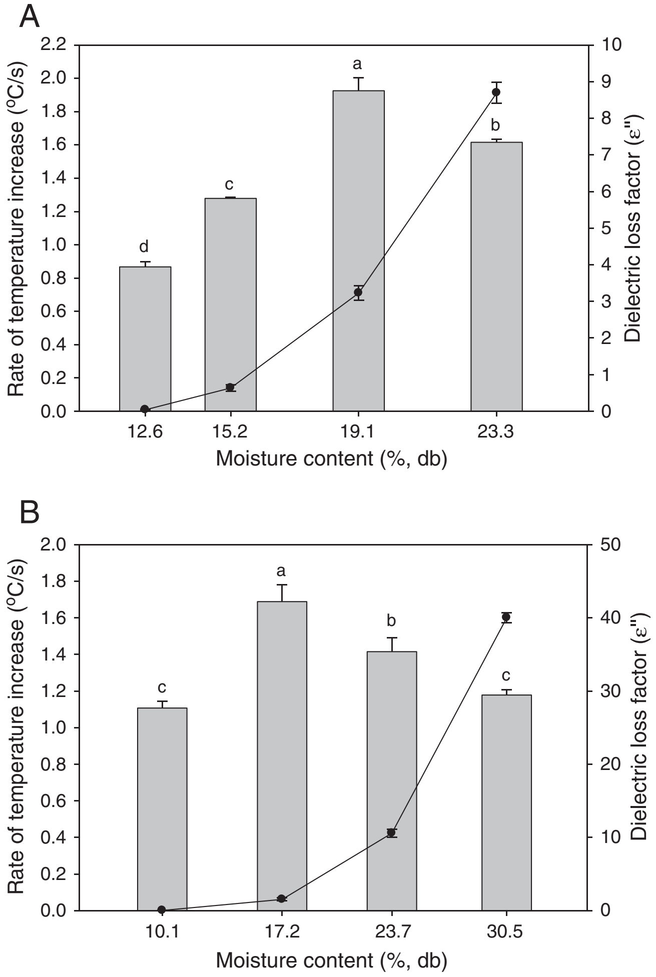

Fig. 3. Relationship between rate of temperature increase and dielectric loss factor of red (A) and black (B) pepper powder at controlled moisture levels during RF heating. The results are means from three experiments, and error bars indicate standard deviations. █, rate of temperature increase; ●, dielectric loss factor.

