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Radiofrequency pasteurization process for inactivation of *Salmonella* spp. and *Enterococcus faecium* NRRL B-2354 on ground black pepper

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ABSTRACT

Salmonella persistence in ground black pepper has caused several foodborne outbreaks and created public concern about the safety of low water activity (a_w) foods. In this study, radiofrequency (RF) processing was evaluated for pasteurization of ground black pepper. Stability and homogeneity tests were done for both *Salmonella* spp. and *E. faecium* during moisture equilibration before RF heating to evaluate the inoculation method. Moisture content of samples were conditioned such that the final moisture content after RF heating reached the optimal storage moisture. RF heating was shown to provide more than 5.98 log CFU/g reduction for *Salmonella* spp. and the reduction of 3.89 log CFU/g for *E. faecium* with a 130 s of treatment time. The higher thermal resistance of *E. faecium* indicated its suitability as surrogate for *Salmonella* spp. during RF heating of ground black pepper. Piperine, total phenolics, volatile compounds, and antioxidant activity were assessed as quality parameters for ground black pepper. The results demonstrated that the RF processing provided effective inactivation of *Salmonella* spp. with insignificant ($p > 0.05$) quality deterioration.

1. Introduction

Known as “King of spice” or “Black Gold”, black pepper is the fruit of *Piper nigrum* and one of the most popular spices in the world (Nisha et al., 2009). Because of its pungency and aromatic odor, black pepper is commonly used as food flavoring and seasoning agent to enhance food flavor. The pungency of black pepper is attributed to piperine and phenolics (Srinivasan, 2007), while the volatile oils contribute to the characteristic aromatic odor of black pepper (Murthy and Bhattacharya, 2008). In addition to its pungency, piperine provides black pepper with high antioxidant activity which protects against oxidative damage by inhibiting or quenching free radicals (Srinivasan, 2007).

Moisture content is an important quality parameter that needs to be continuously monitored during the manufacturing and storage of black pepper (Dhas and Korikanthimath, 2003). Black pepper could be classified as a low water activity (a_w) food with typical a_w lower than 0.70. Foods with a_w levels lower than 0.70 provide a natural barrier for growth of many microorganisms (Blessington et al., 2013).

However, black pepper contaminated with *Salmonella* has been reported to be the cause of several outbreaks (Centers for Disease Control, 1982; Centers for Disease Control and Prevention, 2010) and numerous recalls (Dey et al., 2013) which not only causes food safety issues, but also results in enormous economic losses. Although it has been reported that *Salmonella* can only reproduce themselves in black pepper at a_w higher than 0.94, they are still able to survive for several years at low a_w storage conditions (Keller et al., 2013). As a_w decreases, *Salmonella* exhibits increasing thermal resistance which makes it difficult to be inactivated (Beuchat, 1981; Liu et al., 2018b; 2018c). Because black pepper is frequently added as a raw ingredient in many ready-to-eat foods which are not subjected to further thermal treatment, *Salmonella* persistence could potentially create a public health risk (Little et al., 2003, p.). Thus, it is necessary for the spice industry to develop effective decontamination methods to ensure the microbiological safety of black pepper.

Current decontamination methods such as ozone treatment, fumigation by ethylene oxide, irradiation with gamma rays and steam treatment have been developed to reduce microbial load in black pepper, but these methods come with limitations. Ozone treatment has been reported to reduce the microbial load in ground black pepper by 3–6 log CFU/g at ozone concentration of 6.7 mg/L for 10 min, but resulted in the oxidation of certain volatile oil constituents (Zhao and Cranston, 1995). Ethylene oxide has been shown to significantly reduce

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microbial load in black pepper (Leistritz, 1997; Toofanian, 1986), however, this toxic and inflammable chemical has been banned in the European Union (Uijl, 1992). Gamma ray irradiation is not well-accepted by consumers, although it has been proven to significantly reduce *S.* Typhimurium in black pepper (Song et al., 2014). Steam treatment is commonly applied to black pepper as a decontamination method in the European spice industry (Schweiggert et al., 2007), but it has been reported to cause significant color loss and quality deterioration in addition to increased moisture levels which affect the shelf life of black pepper (Schneider, 1993; Waje et al., 2008). Therefore, the development of an innovative technology becomes necessary for decontamination of black pepper while maintaining product quality.

Radiofrequency (RF) processing is a novel thermal processing method based on dielectric heating using electromagnetic waves. RF heating is based on volumetric heating and transfers the electromagnetic energy directly into the food product which generates the heat due to vibration of water and ionic molecules friction. The advantages of RF heating compared to conventional thermal treatments are the rapid heating rate, better heating uniformity and higher penetration depth (Boreddy et al., 2016; Boreddy and Subbiah, 2016; Chen et al., 2017, 2013; Lau et al., 2016; Lau and Subbiah, 2017; Piyasena et al., 2003). RF heating has been widely applied by the food industry for post-baking of cookies (Palazoğlu et al., 2012). It has also been investigated in the research laboratories for various applications such as drying of grain products (Jumah, 2005) and pet food (McCulloch and Nelson, 1977), pest control of walnuts (Mitcham et al., 2004) and grain (Nelson and Whitney, 1960), food pasteurization (Al-Holy et al., 2005; Houben et al., 1991) and inactivation of *Salmonella* in many low-moisture foods (Li et al., 2017; Liu et al., 2018a; Villa-Rojas et al., 2017).

As required by the Food Safety Modernization Act, food manufacturers are responsible for the validation of their process controls to ensure food safety which means showing scientific proof that their process is effective in controlling hazards (Food and Drug Administration et al., 2013). Food manufacturers cannot directly introduce food pathogen like *Salmonella* into their facilities for process validation as it can become very hard to eliminate them from the facilities. Therefore, it is necessary to find a surrogate which behaves the same as or has a higher resistance than *Salmonella* for validation studies within food facilities. *Enterococcus faecium* NRRL B-2354 has been commonly applied as a surrogate for *Salmonella* in testing of thermal treatments (Almond Board of California, 2007), extrusion of carbohydrate-protein meal (Bianchini et al., 2014) and oat flour (Verma et al., 2018), RF heating of wheat flour (Villa-Rojas et al., 2017), and infrared pasteurization of raw almonds (Bingol et al., 2011). However, it is important to evaluate the surrogate for its suitability for a specific food product and process treatment (Food and Drug Administration, 2015a, 2015b).

This study aimed to develop a practical RF heating process which could effectively pasteurize ground black pepper with minimal deterioration in product quality. The specific objectives of this study were to: 1) investigate RF heating for inactivation of *Salmonella* spp. in ground black pepper, 2) evaluate *E. faecium* as a suitable surrogate for this process and 3) assess the effect of RF heating on the quality of ground black pepper.

