Food Microbiology 63 (2017) 22–27

Contents lists available at ScienceDirect

Food Microbiology

journal homepage: www.elsevier.com/locate/fm

Application of low frequency pulsed ohmic heating for inactivation of foodborne pathogens and MS-2 phage in buffered peptone water and tomato juice

Sang-Soon Kim [a, b], Won Choi [c], Dong-Hyun Kang [a, b, *]

[a] Department of Agricultural Biotechnology, Center for Food and Bioconvergence, and Research Institute for Agricultural and Life Sciences, Seoul National University, Seoul 08826, Republic of Korea
[b] Institutes of Green Bio Science & Technology, Seoul National University, Pyeongchang-gun, Gangwon-do, 25354, Republic of Korea
[c] Department of Rural Systems Engineering, College of Agriculture and Life Sciences, Seoul National University, Seoul 08826, Republic of Korea

ARTICLE INFO

Article history:
Received 3 December 2015
Received in revised form
6 September 2016
Accepted 9 October 2016
Available online 11 October 2016

Keywords:
Frequency
Pulsed ohmic heating
Pathogens inactivation
Sub-lethal injury
Tomato juice
Electrode corrosion

ABSTRACT

The purpose of this study was to inactivate foodborne pathogens effectively by ohmic heating in buffered peptone water and tomato juice without causing electrode corrosion and quality degradation. *Escherichia coli* O157:H7, *Salmonella* Typhimurium, and *Listeria monocytogenes* were used as representative foodborne pathogens and MS-2 phage was used as a norovirus surrogate. Buffered peptone water and tomato juice inoculated with pathogens were treated with pulsed ohmic heating at different frequencies (0.06–1 kHz). Propidium iodide uptake values of bacterial pathogens were significantly ($p < 0.05$) larger at 0.06–0.5 kHz than at 1 kHz, and sub-lethal injury of pathogenic bacteria was reduced by decreasing frequency. MS-2 phage was inactivated more effectively at low frequency, and was more sensitive to acidic conditions than pathogenic bacteria. Electrode corrosion and quality degradation of tomato juice were not observed regardless of frequency. This study suggests that low frequency pulsed ohmic heating is applicable to inactivate foodborne pathogens effectively without causing electrode corrosion and quality degradation in tomato juice.

1. Introduction

Foodborne illness has long been a worldwide public health problem. The US Centers for Disease Control and Prevention (CDC) indicates that 48 million people become ill, 128,000 are hospitalized, and 3000 die of foodborne illness in the United States each year (CDC, 2010). Harmful substances causing foodborne illness include biological, chemical, and physical hazards (NACMCF, 1992). *Escherichia coli* O157:H7, *Salmonella* Typhimurium, *Listeria monocytogenes*, and norovirus are representative biological hazards related to foodborne outbreaks (CDC, 2011; Scallan et al., 2011). Raw ingredients such as vegetables can become contaminated with pathogenic microorganisms via soil, water, and fertilizer, and Sun (2011) indicated that cross-contamination during processing commonly occurs from utensils/surfaces and infected food handlers.

Thermal processing is widely employed to eliminate biological hazards in food. In conventional heating, heat transfer is achieved via conduction and convection, which cause cold spots in samples. Tewari and Juneja (2008) indicated that pathogenic microorganisms can survive in cold spots. Therefore, novel thermal technologies such as radio-frequency heating (Awuah et al., 2014), microwave heating, and ohmic heating have been proposed as alternatives, which enable rapid and uniform heating. Among these alternative technologies, ohmic heating especially has advantages in heating uniformity, cost, and energy efficiency (Sastry et al., 2014). By virtue of these advantages, ohmic heating can be used in various food processes such as blanching, evaporation, dehydration, fermentation, and extraction (Sastry and Barach, 2000).