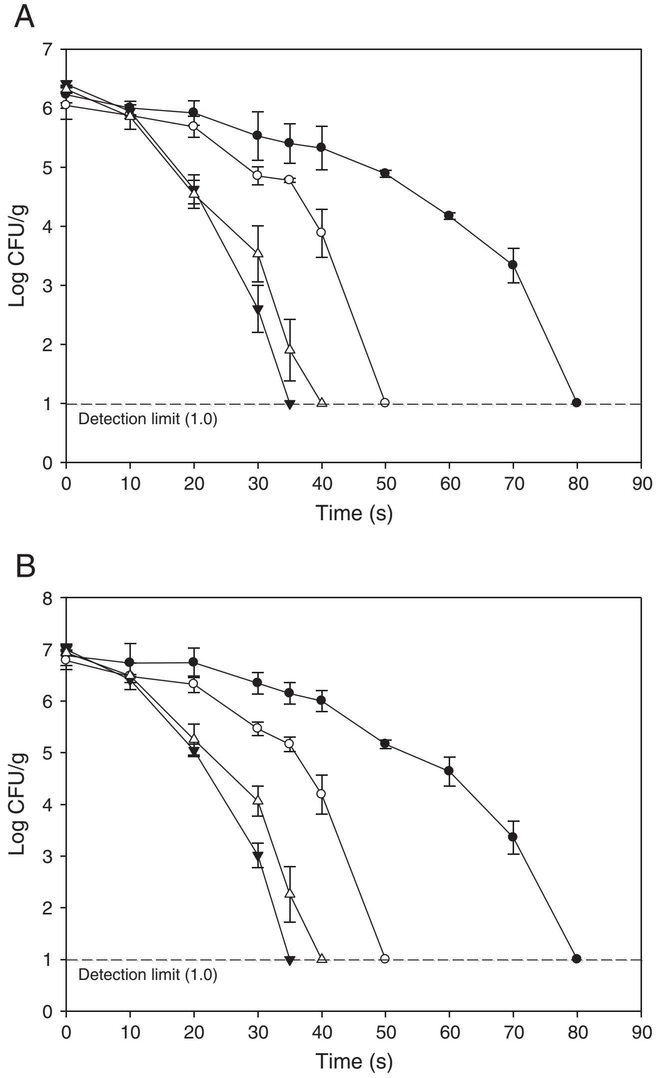

Fig. 4. Survival curves for *Escherichia coli* O157:H7 (A) and *Salmonella* Typhimurium (B) on red pepper powder with moisture contents of 12.6% (●), 15.2% (○), 19.1% (▼), and 23.3% (△) during RF heating. All moisture contents are expressed on a dry basis. The results are means from three experiments, and error bars indicate standard deviations.

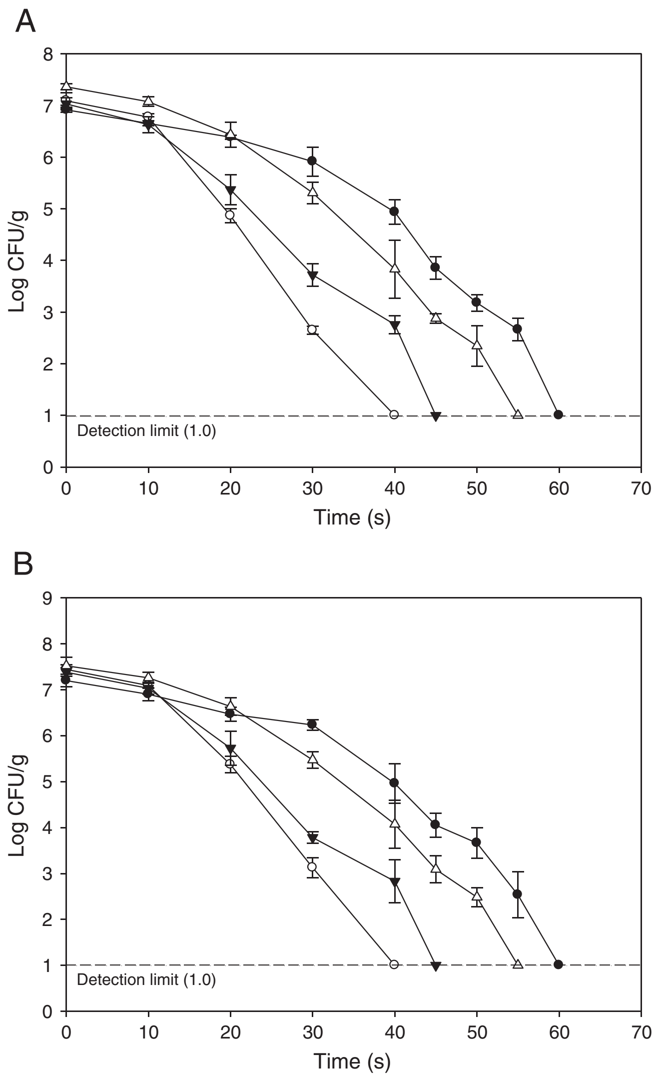

Fig. 5. Survival curves for *Escherichia coli* O157:H7 (A) and *Salmonella* Typhimurium (B) on black pepper powder with moisture contents of 10.1% (●), 17.2% (○), 23.7% (▼), and 30.5% (△) during RF heating. All moisture contents are expressed on a dry basis. The results are means from three experiments, and error bars indicate standard deviations.

RF heating at 17.2% (db) moisture content reduced both pathogens to below the detection limit after 40 s. At 23.7% and 30.5% (db), *E. coli* O157:H7 and *S.* Typhimurium were reduced to below detectable levels after 45 s and 55 s of treatment, respectively. At the lowest moisture content, the treatment time required to reduce both pathogens to below the detection limit in black pepper was less than that in red pepper. Populations of *E. coli* O157:H7 and *S.* Typhimurium decreased by 5.9 log CFU/g and 6.2 log CFU/g after 60 s, respectively.

