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A THESIS FOR THE DEGREE OF MASTER OF SCIENCE

**Influence of moisture content on inactivation of
foodborne pathogens in powdered foods by radio-
frequency heating**

고주파가열을 이용한 건조분말식품의
병원성 미생물 저감화에 대한 수분함량의 영향

February, 2014

Department of Agricultural Biotechnology

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석사학위논문

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이 논문을 석사학위 논문으로 제출함

2014 년 2 월

서울대학교 대학원 농생명공학부

정 슬 기

정슬기의 석사 학위논문을 인준함

2014 년 2 월

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ABSTRACT

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. It has potential for thermal processing of powdered foods which have trouble to be sterilized with conventional heating due to their low thermal conductivities. However, commercial RF heating is rather limited due to a lack of in-depth technical information. In this study, RF heating and dielectric measurement system was developed to provide information not only about reducing thermal abuse in the food product but also about maximizing process effectiveness. The influence of moisture content during RF heating on heating rate, dielectric properties, and inactivation of foodborne pathogens was investigated. The effect of RF heating on quality of red and black pepper spice within different moisture ranges was also investigated. Red peppers (12.58%, 15.16%, 19.07%, and 23.31% dry basis moisture content, db) and black pepper (10.11%, 17.17%, 23.73%, and 30.52% db) inoculated with *Escherichia coli*

O157:H7 and *Salmonella enterica* serovar Typhimurium were treated in a RF heating system with a constant frequency of 27.12 MHz. The heating rate of the sample was dependent on moisture content up to 19.07% (db) of red peppers and 17.17% (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* to below the detection limit (1 log CFU/g) decreased and then increased again when the moisture content exceeded a level corresponding to the peak heating rate. Color values and volatile flavor components of RF treated red and black pepper spice of various moisture contents were not significantly ($P > 0.05$) different from those of nontreated samples. RF treatment significantly ($P < 0.05$) reduced moisture content of red and black pepper by up to 3.01% (db) and 4.65% (db), respectively. These results suggest that RF heating can be effectively used to not only control pathogens but also reduce moisture levels in spices

without affecting product quality and that the effect of inactivation is dependent on moisture content.

***Keywords:* Radio-frequency heating; Moisture content; Dielectric properties; Foodborne pathogen; Powdered foods**

***Student Number:* 2012-21181**

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I. 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 (Luechapattanaorn 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 sample properties affects 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 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., 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.

Red (*Capsicum* spp.) and black (*Piper nigrum*) pepper spice was selected as a model to investigate the effects of RF heating on a solid food. Because of their low moisture content, spices are non-perishable 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 spice. 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. Only few decontamination methods exist for reducing microbial loads of spices; 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 spice 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 red and black pepper spice. The effects of RF heating for inactivating *E. coli* O157:H7 and *S. Typhimurium* in red and black pepper 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.

II. 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, DT 104) 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 $4,000 \times g$ 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 spice, 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 peppers and processed black pepper 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 $10.61 \pm 0.13\%$ (dry basis, db) and $6.83 \pm 0.10\%$ (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 $22 \pm 2^\circ\text{C}$ in ziplock bags prior to any conditioning. In this study, four moisture levels each of red peppers (12.58%, 15.16%, 19.07%, and 23.31% db) and black pepper (10.11%, 17.17%, 23.73%, and 30.52% 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 red and black pepper spice in polyethylene bags at room temperature ($22 \pm 2^\circ\text{C}$), 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.

