



Radio-frequency heating to inactivate *Salmonella* Typhimurium and *Escherichia coli* O157:H7 on black and red pepper spice

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ABSTRACT

The efficacy of radio-frequency (RF) heating to inactivate *Salmonella* Typhimurium and *Escherichia coli* O157:H7 on black and red pepper spice was investigated. A 27.12 MHz RF heating system consisted of two parallel-plate electrodes was used, with the sample being placed between them. Black peppers (whole and ground) and red peppers (+16 mesh, −16 +25 mesh, and −25 mesh) inoculated with *S. Typhimurium* and *E. coli* O157:H7 were treated with RF energy during 50 s for black peppers and 40 s for red peppers, and color change of samples was evaluated after treatment. RF heating for 50 s resulted in 2.80 to 4.29 log CFU/g reductions of *S. Typhimurium* and *E. coli* O157:H7 in black peppers and RF heating of red peppers for 40 s reduced pathogens by 3.38 log CFU/g to more than 5 log CFU/g (below the detection limit) without affecting the color quality change. The results suggest that RF heating has the potential for novel thermal process to control foodborne pathogens in spice.

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1. Introduction

Foodborne pathogens such as *Salmonella* Typhimurium and *Escherichia coli* O157:H7 are recognized as a major cause of foodborne outbreaks. *S. Typhimurium* is the most commonly isolated *Salmonella* serotype, and the symptoms of infection are diarrhea, abdominal pain, mild fever, and chills (Baird-Parker, 1990; Rhee et al., 2003). *E. coli* O157:H7 has emerged as an important foodborne pathogen, causing hemorrhagic colitis and hemolytic uremic syndrome (Doyle, 1991; Griffin and Tauxe, 1991; Wang et al., 1996). These pathogens were frequently found in animal manures and could be an important source of contamination of agricultural commodities grown in fields (Islam et al., 2004; Wang et al., 1996).

Spices have been used as flavoring and seasoning agents in foods. However, they are of natural origin and may be burdened with high levels of molds, yeasts, and bacteria (Rico et al., 2010; Schweiggert et al., 2007). Contaminated spices can lead to a rapid spoilage of the foods and a serious foodborne illness when they are added to foods that do not undergo further cooking (Rico et al., 2010; Waje et al., 2008). In 2009, a multistate outbreak of *Salmonella* Montevideo infections occurred in USA which was traced to salami products made with contaminated black and red pepper spice (CDC, 2010). By the April

2010, a reported 272 persons in 44 states and DC had become ill due to the outbreak (CDC, 2010).

Several decontamination methods have been developed to reduce the microbial loads of spices, such as fumigation with ethylene oxide, irradiation, and steam treatment. However, use of ethylene oxide which is generally regarded as a carcinogen is restricted and even banned in the European Union, and irradiation has not found acceptance by the consumer although it is allowed for the decontamination of dried spices in numerous countries (Farkas, 2006; Schweiggert et al., 2007). Waje et al. (2008) reported that commercial steam treatment (at 1020 mbar and 100 °C for 16 min) of black pepper led to a quality deterioration with significant loss of color and flavor. Accordingly, there is a need for the development of innovative technologies for disinfecting spices while maintaining the quality.

Radio-frequency (RF) heating implies the use of electromagnetic energy at frequencies between 1 and 300 MHz to generate heat in a dielectric material (Orfeuill, 1987). Recently, RF heating in food processing offers the possibility to replace the conventional method of heat processing (Pereira and Vicente, 2009). There have been several researches implicating RF heating as a new thermal treatment method for nuts (Gao et al., 2010; 2011; Wang et al., 2001) and fruits (Casals et al., 2010; Ikediala et al., 2002; Wang et al., 2006). In conventional heating, produced heat is transferred from the exterior of the food to the interior by convection or conduction (Doores, 2002). On the contrary, RF generates heat within the food and throughout its mass due to the friction resulting from the rotational movement of the molecules and the space charge displacement in response to

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an alternating electric field (Orsat et al., 2004; Zhao, 2000). Thus, RF heating has the potential for fast heating in solid and semi-solid foods which have low thermal conductivities (Casals et al., 2010; Luechapattananorn et al., 2005). In addition, RF heating offers advantages of more uniform heating than microwave heating due to its lower frequencies (13.56, 27.12, and 40.68 MHz), longer wave lengths, and deep penetration depth compared with those of microwaves at 915 or 2450 MHz (Luechapattananorn et al., 2005; Marra et al., 2009), but is not completely homogeneous (Tang et al., 2000).

