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Identifying possible non-thermal effects of radio frequency energy on inactivating food microorganisms

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ABSTRACT

Radio frequency (RF) heating has been successfully used for inactivating microorganisms in agricultural and food products. Athermal (non-thermal) effects of RF energy on microorganisms have been frequently proposed in the literature, resulting in difficulties for developing effective thermal treatment protocols. The purpose of this study was to identify if the athermal inactivation of microorganisms existed during RF treatments. *Escherichia coli* and *Staphylococcus aureus* in apple juice and mashed potato were exposed to both RF and conventional thermal energies to compare their inactivation populations. A thermal death time (TDT) heating block system was used as conventional thermal energy source to simulate the same heating treatment conditions, involving heating temperature, heating rate and uniformity, of a RF treatment at a frequency of 27.12 MHz. Results showed that a similar and uniform temperature distribution in tested samples was achieved in both heating systems, so that the central sample temperature could be used as representative one for evaluating thermal inactivation of microorganisms. The survival patterns of two target microorganisms in two food samples were similar both for RF and heating block treatments since their absolute difference of survival populations was < 1 log CFU/ml. The statistical analysis indicated no significant difference ($P > 0.05$) in inactivating bacteria between the RF and the heating block treatments at each set of temperatures. The solid temperature and microbial inactivation data demonstrated that only thermal effect of RF energy at 27.12 MHz was observed on inactivating microorganisms in foods.

1. Introduction

Radio frequency (RF) treatments have been proposed as a novel dielectric heating technology for rapidly heating agricultural and food products based on electromagnetic waves ranging from 3 kHz to 300 MHz. It has been successfully used for inactivating food microorganisms, such as *Escherichia coli* (Geveke and Brunkhorst, 2004; Kim et al., 2012; Li et al., 2017a), *Salmonella* (Gao et al., 2011; Ha et al., 2013; Nelson et al., 2002), and *Listeria* (Al-Holy et al., 2004; Awuah et al., 2005). The pasteurization mechanism with dielectric heating, including RF and microwave energy, has been debated and athermal (non-thermal) effects of electromagnetic energy are often proposed in the literature (Banik et al., 2003; Jacob et al., 1995; Shazman et al., 2007; Soghomonyan et al., 2016; Velizarov et al., 1999; Wang and Wang, 2016). Thermal effects are mostly considered on inactivating food microorganism since the cell death is only related to the heat generated by frictional interaction between the polarized molecules and charged ions in a product in response to the RF and microwave fields (Awuah et al., 2005; Shazman et al., 2007). Athermal effects are also claimed in many reports since high inactivation rates of microorganisms are observed at a low sample temperature under the electric fields (Saadi et al., 2014; Velizarov et al., 1999).

Thermal effects of RF or microwave energy are generally considered to be the only cause of inactivating food microorganisms (Carroll and Lopez, 1969; Gandhi, 1987; Gedye, 1997; Geveke et al., 2002; Hamoud-Agha et al., 2013; Hamoud-Agha et al., 2014; Ingram and Page, 2010; Shazman et al., 2007). However, there still has been a controversial discussion regarding the existence of athermal effects of RF and microwave energy on microorganism inactivation (Banik et al., 2003; Latorre et al., 2012; Shamis et al., 2012). Early researches report that microorganisms are inactivated more in RF or microwave systems than in water or oil bath methods (Culkin and Fung, 1975; Jacob et al., 1995). But the results cannot be repeated due to lack of the detailed heating parameters. The death rates of *Escherichia coli* in microwave heating are also higher than those obtained in conventional heat environment at the same temperature (Banik et al., 2003). Similar results were published by Dreyfuss and Chipley (1980) using *Staphylococcus aureus*, Singh et al. (1994) using *Cyano-bacteruim Nostoc*, and

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Schlisselberg et al. (2013) using *Salmonella* and *Listeria monocytogenes*. However, the differences of dynamic heating process, such as heating rate and uniformity between the two heating treatments, are not considered, making thermal and athermal effects difficult to distinguish. Cvengros et al. (2004) developed a thermostated oil bath to perform a same time–temperature profile with microwave heating treatment, but only the surface temperature of the samples measured using infrared sensors. Thus, it's extremely difficult to make a solid conclusion on existing athermal effects in RF or microwave treatments due to the lack of common experimental conditions (Banik et al., 2003; Jacob et al., 1995; Shamis et al., 2012; Velizarov et al., 1999; Wang and Wang, 2016).

Heating treatment conditions, including setpoint temperature, heating rate, and heating uniformity, have an important effect on thermal inactivation rates of given food microorganisms. For example, the different sample temperature results in various thermal inactivation rates since *D*-values of *E. coli* in ground beef are 11.85 and 1.63 min at 55 and 60 °C, respectively (Juneja et al., 1997). Even at the same temperature of 57 °C, the *D*-value of *E. coli* in mashed potato at the heating rates of 0.1 and 0.5 °C/min is significantly higher than that at 1, 5, and 10 °C/min (Chung et al., 2007; Chung et al., 2008; Kou et al., 2016). Beside these, the major challenge of the RF treatment is heating uniformity (Huang et al., 2016b; Jiao et al., 2015), which is a key factor related to the observed athermal phenomena (Hamoud-Agha et al., 2013). The non-uniform experimental temperature distribution is a major obstacle to identify athermal effects of RF energy on inactivating food microorganisms.

There is a need for reliable data that should be obtained in well-controlled conventional heating systems to simulate the RF heating process with the acceptable heating uniformity. A unique experimental thermal death time (TDT) heating block system has been successfully developed in our lab with a conventional source of heating energy for simulating RF treatments (Kou et al., 2016). The heating rates, setpoint temperatures, and holding times of samples can be precisely controlled by Visual-Basic software with proportional-integral-derivative (PID) algorithms in this TDT heating block system (Kou et al., 2016). This block system holds the potential to study the sole thermal inactivation rates of microorganism under the same heating conditions recorded during the RF heating process.