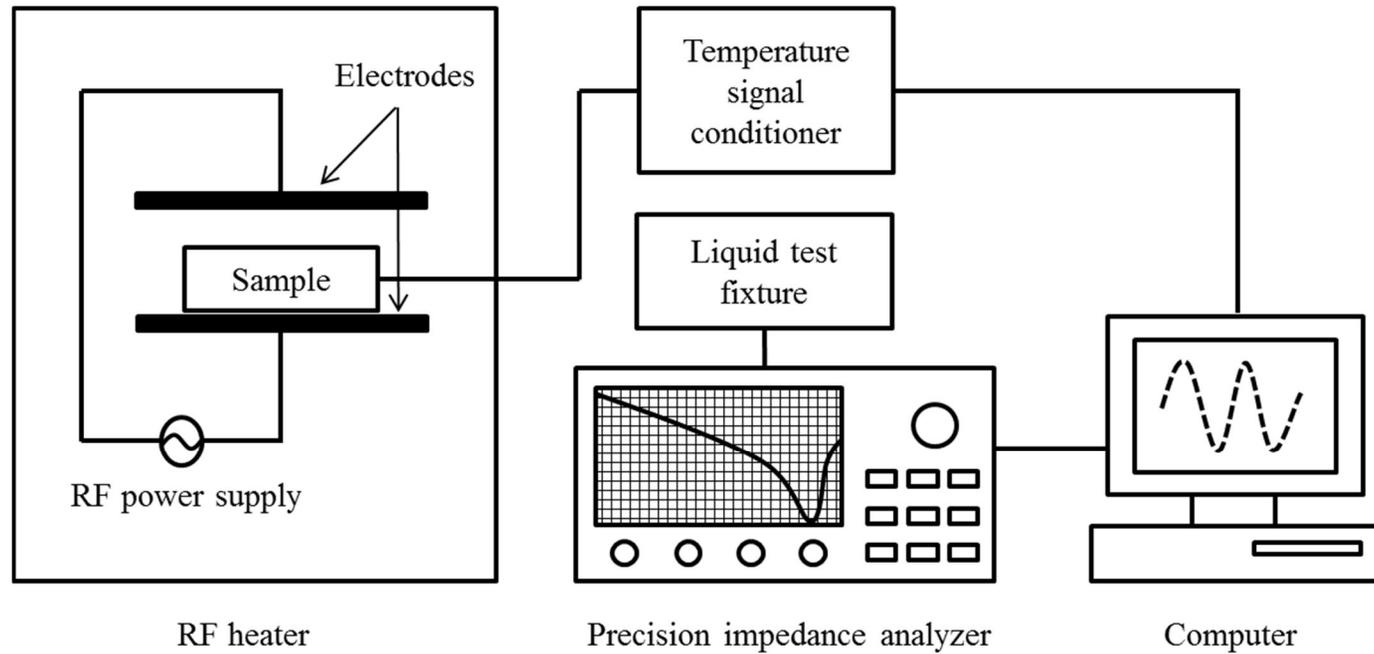


Fig. 1. Schematic diagram of RF heating and dielectric measurement system at Seoul National University (Seoul, South Korea).

2.5. RF heating treatment

For the RF heating treatment, 25 g of inoculated red and black pepper spice 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. The sensor was directly inserted at the center of the treated red and black pepper spice 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 ($22 \pm 2^\circ\text{C}$). The

dielectric properties of red and black pepper spice were calculated as follows (equation 1 and 2):

$$\varepsilon' = \frac{C_p}{C_0} \quad (1)$$

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

$$\varepsilon'' = \frac{1}{C_0 R_p \omega} \quad (2)$$

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 ($2\pi f$).

2.8. Bacterial enumeration

At selected time intervals, treated 25 g samples were immediately transferred into sterile stomacher bags (Labphas, Inc., Sainte-Julie, Quebec, Canada) containing 225 ml of 0.2% PW, which were pre-chilled 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 (Difco) and xylose lysine desoxycholate agar (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, Ogdensburg, NY) and *Salmonella* latex agglutination assay (Oxoid) for *E. coli* O157:H7 and *S. Typhimurium*, respectively.

2.9. Color and post-treatment moisture content measurement

To measure the effect of RF heating on the color of red and black pepper spice 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.

2.10. Volatile flavor component measurement

Capsaicinoids and piperine were measured as volatile flavor components in this study for red peppers and black pepper, respectively. The total capsaicinoids content was tested according to the method described by

Vincent and Ken (19). 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). One ml of the sample extract was diluted with 9 ml of distilled water and passed into a Sep-pak (WAT054945; Waters, Milford, MA) conditioned with 5 ml of acetonitrile and 5 ml of triple-distilled water applied serially. The capsaicinoids were then eluted with 4 ml of acetonitrile followed by 1 ml of acetonitrile containing 1% acetic acid. 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 C₁₈ 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. The injection volume was 20 ml, and the retention time was 10 min for capsaicin and 14 min for dihydrocapsaicin.

Piperine concentration in black pepper was also determined using HPLC set at 340 nm. The column which was the same one we used to separate the capsaicinoid in red peppers was utilized using 45% acetonitrile in 0.3% phosphate (pH 4.0) as a mobile phase. Its preparation was as follow: 3 g of monopotassium phosphate was stirred in 500 ml of triple-distilled water in a 1L beaker until completely dissolved. Acetonitrile (450 ml) was added to the mixture and pH adjusted to 4.0 with acetic acid. The solution was transferred to a 1 L volumetric flask, made up to volume with triple-distilled water, filtered using a 0.45 mm pore size membrane filter (Sartorius, Goettingen, Germany), and degassed via vacuum before being applied to the column. A flow rate of 1.5 ml/min was used, and the retention time was 14 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-ml portion of filtrate was injected into the column using the HPLC autosampler.