The objectives of this study were to evaluate the efficacy of RF heating to inactivate *S. Typhimurium* and *E. coli* O157:H7 on black and red pepper spice and determine the effect of RF heating on the quality of spices by color measurements.

2. Materials and methods

2.1. Cultures and cell suspension

Three strains each of *S. Typhimurium* (ATCC 19586, ATCC 43174, DT 104) and *E. coli* O157:H7 (ATCC 35150, ATCC 43889, ATCC 43890) were obtained from the bacterial culture collection of Seoul National University (Seoul, Korea). Each strain of *S. Typhimurium* and *E. coli* O157:H7 was cultured in tryptic soy broth (Difco, Becton Dickinson, Sparks, MD, USA) at 37 °C for 24 h, harvested by centrifugation at 4000×g for 20 min at 4 °C, and washed three times with buffered peptone water (BPW; Difco). The final pellets were resuspended in BPW, corresponding to approximately 10⁷ to 10⁸ CFU/ml. Subsequently, suspended pellet of each species of the two pathogens were mixed to produce culture cocktails. These culture cocktails at a final concentration of approximately 10⁸ CFU/ml were used in this study.

2.2. Sample preparation and inoculation

Commercially processed black peppers and dried red peppers were purchased at a local grocery store (Seoul, Korea). Two types of black pepper, whole (−5 + 6 mesh) and ground (−20 + 40 mesh), and three types of dried red pepper (+ 16 mesh, −16 + 25 mesh, and −25 mesh) divided by differences in particle size determined using a sieve shaker (Chung Gye Industrial Mfg., Co., Gyeonggi, Korea) were used. For inoculation, 0.3 ml of the culture cocktail was applied to each 25 g of samples filled about 1 cm in glass beakers (7.5 cm in diameter and 10.0 cm deep). The inoculated samples were mixed to ensure even distribution of the pathogens and dried for 1 h inside a biosafety hood (22 ± 2 °C) (Akbas and Ozdemir, 2008; Emer et al., 2008).

2.3. RF heating system

RF heating system (Fig. 1) with a maximum power of 9 kW at the frequency of 27.12 MHz was used in this study. This system was developed and constructed at Seoul National University (Seoul, Korea) and Dong Young Engineering Co., Ltd. (Gyeongnam, Korea). The RF electric field was generated between two parallel-plate electrodes (30.0 × 35.0 cm; 0.6 cm thick) and the distance between the two electrodes was 11.0 cm. A sample filled 1 cm in glass beaker was placed on the center of the bottom electrode. The RF treatments consisted of 50 s treatments on black peppers and 40 s treatments on dried red peppers in order to achieve the sample temperatures of 60 °C while maintaining the color quality. Non-treated sample was used as the control. All experiments were repeated three times.

2.4. Temperature measurement

Fiber optic temperature sensor (FOT-L, FISO Technologies Inc., Quebec, Canada) connected to a signal conditioner (FTI-10, FISO Technologies



Fig. 1. Radio-frequency (27.12 MHz) heating system at Seoul National University (Seoul, Korea).

Inc., Quebec, Canada) was used to measure real-time temperatures in samples during RF heating. The sensor was directly inserted 1 cm inside the core and perimeter of the treated sample and the temperature was manually recorded every 5 s. Since fiber optic sensor is consisted of electric insulating material, it does not interfere with the temperature profile of the treated sample (Wang et al., 2003). Average and standard deviation values of RF treated sample temperatures at the core and perimeter were compared to determine the heating rate of samples. All experiments were repeated three times.