3.5. Recovery of heat-injured cells

Surviving cells and heat-injured cells of *E. coli* O157:H7 and *S.* Typhimurium from red and black pepper powders following RF heating were compared (data not shown). When inoculated red and black pepper powders were treated with RF heating, slightly higher numbers of both pathogens were detected on the agar for recovery (SPRAB and OV-XLD) than on the selective agar (SMAC and XLD). However, during the entire treatment time, no significant ($P > 0.05$) differences in the levels of cells enumerated between the agar for recovery and the selective agar were observed in powdered red and black pepper spices with different moisture ranges.

3.6. Effect of RF heating within different moisture range on product quality

The color and volatile flavor components (capsaicinoids for red pepper and piperine for black pepper) of spice samples of various moisture contents after RF heating for time intervals required to reduce *E. coli* O157:H7 and *S.* Typhimurium to below the detection limit (1 log CFU/g) are summarized in Tables 2 and 3. Within all tested levels of moisture content, L^*, a^*, and b^* values of RF-treated samples were not significantly ($P > 0.05$) different from those of untreated samples. There were also no significant ($P > 0.05$) differences in volatile flavor components, capsaicinoids and piperine, between untreated and treated red and black pepper powders. Although they varied slightly in accordance with RF heating treatment at several moisture levels, statistically significant differences were not detected between any of the tested samples (Tables 2 and 3). Thus, RF heating did not affect the color or amount of volatile flavor components of powdered red and black pepper spices of differing moisture content ($P > 0.05$). Table 4 shows the moisture content of powdered red and black pepper following RF treatment. There were statistically significant ($P < 0.05$) differences between untreated and treated samples. The moisture content of red and black pepper powders subjected to RF heating was reduced by 2% to 18% and 12% to 27% (Table 4), respectively. Except for red pepper of 12.6% db, all samples of different moisture levels experienced significantly drying ($P < 0.05$).

4. Discussion

The objectives of the present study were to examine the influence of moisture content of red and black pepper powders during RF heating on heating rate, dielectric properties, and inactivation of foodborne pathogens. The impact of RF heating on the quality of red and black pepper was also investigated. Increasing the moisture content of both spices not only had an effect on the heating rate but also increased the dielectric properties. These results imply that RF heating is influenced by dielectric properties of the product which depend on its moisture content. The dielectric properties of food materials determine how they react to an external electromagnetic wave and indicate permeability, permittivity and electrical conductivity (Kuang and Nelson, 1998). Of these, the permittivity which determines the dielectric constant and the dielectric loss factor affects RF heating (Marra et al., 2009). The dielectric constant is a characteristic of the ability of a material to store electric energy and a measure of the polarizing effect from an external electric field; in other words, how easily the medium is polarized. The dielectric loss factor reflects the amount of electric energy lost as heat, which is related to how the energy from an applied electric field is absorbed and converted to heat (Gao et al., 2012; Zhang and Datta, 2001). Based on the theory, there has been a need for knowledge of the dielectric properties of foods in order to develop effective RF heating processes.

Specific factors such as frequency of the electromagnetic waves, temperature, moisture content, and salt content of food materials affect the related dielectric properties and the resultant RF heating (Galema, 1997). Moisture content can be one of the key parameters for rapid heating by increasing the dielectric loss factor of red and black pepper. The electrical power transferred to the food as heat (P) is given by the equation $P = 2\pi f V^2 \varepsilon_0 \varepsilon''$, where f is the frequency, V is the electric field strength, ε_0 is the dielectric constant of a vacuum considered equal to 8.85×10^{-12} F/m, and ε'' is the dielectric loss factor of the sample. From this equation, the heat generated is proportional to the frequency, the electric field strength, and the dielectric loss factor. In the present study, frequency and electric field strength were fixed at 27.12 MHz and 0.3 kV/cm, respectively, leaving only the impact of the dielectric loss factor to be examined, which depends on moisture content during RF heating. Our results agree with the equation; more heat was generated as a result of a higher dielectric loss factor of powdered red and black pepper spices resulting from higher moisture levels. However, above