2.11. Statistical analysis

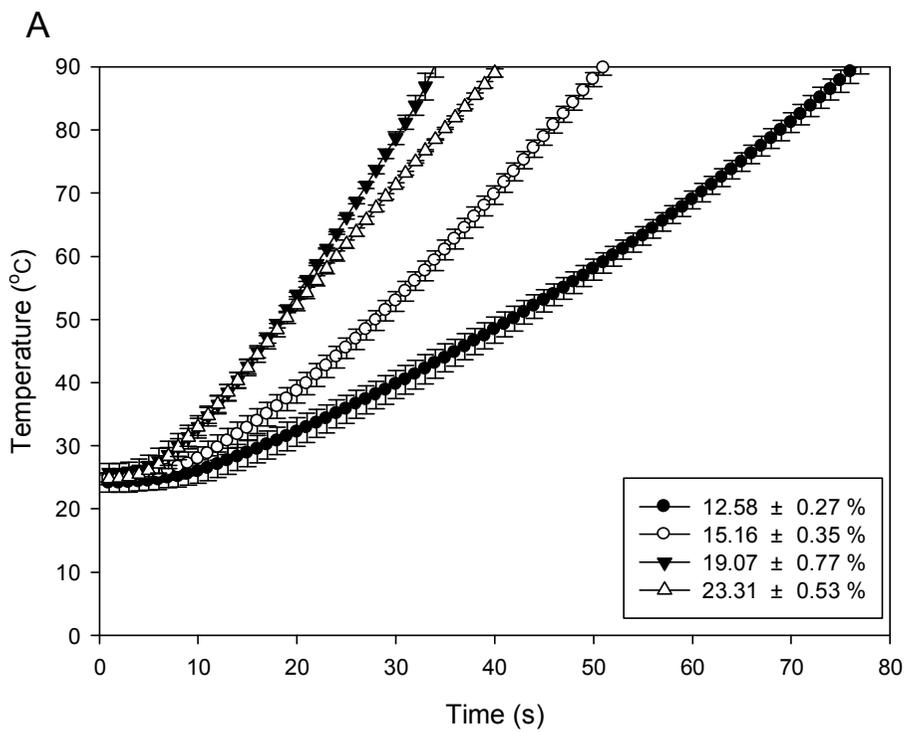
All experiments were repeated three times with duplicate samples. Data were analyzed by the analysis of variance procedure of Statistical Analysis System (SAS Institute, Cary, NC), and mean values were separated using Duncan's multiple-range test. $P < 0.05$ was used to determine significant differences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

III. RESULTS

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

Average temperatures of red peppers and black pepper of various moisture contents, respectively, from 12.58% to 23.31% (db), and 10.11% to 30.52% (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 19.07% (dry basis, db) for red peppers and 17.17% (db) for black pepper, but there was a significant decrease ($P < 0.05$) when moisture content exceeded the above levels. Red peppers with 19.07% (db) and black pepper spice with 17.17% (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.



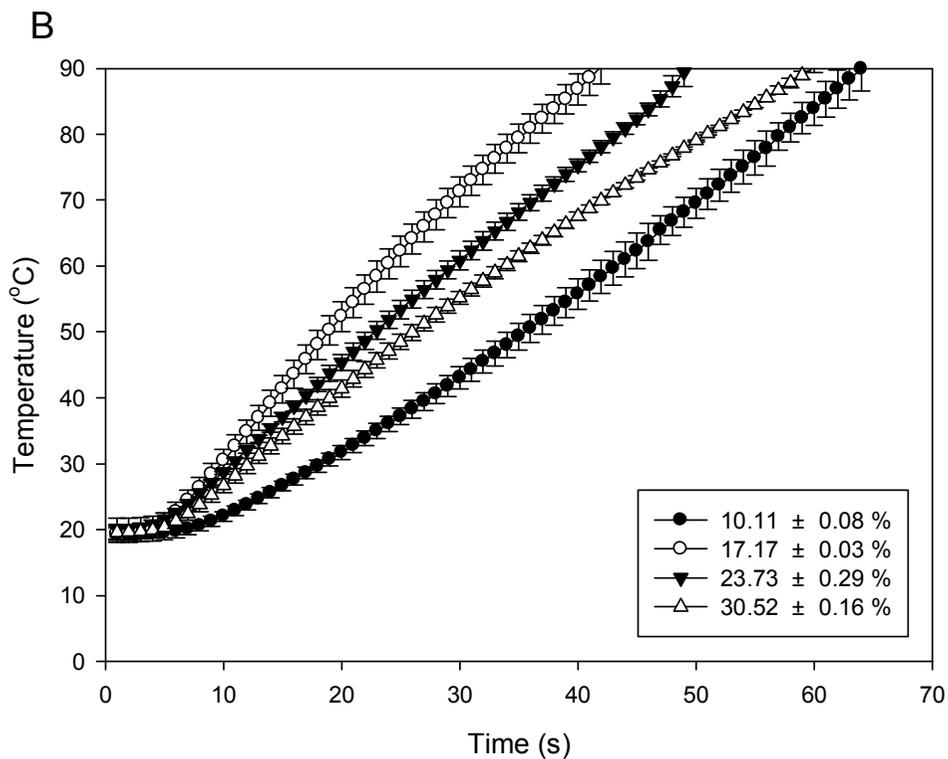


Fig. 2. Temperature curves of red peppers (A) and black pepper (B) at controlled moisture levels during RF heating. All moisture contents are expressed on a dry basis. The results are means from three experiments, and error bars indicate standard deviations.