Electrode corrosion is a crucial obstacle when ohmic heating is used for food processing. Metal ions, which are contaminants and have toxic potential, can migrate into a food sample due to electrode corrosion. Moreover, oxygen produced during electrolysis can oxidize lipids and vitamin C (Tola et al., 2014). Several interventions have been proposed to prevent electrode corrosion. At first, inert electrodes such as titanium and platinum were introduced instead

* Corresponding author. Department of Agricultural Biotechnology, Seoul National University, Seoul, 08826, Republic of Korea.
E-mail address: kang7820@snu.ac.kr (D.-H. Kang).

http://dx.doi.org/10.1016/j.fm.2016.10.021
0740-0020/

of stainless steel (Tzedakis et al., 1999). Secondly, high frequency was adopted to minimize undesired chemical reactions (Lee et al., 2013). Finally, pulsed ohmic heating with high frequency and shorter pulse width was suggested as a solution to reduce electrode corrosion (Samaranayake et al., 2005).

The level of frequency varies from a few Hz to dozens of kHz in ohmic heating. Even though the usual frequency of the general electrical system is 50 or 60 Hz, low frequencies have been avoided in ohmic heating because of electrolytic effects (Lee et al., 2013). However, development of inert electrode material and pulse waveforms enable the use of a wide range of frequency. Accordingly, the effect of frequency has been studied from various points of view recently. In particular, the relation between frequency and non-thermal effect was identified for drying rate, spore inactivation, and diffusion in relation to the food industry (Kulshrestha and Sastry, 2003; Lima and Sastry, 1999; Somavat et al., 2012). Even though pathogenic bacteria and viruses are major biological hazards causing foodborne illness, research investigations about the effect of frequency on inactivation of foodborne pathogens is limited.

The aim of the present study was to identify the effect of frequency in pulsed ohmic heating for inactivation of *E. coli* O157:H7, *S.* Typhimurium, *L. monocytogenes* and MS-2 bacteriophage (norovirus surrogate). Electrode corrosion and the change of color and lycopene content of tomato juice were also investigated in this study.

2. Materials and methods

2.1. Analysis of electrode corrosion

Analysis of electrode corrosion was conducted according to the method performed by Lee et al. (2013). Tomato juice samples were subjected to ohmic heating with various waveforms (sine, square, triangle, and pulse) and frequencies (0.06, 0.1, and 0.2 kHz). After tomato juice reached 90 °C, a 1-g sample was collected into a polypropylene bottle and then stabilized by adding 10 ml of concentrated nitric acid (60% v/v). Concentrations of Ti (mg/kg) migrating into the tomato juice were taken as measures of electrode corrosion. Quantitative analyses of Ti were conducted by an inductively coupled plasma-mass spectrometer (820-MS; Varian, Australia). An untreated sample was used as a blank (control).

2.2. Bacterial cultures and cell suspension

Three strains each of *E. coli* O157:H7 (ATCC 35150, ATCC 43889, ATCC 43890), *S.* Typhimurium (ATCC 19585, ATCC 43971, DT 104), and *L. monocytogenes* (ATCC 19111, ATCC 19115, ATCC 15313) were obtained from the bacteria culture collection of Seoul National University (Seoul, Korea). Stock cultures and working cultures were prepared by the method described previously (Kim and Kang, 2015a,b). A single colony cultivated from frozen stocks on tryptic soy agar (TSA; Difco, Becton, Dickinson, Sparks, MD) was inoculated into 5 ml of tryptic soy broth (TSB; Difco, Becton, Dickinson, Sparks, MD), incubated at 37 °C for 24 h, collected by centrifugation at 4000 × g for 20 min at 4 °C, and washed three times with 0.2% peptone water (PW; Bacto, Becton, Dickinson, Sparks, MD). The final pellets were resuspended in 0.2% PW, corresponding to approximately 10^8–10^9 CFU/ml. Afterwards, suspended pellets of the three pathogens were combined to comprise a mixed culture cocktail containing approximately equal numbers of cells of each strain of *E. coli* O157:H7 (10^7 CFU/ml), *S.* Typhimurium (10^7 CFU/ml), and *L. monocytogenes* (10^6 CFU/ml).

2.3. Sample preparation and inoculation

Sterile buffered peptone water (BPW; Difco, Sparks, MD, pH 7.2) and pasteurized tomato juice (pH 3.6; 11.8 °Brix) were used in this experiment. Each sample was stored under refrigeration (4 °C) and removed, 1 h prior to inoculation, and allowed to equilibrate to room temperature (22 ± 1 °C). Twenty five ml of each sample were put in the ohmic heating chamber. Each pathogen culture (0.2 ml) was inoculated respectively for the propidium iodide uptake test whereas the three pathogens were combined to comprise a mixed culture cocktail for inactivation experiments.