Table 2
Color values and capsaicinoid content of treated and untreated red pepper powder of varying moisture levels subjected to RF heating.[a]

Moisture content (%)[b]	Treatment time (s)	Color[c]			Capsaicinoids (mg/100 g)		
		L*	a*	b*	Capsaicin	Dihydrocapsaicin	Total[d]
12.6	0	33.62 ± 0.49 a	22.10 ± 0.22 a	17.18 ± 0.40 a	36.87 ± 0.66 a	36.22 ± 0.90 a	73.09 ± 1.20 a
	80	33.60 ± 0.45 a	22.07 ± 0.40 a	17.60 ± 0.58 a	36.01 ± 0.87 a	36.91 ± 1.43 a	72.92 ± 0.56 a
15.2	0	32.54 ± 0.24 a	22.19 ± 0.46 a	17.24 ± 0.37 a	36.41 ± 1.34 a	35.15 ± 0.26 a	71.56 ± 1.53 a
	50	32.95 ± 0.24 a	21.93 ± 0.27 a	17.61 ± 0.19 a	35.88 ± 0.47 a	35.80 ± 0.37 a	71.68 ± 0.20 a
19.1	0	30.58 ± 0.09 a	20.00 ± 0.21 a	14.94 ± 0.24 a	31.46 ± 0.40 a	32.66 ± 0.95 a	64.12 ± 1.20 a
	35	30.72 ± 0.42 a	20.25 ± 0.45 a	15.73 ± 0.44 a	32.19 ± 0.81 a	33.53 ± 0.64 a	65.72 ± 1.26 a
23.3	0	29.25 ± 0.49 a	18.43 ± 0.40 a	13.38 ± 0.36 a	23.44 ± 0.62 a	26.09 ± 0.10 a	49.53 ± 0.65 a
	40	29.61 ± 0.71 a	18.47 ± 0.26 a	14.16 ± 0.43 a	23.91 ± 0.16 a	25.26 ± 0.31 a	49.17 ± 0.16 a

[a] Means ± standard deviations from three replications. Values followed by the same letters within the column per moisture content are not significantly different ($P > 0.05$).
[b] All moisture contents are expressed on a dry basis.
[c] Color parameters are L* (lightness), a* (redness), b* (yellowness).
[d] Total capsaicinoids; capsaicin + dihydrocapsaicin.

threshold dielectric loss factors which are 3.23 at 19.1% (db) for red pepper and 1.53 at 17.2% (db) for black pepper, the heating rate decreased. This result is in agreement with earlier research by Orfeuil (1987) which suggested that if the dielectric loss factor was too high, current leakage took place through the material. Conversely, if it was too low, heating took place slowly and it became difficult to reach the desired temperature. It has been proposed that the dielectric loss factor should be within proper range for successful RF heating (Birla et al., 2008). Therefore, it is important to consider thoroughly the relationships between moisture content, dielectric loss factor, and heating rates of foods to be heated in order to maximize the effectiveness of RF heating.

Although the number of research studies on RF heating inactivation of foodborne pathogens has grown in recent years (Ha et al., 2013; Kim et al., 2012; Byrne et al., 2010; Uemura et al., 2010; Schlisselberg et al., 2013), to date, there is no published information concerning the inactivation of *E. coli* O157:H7 and *S.* Typhimurium in spices of varying moisture content using RF heating. In the present study, moisture content of powdered red and black pepper had a profound effect on inactivating *E. coli* O157:H7 and *S.* Typhimurium. At a moisture content of 12.6% (db), 80 s was required to reduce the levels of *E. coli* O157:H7 and *S.* Typhimurium in red pepper to undetectable levels, but only 35 s was required at 19.1% (db). As for black pepper, 60 and 40 s were required to reduce both pathogens to below the detection limit (1 log/CFU) at 10.1% (db) and 17.2% (db), respectively. As with the relationship between moisture content and heating rate, there was a threshold at which increasing moisture content led to an extension of RF treatment time required to reduce *E. coli* O157:H7 and *S.* Typhimurium to below detectable levels; this was true for both red and black pepper spices.