Objective of this study were to: (1) analyze the sample temperature profiles and heating uniformity in both heating block and RF treatments, (2) use the heating block system to simulate the RF heating process, and (3) compare the inactivation results of two bacteria strains in two food samples between the conventional heating and RF treatments.

2. Materials and methods

2.1. Equipment

A parallel plate RF heating system (COMBI 6-S, Strayfield International Limited, Wokingham, UK) of 6 kW at a frequency of 27.12 MHz with a free-running oscillator was used in this study. The system included a RF generator and an applicator containing two parallel electrodes inside a large rectangular oven. More detailed descriptions of the system can be found in Wang et al. (2010). The RF power was adjusted by changing the electrodes gap, and the RF power and heating rate increased as decreasing gap. A small polypropylene cylindrical test cell with dimensions of ϕ3.0 cm × 1.5 cm was selected as the sample holder, and the center temperature of the sample was measured using a fiber-optic temperature sensor system (HQ-FTS-D120, Heqi Technologies Inc., Xian, China) with an accuracy of ± 0.5 °C (Fig. 1).

A computer-controlled heating block system was used as a conventional thermal energy device. The heating block system consisted of a heating unit with 6 cells, a data acquisition/control unit, and a computer (Fig. 2). The setpoint temperature, heating rate, and holding time of the system were controlled by the customized Visual Basic software and two PID controllers (I32, Omega Engineering, Inc., Stamford, CT, USA), and the central sample temperature in the cell was measured by type-T thermocouple sensors (TMQSS-020-6, Omega Engineering Ltd., Stamford, CT, USA) with an accuracy of ± 0.5 °C, which were pre-calibrated by the program to keep consistent with the data from the fiber-optic sensor system. Detailed descriptions of the heating block system can be found in Kou et al. (2016).

2.2. Bacteria strains and cultivation

Two bacteria strains, *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 25923), from the College of Food Science and Engineering, Northwest A&F University (Yangling, China), were selected for the study. The cell suspensions of these two microbial strains were obtained and handled according to the previous study described in Li et al. (2017b). The final cell population was adjusted to a level of 10^9 CFU/ml and refrigerated (3 ± 1 °C) for no > 3 d before inoculation.

2.3. Sample preparation

Apple juice and mashed potato were used to study the possible athermal effects of RF energy on inactivating microorganisms. Apple juice (Huiyuan 100% Apple Juice, Yangling, China), as a representative acid and liquid food, was purchased from a local supermarket and stored in a refrigerator (BD/BC-297KMQ, Midea Refrigeration Division, Hefei, China) at 3 ± 1 °C and used within the expiration date. Mashed potato is often used as a semi-solid model food, because of its simple preparation, consistent chemical composition, and relatively homogeneous structure (Bornhorst et al., 2017; Campañone and Zaritzky, 2005). Dry mashed potato flakes (Simplot Australia Ltd., Tasmania, Australia) were mixed with distilled water to formulate into 15.38% wet basis (w.b.) mashed potato.

Each of 2 ml *E. coli* and *S. aureus* suspensions was inoculated into 200 ± 5 ml apple juice or 80 ± 1 g mashed potato to form composite samples with an initial population of 10^7–10^8 CFU/ml (CFU/g) for testing. All samples were kept at 30 °C in a water bath (SC-15, Ningbo Scientz Biotechnology Co., Ltd., Ningbo, China) to get the same initial temperature before pasteurization experiments.

2.4. RF treatments

For apple juice, five cylindrical test cells were placed vertically in the middle of the electrodes, as shown in Fig. 1(a). The test cell at the center position was filled with 5 ml of inoculated samples and others with 5 ml of non-inoculated samples. The electrode gap of 9 cm was chosen based on preliminary experiments to obtain a fast heating rate. The sample core temperature was monitored and recorded using the fiber-optic sensor during the RF heating. After the temperature reached 35, 40, 45, 50, 55, 60, and 65 °C, the inoculated test cells were taken out from the RF system and placed in a sealed plastic bag, then put into ice water (≈4 °C, for at least 2 min) immediately for cooling, and then ready for enumeration.

For mashed potato, 130 g samples were added into 150 ml glass beaker, and a single cylindrical test cell filled with 5 g inoculated mashed potato was placed at the center of the upper layer in the beaker, as shown in Fig. 1(b). The electrode gap of 9.5 cm was chosen based on no arcing and fast heating rates during the preliminary tests. The other experimental conditions were the same as those with the apple juice.

After RF heating treatments, the samples were mixed with 20 ml sterile physiological saline in sterile flasks. Then, the mixture was 10-fold serially diluted in 0.9 ml of sterile physiological saline, and 0.1 ml of the diluent was spread onto LB agar. Where low levels of surviving cells were anticipated, 0.1 ml of undiluted homogenate was spread-