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

The dielectric properties of conditioned red pepper and black pepper spice 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 peppers significantly ($P < 0.05$) increased with increasing moisture content from 12.58% to 23.31% (db). Similar patterns of the effect of moisture content were obtained for black pepper in the range of 10.11% to 30.52% (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 peppers. 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.

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

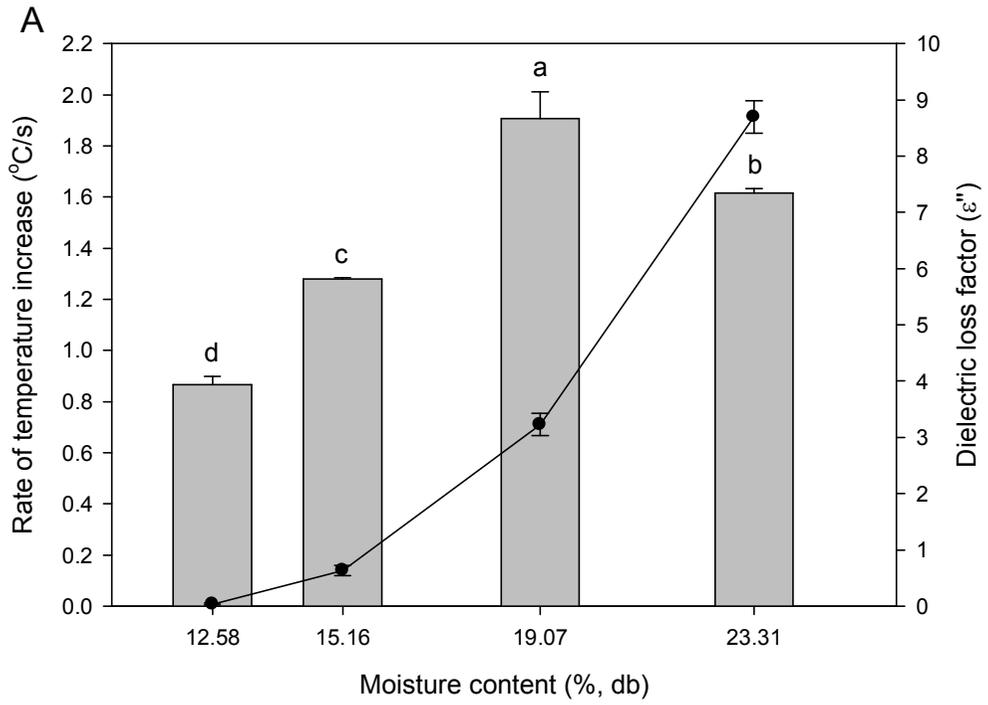
Moisture Content (% db)	Dielectric properties	
	ϵ' ^b	ϵ''
Red pepper		
12.58	3.17 ± 0.14 d	0.03 ± 0.02 d
15.16	4.64 ± 0.30 c	0.63 ± 0.09 c
19.07	8.78 ± 0.28 b	3.23 ± 0.20 b
23.31	13.80 ± 0.28 a	8.69 ± 0.29 a
Black pepper		
10.11	1.72 ± 0.03 d	0.02 ± 0.00 d
17.17	4.45 ± 0.32 c	1.53 ± 0.22 c
23.73	11.11 ± 0.21 b	10.58 ± 0.56 b
30.52	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.

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

The results of the rate of temperature increase, dielectric loss factor, and moisture contents of 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. 19.07% (db) for red peppers and 17.17% (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 peppers and black pepper, respectively.