2.5. Microbial enumeration

At the selected time intervals, each treated 25 g of black and red pepper were immediately transferred into sterile stomacher bags (Labplas Inc., Sainte-Julie, Quebec, Canada) containing 225 ml of BPW and homogenized for 2 min with a stomacher (EASY MIX, AES Chemunex, Rennes, France). After homogenization, 1 ml aliquots of sample were serially diluted in 9 ml of BPW, and 0.1 ml of sample or diluent was spread-plated onto each selective medium. Xylose Lysine Desoxycholate agar (Difco) and Sorbitol MacConkey agar (Difco) were used as selective media for the enumeration of *S. Typhimurium* and *E. coli* O157:H7, respectively. Where low levels of surviving cells were anticipated, 1 ml of undiluted stomacher bag contents was equally distributed into four plates of each medium and spread-plated. All agar media were incubated at 37 °C for 24 h and colonies were counted.

2.6. Color measurement

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

2.7. Statistical analysis

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

3. Results

Average temperatures of core and perimeter of whole and ground black pepper during treatment with 27.12 MHz RF energy are shown in Fig. 2. Temperatures of both samples rapidly increased with increasing treatment time, and there were no significant differences ($P \geq 0.05$) between the average heating rate of ground black pepper and that of whole black pepper. After 50 s of RF heating, each sample reached 67 and 62 °C, respectively.

Fig. 3 shows average temperatures of three types of dried red pepper (+16 mesh, -16+25 mesh, and -25 mesh) during RF treatment. The average heating rates were significantly different ($P \leq 0.05$) in each of dried red peppers, +16 mesh sample was heated at the most rapid rate, followed by -16+25 mesh and -25 mesh. Three types of dried red pepper had higher heating rates than whole and ground black pepper at all temperatures. After 40 s of treatment, each sample reached 79, 70, and 57 °C, respectively.

The survival of *S. Typhimurium* and *E. coli* O157:H7 in black peppers during 50 s of RF heating is shown in Fig. 4. Survival of both pathogens decreased with increasing treatment time. For *S. Typhimurium* (Fig. 4a), there were no significant reductions ($P > 0.05$) in microbial levels for 20 s regardless of sample types compared to the control. However, treatment for 30 s significantly reduced ($P < 0.05$) levels of this pathogen by 1.28 and 1.97 log CFU/g in whole and ground black pepper, respectively. After 50 s of RF treatment, levels of *S. Typhimurium* were greatly reduced by 3.18 and 4.29 log CFU/g in whole and ground black pepper, respectively. Reduction patterns of *E. coli* O157:H7 in black peppers were similar to those of *S. Typhimurium* (Fig. 4b). After 50 s of treatment, levels of *E. coli* O157:H7 were reduced by 2.80 and 3.74 log CFU/g in whole and ground black pepper, respectively.

The survival of *S. Typhimurium* and *E. coli* O157:H7 in dried red peppers during 40 s of RF heating is shown in Fig. 5. For *S. Typhimurium* (Fig. 5a), there were no significant differences ($P > 0.05$) in microbial levels between control and 20 s of treatment regardless of sample types. After 30 s, each significant reduction ($P < 0.05$) of 2.55 and 1.56 log CFU/g were observed in +16 mesh and -16+25 mesh samples, and there was not a significant reduction ($P > 0.05$) of 0.63 log CFU/g in -25 mesh sample. Treatment for 40 s significantly ($P < 0.05$) reduced levels of *S. Typhimurium* by 3.38 log CFU/g in -25 mesh and to below the detection limit (1 log) in +16 mesh and -16+25 mesh. *E. coli* O157:H7 in dried red peppers (Fig. 5b) presented similar reduction patterns to *S. Typhimurium*, and were reduced by 3.50 log CFU/g in -25 mesh and to below the detection limit in +16 mesh and -16+25 mesh after 40 s.