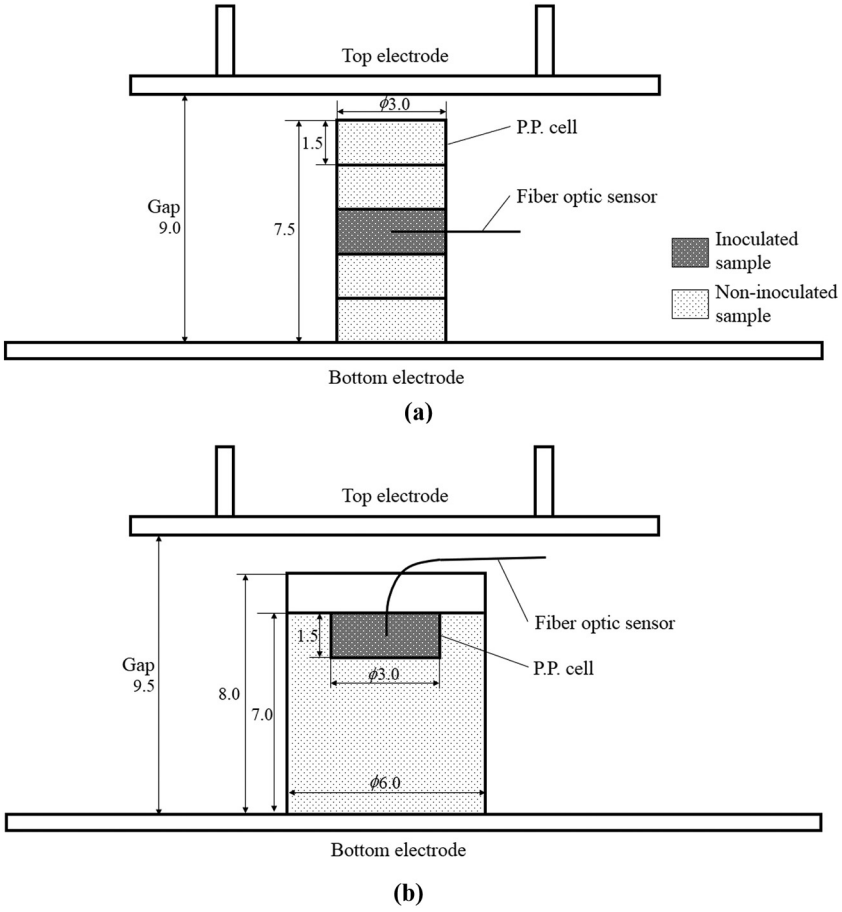


Fig. 1. Schematic diagram of sample arrangements for apple juice (a) and mashed potato (b) in polypropylene (P.P.) cylindrical test cells subjected to the pilot-scale 6 kW, 27.12 MHz RF cavity with all dimensions in cm.

plated (Jeong et al., 2017). All plates were incubated at 37 °C with 24 h for enumeration.

2.5. Heating block thermal treatments

1 ml inoculated apple juice or 1 g mashed potato was placed in a TDT cell in heating block system. Several heating processes with different setpoint temperatures and heating rates were chosen based on the heating process with RF treatments, and continuously operated by the heating block system. Once each sample was heated at the temperatures of 35, 40, 45, 50, 55, 60, and 65 °C, following the real temperature-time curve obtained from the RF heating process, the TDT cells were immediately placed in ice water until further analysis was performed.

After the heating block treatments, the samples were mixed with 9 ml sterile physiological saline in sterile flasks. Then, the procedures of subsequent 10-fold serial dilution and cell enumeration were the same as those with RF treatments.

The mean viable colony counts of two samples at each temperature for both heating methods were log transformed and plotted against the sample temperature in °C. Each treatment was repeated three times.

2.6. Heating uniformity tests

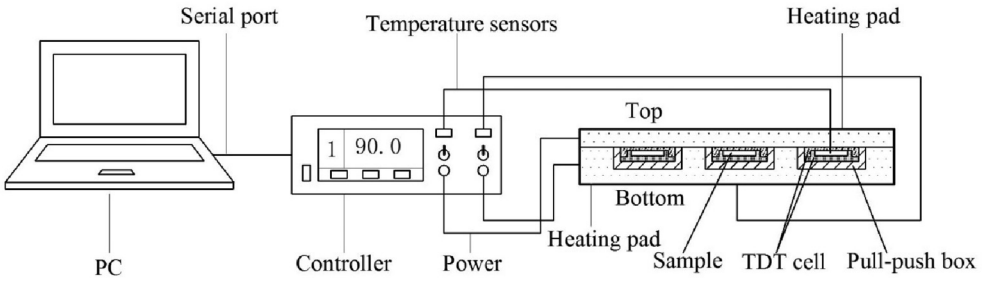
Heating uniformity is an important factor during the comparative experiments. The uniformity index (UI) is one of the useful indexes to evaluate the heating uniformity of treated samples (Hou et al., 2014; Jiao et al., 2012; Wang et al., 2008; Zhang et al., 2017), and is defined as the ratio of rise in standard deviation of sample temperatures to the rise in mean temperatures over the treatment time (Wang et al., 2008). The smaller UI values represent the better heating uniformity (Zhou and Wang, 2016).

Firstly, heating uniformity was examined after the RF heating. For apple juice, immediately after the sample temperature obtained by the fiber-optic system achieving 40, 50 and 60 °C, the surface temperatures of the upper layer were measured by an infrared (IR) thermal imaging camera (ThermaCam A3X0, FLIR Systems AB, Stockholm, Sweden) with accuracy of ± 2 °C. In each of the thermal images, 249 individual temperature data were collected for statistical analyses. The temperatures of selected 9 positions in the middle layers were obtained by a thin type-T thermocouple thermometer (TMQSS-020-6, Omega Engineering Ltd., Stamford, CT, USA). The 9 positions were equally distributed at middle layer in the sample holder and specifically located as shown in Fig. 3. The total time spent in measuring 9 temperatures was < 15 s. For mashed potato, samples were divided into upper and middle layers using a thin-plastic film (thickness of 0.28 mm). The heating procedures were the same as those of the apple juice, except for that the mashed potato temperature distributions in the middle layer were mapped sequentially after the upper layer using the IR system. The mean value and standard deviation of surface and interior sample temperatures were used to determine the heating uniformity index.

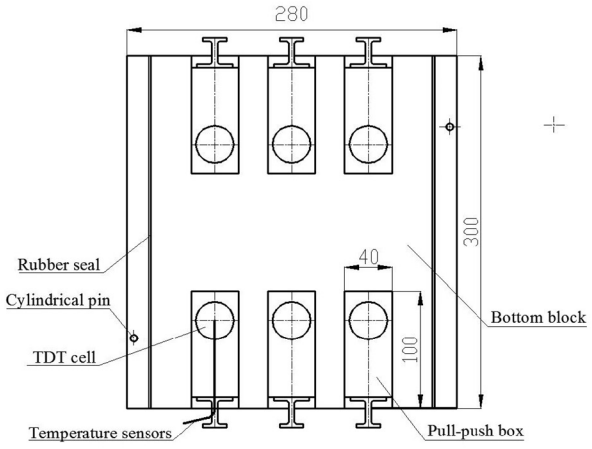
To compare the heating environment between RF and heating block treatments, surface thermal images of apple juice and mashed potato samples with the heating block treatments were taken at the same temperatures of 40, 50 and 60 °C by the IR system. In each of the thermal images, 256 individual surface temperature data points were collected and used for data analyses. Each experiment was replicated three times.