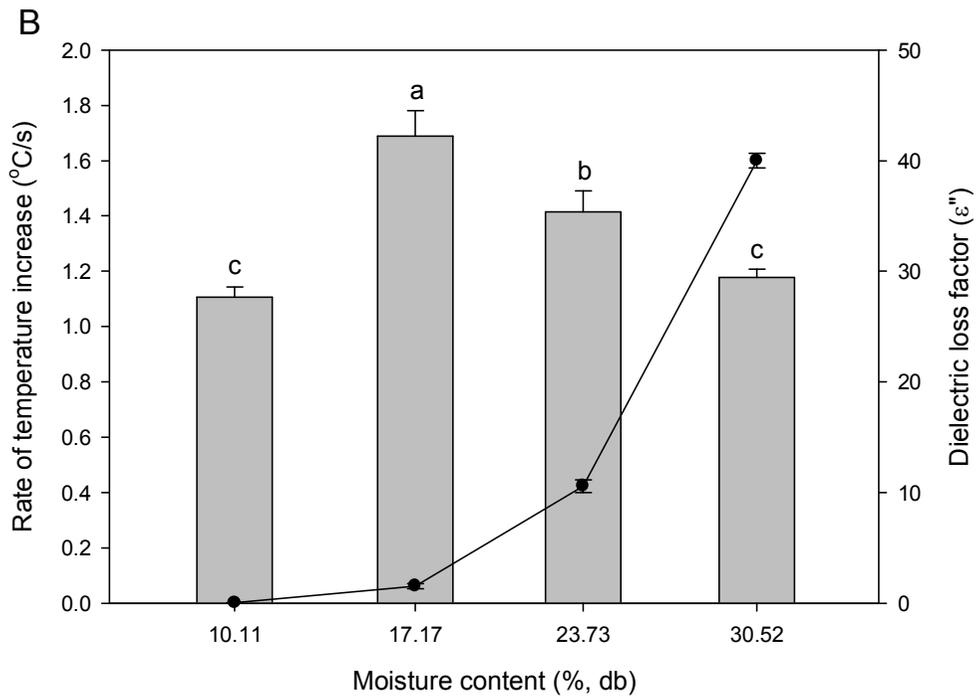


Fig. 3. Relationship between rate of temperature increase and dielectric loss factor of red peppers (A) and black pepper (B) at controlled moisture levels during RF heating. The results are means from three experiments, and error bars indicate standard deviations. ■, rate of temperature increase; ●,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

† Different letters between rates of temperature increase indicate significant differences ($P < 0.05$).

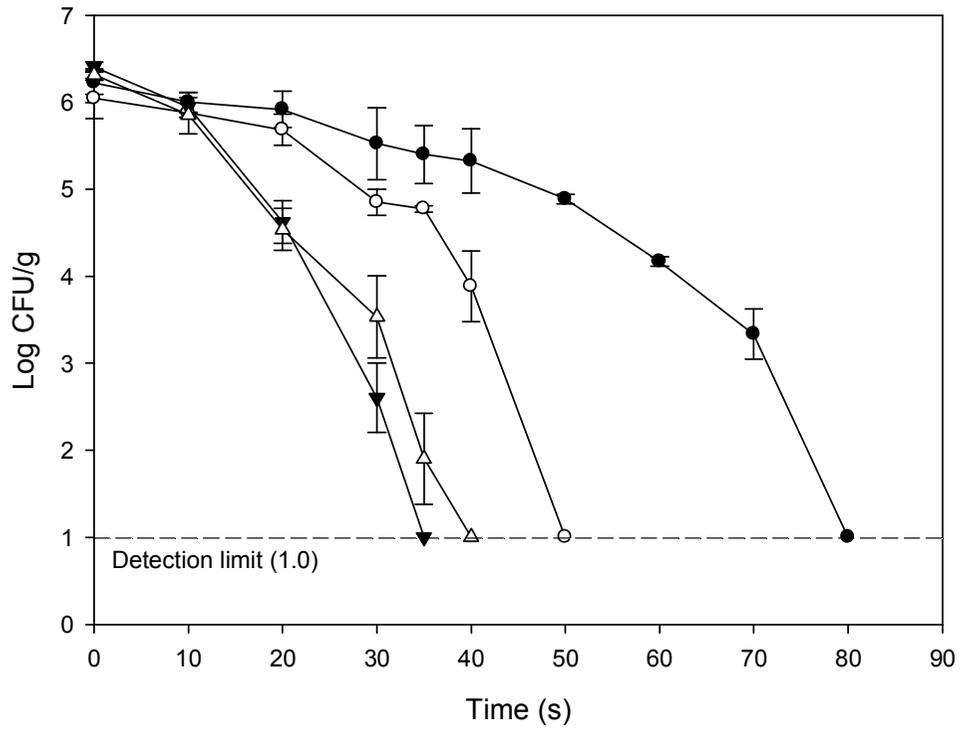
3.4. Influence of moisture content on inactivation of foodborne pathogens in red and black pepper spice

Populations (log CFU/g) of *E. coli* O157:H7 and *S. Typhimurium* in red peppers during RF heating are depicted in Fig. 4. As the moisture content increased from 12.58% to 19.07% (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 19.07% (db). The levels of surviving cells of both pathogens were reduced to below the detection limit within 35 s at a moisture content of 19.07% (db). At 23.31% (db), levels of *E. coli* O157:H7 experienced a significant reduction of 4.41 log CFU/g after 35 s and a > 5.32-log reduction to below the detection limit after 40 s of treatment. Cell numbers of *S. Typhimurium* were reduced by 2.26 log CFU/g after 35 s and to below the detection limit after 40 s of treatment. At 15.16% (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 peppers were greatly reduced to undetectable levels after 80 s at 12.58% (db).