2.4. Propidium iodide uptake test

The fluorescent dye propidium iodide (PI; Sigma-Aldrich, P4170) was used to determine cell membrane damage. The PI uptake test was conducted according to the method described by Park and Kang (2013). BPW was subjected to ohmic heating at various frequencies until reaching 75 °C. Untreated and ohmic treated BPW were centrifuged at 10,000*g* for 10 min at 4 °C. Supernatants were discarded, and the cell pellets were resuspended in 5 ml phosphate-buffered saline (PBS; Corning, pH 7.4) to an optical density at 680 nm of approximately 0.2 (Spectramax M2e; Molecular Devices, Sunnyvale, CA) for *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes*, corresponding to approximately 10^7 CFU/ml. PI was added to a final concentration of 2.9 μM and incubated for 10 min. After incubation, samples were centrifuged two times under the same conditions. The final cell pellets were resuspended in 5 ml PBS and fluorescence was measured with a spectrofluorophotometer (Spectramax M2e; Molecular Devices, Sunnyvale, CA) at an excitation wavelength of 493 nm and an emission wavelength of 630 nm. Fluorescence data obtained for untreated cells were subtracted from all treated values and then normalized for OD_{680}.

$$\text{PI value} = (\text{fluorescence value of treated cells} - \text{fluorescence value of untreated cells})/OD_{680}$$

2.5. Ohmic heating treatment

Ohmic heating treatments were carried out in a previously described apparatus (Kim and Kang, 2015c). The ohmic heating system consisted of a function generator (catalog number 33210A; Agilent Technologies, Palo Alto, CA), a precision power amplifier (catalog number 4510; NF Corp., Yokohama, Japan), a two-channel digital-storage oscilloscope (catalog number TDS2001C; Tektronix, Inc., Beaverton, CO), a data logger (catalog number 34970A; Agilent Technologies), and an ohmic heating chamber. BPW and tomato juice inoculated with pathogens were treated by ohmic heating with pulse waveform (0.1 duty ratio) and different frequencies (0.06, 0.2, 0.5, and 1 kHz). The electric field strength was fixed at 47.7 V_{pp}/cm. The target temperature was 70 °C and 80 °C for BPW and tomato juice, respectively.

2.6. Bacterial enumeration

2.6.1. Enumeration with selective medium

For microbial enumeration, each treated 25 g sample was immediately transferred into a sterile stomacher bag (Labplas, Inc., Sainte-Julie, Quebec, Canada) containing 225 ml of sterile 0.2% peptone water and homogenized for 2 min using a stomacher (Easy Mix; AES Chemunex, Rennes, France). After homogenization, 1 ml samples were 10-fold serially diluted with 9 ml of sterile 0.2%

peptone water and 0.1 ml of stomached samples or diluents were spread plated onto each selective medium. Sorbitol MacConkey (SMAC) agar (Difco), xylose lysine deoxycholate (XLD) agar (Difco), and Oxford agar base (OAB; Difco) with antimicrobial supplement (Bacto Oxford antimicrobial supplement; Difco) were used as selective media for enumeration of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes*, respectively. All plates were incubated at 37 °C for 24–48 h before counting colonies characteristic of the pathogens.

2.6.2. Enumeration of sub-lethally injured bacterial cells

The overlay (OV) method, developed by Hartman et al. (1975) and verified by Wu and Fung (2001), was used to enumerate sub-lethally injured cells of *S.* Typhimurium and *L. monocytogenes*. After cells resuscitated on tryptic soy agar (TSA; Difco) at 37 °C for 2 h, plates were overlaid with 7–8 ml of selective medium (XLD or OAB). The plates were further incubated for 22–46 h at 37 °C before colonies were counted. Phenol red agar base with 1% sorbitol (SPRAB; Difco) was used to enumerate injured cells of *E. coli* O157:H7. After incubation at 37 °C for 24 h, typical white colonies characteristic of *E. coli* O157:H7 were enumerated. Randomly selected isolates from SPRAB plates were subjected to serological confirmation as *E. coli* O157:H7 (RIM, *E. coli* O157:H7 latex agglutination test; Remel, Lenexa, KS), because SPRAB is not typically used as selective agar for enumerating *E. coli* O157:H7.