2. Materials and methods

2.1. Black peppercorn samples

Three batches of commercially steam-sterilized black peppercorns were obtained from three different production lots from McCormick & Company, Inc (Sparks, MD, USA) and stored in a walk-in cooler at −12 °C to maintain the quality.

2.2. Sample preparation

Black peppercorn samples were placed in an equilibration chamber which consisted of a small glove box and a humidity control system. The humidity control system consisted of a humidity sensor (AM2303, Aosong Electronics Co., Ltd., Guangzhou, China), a fan, solenoid valves, an air pump (Fusion 700, JW Pet, Teterboro, NJ), a wet column consisting of water and humidifier wicks, a dry column filled with silica beads (640SGO55, Sorbent Systems, Los Angeles, CA), and a microcontroller (Mega 2560 R3, SainSmart Technology, Inc., Lenexa, KS) which maintained relative humidity within ± 0.3% of the set point.

Moisture content and $a_{w,25°C}$ of the samples were measured using a Halogen Moisture Analyzer HR73 (Mettler Toledo Laboratory and Weighing Technologies, Greifensee, Switzerland) and a dew point water activity meter (Aqualab Series 4 TE, Decagon Devices Inc., Pullman, WA, USA) at 25 °C, respectively.

The spice board (Spices Board, 2007) defined that the optimal storage moisture content of ground black pepper must be less than 10.5% (wet basis, wb). Because RF heating reduces the moisture content, the industry would lose the mass. During RF heating experiments, a typical amount of moisture loss was identified, and the samples were preconditioned to a higher moisture content by placing them in the relative humidity chamber for 5 days so that after RF heating, the moisture content would be just below 10.5%. The increased moisture should also assist in reducing the thermal resistance of microorganisms resulting in higher log reductions (Syamaladevi et al., 2016).

Three 3-g samples were randomly taken from the equilibration chamber for measuring moisture content and $a_{w,25°C}$ every day for one week to evaluate the stability. On the second day after equilibration, black peppercorn samples were ground using a Waring Commercial Spice Grinder (WSG60, Conair Corporation, CT, USA) for 30 s and passed through a U.S. No. 20 sieve (0.841 mm sieve opening) to achieve a consistent particle size.

2.3. RF heating of ground black pepper

A total of 400 ± 0.1 g of ground black pepper sample was placed uniformly in a laminated paper tray (Fig. 1) sealed with a plastic film (Press'n Seal, The Glad Products Company, Oakland, CA, USA) to minimize heat and moisture loss from the surface. A vented nut was fixed onto the center of the film for controlled release of water vapor. Six fiber optic temperature probes (Neoptix, Inc., Quebec City, Quebec, Canada) with an accuracy of ± 0.6 °C were inserted into the tray through pre-drilled holes on the short edge of the tray as described below: (Fig. 1):

- probes 1, 2, 3 were inserted to the center and arranged from the top to the bottom vertically with a 1 cm interval to each other;
- probes 4, 5, 6 were inserted close to both edge from the top to the

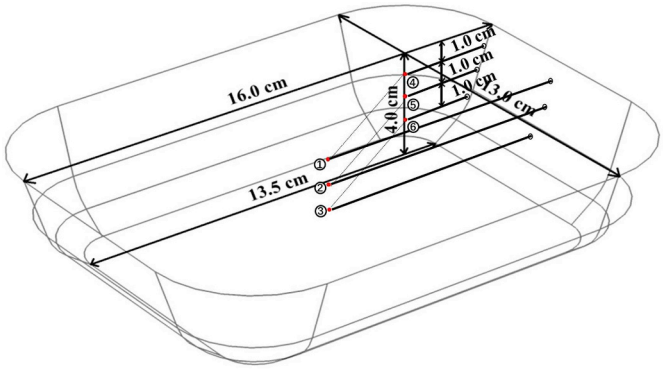

Fig. 1. Dimensions for the laminated paper tray; locations of the six fiber optic sensors.

bottom vertically with a 1 cm interval to each other.

Each fiber optic temperature probe was held in place with a plastic holder in the tray to ensure the temperature of same location was measured each time. The temperature history during RF heating was recorded by the probes every second.

A 6 kW, 27.12 MHz pilot-scale parallel-plate RF heating system (Model SO-6B, Monga Strayfield Pvt. Ltd., Pune, India) was used to heat ground black pepper in this study. The sample tray was placed inside the RF heating chamber at the center of the bottom electrode and the electrode gap was adjusted to 10.5 cm which gave the fastest heating rate without arcing. RF heating was conducted for 120 and 130 s in duplicates. Immediately after RF heating, the top surface temperature profiles were recorded by an infrared camera (Thermal CAMTM SC-640, FLIR Systems, Inc., North Billerica, MA) with an accuracy of ± 2 °C after removing the film. The cold spot was determined according to the results from the fiber optic temperature probes and infrared camera for the subsequent microbial challenge pack studies, which involves placing a small bag of inoculated samples at the cold spot. To evaluate whether the placement of bag affected the heating pattern, a small heating study was conducted by heating the product with or without the bags for 120 s in duplicates.

The inoculated pack method used in Liu et al. (2018a) was followed and improved in this study. The inoculated pack was placed at the determined cold spot with a larger amount of inoculated sample to evaluate the microbial inactivation. A small polyethylene bag (60 mm × 80 mm x 5 mm) was filled with 20 ± 0.1 g of ground black pepper sample, then placed in the determined cold spot in a tray filled with 380 ± 0.1 g of ground black pepper sample so that the total mass was the same. To allow the release of vapor from the bag, a tiny hole was cut at the corner of the bag. Then, the tray was sealed and processed by RF using the same settings described above. One fiber optic temperature probe was inserted into the bag to trace the temperature history during RF heating. In the subsequent microbial challenge studies, the inoculated sample would be placed at the same location without fiber optic sensor to estimate the inactivation of RF heating.

To evaluate the RF heating of ground black pepper, a heating non-uniformity index (λ) was used, defined by the following equation:

$$\lambda = \frac{\sqrt{\sigma^2 - \sigma_0^2}}{\mu - \mu_0}$$

where μ and μ_0 are final and initial average sample top surface temperatures (°C) measured using a thermal imaging camera, and σ and σ_0 are final and initial standard deviation of sample top surface temperatures (Wang et al., 2008).

2.4. Background microflora

Upon receiving the black peppercorn samples, background microflora tests were performed to quantify the aerobic bacteria. The test was conducted by first diluting three random 10 g samples from each batch into 90 mL of 0.1% buffered peptone water (BPW; Becton, Dickinson and Company, Sparks, MD). The diluted sample was then stomached for 1 min in a stomacher (Neutec Group Inc, NY, USA), diluted in 9 mL 0.1% BPW blanks, plated on 3M™ Petrifilm™ Aerobic Count Plates (3M Microbiology, St Paul, MN) and incubated for 24 h at 37 °C for enumeration.