Figure 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 peppers. RF heating at 17.17% (db) moisture content reduced both pathogens to below the detection limit after 40 s. At 23.73% and 30.52% (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 peppers. Populations of *E. coli* O157:H7 and *S. Typhimurium* decreased by 5.91 log CFU/g and 6.20 log CFU/g after 60 s, respectively.

A



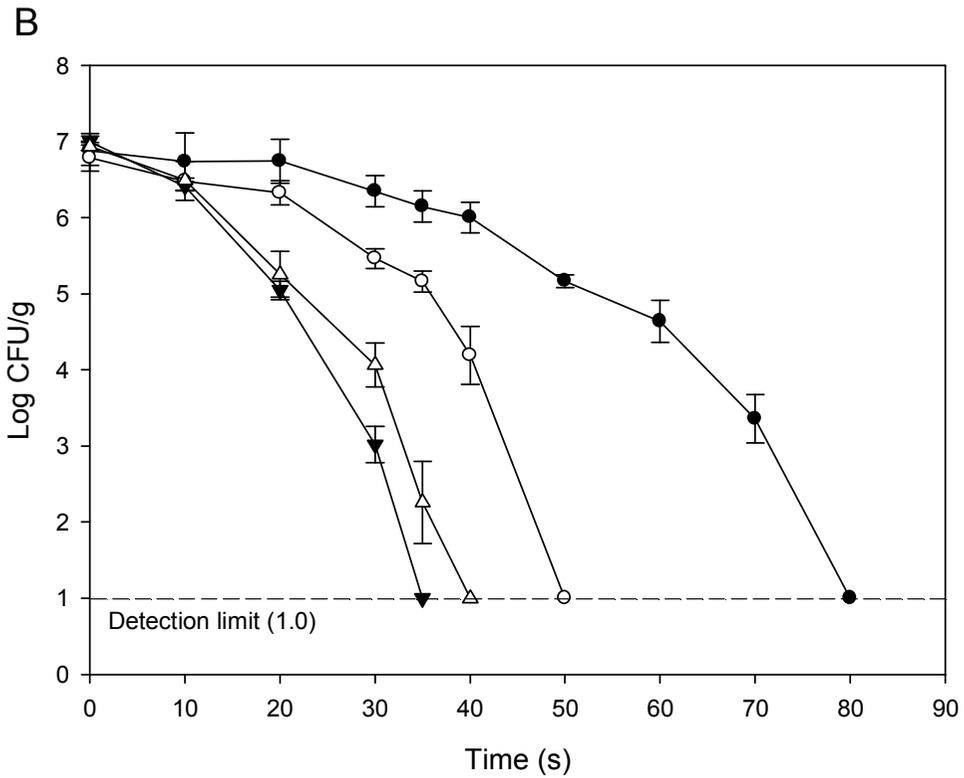
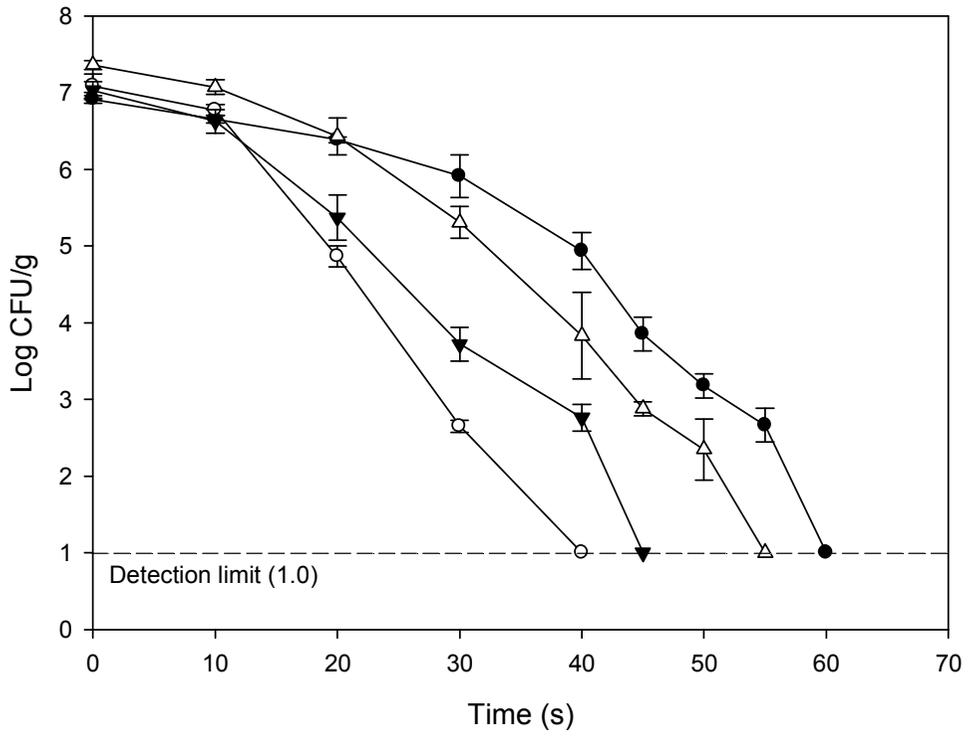