2.7. Experimental procedures for MS-2 phage inactivation

MS-2 bacteriophage ATCC 15597 stocks were prepared to have an initial population of approximately 4.2×10^{11} PFU/ml. A single colony of *E. coli* C3000 from frozen stocks on TSA was inoculated into 5 ml TSB and incubated at 37 °C until attaining exponential to early stationary phase. BPW and tomato juice inoculated with 0.1 ml of phage culture were treated with ohmic heating until reaching 70 °C and 80 °C, respectively. Each treated sample was immediately transferred into a sterile stomacher bag and homogenized with a stomacher. For enumeration, the soft-agar overlay (double-agar layer) plaque assay method was used (Cho et al., 2011). The medium for bottom agar used in the plaque assays contained 1 g/L yeast extract, 1 g/L glucose, 8 g/L sodium chloride, 0.22 g/L calcium chloride, 10 g/L tryptone, and 15 g/L agar. Lactose Broth (LB; Difco) with 7 g/L agar was used as the top agar medium.

2.8. Color, lycopene measurement

Color and lycopene content of treated and untreated tomato juice (control) were measured. All treated samples were cooled immediately in a crushed ice water mixture. Color values were measured with a Minolta colorimeter (model CR400; Minolta Co., Osaka, Japan). The values for L^*, a^*, and b^* were measured to evaluate color changes of tomato juice. The parameter L^* is a measure of lightness, a^* is an indicator of redness, and b^* is a measure of yellowness. Lycopene content in tomato juice was measured according to the previously described method (Lee et al., 2013). The concentrations of lycopene in tomato juice were determined using absorbance and sample weight with equation (1). Absorbance was measured with a spectrofluorophotometer (Spectramax M2e; Molecular Devices, Sunnyvale, CA) at 503 nm.

$$\text{Lycopene (mg/kg tissue)} = A_{503} * 0.0312/\text{kg sample} \quad (1)$$

2.9. Statistical analysis

All experiments were replicated three times. All experiments for pathogen inactivation were duplicate-plated. All data were analyzed by the analysis of variance procedure of the Statistical Analysis System (version 9.3, SAS Institute, Cary, NC) and mean values were separated using Duncan's multiple-range test. Significant differences in the processing treatments were determined at a significance level of $p = 0.05$.

3. Results

3.1. Effect of waveform and frequency on the electrode corrosion

The level of electrode corrosion increased significantly ($p < 0.05$) with decreasing frequency in sine, square, and triangle waveforms (Fig. 1). On the other hand, frequency did not have a significant effect ($p > 0.05$) on the level of electrode corrosion when using a pulse waveform. The concentration of titanium ions was less than 0.25 mg/kg when using a pulse waveform regardless of frequency. Therefore, the pulse waveform was utilized in subsequent experiments.

3.2. PI uptake ability

PI uptake values in BPW varied with frequency and type of pathogen (Table 1). The values for *L. monocytogenes* were larger than those for *E. coli* O157:H7 and *S.* Typhimurium regardless of frequency. For each pathogen, PI uptake values at 1 kHz were significantly ($p < 0.05$) lower than those at 0.06–0.5 kHz.

3.3. Inactivation of bacterial pathogens and MS-2 bacteriophage

Reductions (log CFU/ml) of bacterial pathogens differed depending on frequency and type of sample (Table 2). Reductions in BPW were larger than those in tomato juice for all three bacterial pathogens. For each pathogen, the levels of inactivation were not significantly different among frequencies when enumerated on the selective agar ($p > 0.05$). On the other hand, reductions decreased significantly at 1 kHz when enumerated on the recovery medium ($p < 0.05$). Resuscitation levels of sub-lethally injured cells were calculated from the inactivation data (Table 3). The level of resuscitation increased as frequency increased for all three pathogens and the values at 1 kHz was significantly larger those of lower frequencies ($p < 0.05$).

Reductions (log PFU/ml) of MS-2 bacteriophage in BPW and tomato juice are shown in Table 4. The reductions in tomato juice were larger than those in BPW regardless of frequency. For each sample, the level of inactivation significantly increased ($p < 0.05$) as frequency decreased.