2.5. Bacteria strains

Five different strains of *Salmonella enterica* associated with different low moisture foodborne outbreaks were selected for these microbiological studies, namely, *Salmonella* Agona 447967; *Salmonella* Reading; *Salmonella* Tennessee K4643; *Salmonella* Montevideo 488275; and *Salmonella* Mbandaka 698538. *Enterococcus faecium* NRRL B-2354 was selected as the non-pathogenic surrogate for the validations. *Salmonella* Agona 447967, *Salmonella* Montevideo 488275 and *Salmonella* Mbandaka 698538 were obtained from FDA, ORA Regional Lab in Jefferson, AR. *Salmonella* Reading was obtained from FDA Culture Collection in Bedford Park, IL. *Salmonella* Tennessee K4643 were obtained from the University of Georgia, Athens. *Enterococcus faecium* NRRL B-2354 was obtained from USDA, ARS (Peoria, IL). All the bacteria were kept in trypticase soy broth (TSB; Becton, Dickinson and Company, Sparks, MD) with 0.6% (w/v) yeast extract (YE; Becton, Dickinson and Company, Sparks, MD) (TSBYE) supplemented with 40% glycerol and stored at −80 °C until used.

2.6. Inoculum preparation and inoculation

For each bacterial strain, 1 mL of the frozen stock was transferred into 10 mL of TSBYE and vortexed for 10 s, then incubated for 24 h at 37 °C. For isolation, the overnight culture was streaked onto the surface of tryptic soy agar (TSA; Becton, Dickinson and Company, Sparks, MD) supplemented with 0.6% (w/v) yeast extract (TSAYE) using a 10 μL sterile loop, then plates were incubated upside down at 37 °C for 24 h. After incubation, a loopful (~10 μL) of one isolated colony was taken from the overnight plate, transferred into TSBYE and then incubated for 24 h at 37 °C. Next, 100 μL of the overnight broth culture was spread plated onto a TSAYE plate and incubated upside down at 37 °C for 24 h. Finally, the bacterial lawn on TSAYE was harvested with 3 mL of 0.1% BPW. This process was repeated for each strain. To prepare the *Salmonella* cocktail, 2 mL of the inoculum of each *Salmonella* strain was aseptically pipetted into a sterile conical tube, and vortexed for 30 s. The prepared *Salmonella* cocktail and *E. faecium* inoculum would be used for inoculation within 2 h. The initial microbial load of the *Salmonella* cocktail and *E. faecium* inoculum was ca. 10^{10} CFU/mL and ca. 10^9 CFU/mL, respectively.

In the real case, most of the cross-contaminations could happen during the cultivation and harvest of black peppercorn. The contaminated black peppercorn would be ground later, during which *Salmonella* could survive on the surface of black peppercorn. Therefore, to simulate the real-case scenario, the inoculated ground black pepper samples were prepared by inoculating black peppercorn samples first, and then grinding the black peppercorn samples into ground black pepper.

One day before the inoculation, 500 g of black peppercorn stored in the walk-in cooler were taken out, aseptically transferred to a sterile Whirl-Pak style bag and stored at room temperature overnight. Then, 10 mL of the *Salmonella* cocktail was sprayed onto the black peppercorn sample, and the bag was shaken for 10 min to achieve a proper homogeneity. The inoculated sample was then transferred into the equilibration chamber to reach the target water activity. On the second day after inoculation, the inoculated sample was ground and then placed in the equilibration chamber to re-equilibrate until the RF heating treatment. The same procedure was used to prepare samples inoculated with *E. faecium*.

2.7. Microbial stability and homogeneity tests

The stability and homogeneity tests were conducted by microbial enumeration of the inoculated samples every day for one week. Briefly, three 3-g of sample was randomly taken from the inoculated batch in the equilibration chamber, aseptically transferred into Whirl-Pak bags, diluted with 27 mL of 0.1% BPW, and stomached for 1 min. The diluted sample was then tenfold serially diluted in 9 mL 0.1% BPW blanks and spread plated onto TSAYE supplemented with 0.05% (w/v) ammonium iron citrate (SIGMA-ALDRICH, Co., MO, USA), and 0.03% (w/v) sodium thiosulfate (Fisher Chemical, NJ, USA) (mTSA) for *Salmonella* spp. or TSAYE supplemented with 0.05% (w/v) ammonium iron citrate, and 0.025% (w/v) esculin hydrate (ACROS, NJ, USA) (eTSA) for *E. faecium*, and plates were incubated for 24 h at 37 °C. Colonies with a black center

were enumerated as *Salmonella* spp., while black colonies were enumerated as *E. faecium*. The moisture content and $a_{w,25°C}$ were also measured every day during the microbial stability test.

2.8. Inactivation of Salmonella spp. and Enterococcus faecium

On the day of the RF heating challenge studies, the $a_{w,25°C}$ of the inoculated ground black pepper sample was measured to confirm that it reached the target $a_{w,25°C}$ before RF heating. The RF heating of ground black pepper sample was conducted for 120 and 130 s. Immediately after RF heating, the packed sample was soaked into an ice-water bath for 3 min to prevent further thermal inactivation.

The packed inoculated sample was then transferred to a sterile Whirl-Pak bag, serially diluted and spread plated onto mTSA for *Salmonella* spp. and eTSA for *E. faecium* and incubated for 24 h at 37 °C. Untreated inoculated samples were enumerated as the control (log N_0). The total bacterial reduction was obtained by subtracting the number of survivors to the RF heating (log N) from the control. The RF heating time which gave more than 5 log reduction of *Salmonella* spp. was chosen as the optimal RF heating configuration and was used for quality analysis of uninoculated samples.

2.9. Quality analysis

Three batches of uninoculated ground black pepper were equilibrated to the target $a_{w,25°C}$ and heated by RF using the optimal RF heating configuration which provides more than 5 log reduction of *Salmonella* in ground black pepper. After RF heating, the samples were transferred to separate Ziploc bags and sealed. Then, the samples were allowed to cool down at room temperature instead of chilling in the ice-water bath for estimating the worst-case scenario of quality deterioration.

To prepare the ethanol extract for the quality analysis, 0.400 ± 0.001 g of sample was diluted by 100 mL of 200 proof ethanol (Decon Labs, Inc., PA, USA) and magnetically stirred for 12 h. The solution was filtered, and the ethanol extract was stored in dark condition at 4 °C until analysis.