Fig. 4. Survival curves for *Escherichia coli* O157:H7 (A) and *Salmonella* Typhimurium (B) on red peppers with moisture contents of 12.58% (●), 15.16% (○), 19.07% (▼), and 23.31% (△) during RF heating. All moisture contents are expressed on a dry basis. The results are means from three experiments, and error bars indicate standard deviations.

A



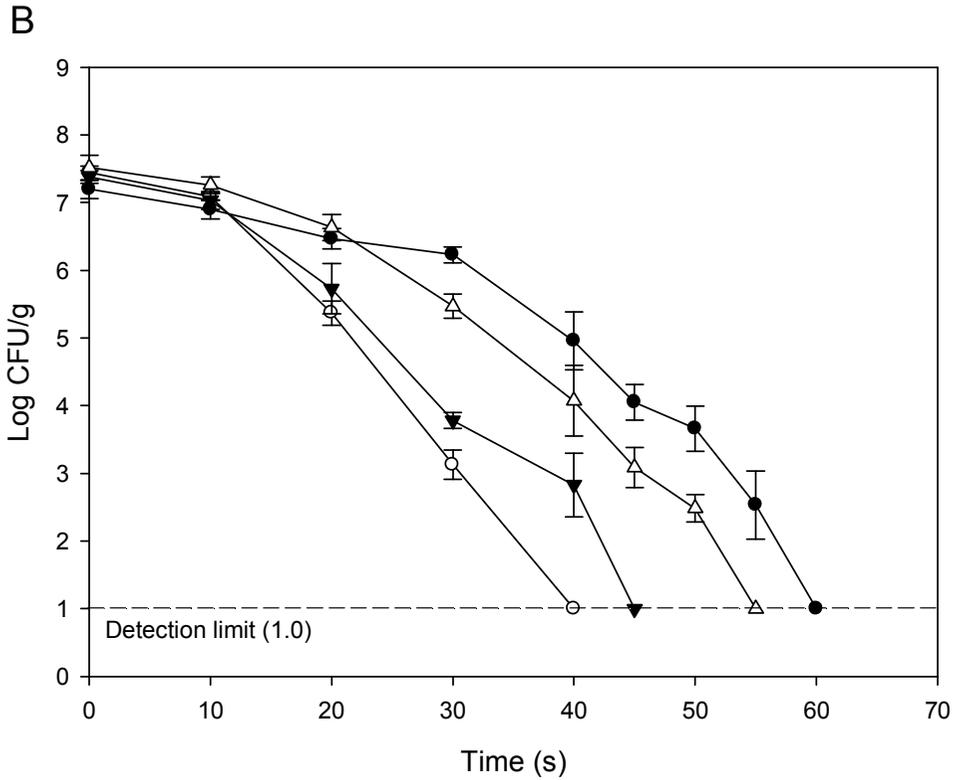


Fig. 5. Survival curves for *Escherichia coli* O157:H7 (A) and *Salmonella* Typhimurium (B) on black pepper with moisture contents of 10.11% (●), 17.17% (○), 23.73% (▼), and 30.52% (△) during RF heating. All moisture contents are expressed on a dry basis. The results are means from three experiments, and error bars indicate standard deviations.

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

The color and volatile flavor components (capsaicinoids for red peppers and piperine for black peppers) 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. 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 red and black pepper spice of differing moisture content ($P > 0.05$). Table 4 shows the moisture content of

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 peppers and black pepper subjected to RF heating was reduced by 2% to 18% and 12% to 27% (Table 4), respectively. Except for red peppers of 12.58% db, all samples of different moisture levels experienced significantly drying ($P < 0.05$).