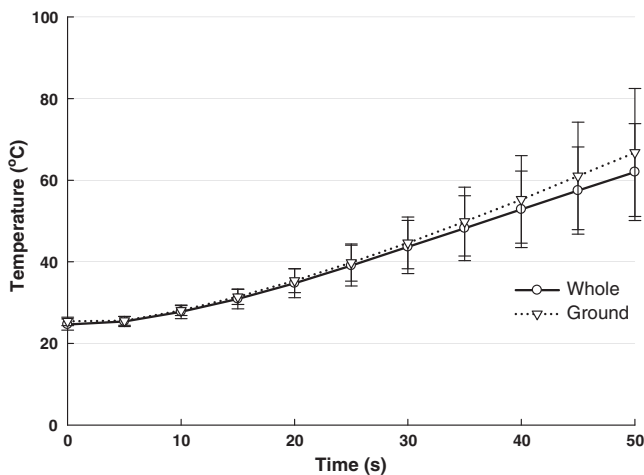


Fig. 2. Average temperature–time histories of core and perimeter of black peppers when subjected to 27.12 MHz RF energy.

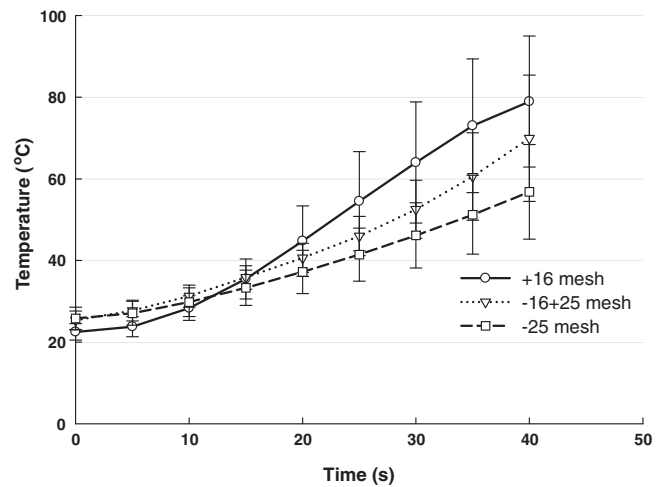


Fig. 3. Average temperature–time histories of core and perimeter of dried red peppers when subjected to 27.12 MHz RF energy.

Color values of black and red pepper after RF treatment are summarized in Table 1. L^* , a^* , and b^* values of RF treated samples were not significantly different ($P > 0.05$) to that of non-treated samples.