2.9.1. Color measurement

A colorimeter (BC-10, Minolta Co. Ltd., Osaka, Japan) was used to determine the color of black pepper by measuring the color values of L* (lightness and darkness), a* (redness and greenness), and b* (yellowness and blueness). Before measurement, the colorimeter was calibrated with a white calibration pad. A petri dish was filled with ground black pepper to attain a flat surface, and then five random locations were measured by the colorimeter. The total color difference (ΔE) was calculated to assess any effect of the RF heating by using the following formula (Robertson, 1977):

$$\Delta E = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}}$$

2.9.2. Total volatile compounds

The volatiles compositions of ground black pepper samples were determined by a Thermo Scientific headspace gas chromatography (Trace 1300) equipped with ISQ Mass Selective Detector (GC-MS). At the agitator temperature of 75 °C, 1 g of ground black pepper sample was incubated for 2 min. An amount of 0.7 mL of gas mixture released from black pepper was injected into GC-MS with 1:80 split ratio. The GC column used in this test was TG-5MS 30 m × 0.25 mm ID x 0.25 dF capillary column. The oven temperature setting was as follows: first it was heated from 40 to 290 °C at a 30 °C/min heating rate, and then maintained at 290 °C for 2 min. The mass range was 10–650 amu and ionization energy was 70 eV. The NIST 11 mass spectral library was used to identify the compositions of the samples.

2.9.3. Total phenolics

The total phenolic contents of the black pepper ethanol extracts were determined by using the Folin-Ciocalteu assay (Singleton and Rossi, 1965). Aliquot of 1 mL of extracts and standard solutions of gallic acid (1, 2, 3, 4, 5 μg/mL) were used in the assay. The blank was ethanol. Two milliliters of Folin-Ciocalteu's phenol reagent were pipetted into the sample tubes and vortexed for 5 s. After 10 min, 2 mL of Na_2CO_3 was added to the mixture and vortexed for 5 s. The sample tube was incubated for 2 h in the dark at 25 °C. An UV-1800 Shimadzu spectrophotometer (Shimadzu Corp., Kyoto, Japan) was used to quantitate the absorbance of each sample solution at 765 nm wavelength.

2.9.4. Antioxidant activity

The antioxidant activity was determined by using the 1,1 Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay. An amount of 3 mg DPPH (Alfa Aesar, 95% purity) was diluted with 100 mL ethanol to obtain a 30 μg/mL of DPPH solution. The 1 mL of DPPH solution was mixed with 0, 2.0, 2.5, 3.0, 3.5, and 4.0 mL of ground black pepper ethanol extract in test tubes and the tubes were filled up to 5 mL using ethanol. The mixtures were vortexed for 5 s, and incubated in the dark for 30 min at room temperature. The tube without sample ethanol extract was used as control and ethanol was used as blank. After incubation, an UV-1800 Shimadzu spectrophotometer was used to determine the absorbance of the remaining DPPH radicals in the solutions. The scavenging of DPPH radical was calculated according to the following equation (Bersuder et al., 1998):

$$\text{DPPH Radical} - \text{scavenging activity } (\%) = [(A_{control} - A_{sample}) / A_{control}] \times 100$$

where $A_{control}$ and A_{sample} are the absorbances of the control and the sample, respectively.

2.9.5. Piperine content

An Agilent 1100 Series High Performance Liquid Chromatograph (HPLC) equipped with variable wavelength detector was used to determine the piperine content of ground black pepper ethanol extracts. Standard piperine (Alfa Aesar, 98% purity) solutions were prepared in ethanol at the concentrations of 25, 50, 100, 200 and 400 μg/mL to create the standard curve. For the test, Eclipse Plus C18 (4.6 × 100 mm, 3.5 μm) column was used as stationary phase and isocratic elution was performed with mobile phase of 50% of methanol and 50% water mixture. The flow rate was 0.7 mL/min and detection wavelength was at 341 nm.

2.10. Statistical analysis

Three biological replicates (batches of different black pepper production lots inoculated with new frozen stock of same bacterial strain) and two technical replicates (RF heating for the single biological replicate) were conducted in this experiment. Data were analyzed using Excel 2016 (Microsoft Corporation, Redmond, WA). The microbial log reduction, moisture content, water activity, color values of L*, a*, and b*, total phenolics, piperine and total volatile compounds following RF treatments were subjected to paired t-test. Significant differences were presented at the 95% confidence level ($P < 0.05$).

3. Results and discussion

3.1. Temperature profile of RF heating

RF heating of ground black pepper was conducted for 120 and 130 s, which resulted in a final average surface temperature of 78.1 and 80.1 °C, respectively (Table 1). From trial-and-error experiments, the initial moisture content and $a_{w,25°C}$ were adjusted to 12.8% (wb) and 0.664, so that the final moisture content and $a_{w,25°C}$ would drop to

Table 1
Temperature profile of the top surface of RF heated ground black pepper for different treatment times.

Matrices on top surface	Treatment time (s)	
	120	130
Average Temperature (°C)	78.1 ± 0.8	80.1 ± 0.8
Maximum Temperature (°C)	99.9 ± 1.0	101.1 ± 0.5
Minimum Temperature (°C)	69.2 ± 0.9	69.5 ± 0.8
Heating uniformity index (λ)	0.075 ± 0.021	0.076 ± 0.004

Table 2
Moisture content, water activity, color parameters, total phenolics and piperine content of untreated and treated black pepper samples subjected to 130 s of RF heating.

Quality Parameter	Control	RF treated
Moisture content (wb) (%)	12.8 ± 0.1 [a]	10.5 ± 0.1 [b]
Water activity at 25 °C	0.664 ± 0.003 [a]	0.592 ± 0.007 [b]
Color (L*)	55.8 ± 0.8 [a]	55.2 ± 0.4 [a]
Color (a*)	4.1 ± 0.1 [a]	4.0 ± 0.1 [a]
Color (b*)	10.8 ± 0.4 [a]	11.0 ± 0.3 [a]
Color difference (ΔE)	0	0.65
Total phenolics (mg/g)	19.5 ± 0.1 [a]	19.4 ± 0.2 [a]
Piperine (ug/g)	37.5 ± 0.3 [a]	37.7 ± 0.4 [a]

[1]Within a row, the numbers with the same alphabet in the superscript are not significantly different from each other ($p > 0.05$).

10.5% (wb) and 0.592 after the RF heating (Table 2). The final moisture of 10.5% (wb) was selected because the Spice Board (Spices Board, 2007) identified this as the optimal moisture content for long term storage of ground black pepper to prevent fungal deterioration. Considerably moisture migration was not observed during RF heating of ground black pepper.

During RF heating, the corners and edges always absorb more electromagnetic energy (Tiwari et al., 2011), which results in the hot spot. Because of the thermal runaway heating (Awuah et al., 2014), the hot spot will be further overheated which leads to higher non-uniformity. Therefore, temperature was measured on edges at the top, middle, and bottom layers. When RF heating is not combined with hot air heating, the outside air is not heated up by RF due to its dielectric properties. Therefore, the product loses heat to the surrounding air and the bottom electrode. Due to this effect and focused heating of RF, the geometric center is usually hotter, if the product depth is much smaller than the penetration depth. This has resulted in top center of the package to be the cold spot in several studies (Liu et al., 2018a; Wei et al., 2018). So, the temperature of the geometric center, top center, and bottom center were also recorded.