2.7. Statistical analysis

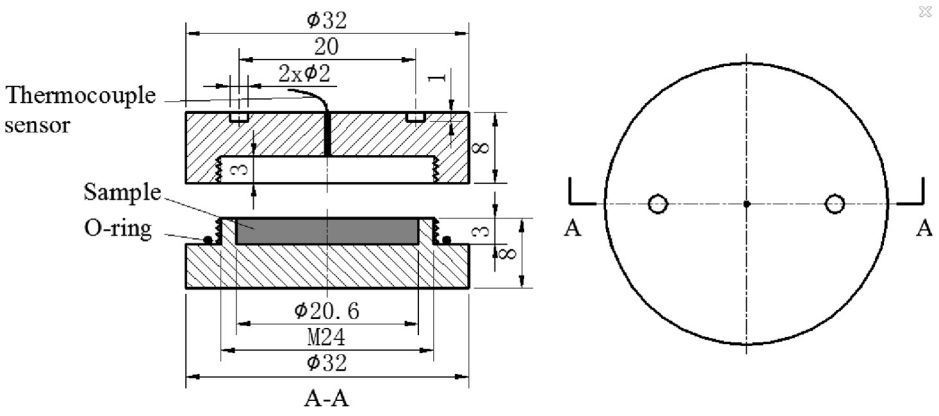
Data were expressed as mean ± standard deviations of triplicate



(a)



(b)



(c)

Fig. 2. Schematic diagram of (a) the TDT heating block system, (b) top view after removing the top block and (c) schematic diagram of a TDT test cell with all dimensions in mm.
(Adapted from Kou et al., 2016).

independent treatments. Significant differences ($P < 0.05$) within means were analyzed using variance and Tukey's honestly significant difference (HSD) test in the statistical software SPSS 16.0 version (SPSS Inc., Chicago, IL, USA) together with the Correlation coefficients (R-value) between the temperature measurement methods.

3. Results

3.1. Heating uniformity evaluation in the RF treatment

The temperatures measured by fiber-optic and IR systems for apple juice and mashed potato at different setpoint temperatures with the RF treatment are listed in Table 1. The sample temperatures using fiber-optic systems were close to those using IR systems, especially for the middle layer, since the average temperature difference between fiber-optic and IR systems was < 0.95 °C and no significant difference ($P > 0.05$) in both sample temperatures was observed among these measurement methods. The Pearson correlation coefficients were 0.996 and 0.999 for fiber-optic and IR temperatures in upper and middle layers for apple juice, and 0.991 and 0.995 for mashed potato, respectively. The temperature difference between the upper and middle layers was < 1.41 °C in apple juice, and 1.96 °C in mashed potato. The

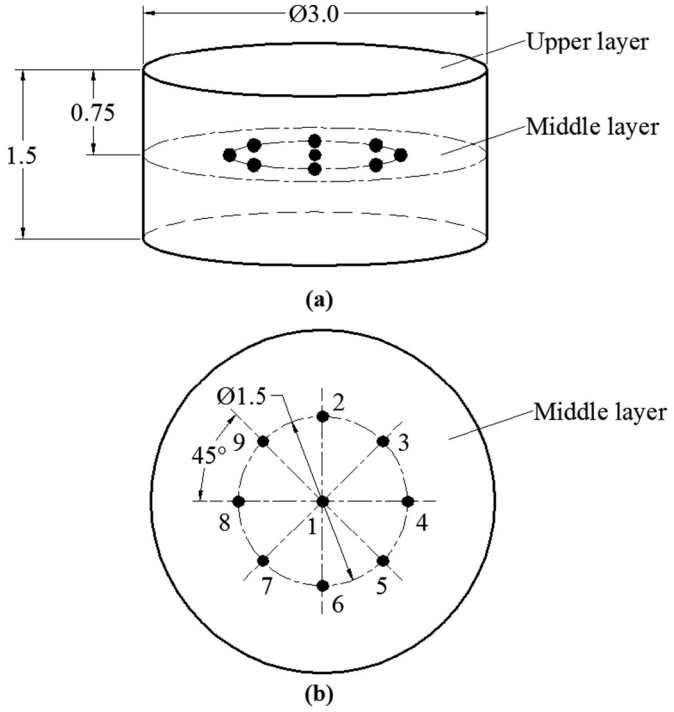


Fig. 3. Nine locations of thermocouples (a) and top view of the middle layer (b) in polypropylene cylindrical test cells for apple juice temperature measurements in RF systems (all dimensions are in cm).

temperatures in the upper layer were a little lower than those in the middle layer since the average temperature in the upper and middle layers over the whole procedure was 49.79 and 50.29 °C in apple juice, and 49.01 and 50.49 °C in mashed potato, respectively. The maximum and average UI values were 0.019 and 0.016 in apple juice, while 0.045 and 0.038 in mashed potato, suggesting the better heating uniformity in apple juice. The heating uniformity in the upper layer was better than in the middle layer, especially for mashed potato, but without significant difference ($P > 0.05$).