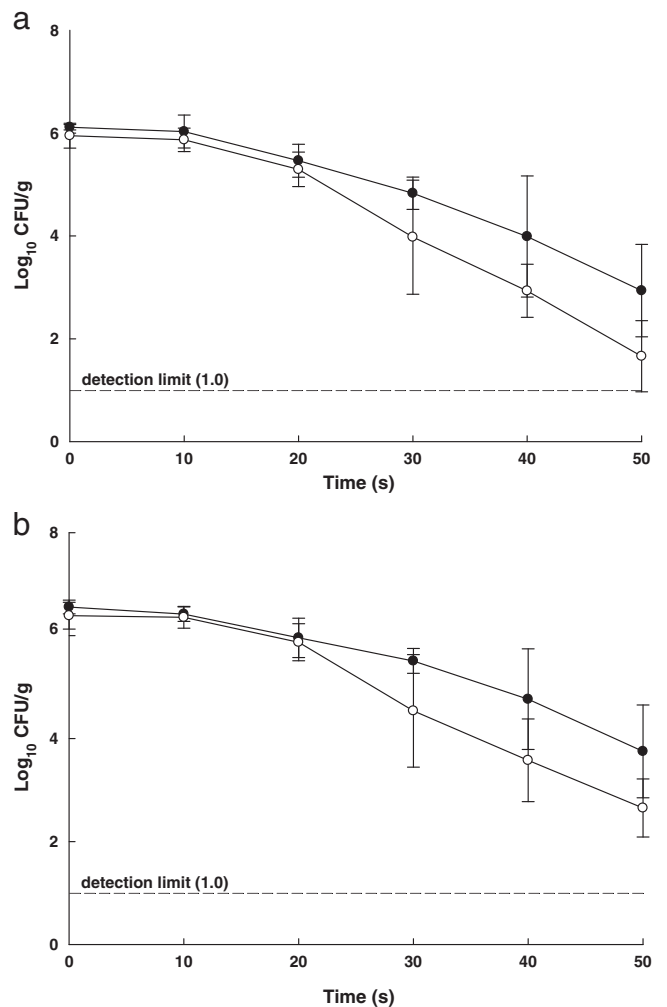


Fig. 4. Survival curves for (a) *S. Typhimurium* and (b) *E. coli* O157:H7 on black peppers during RF heat treatment. Error bars indicate a 95% confidence interval. ●, whole; ○, ground.

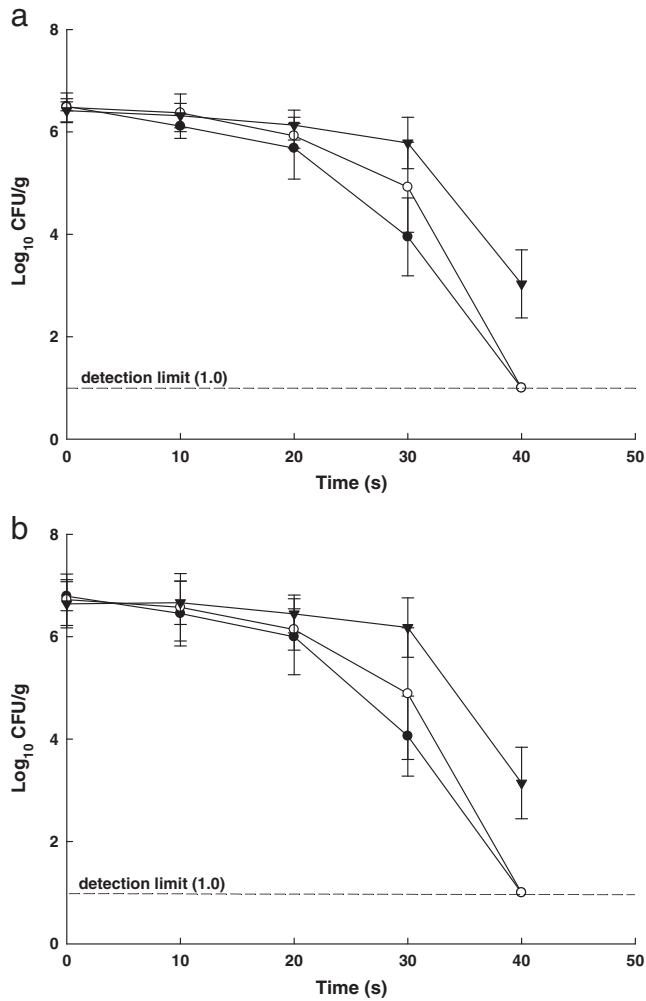


Fig. 5. Survival curves for (a) *S. Typhimurium* and (b) *E. coli* O157:H7 on dried red peppers during RF heat treatment. Error bars indicate a 95% confidence interval. ●, +16 mesh; ○, -16+25 mesh; ▼, -25 mesh.

4. Discussion

With the direct interaction between RF energy and the material, heat is generated within the material and throughout its mass. This can significantly increase heating rates and reduce heating time (Tang and Wang, 2007). Although thermal process based on RF energy has great potential as a food pasteurization and sterilization technique, there are limited studies of effectiveness of RF heating on pathogenic microorganisms in foods. In the present study, RF treatment rapidly increased the temperatures of black and red pepper high enough to inactivate pathogens. RF treatment for two types of

sample (+16 mesh and -16+25 mesh) among all five types could meet the requirements to achieve 5-log reduction of *S. Typhimurium* and *E. coli* O157:H7 without affecting the color quality change.