Fig. 2 shows that during RF heating there was a linear heating rate until the temperature reached around 80–100 °C. After that, the heating rate leveled off due to evaporative cooling and also due to heat transfer to cold spot by movement of steam. At first, the bottom edge location (6) reached the highest temperature, over 100 °C, in 80 s. When the bottom edge reached the leveling state, there was a drastic increase in heating rate of edge center (5) which is just above the edge bottom (6). Thus, the steam from the bottom edge was rising up and then heated the product at the edge center location. Similarly, the top edge then heated up with a higher heating rate within few seconds after the middle center heated with a higher heating rate. The same pattern was shown for the probes in the middle location, where the middle bottom (3) heated faster than middle center (4), while the middle top was the coldest. In general, the central locations heated slower than the edges at all vertical distances.

The fast heating rate provided by RF heating reduced the come-up-time considerably compared to conventional heating (Handa et al., 2001). Faster come-up time allows for high-temperature and short time processing, which could potentially minimize the quality deterioration while achieving the desired lethality. The rapid RF heating of ground black pepper was also observed in other studies. For example, ground black pepper reached 62 °C after 50 s of RF heating (Kim et al., 2012) and the temperature of ground black pepper with a moisture content of 17.2% (db) increased from 22.2 °C to 90.2 °C after 63 s of RF heating (Jeong and Kang, 2014). The variance of the heating rate in different studies could result from the different moisture content of the samples (Jeong and Kang, 2014), different RF heating configurations (Chen et al., 2017; Villa-Rojas et al., 2017) and different sample sizes (Piyasena et al., 2003).

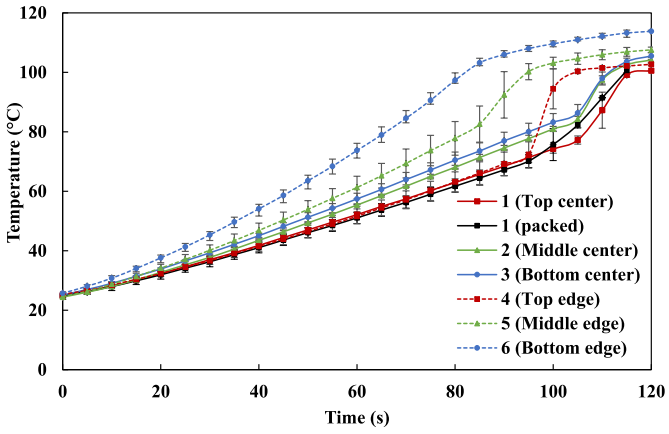

Fig. 2. Temperature histories of ground black pepper during RF heating.

The small deviation of the average temperature measured at the surface indicated that the RF heating is highly replicable (Table 1). The non-uniformity indexes were calculated and the results are similar to other RF heating uniformity optimization studies (Chen et al., 2015, 2017; Jiao et al., 2015; Wang et al., 2007) which means RF heating provides a good heating uniformity during the heating of ground black pepper. According to the results of RF heating pattern, the cold spot was located at location (1) which is the center of the top layer. This location has also been reported in other studies to be a cold spot (Chen et al., 2017; Tiwari et al., 2011; Villa-Rojas et al., 2017). A packed sample was placed at (1), and the temperature of the bag was traced during the RF heating. Its temperature history showed no considerable difference from the sample without a pack (Fig. 2), which indicated that the slim plastic bag did not affect RF heating. Similar results have also been reported in other RF studies (Liu et al., 2018a). Thus, for the microbial challenge pack studies, the packed inoculated sample was placed at location (1) for each tray to account for the worst-case scenario.

3.2. Stability and homogeneity tests

Background microflora was minimal with < 10 CFU/g of aerobic bacteria was detected in all three batches of black peppercorn. As the added population of *Salmonella* spp. and *E. faecium* was considerably higher, the background microflora should not affect the overall results.

The stability and homogeneity tests of *Salmonella* spp. and *E. faecium* after inoculation were conducted at $a_{w,25°C}$ of 0.660 ± 0.025 for one week. In Fig. 3, it can be observed that the moisture content and $a_{w,25°C}$ reached equilibrium on the second day after the inoculation. Therefore, the black peppercorn samples were ground on the second day, then transferred back to the equilibration chamber. The results of the stability tests for both bacteria are shown in Fig. 4. The initial population of *Salmonella* spp. in the black peppercorn samples was more than 8 log CFU/g and it dropped by around 1 log CFU/g after two days. The grinding caused a further reduction of 0.3 log CFU/g but the *Salmonella* spp. population subsequently stabilized, with only 0.2 log CFU/g change after 5 days. *E. faecium* showed a better stability on black pepper samples with less than 0.5 log CFU/g of reduction in one week

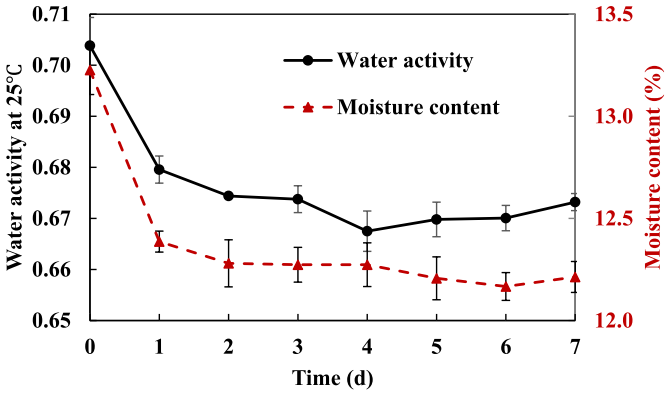

Fig. 3. Stability test of water activity and moisture content for 7 days in equilibration chamber.

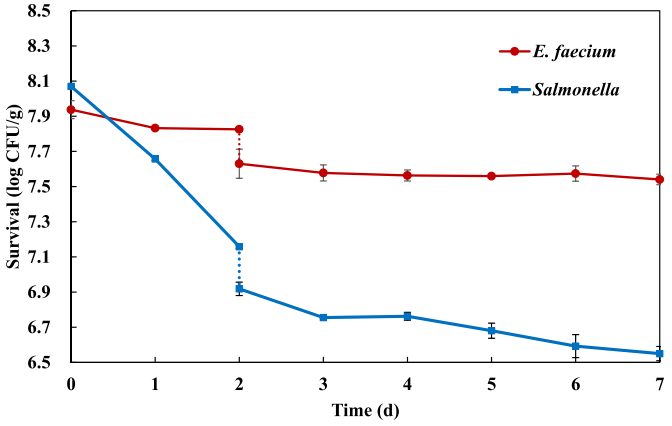

Fig. 4. Stability test of *Salmonella* spp. and of *E. faecium* in ground black pepper. On Day 2, grinding of black peppercorn was performed which resulted in a slight reduction.

including a reduction of 0.2 log CFU/g from the grinding. Both bacteria showed a good stability after the third day and thus the fifth day was selected for microbial challenge studies of RF heating. The observed stability of *Salmonella* was not surprising as *Salmonella* has been previously reported to survive on ground black pepper samples for a long period at low a_w environment (Keller et al., 2013). Small standard deviations of both bacteria (< 0.2 log CFU/g) were observed during these tests indicating that the inoculation method provided a good homogeneity for the ground black pepper samples.