3.2. Comparison of the heating process between the RF and heating block treatments

From the surface thermal images of two samples by IR system, it could be found that a uniform temperature distribution was shown both in apple juice and mashed potato at four setpoint temperatures after the RF and heating block treatments, since the maximum temperature difference was < 2 °C (Fig. 4). Judging from the heating pattern, the samples in the TDT-cells with the heating block treatment had slightly better heating uniformity than in the polypropylene cells with the RF treatment, and the temperature distribution was more uniform in apple juice than in mashed potato. The details of the samples' surface temperature are summarized in Table 2. The differences of the average temperature between the RF and heating block treatments were 0.07, 0.11, 0.26 and 0.50 °C in apple juice at the temperatures of 30, 40, 50 and 60 °C, respectively, and 0.11, 0.91, 1.22 and 1.28 °C in mashed potato. The lower standard deviation (SD) and maximum difference temperature in apple juice also indicated better uniformity than in mashed potato for two heating treatments. Compared with the RF treatment, the heating block treatment had smaller SD and maximum difference values of the surface temperature, resulting in a better heating uniformity. But there was no significant difference of average temperatures between treatments when evaluated over the all temperatures ($P > 0.05$).

Fig. 5 shows the temperature-time histories of two samples under the heating block system and RF treatments with a setpoint of 65 °C. By setting a number of linear regression curves, heating processes with different setpoint temperatures and heating rates were continuously operated by the heating block system to simulate the temperature-time histories obtained by the RF treatment. For example, a heating process with the initial temperature of 30 °C, the setpoint temperatures of 35 °C, with the heating rates of 2.4 °C/min were set by the heating block system, to get the same heating process with the RF treatment from 30 to 35 °C for the apple juice sample. As the setpoint temperature increased, the number of heating processes needed in heating block system also increased. Three heating processes with the setpoint temperatures of 45, 60 and 65 °C, and the heating rates of 2.4, 1.4 and 0.6 °C/min were operated by the heating block system, to simulate the RF heating to 65 °C for apple juice. For mashed potato, the heating process during the RF heating to 65 °C, was simulated by the heating block system with also three heating processes for the setpoint temperatures of 35, 50 and 65 °C, and the heating rates of 2.2, 4.2 and 4.8 °C/min accordingly. The maximum temperature difference between the heating process curves obtained from two systems was 0.8 °C in apple juice, and 0.9 °C in mashed potato. The Pearson correlation coefficients were above 0.998 for the temperature profiles of the heating block and RF treatments.

3.3. Effect of RF and heating block treatments on inactivation of microorganisms

Initial populations of *E. coli* and *S. aureus* counted immediately after inoculation were 8.62 ± 0.25 and 8.12 ± 0.22 log CFU/ml in apple juice, and 8.37 ± 0.12 and 8.55 ± 0.25 log CFU/g in mashed potato, respectively. Fig. 6 shows survival curves of the two microbial strains in samples with the RF and heating block treatments. The survival patterns of *E. coli* and *S. aureus* in apple juice and mashed potato using the heating block treatment were similar to those with the RF treatment. As exhibited in Fig. 6 (a), during an initial period using the RF treatment, no significant ($P > 0.05$) log reduction of *E. coli* in apple juice was

Table 1
The comparison of temperature and heating uniformity (Avg ± SD over three replicates) in two food samples between Fiber optic sensor (FO) and infrared thermal imaging camera (IR) at three setpoint temperatures using RF treatments.

Samples	FO Temperature in the center	IR Temperature			
		Upper Layer		Middle Layer	
Apple juice	Avg ± SD (°C)	Avg ± SD (°C)	UI	Avg ± SD (°C)	UI
	40.73 ± 0.33a[a]	40.09 ± 0.07a	0.017 ± 0.011A	40.11 ± 0.23a	0.018 ± 0.002A
	50.20 ± 0.42a	50.04 ± 0.15a	0.016 ± 0.009A	50.13 ± 0.14a	0.019 ± 0.003A
	60.13 ± 0.28a	59.23 ± 0.35a	0.012 ± 0.004A	60.64 ± 0.17a	0.013 ± 0.005A
Mashed potato	40.26 ± 0.17a	39.02 ± 0.23a	0.045 ± 0.002A	39.94 ± 0.64a	0.045 ± 0.003A
	49.77 ± 0.49a	49.16 ± 0.43a	0.024 ± 0.003A	50.72 ± 0.48a	0.038 ± 0.010A
	60.17 ± 0.33a	58.84 ± 0.53a	0.038 ± 0.016A	60.80 ± 0.89a	0.040 ± 0.014A

[a] Same lower and upper case letters indicate that means of the temperature and uniformity index are not significantly different at $P = 0.05$ among layers in the same row.