When black and red peppers were treated with RF, increased heating rates correlated with increased reduction level of pathogens in sample. RF treatment causes microbial inactivation predominantly through thermal effects, such as denaturation of enzymes, proteins, nucleic acids, or other vital components, as well as disruption of membranes (Datta and Davidson, 2000; Heddleson and Doores, 1994). Geveke et al. (2002) applied RF energy at 18 MHz and an electric field strength of 0.5 kV/cm to the liquids (apple cider, beer, deionized water, liquid whole egg, and tomato juice) containing *E. coli* K-12, *Listeria innocua*, or yeast while heat was simultaneously removed to control temperature. They concluded that there was no evidence to non-thermal effects of RF energy on microbial inactivation.

However, RF heating is a complex physical process, thus heating rates were different through each kind of sample. Heating rate during RF treatment is affected by the food composition, the size of the material, its salt content, its moisture content, its density, its temperature and a few other factors (Orsat and Raghavan, 2005). There is a need to fully understand the influence of various factors on heating rates in materials to maximize the effectiveness of RF heating.

Numerous studies have been performed to investigate the effect of microwave heating on pathogenic microorganisms in foods (Datta and Davidson, 2000). Foodborne pathogens such as *Bacillus cereus*, *Campylobacter jejuni*, *Clostridium perfringens*, pathogenic *E. coli*, *Listeria monocytogenes*, *Staphylococcus aureus*, and *Salmonella* were reported to be inactivated by microwave heating (Chipley, 1980; Datta and Davidson, 2000; Heddleson and Doores, 1994; Heddleson et al., 1994; Knutson et al., 1987; Rosenberg and Bogl, 1987). As a potential pasteurization and sterilization technique, more work needs to be published on the effectiveness of RF for inactivating microorganisms and its impact on product quality and shelf life (Marra et al., 2009).

RF heating has been used in various industrial applications for many years (Orsat and Raghavan, 2005). There is a definite advantage in using RF heat in dried commodities, especially in spice. Since electromagnetic energy tends to act on the water and aqueous ions in spice, RF heating brings a significant performance increase to a heating rate with a drying process (Marshall and Metaxas, 1999; Orsat and Raghavan, 2005; Tang and Wang, 2007). In conclusion, this study indicates that the RF heating is highly efficacious in reducing foodborne pathogens on black and red pepper spice without affecting the color quality change. RF treatment could be applied to control of foodborne pathogens in spice over conventional decontamination methods.

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Table 1
Color values^a of RF treated black pepper and red pepper.

Parameter ^b	Treatment time (s)	Black pepper		Treatment time (s)	Red pepper		
		Whole	Ground		+16 mesh	-16+25 mesh	-25 mesh
L*	0	20.05 ± 0.64 a	42.66 ± 1.88 a	0	41.03 ± 0.62 a	41.48 ± 0.11 a	44.78 ± 0.67 a
	50	17.80 ± 1.82 a	41.60 ± 0.79 a	40	41.04 ± 0.34 a	41.56 ± 0.19 a	44.90 ± 0.29 a
a*	0	5.10 ± 1.06 a	2.58 ± 0.39 a	0	14.71 ± 1.49 a	15.64 ± 0.54 a	22.89 ± 0.56 a
	50	4.40 ± 1.23 a	2.56 ± 0.10 a	40	13.85 ± 0.77 a	15.71 ± 0.25 a	22.71 ± 0.16 a
b*	0	8.87 ± 1.73 a	16.98 ± 1.33 a	0	13.19 ± 1.25 a	13.44 ± 0.35 a	20.62 ± 0.29 a
	50	7.29 ± 1.63 a	17.02 ± 0.78 a	40	12.71 ± 0.68 a	13.52 ± 0.31 a	20.54 ± 0.45 a

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

^b Color parameters are L* (lightness), a* (redness), and b* (yellowness).

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