It is important to evaluate the inoculation method and monitor the equilibration process especially for low a_w food. When the liquid inoculum was mixed with black pepper samples, the added moisture could result in the release of water-soluble antimicrobials which may decrease the population level before the microbial challenge studies (Waje et al., 2008). After inoculation, the change in environment could cause a shock to the bacteria (Palipane and Driscoll, 1993). The bacteria need to adapt themselves to the low a_w environment and it is necessary for the bacteria to reach a stable status before conducting microbial challenge studies to remove the influences brought by the new environment (Bowman et al., 2015). When exposed to a low aw environment, *Salmonella* has been reported to develop enhanced tolerance to heat treatment (Gruzdev et al., 2011) which increased the difficulty for thermal inactivation. In general, black pepper could get contaminated during cultivation, harvesting, and drying (Nair, 2004) before processing which gave bacteria enough time to adjust themselves to the low a_w environment and build up thermal resistance. Thus, it is necessary to equilibrate the sample until the bacteria reach a stable status before RF heating to simulate the real scenario.

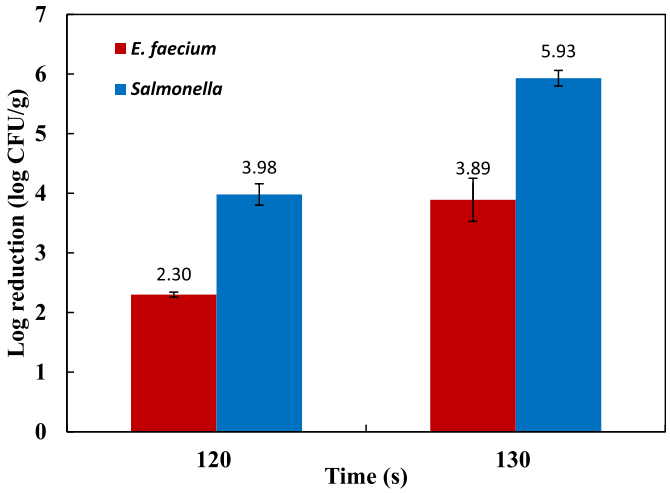

Fig. 5. Comparison of the log reduction between *Salmonella* spp. and *E. faecium* at 120 s and 130 s of RF heating. Error bars indicate the ± 1 standard deviation between the biological replications.

3.3. Microbial challenge pack studies of RF heating

The RF heating treatments were conducted for both pre-packed ground black pepper samples inoculated with *Salmonella* spp. or *E. faecium* for 120 and 130 s, respectively. The inoculated pack method developed in Liu et al., (2018a) was followed. Liu et al., (2018b) used 5 g of inculcated samples in a tray containing 3 kg of uninoculated sample. In this study, we used 20 g of samples to cover larger area in a tray containing 400 g of uninoculated samples to take in to account of variability in cold spot determination.

The inoculated black pepper samples in the small packs were enumerated, and the comparison of log reduction between *Salmonella* spp. and *E. faecium* at 120 s and 130 s of RF heating is shown in Fig. 5. The error bars indicate the ± 1 standard deviation between the biological replications. At the RF heating time of 120 s, the variance introduced by RF processing systems and associated microbial enumeration method can be estimated by calculating the average of standard deviation between two technical replications for three batches and was found to be 0.47 and 0.04 log CFU/g for *Salmonella* and *E. faecium*. The variance introduced by different batches of samples (replications) and associated microbial enumeration method can be estimated by the standard deviation between three biological replications (after averaging the values for two technical replications) and was found to be 0.30 and 0.06 log CFU/g. Therefore, the variability introduced by RF processing is similar to the variance introduced by different batches of samples and the error might be introduced primarily by the microbial enumeration method rather than RF processing or samples.

At 120 s of RF heating, reductions of 3.98 and 2.30 log CFU/g were achieved for *Salmonella* spp. and *E. faecium*, respectively. At 130 s of RF heating, more than 5.93 log CFU/g reduction was obtained for *Salmonella* spp., while *E. faecium* only experienced a reduction of 3.89 log CFU/g. For both bacteria, the log reductions at 130 s were significantly higher than 120 s ($P < 0.05$) and within this 10 s, an extra 2 log CFU/g and an extra 1.6 log CFU/g was effectively attained for *Salmonella* spp. and *E. faecium*, respectively, due to the high temperature achieved. Kim et al. (2012) reported that 50 s of RF heating with a final average temperature of 60 °C could provide a 4.29 log CFU/g reduction of *Salmonella* Typhimurium in ground black pepper (Kim et al., 2012) and 1 min of vacuum steam treatment of black pepper at 75 °C would result in 6.10 log CFU/g reduction of *Salmonella* PT 30 (Shah et al., 2017). The *Salmonella* strains in those studies seem to be less thermally resistant compared to the ones used here which could be the result from the different inoculation methods, moisture equilibration (Bowman et al., 2015), different *Salmonella* strains used and different

initial a_w of the samples (Jeong and Kang, 2014; Kim et al., 2012). Specifically, both studies (Jeong and Kang, 2014; Kim et al., 2012) inoculated the ground black pepper samples, and immediately treated the samples using RF heating without any equilibration which prevented the bacteria to adapt to low moisture environment. In addition, they also used only one serotype of *Salmonella*, while this study used a *Salmonella* cocktail of five strains.

RF heating was shown to effectively inactive *Salmonella* spp. in ground black pepper by providing more than 5.93 log CFU/g reduction within 130 s. This RF heating adequately reduced the presence of *Salmonella* spp. by 5 logs which met the 5-log pathogen reduction performance standard (Food and Drug Administration et al., 2012, 2013) and thus the quality analysis of ground black pepper was conducted at this condition. Because of the short come-up time, RF heating could be considered as a high-temperature-short-time processing which potentially minimizes the quality loss. RF heating was shown to provide a more rapid inactivation of *Salmonella* spp. than other methods like ozone treatment which took 10 min to achieved a 4 log CFU/g reduction (Zhao and Cranston, 1995) and cold plasma which provided a 4.1 log CFU/g reduction after a treatment of 30 min (Hertwig et al., 2015). In this study, ground black pepper samples were immediately cooled down after RF heating which represented a conservative estimate of microbial log reduction, because the products may not be chilled immediately after processing in the real industrial setting and thus RF heating could potentially provide additional inactivation.