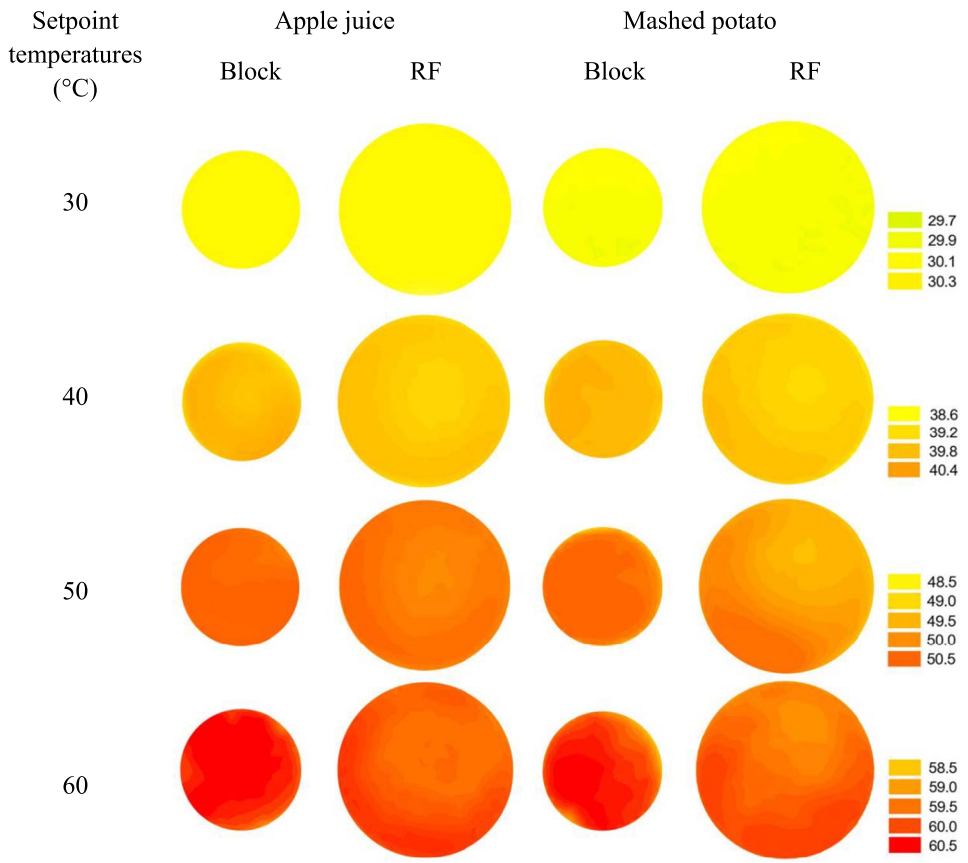


Fig. 4. Temperature distribution on the top surface of apple juice and mashed potato at different setpoint temperatures after RF and heating block treatments (T, °C).

Table 2
The detailed surface temperatures of apple juice and mashed potato at different setpoint temperatures after RF and heating block treatments, including average (Avg), standard deviation (SD) and maximum difference (Max dif) temperatures (°C) based on the thermal imaging data.

Treat	Setpoint temperature	Apple juice			Mashed potato		
		Avg	Std	Max dif	Avg	Std	Max dif
RF	30	30.25[a]	0.026	0.56	30.26	0.056	0.60
	40	40.09	0.071	0.77	39.02	0.231	1.60
	50	50.04	0.146	1.31	49.16	0.427	1.63
	60	59.23	0.350	1.90	58.84	0.534	1.98
Block	30	30.18	0.015	0.31	30.15	0.044	0.35
	40	40.20	0.036	0.37	39.93	0.188	0.91
	50	50.30	0.128	0.98	50.38	0.386	1.15
	60	59.73	0.260	1.58	60.12	0.409	1.65

[a] No significant difference of average temperatures was observed between treatments when evaluated over the all temperatures ($P > 0.05$).

observed when the temperature heated from 30 to 50 °C. At temperature > 50 °C, survival populations started to drop down and decreased almost log-linearly with the temperature. The RF treatment inactivated populations of *E. coli* in apple juice by about 1.79 and 5.38 log at temperatures of 55 and 60 °C, respectively, and viable cell count was not detectable as the sample temperature achieved 65 °C. When using the heating block treatment, survival populations of *E. coli* in apple juice were also reduced slightly by < 0.5 log from the initial level as temperature was below 50 °C. Reduced cell count was about 2.34 and 5.77 log at temperatures of 55 and 60 °C, respectively, and *E. coli* was also no longer detectable when the sample temperature reached 65 °C. For *E. coli* in mashed potato, the survival populations were 3.82 and 3.68 log CFU/g with the RF and heating block treatment at 65 °C, respectively. For *S. aureus*, at finial temperature of 65 °C, the RF heating achieved 5.65 and 4.33 log reductions in apple juice and mashed potato, respectively, while 5.96 log CFU/ml and 4.80 log CFU/g reductions were correspondingly obtained in the heating block treatment.

Table 3 lists the absolute difference of survival populations in two samples between the RF and heating block treatments at different setpoint temperatures. The difference was no > 1 log CFU/ml (CFU/g), and 92.9% of the absolute difference values were < 0.5 log CFU/ml (CFU/g), in which 38.5% data were equal or < 0.1 log CFU/ml (CFU/g). According to Tukey's HSD test, the lowest *P*-value in four cases comparison between the two treatments at each setpoint temperature was 0.09 (Table 3), and the *P*-value over the whole temperatures was 0.87, 0.67, 0.80 and 0.85 for the cases of *E. coli* in apple juice, *E. coli* in mashed potato, *S. aureus* in apple juice, and *S. aureus* in mashed potato, respectively.

4. Discussion

In the present study, a uniform temperature distribution and a real-time temperature control in samples were achieved in comparative experiments between the RF heating and conventional heating. From the surface temperature comparison of two sample layers, the maximum temperature difference within the samples was 1.98 °C during RF treatment, which were better than the earlier results (3.3 °C) in microwave heated gel model samples (1 cm diameter) reported by Hamoud-Agha et al. (2013), and a temperature difference over 5 °C with RF heating using a potato cube sample of 3 cm per side published by Ferrari-John et al. (2016). Meanwhile, the maximum temperature difference during the heating block treatment was 1.65 °C at the same temperature with the same samples, indicating that the similar trend of temperature gradients appeared in two samples at each setpoint temperature between both treatments (Table 2). The sample temperatures were more uniform in apple juice than in mashed potato due to free heat convection existed in liquid food, which was in good agreement