Table 2. Color values and capsaicinoids content of treated and untreated red peppers of varying moisture levels subjected to RF heating^a

Moisture content (%) ^b	Treatment time (s)	Color ^c			Capsaicinoids (mg/100g)		
		L [*]	a [*]	b [*]	CAP ^d	DHC ^e	Total ^f
12.58	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
15.16	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
19.07	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
23.31	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 CAP; capsaicin

^e DHC; dihydrocapsaicin

^f Total capsaicinoids; capsaicin + dihydrocapsaicin

Table 3. Color values and piperines content of treated and untreated black pepper of varying moisture levels subjected to RF heating^a

Moisture content (%) ^b	Treatment time (s)	Color ^c			Piperine (mg/100g)
		L*	a*	b*	
10.11	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
17.17	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
23.73	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
30.52	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 peppers and black pepper before and after RF heating^a

Product	Moisture content (% db)	
	Control	RF treated
Red pepper	12.58 ± 0.27 a	12.37 ± 0.11 a
	15.16 ± 0.35 a	14.01 ± 0.13 b
	19.07 ± 0.77 a	17.45 ± 0.13 b
	23.31 ± 0.53 a	20.30 ± 0.24 b
Black pepper	10.11 ± 0.08 a	5.60 ± 0.33 b
	17.17 ± 0.03 a	12.52 ± 0.34 b
	23.73 ± 0.29 a	21.03 ± 0.22 b
	30.52 ± 0.16 a	27.71 ± 0.07 b

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

IV. DISCUSSION

The objectives of the present study were to examine the influence of moisture content of red peppers and black pepper 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 this 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 (Galama, 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 $P = 2\pi fV^2\varepsilon_0\varepsilon''$, 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 red and black pepper spice resulting from higher moisture levels. However, above threshold dielectric loss factors which are 3.23 at 19.07% (db) for red peppers and 1.53 at 17.17% (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 red and black pepper had a profound effect on inactivating *E. coli* O157:H7 and *S. Typhimurium*. At a moisture content of 12.58% (db), 80 s was required to reduce the levels of *E. coli* O157:H7 and *S. Typhimurium* in red peppers to undetectable levels, but only 35 s was required at 19.07% (db). As for black pepper, 60 and 40 s were required to reduce both pathogens to below the detection limit (1 log/CFU) at 10.11% (db) and 17.17% (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 peppers and black pepper. 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 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 red and black pepper spice 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 12.58% (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 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. Red and black pepper spice 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 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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VI. 국문초록

고주파가열은 식품에 교류전류를 통과시켜 식품 분자의 진동에 의해 식품 내부에서 급속하게 열을 발생시키는 기술이다. 고주파가열 기술은 낮은 열 전도도로 인해 기존 열처리 방법으로 살균이 어려운 건조분말식품에 효과적으로 적용할 수 있다. 그러나 아직까지 고주파가열 기술에 대한 연구가 미비하기 때문에 상업적 살균 시스템에 적용하는 데 한계가 있다. 본 연구에서는 식품의 열 손상을 최소화하고 가공 효과를 최대화하는 처리 조건을 찾고자 고주파가열 시스템과 유전율 측정 시스템을 구축하였다. 고춧가루와 후춧가루의 고주파가열에 있어 수분함량이 가열 속도, 유전율, 식품 유래 병원성균의 저감화에 미치는 영향에 대한 연구를 수행하였다. 또한, 다양한 수분함량의 고춧가루와 후춧가루에 고주파가열을 처리했을 때의 품질 변화를 연구하였다.

주파수를 27.12 MHz 로 고정한 상태에서, *Escherichia coli* O157:H7 와 *Salmonella* Typhimurium 이 접종된 다양한 수분함량의 고춧가루 (12.58%, 15.16%, 19.07%, 23.31%)와 후춧가루 (10.11%, 17.17%, 23.73%, 30.52%)에 고주파가열을 처리하였다. 수분함량이 증가함에 따라 샘플의 가열 속도가 증가하였지만, 고춧가루의 경우에는 19.07%, 후춧가루의 경우에는 17.17% 이상에서는 가열 속도가 유의적으로 감소하였다. 수분함량이 증가함에 따라 고춧가루와 후춧가루의 유전율이 증가하였다. 수분함량이 증가함에 따라 *E. coli* O157:H7 과 *S. Typhimurium* 을 검출 한계 (1 log CFU/g) 이하로 저감화하는 데 필요로 하는 시간이 감소하다가 고춧가루의 경우에는 19.07%, 후춧가루의 경우에는 17.17% 이상에서는 다시 증가하였다. 고주파가열 처리 후 시료의 색과 휘발성 향미 성분 변화는 유의적인 차이를 나타내지 않았다 ($P > 0.05$). 고주파가열 처리는 고춧가루 및 후춧가루에서 각각 수분함량을 최대 3.01% 및 4.65% 감소시켰다 ($P < 0.05$). 이러한

결과는 고주파가열 기술이 건조분말식품의 품질 손상 없이 병원성균을 저감화함과 동시에 건조하는 데 효과적으로 이용될 수 있음을 보여주고, 병원성균의 저감화 효과는 수분함량에 영향을 받음을 나타낸다.

주요어: 고주파가열, 수분함량, 유전율, 식중독균, 건조분말식품

학번: 2012-21181