At both heating times, a significantly higher log reduction was obtained for *Salmonella* spp. than *E. faecium* which indicated that *E. faecium* was more thermally resistant during RF heating of ground black pepper. Hence, *E. faecium* is a suitable surrogate for *Salmonella* spp. for RF heating of ground black pepper. Although *E. faecium* has been demonstrated to be a good surrogate for *Salmonella* in many thermal processing of low-moisture food (Almond Board of California, 2007; Bianchini et al., 2014, 2014; Kopit et al., 2014; Ma et al., 2007), it still necessary to conduct the validation because under different product matrixes and processing methods, the surrogate may not accurately represent the behavior of the pathogen of interest (Rachon et al., 2016).

3.4. Quality analysis

The quality of the ground black pepper sample was mainly accessed by its moisture content, $a_{w,25°C}$, color, piperine, total phenolics, antioxidant activity and volatile compounds. The $a_{w,25°C}$ of ground black pepper sample was equilibrated to 0.660 ± 0.025 with a moisture of 12.8 ± 0.1% (wb) which would result in $a_{w,25°C}$ significantly dropped to 0.595 ± 0.025 with a moisture of 10.5 ± 0.1% (wb) after RF heating for 130 s (Table 2). According to American Spice Trade Association (American Spice Trade Association and others, 2011), black peppercorn should achieve a uniform moisture content that is certainly not higher than 12.0% (wb), while the optimal storage moisture content of ground black pepper is below 10.5% (wb). RF heating has shown to adequately reduce the moisture of ground black pepper to the optimal storage moisture of 10.5% (wb). Moisture content not only affects the quality but also safety of black pepper as microbial resistance changes with a_w. The a_w assesses the amount of available free water that could be used to support food spoilage, thus it plays an important role in microbial control and food safety (Beuchat, 1981).

The color of ground black pepper samples did not experience a significant change after the RF heating. The calculated ΔE of 0.65 indicated a normally invisible difference between the control and RF treated samples (Mokrzycki and Tatol, 2011). The color of black pepper is important to be accessed for thermal process, because the thermal decontamination process like commercial steam treatment has been reported to cause significant color loss of black pepper (Schneider, 1993). Although RF heating heated up the samples to a high temperature, it did not induce significant color loss to ground black pepper, which was attributed to the short treatment time.

Table 3
Total volatile compounds of untreated and treated black pepper samples subjected to 130 s of RF heating.

Compound	Area (%)	
	Control	RF treated (% difference)
α-Thujene	1.37 ± 0.13 [a]	0.91 ± 0.04 [b] (- 33.58%)
α-Pinene	4.89 ± 0.42 [a]	3.68 ± 0.15 [b] (- 24.77%)
Camphene	1.76 ± 0.14 [a]	1.07 ± 0.02 [b] (- 39.20%)
Sabinene	5.57 ± 0.48 [a]	4.9 ± 0.5 [a]
β-Pinene	8.57 ± 0.63 [a]	7.99 ± 0.15 [a]
α-Phellandrene	6.1 ± 0.75 [a]	5.56 ± 0.35 [a]
β-Mycene/4(10)-Thujene	1.06 ± 0.09 [a]	1.08 ± 0.02 [a]
β-Phellandrene	9.62 ± 0.8 [a]	8.06 ± 1.26 [a]
Camphene, (1R, 4S)	8.22 ± 0.62 [a]	6.77 ± 0.2 [b] (- 17.64%)
γ-Terpinene	0.94 ± 0.06 [a]	0.91 ± 0.06 [a]
3-Carene	5.88 ± 0.55 [a]	5.72 ± 0.05 [a]
2-Carene	1.23 ± 0.15 [a]	1.06 ± 0.04 [a] (- 13.82%)
β-Ocimene	16.76 ± 1.64 [a]	17.92 ± 0.28 [a]
D-Limonene	11.17 ± 1.17 [a]	12.34 ± 0.49 [a]
p-Menth-3-en-1-ol	0.63 ± 0.09 [a]	0.61 ± 0.02 [a]
Terpineol, cis-β-	0.55 ± 0.08 [a]	0.49 ± 0.02 [a]
p-Mentha-1,4(8)-diene	0.22 ± 0.02 [a]	0.25 ± 0.02 [a] (+13.64%)
β-Linalool	0.33 ± 0.04 [a]	0.3 ± 0.02 [a]
p-Menth-3-ene, 2-isopropenyl-1-vinyl-, (1S, 2R)	0.75 ± 0.12 [a]	0.76 ± 0.02 [a]
β-Copaene	0.09 ± 0.01 [a]	0.09 ± 0.01 [a]
α-Copaene	1.2 ± 0.21 [a]	1.14 ± 0.04 [a]
β-Elemene	0.26 ± 0.02 [a]	0.25 ± 0.01 [a]
Caryophyllene	11.46 ± 8.18 [a]	16.78 ± 0.82 [a]
Humulene	0.55 ± 0.08 [a]	0.53 ± 0.03 [a]
trans-α-bargamotene	0.2 ± 0.01 [a]	0.19 ± 0.02 [a]
δ-Cadinene	0.09 ± 0.01 [a]	0.09 ± 0.01 [a]
Isocaryophyllene	0.31 ± 0.04 [a]	0.33 ± 0.03 [a]
(Z,E)-α-Farnesene	0.22 ± 0.03 [a]	0.21 ± 0.02 [a]
Caryophyllene oxide	0.03 ± 0.01 [a]	0.03 ± 0.01 [a]

[1]Within a row, the numbers with the same alphabet in the superscript are not significantly different from each other ($p > 0.05$).