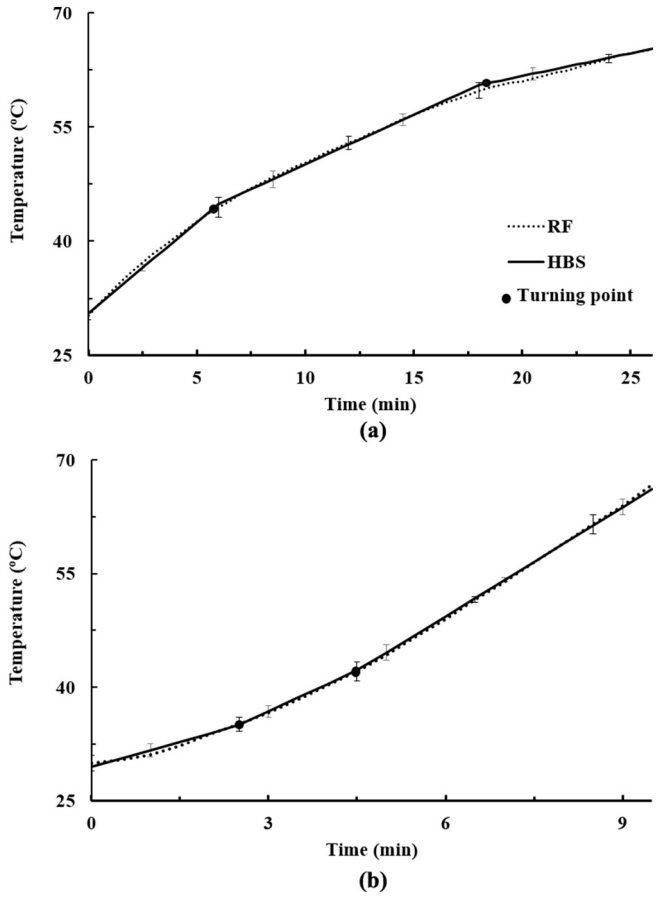


Fig. 5. The center temperature-time histories of (a) apple juice and (b) mashed potato with RF and the block heating.

with the data in James et al. (2002) and Vadivambal and Jayas (2010). The relatively lower temperature in the upper layer of two samples was mainly because of the heat loss between the outer surfaces of the samples and the surrounding air (≈25 °C). The better uniformity in the upper layer was caused by the field bending all-around of the sample and concentrating on the middle part, which was in good agreement with the results in Huang et al. (2015), Zheng et al. (2016), and Zhou and Wang (2016). The different temperature distribution on the sample surface in heating block and RF treatment was due to the different heat transfer modes. The energy conversion from RF energy to heat occurs within the product itself, but conventional heat is transferred by convection, conduction, and infrared radiation (Jeong et al., 2017). The heating uniformity was slightly better in TDT-cell than in polypropylene cell, mainly caused by the smaller capacity in the TDT-cell. The acceptable temperature distribution in apple juice and mashed potato demonstrated that the central sample temperature could be used as representative one for evaluating thermal inactivation of microorganisms over the whole geometrical volume. The difference of the core temperature obtained by the fiber-optic and thermocouples between RF and heating block treatments was < 1.0 °C in two samples over the whole heating process, guaranteeing the rates of temperature rise in the RF and heating block treatments be identical all the time, so as to distinguish the thermal and athermal effects of RF heating on inactivating microorganisms.

Based on the repeatable temperature data, the present study investigated the effect of RF heating on inactivating microorganisms in foods. Below the sublethal temperature, the viable cell count in all cases reduced slightly, and the maximum survival populations appeared at temperature of 40–45 °C with both heating block and RF treatments, which is in accordance with Li et al. (2017b) and Yuk and Marshall

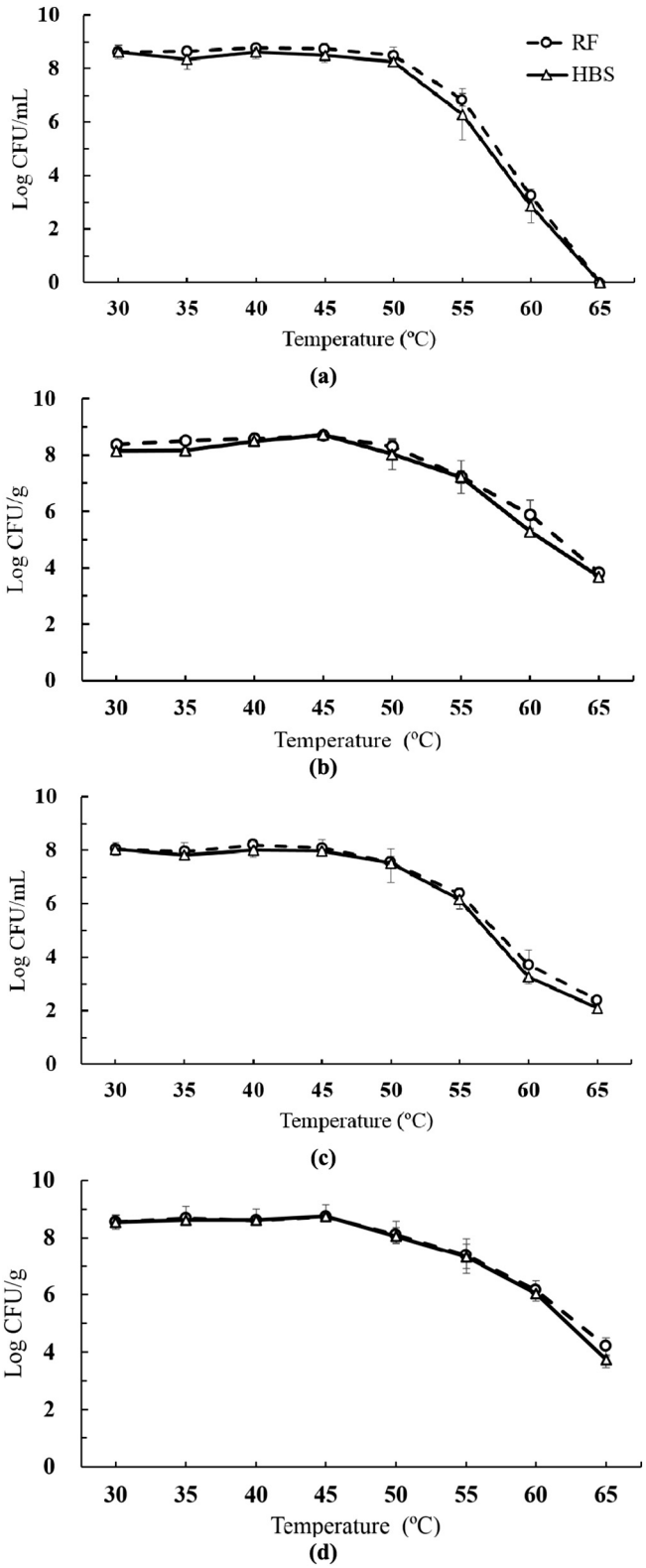


Fig. 6. Survival populations (Avg ± SD) of microbial strains in two representative samples during RF and block heating. (a) *E. coli* in apple juice, (b) *E. coli* in mashed potato, (c) *S. aureus* in apple juice, and (d) *S. aureus* in mashed potato.