3.4. Color and lycopene content of tomato juice

Color values of L*, a*, and b* and lycopene content were chosen to represent tomato juice quality (Table 5). Color values of treated samples were not significantly different from those of untreated samples ($p > 0.05$). Lycopene content of treated samples also did not significantly differ from that of untreated samples ($p > 0.05$).

4. Discussion

The objectives of the present study were to identify the effect of frequency of pulsed ohmic heating on electrode corrosion, PI uptake, pathogen inactivation, and the quality of food. The electrode corrosion rate of all waveforms, except pulse, increased as

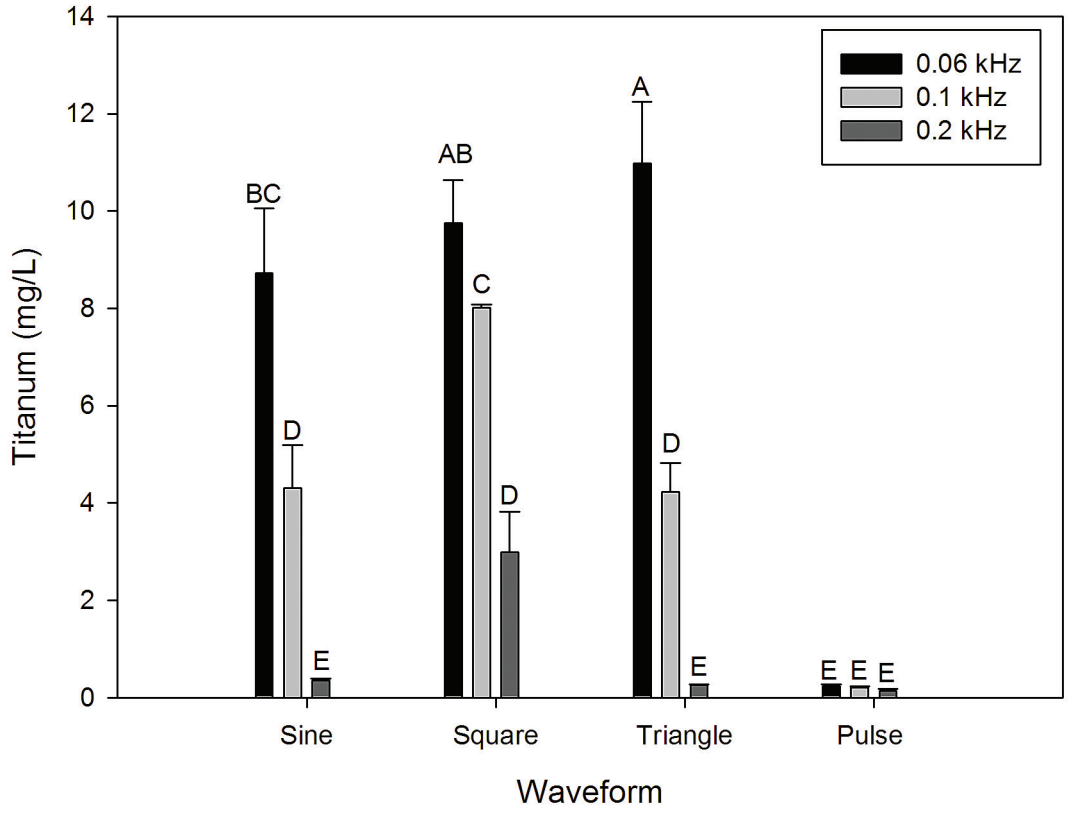

Fig. 1. Concentration of titanium ions migrated into tomato juice subjected to ohmic heating at different waveforms and frequencies. The results are means from three replications, and error bars indicate standard errors. Bars with different letters are significantly different ($p < 0.05$).

Table 1
PI uptake value of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* subjected to pulsed ohmic heating at different frequencies[a].