The main odor, flavor and antioxidant activity contributors to black pepper: piperine, total phenolic and volatiles, were evaluated in this study. Table 2 shows that the total phenolics and piperine contents of RF treated samples were not significantly different from the control samples. In this study, there were 29 volatile compounds identified and the 9 major components (> 5% peak area) were β-ocimene (16.76%), followed by caryophyllene (11.46%), D-limonene (11.17%), β-phellandrene (9.62%), β-pinene (8.57%), camphene 1R, 4S (8.22%), α-phellandrene (6.1%), 3-carene (5.88%) and sabinene (5.57%) which accounted for 83.3% of the total amount of volatile compounds in the untreated ground black pepper samples (Table 3). In Fig. 6, it shows that the same 29 volatile compounds were detected in both control and RF treated samples, and there was no any new compounds found after RF processing compared to the volatiles tests in other studies (Ferreira et al., 1999; Zhao and Cranston, 1995). Among those major compounds, only camphene experienced a significant decrease with a decline of 17.64% after RF heating. The decline of camphene, α-thujene, and α-pinene resulted from volatilization of compounds due to high temperature during RF heating. It has been reported the camphene was undetectable after ozone treatment (Zhao and Cranston, 1995). Sabinene and terpinene, reported to be the major contributors to the odor of black pepper (Pino et al., 1990), did not show significant reduction after RF heating. It has been reported that if the amount of monoterpenes (excluding α- and β-pinene) is high and amount of pinenes was low, the pepper aroma quality is optimal. On the other hand, sesquiterpenes defines the “pepper odor” (Schulz et al., 2005). It was good to note that major monoterpenes such as sabinene, α-phellandrene, 3-carene, β-ocimene and D-limonene and sesquiterpenes such as caryophyllene did not significantly change in black pepper samples after RF heat treatment. Any changes in relative composition of the major

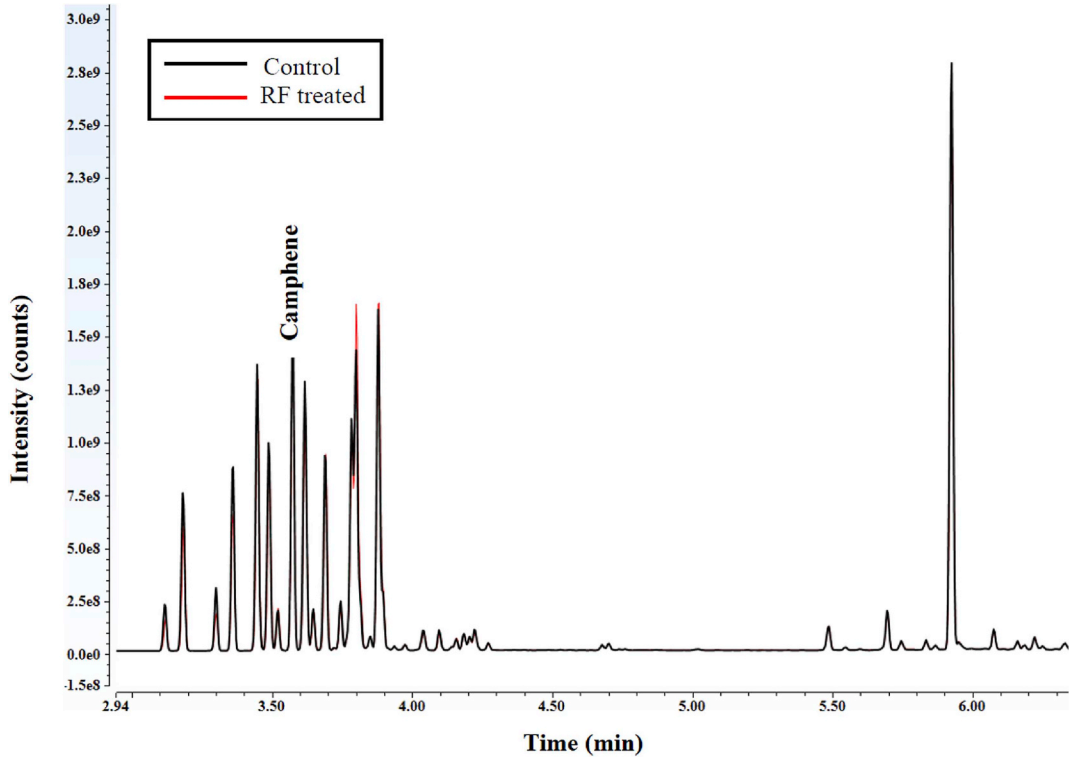

Fig. 6. GC chromatograms of ground black pepper volatile compounds. Labeled peaks represent the major compound which significantly dropped during RF heating.

volatile compounds affect the overall flavor of black pepper (Chacko et al., 1996). As RF heating resulted only in minor changes in major volatile compounds, the overall impact on aroma of ground black pepper is minimal.

The DPPH is commonly used to determine the free radical-scavenging activity of an antioxidant, and it has a characteristic absorbance at 517 nm (Yamaguchi et al., 1998; Zarai et al., 2013). The antioxidant activity of ground black pepper was evaluated using the DPPH radical-scavenging assay and the results are shown in Fig. 7. As it could be deduced from this figure, there was no significant difference between RF treated sample and control and thus RF heating did not affect overall antioxidant activity of ground black pepper.

This study involving stationary RF treatment demonstrated that RF is a feasible technology and *E. faecium* could be used for validation at the industrial scale. Other studies have scaled-up RF process by moving the product on a conveyor belt (Jiao et al., 2012; Wang et al., 2007). To scale up RF heating of ground black pepper, a continuous RF processing could be performed by placing the retail ready packages on a conveyor belt. However, water evaporation during RF heating could cause condensation issues, which may result in caking of powder products or expansion of packages. In such instances, one-way degassing valve can be placed in the package for releasing gas during RF while preventing cross contamination from outside air. Another way to scale-up RF process of black pepper is by directly placing the unpacked products on a conveyor belt, which is more common in wholesale treatment. When the product is placed in a container, there is an edge heating on all four sides. However, when the product is conveyed in a bed, the hot spots will be only on two sides of the bed. To improve the heating uniformity of RF process, hot air assistance, intermittent stirring, electrode modification could be applied for the process (Jiao et al., 2015; Liu et al., 2011; Wang et al., 2005).

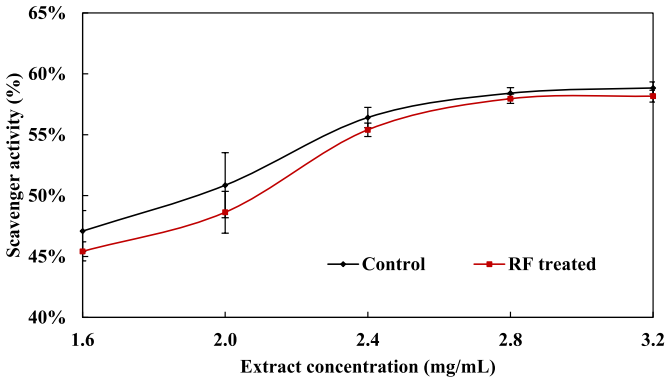

Fig. 7. Antioxidant activities of untreated and treated black peppercorn samples subjected to RF heating.

4. Conclusions

In this study, RF heating was shown to effectively inactivate *Salmonella* spp. in ground black pepper and provided more than 5.93 log CFU/g reduction with only 130 s of heating. *E. faecium* was concluded to be as a suitable surrogate for *Salmonella* spp. after the RF treatments. At the same RF heating time of 130 s, it was also demonstrated that there was no significant quality loss and RF heating was able to dry the ground black pepper to the optimal storage moisture.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fm.2019.03.007.

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