(2003). During the inactivation periods, a > 7 log reduction of *E. coli* was confirmed in apple juice at 65 °C, which was similar to the result published by Awuah et al. (2005) reporting a RF treatment inactivation of > 7.45 log reduction for *E. coli* K-12 in milk at temperature of 62.2 °C with holding time of 24 s. The microbial population reduction in apple

Table 3
The absolute difference of survival population and *P*-value in two samples between the RF and heating block treatments at different setpoint temperatures.

Experiment	Setpoint temperatures (°C)						
	35	40	45	50	55	60	65
Difference of survival population (CFU/ml for apple juice, CFU/g for mashed potato)							
E. coli in apple juice	0.29	0.15	0.23	0.24	0.55	0.38	0.00
E. coli in mashed potato	0.18	0.09	0.03	0.28	0.00	0.21	0.14
S. aureus in apple juice	0.33	0.25	0.20	0.10	0.19	0.61	0.16
S. aureus in mashed potato	0.06	0.01	0.02	0.06	0.07	0.13	0.47
P-value							
E. coli in apple juice	0.40	0.53	0.39	0.36	0.48	0.46	NA[a]
E. coli in mashed potato	0.09	0.35	0.60	0.55	0.99	0.12	0.22
S. aureus in apple juice	0.36	0.46	0.51	0.87	0.65	0.13	0.20
S. aureus in mashed potato	0.66	0.91	0.82	0.85	0.91	0.70	0.19

[a] NA means that *P*-value cannot be computed because the standard deviations of both groups are 0.

juice was a little higher than that in mashed potato at a treatment temperature of 65 °C using the RF or heating block treatments. Similar results are observed by Yuk et al. (2009) and Chung et al. (2007), indicating that the *D*-value for *E. coli* K12 in apple cider was 0.05 min at 62 °C, and in mashed potato was 0.51 min at 63 °C, respectively. *S. aureus* strains showed a higher thermotolerance than *E. coli* in two samples, which was in accordance with the data reported by Buzrul and Alpas (2007). Statistically, however, there was no significant difference ($P > 0.05$) in inactivating these bacteria strains between the RF and the heating block treatments during the whole inactivation periods.

The results in this study were close to those in a well-controlled heating system reported by Shazman et al. (2007). In their study, a combined microwave and conventional heating equipment was designed, resulting in the required time-temperature curve. They clearly demonstrated no athermal effect in Maillard reaction, protein denaturation, and mutagenesis of bacteria. It has shown that there is necessary to develop and maintain precise temperature control, including a uniform temperature distribution and the real-time temperature measurement and simulation in samples during the heating treatment, in order to confirm the existence of athermal effect in dielectric heating processes on inactivating microorganisms. Although there were still studies reported the dielectric heating produced different reductions of tested pathogens at the same temperature using convective heating (Boldor et al., 2008; Latorre et al., 2012; Schlisselberg et al., 2013; Shamis et al., 2012), the application limitation of these studies is the lack of the specific temperature analysis between the treatments, which acts as obstacles to distinguish the thermal and athermal effects of the dielectric heating. Hamoud-Agha et al. (2014) and Wang and Wang (2016) also noted that it would be a beneficial start for the industrial application of RF and microwave treatment processes, when achieving the required real-time temperature control and the heating uniformity of the treatment methods. According to the above analysis, the results of this experiment clearly supported the conclusion that only thermal effect of RF energy at 27.12 MHz was observed on inactivating microorganisms in foods.

Lack of athermal effects of RF treatments from this study might be because the electric field intensity was too lower to rupture the cell membrane. Hülsheger et al. (1981) compared the killing rate of Escherichia coli K-12 by using electric pulses with different field intensity, and proved that 5–6 kV/cm was a minimum electric field intensity for inactivation of *E.coli*. Ukuku et al. (2008) found that the inactivation of *Escherichia coli* K-12 in apple juice at a frequency of 20 kHz was higher at electric intensity of 15 kV/cm than at 0.15 kV/cm at the same temperature due to the athermal effects. In the present study, the field intensity of the 27.12 MHz RF system was < 0.3 kV/cm simulated by Huang et al. (2016a), which was less than electric field intensity of approximately 0.5 kV/cm in the 18 MHz RF treatment used by Geveke et al. (2002) who reported only thermal effect on the inactivation of microorganisms. Another possible influence factor for athermal effects is the treatment frequency. Many studies reported the athermal effects at low frequency range (Saadi et al., 2014; Ukuku et al., 2008). Voina et al. (2016) revealed that the biochemical processes had significant changes in certain frequency range of 1–160 Hz and Geveke et al. (2002) also indicated a new RF system with higher electric field intensity and/or lower frequency should be developed to identify non-thermal effects on inactivating microorganisms. Therefore, no athermal effects could be identified on inactivating microorganisms in RF systems due to the lower electric field intensity (< 0.3 kV/cm) and high frequency range (27 MHz).

5. Conclusion

This study examined the thermal and athermal effects of RF energy on inactivating microorganisms in two representative samples. A unique TDT heating block system providing only thermal energy to food materials was developed to simulate the same RF heating process, including the heating uniformity, final temperature and heating rate. Two microbial strains in apple juice and mashed potato were selected for the inactivation comparison experiments using RF and block system under the same heating conditions. After achieving the same heating conditions, the inactivation results demonstrated that no significant difference in inactivating two bacteria strains in two samples was observed between RF and block heating treatments. The results suggested that athermal effects do not exist in RF systems at 27.12 MHz for microbial inactivation and the practical RF pasteurization process should be developed based only on thermal effects.

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