Following heating treatment, sub-lethally injured foodborne pathogens are potentially as dangerous as their uninjured counterparts (McCleer and Rowe, 1995; Lee and Kang, 2001). This is because heat-injured cells could undergo resuscitation and become normal. Therefore, the cell numbers enumerated on selective agar are not good enough to represent the total surviving populations in the samples. After RF heating within different moisture range, there were no significant ($P > 0.05$) differences between injured and uninjured cells in powdered red and black pepper spices. This suggests that RF heating effectively inactivated *E. coli* O157:H7 and *S.* Typhimurium in red and black pepper powders without generating heat-injured cells which could recover.

In addition, after the maximum treatment applied for inactivation of foodborne pathogens, color values (L*, a*, and b*) and volatile flavor component values of samples of varying moisture content were not significantly ($P > 0.05$) different from those of the control. Other researchers also reported that RF heating treatment resulted in food products of superior quality. Ha et al. (2013) found that the RF heated peanut butter crackers maintained color, flavor, texture, and overall acceptability. Geveke et al. (2007) reported that the RF heating method produced no loss in ascorbic acid content and caused little enzymatic browning in orange juice. Based on our results, no significant quality differences were observed between untreated and RF-treated red and black pepper powders of various moisture content, but treatment time required to reduce both pathogens to below the detection limit was different depending on moisture content.

Besides pasteurization and sterilization, RF heating has an excellent drying effect and it has been used in some drying applications such as post-baking drying of cookies, crackers, and snack foods (Piyasena et al., 2003). This is because the electromagnetic energy of RF electric field tends to act on water and aqueous ions (Orfeuil, 1987). In the present study, the drying performance during RF heating of powdered red and black pepper spices was evaluated in terms of the final moisture content. A statistically significant ($P < 0.05$) drying effect was shown in all tested samples except for 12.6% (db) red pepper. Although most samples subjected to RF heating did not meet the microbiologically safe moisture level (around 10%) for spice products, drying efficiency

Table 3
Color values and piperine content of treated and untreated black pepper powder of varying moisture levels subjected to RF heating.[a]

Moisture content (%)[b]	Treatment time (s)	Color[c]			Piperine (mg/100 g)
		L*	a*	b*	
10.1	0	46.77 ± 0.45 a	0.67 ± 0.14 a	13.51 ± 0.10 a	29.72 ± 3.22 a
	60	45.58 ± 1.11 a	0.91 ± 0.13 a	13.25 ± 0.28 a	30.29 ± 0.85 a
17.2	0	44.37 ± 1.20 a	1.20 ± 0.11 a	14.34 ± 0.55 a	30.68 ± 0.28 a
	40	43.40 ± 1.36 a	1.30 ± 0.19 a	13.56 ± 0.87 a	27.71 ± 2.90 a
23.7	0	39.63 ± 0.72 a	2.11 ± 0.16 a	12.91 ± 0.50 a	27.39 ± 0.61 a
	45	40.19 ± 0.60 a	2.03 ± 0.07 a	13.31 ± 0.64 a	28.09 ± 2.37 a
30.5	0	36.77 ± 0.86 a	2.39 ± 0.18 a	11.79 ± 0.84 a	25.34 ± 3.02 a
	55	37.58 ± 0.91 a	2.26 ± 0.20 a	12.01 ± 0.84 a	24.13 ± 2.31 a

[a] Means ± standard deviations from three replications. Values followed by the same letters within the column per moisture content are not significantly different ($P > 0.05$).
[b] All moisture contents are expressed on a dry basis.
[c] Color parameters are L* (lightness), a* (redness), b* (yellowness).