Frequency (kHz)	*E. coli* O157:H7	*S.* Typhimurium	*L. monocytogenes*
0.06	4.40 ± 0.28 A	4.55 ± 0.35 A	9.09 ± 1.30 A
0.2	4.88 ± 0.90 A	4.68 ± 0.31 A	9.35 ± 0.74 A
0.5	4.29 ± 0.13 A	4.84 ± 0.31 A	9.08 ± 0.87 A
1	2.83 ± 0.14 B	3.61 ± 0.11 B	7.38 ± 0.25 B

Means ± standard deviation.
[a]Values in the same column followed by the same letter are not significantly different ($p > 0.05$).

frequency decreased in the present study. Electrode corrosion occurs when the electrical double-layer capacitor is fully charged and faradic current is generated (Samaranayake and Sastry, 2014). Because electrochemical reactions are considered undesirable in food processing, several solutions have been proposed. Even though high frequency above 1 kHz is suggested as one solution to inhibit electrode corrosion in salsa processing (Lee et al., 2013), high frequency equipment is relatively expensive, heavy, and large in size (Samaranayake et al., 2005). Pulsed ohmic heating has been suggested as another solution, but studies about the application of low frequency pulsed ohmic heating for tomato juice processing are limited. In the present study, pulsed ohmic heating prevented electrode corrosion sufficiently at low frequencies (0.06–0.2 kHz), different from the other waveforms. Therefore, we used pulse waveform in subsequent experiments.

Table 2
Reduction (log CFU/ml) of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* in buffered peptone water (BPW) and tomato juice subjected to pulsed ohmic heating at different frequencies[a,b].

	Frequency (kHz)	*E. coli* O157:H7		*S.* Typhimurium		*L. monocytogenes*	
		SMAC	SPRAB	XLD	XLD-OV	OAB	OAB-OV
BPW	0.06	3.66 ± 0.11 Abc	3.33 ± 0.35 Abc	4.68 ± 0.55 Aa	4.11 ± 0.44 Aab	3.58 ± 0.68 Abc	2.95 ± 0.33 Ac
	0.2	3.72 ± 0.05 Abc	3.37 ± 0.35 Ac	4.54 ± 0.20 Aa	3.94 ± 0.17 Ab	3.62 ± 0.25 Abc	2.59 ± 0.47 Ad
	0.5	3.83 ± 0.15 Ab	3.22 ± 0.42 Ab	4.74 ± 0.49 Aa	3.87 ± 0.06 ABb	3.34 ± 0.65 Ab	2.14 ± 0.15 ABc
	1	3.64 ± 0.31 Ab	2.27 ± 0.23 Bc	4.84 ± 0.43 Aa	3.45 ± 0.14 Bb	3.97 ± 0.45 Ab	1.69 ± 0.62 Bc
Tomato juice	0.06	2.61 ± 0.15 Abc	2.32 ± 0.31 Ac	3.37 ± 0.06 Aa	2.54 ± 0.12 Abc	2.87 ± 0.53 Ab	2.49 ± 0.16 Abc
	0.2	2.77 ± 0.24 Abc	2.43 ± 0.07 ABbc	3.48 ± 0.12 Aa	2.30 ± 0.43 Abc	2.87 ± 0.53 Ab	2.19 ± 0.32 Ac
	0.5	2.65 ± 0.05 Ab	2.16 ± 0.27 ABb	3.64 ± 0.25 Aa	2.62 ± 0.32 Ab	3.41 ± 0.42 Aa	2.54 ± 0.47 ABb
	1	2.82 ± 0.09 Aa	1.97 ± 0.09 Bb	3.33 ± 0.18 Aa	1.52 ± 0.02 Bb	3.41 ± 0.93 Aa	1.53 ± 0.34 Bb

Means ± standard deviation.
[a]Values in the same column for each sample followed by the same uppercase letter are not significantly different ($p > 0.05$).
[b]Values in the same row followed by the same lowercase letter are not significantly different ($p > 0.05$).

Table 3
Resuscitated injured cells (log CFU/ml)[a] of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* in buffered peptone water (BPW) or tomato juice subjected to pulsed ohmic heating at different frequencies[b].