Table 4
Moisture content of red and black pepper powders before and after RF heating.[a]

Product	Moisture content (%, db)	
	Control	RF treated
Red pepper	12.6 ± 0.3 a	12.4 ± 0.1 a
	15.2 ± 0.4 a	14.0 ± 0.1 b
	19.1 ± 0.8 a	17.5 ± 0.1 b
	23.3 ± 0.5 a	20.3 ± 0.2 b
Black pepper	10.1 ± 0.1 a	5.6 ± 0.3 b
	17.2 ± 0.0 a	12.5 ± 0.3 b
	23.7 ± 0.3 a	21.0 ± 0.2 b
	30.5 ± 0.2 a	27.7 ± 0.1 b

[a] Means ± standard deviations from three replications. Values followed by different letters within the row are significantly different ($P < 0.05$).

could be improved by using a screw conveyor in conjunction with an industrial RF heater (Waje et al., 2006). Therefore, RF heating is suitable for drying as well as sterilization of spices.

Industrial scale RF heating for controlling foodborne pathogens and reducing moisture in dried commodities can be performed using a continuous system. Powdered red and black pepper spices moving in a screw conveyor could be exposed to RF energy from two electrodes oriented horizontally on both sides of the conveyor. Treatment time could be selected by adjusting the flow rate. There is a need to model the effect of moisture content on inactivation of foodborne pathogens in both spices by RF heating. This can assist the industry in developing appropriate treatment times at certain moisture contents of spice products. Optimization of this process is essential to prevent insufficient heating or overheating which could permit survival of microorganisms or result in quality deterioration of food products. Therefore, in order to simultaneously guarantee product safety and quality, further studies involving modeling inactivation kinetics are required.

Our results indicate that RF heating leads to effective inactivation of *E. coli* O157:H7 and *S.* Typhimurium in powdered red and black pepper of varying moisture content, as well as producing spices of superior quality and simultaneously having an ideal drying effect. The results of this study are fundamental in order to understand and model the response of spices to the RF electromagnetic field at certain moisture contents, and by extension, to apply commercial RF sterilization. With a fuller understanding of the influence of moisture content on heating rates in red and black pepper, RF heating could be a very promising alternative technology to control microbiological contamination in spices.

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References

Birla, S.L., Wang, S., Tang, J., 2008. Computer simulation of radio frequency heating of model fruit immersed in water. J. Food Eng. 84, 270–280.

Byrne, B., Lyng, J.G., Dunne, G., Bolton, D.J., 2010. Radio frequency heating of comminuted meats — considerations in relation to microbial challenge studies. Food Control 21, 125–131.

Centers for Disease Control and Prevention, 2010. *Salmonella* Montevideo infections associated with salami products made with contaminated imported black and red pepper — United States, July 2009–April 2010. Available at: http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5950a3.htm.

Chiang, G.H., 1986. HPLC analysis of capsaicins and simultaneous determination of capsaicins and piperine by HPLC-ECD and UV. J. Food Sci. 51, 499–503.

Demeczky, M., 1974. Continuous pasteurization of bottled fruit juices by high frequency energy. Proceedings of IV International Congress on Food Science and Technology, pp. 11–20.

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.

Galema, S.A., 1997. Microwave chemistry. Chem. Soc. Rev. 29, 233–283.

Gao, M., Tang, J., Johnson, J.A., Wang, S., 2012. Dielectric properties of ground almond shells in the development of radio frequency and microwave pasteurization. J. Food Eng. 112, 282–287.

Geveke, D.J., Brunkhorst, C., Fan, X., 2007. Radio frequency electric fields processing of orange juice. Innov. Food Sci. Emerg. Technol. 8, 549–554.

Guan, D., Cheng, M., Wang, Y., Tang, J., 2004. Dielectric properties of mashed potatoes relevant to microwave and radio-frequency pasteurization and sterilization processes. J. Food Sci. 69, FEP30–FEP37.

Ha, J.W., Kim, S.Y., Ryu, S.R., Kang, D.H., 2013. Inactivation of *Salmonella enterica* serovar Typhimurium and *Escherichia coli* O157:H7 in peanut butter cracker sandwiches by radio-frequency heating. Food Microbiol. 34, 145–150.

Houben, J., Schoenmakers, L., Van Putten, E., Van Roon, P., Krol, B., 1991. Radio frequency pasteurization of sausage emulsions as a continuous process. J. Microw. Power Electromagn. Energy. 26, 202–205.

Kim, S.Y., Sagong, H.G., Choi, S.H., Ryu, S.R., Kang, D.H., 2012. Radio-frequency heating to inactivate *Salmonella* Typhimurium and *Escherichia coli* O157:H7 on black and red pepper spice. Int. J. Food Microbiol. 153, 171–175.

Kuang, W., Nelson, S.O., 1998. Low-frequency dielectric properties of biological tissues: a review with some new insights. Trans. ASAE 41, 173–184.

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.

Little, C.L., Omotoye, R., Mitchell, R.T., 2003. The microbiological quality of ready-to-eat foods with added spices. Int. J. Environ. Health Res. 13, 31–42.

Luechapattanaporn, K., Wang, Y., Wang, J., Tang, J., Hallberg, L.M., Dunne, C.P., 2005. Sterilization of scrambled eggs in military polymeric trays by radio frequency energy. J. Food Sci. 70, 288–294.

Marra, F., Zhang, L., Lyng, J.G., 2009. Radio frequency treatment of foods: review of recent advances. J. Food Eng. 91, 497–508.

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.

Orfeuil, M., 1987. Electric Process Heating: Technologies/Equipment/Applications. Battelle Press, Columbus 519–557.

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.

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.

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.

Rocelle, M., Clavero, S., Beuchat, L.R., 1995. Suitability of selective plating media for recovering heat- or freeze-stressed *Escherichia coli* O157:H7 from tryptic soy broth and ground beef. Appl. Environ. Microbiol. 61, 3268–3273.

Roebuck, B.D., Goldblith, S.A., 1972. Dielectric properties of carbohydrate–water mixtures at microwave frequencies. J. Food Sci. 37, 199–204.

Sacilik, K., Tarimci, C., Colak, A., 2007. Moisture content and bulk density dependence of dielectric properties of safflower seed in the radio frequency range. J. Food Eng. 78, 1111–1116.

Schlisselberg, D.B., Kler, E., Kalily, E., Kisluk, G., Karniel, O., Yaron, S., 2013. Inactivation of foodborne pathogens in ground beef by cooking with highly controlled radio frequency energy. Int. J. Food Microbiol. 160, 219–226.

Schweiggert, U., Carle, R., Schieber, A., 2007. Conventional and alternative processes for spice production — a review. Trends Food Sci. Technol. 18, 260–268.

Tang, J., 2005. Dielectric properties of foods. In: Schubert, H., Regier, M. (Eds.), The Microwave Processing of Foods. Woodhead Publishing Cambridge, Inc., United Kingdom, pp. 22–40.

Uemura, K., Takahashi, C., Kobayashi, I., 2010. Inactivation of *Bacillus subtilis* spores in soybean milk by radio-frequency flash heating. J. Food Eng. 100, 622–626.

Vincent, K.A., Ken, A.B., 1987. Rapid sample preparation method for HPLC analysis of capsaicinoids in Capsicum fruits and oleoresins. J. Agric. Food Chem. 35, 777–779.

Waje, S.S., Thorat, B.N., Mujumdar, A.S., 2006. An experimental study of the thermal performance of a screw conveyor dryer. Dry. Technol. 24, 293–301.

Waje, C.K., Kim, H.K., Kim, K.S., Todoriki, S., Kwon, J.H., 2008. Physicochemical and microbiological qualities of steamed and irradiated ground black pepper (*Piper nigrum* L.). J. Agric. Food Chem. 56, 4592–4596.

Wang, Y., Wig, T.D., Tang, J., Hallberg, L.M., 2003. Sterilization of foodstuffs using radio frequency heating. J. Food Sci. 68, 539–543.

Zhang, H., Datta, A.K., 2001. Electromagnetics of microwave heating: magnitude and uniformity of energy absorption in an oven. In: Datta, A.K., Anantheswaran, R.C. (Eds.), Handbook of Microwave Technology for Food Applications. Marcel Dekker, New York, pp. 1–28.

Zhao, Y., Flugstad, B., Kolbe, E., Park, J.E., Wells, J.H., 2008. Using capacitive (radio frequency) dielectric heating in food processing and preservation — a review. J. Food Process Eng. 23, 25–55.