	Frequency (kHz)	*E. coli* O157:H7	*S.* Typhimurium	*L. monocytogenes*
BPW[c]	0.06	0.33 ± 0.32 Aa	0.57 ± 0.16 Aa	0.63 ± 0.36 Aa
	0.2	0.35 ± 0.34 Aa	0.60 ± 0.34 Aa	1.03 ± 0.37 Aa
	0.5	0.61 ± 0.28 Aa	0.86 ± 0.46 ABa	1.20 ± 0.52 Aa
	1	1.38 ± 0.27 Ba	1.39 ± 0.44 Ba	2.28 ± 0.32 Bb
Tomato juice	0.06	0.30 ± 0.22 Aa	0.90 ± 0.18 Aa	0.38 ± 0.48 Aa
	0.2	0.34 ± 0.32 ABa	1.18 ± 0.49 ABa	0.68 ± 0.41 Aa
	0.5	0.50 ± 0.22 ABa	1.02 ± 0.57 Aa	0.87 ± 0.26 Aa
	1	0.85 ± 0.16 Ba	1.81 ± 0.19 Bb	1.88 ± 0.59 Bb

Means ± standard deviation.
[a]Resuscitated injured cell levels were calculated by subtracting the populations enumerated on selective media (SMAC, XLD, OAB) from those of media used for recovery (SPRAB, XLD-OV, OAB-OV).
[b]Values in the same column for each sample followed by the same uppercase letter are not significantly different ($p > 0.05$).
[c]Values in the same row followed by the same lowercase letter are not significantly different ($p > 0.05$).

Table 4
Reduction (log PFU/ml) of MS-2 phage in buffered peptone water (BPW) and tomato juice subjected to pulsed ohmic heating at different frequencies[a].

Frequency (kHz)	BPW[b]	Tomato juice
0.06	2.35 ± 0.09 Aa	5.80 ± 0.38 Ab
0.2	2.05 ± 0.34 ABa	5.20 ± 0.14 Bb
0.5	1.71 ± 0.15 BCa	5.17 ± 0.21 Bb
1	1.45 ± 0.27 Ca	5.13 ± 0.59 Bb

Means ± standard deviation.
[a]Values in the same column followed by the same uppercase letter are not significantly different ($p > 0.05$).
[b]Values in the same row followed by the same lowercase letter are not significantly different ($p > 0.05$).

Table 5
Quality aspects of tomato juice subjected to pulsed ohmic heating at different frequencies[a].

Frequency (kHz)	Color			Lycopene content (mg/kg tissue)
	L*	a*	b*	
Untreated	34.59 ± 0.51 A	0.71 ± 0.25 A	3.56 ± 0.42 A	23.27 ± 3.05 A
0.06	34.42 ± 0.50 A	0.55 ± 0.39 A	3.52 ± 0.44 A	22.12 ± 0.19 A
0.2	34.26 ± 0.27 A	0.49 ± 0.18 A	3.49 ± 0.05 A	21.46 ± 1.66 A
0.5	34.47 ± 0.71 A	0.52 ± 0.22 A	3.41 ± 0.08 A	22.03 ± 0.47 A
1	34.26 ± 0.55 A	0.52 ± 0.31 A	3.54 ± 0.38 A	23.26 ± 1.51 A

Means ± standard deviation.
[a]Values in the same column followed by the same letter are not significantly different ($p > 0.05$).

PI uptake, which is intimately related to pore formation in the cell membrane (Park and Kang, 2013), decreased significantly at 1 kHz ($p < 0.05$) in the present study. When an electric field is applied to bacteria, pores can form in the cell membrane. Pore formation is dependent on electric field strength, and a critical level is needed for pore formation to lead to membrane destruction. It is assumed a voltage drop across the cell membrane exceeding 1V leads to microbe inactivation (Tsong, 1990). The electric field used in the present study (47.7 V_{pp}/cm) was too low of itself to cause irreversible poration. However, reversible poration caused by such a low electric field could become irreversible by means of heating (Tsong, 1990). We determined that pore formation was also related to frequency in the present study. Decreased pore formation at high frequency was also observed in previous studies, and insufficient time for charging the cell membrane at high frequency was presented as a reason (Kulshrestha and Sastry, 2003; Lima and Sastry, 1999; Somavat et al., 2012). We also assume that limited charging time at high frequency is the reason for the weakened electroporation effect.

Bacterial pathogen inactivation, as demonstrated through enumeration on selective media, did not significantly differ with frequency ($p > 0.05$) whereas numbers of sub-lethally injured cells significantly decreased with decreasing frequency ($p < 0.05$). Sub-lethally injured pathogens generated during food processing are considered a potential biological hazard because they could recover into normal cells (Wu, 2008). Several efforts to reduce generation of injured pathogens have been reported (Ha and Kang, 2013; Kalchayanand et al., 1998) including the NIR-LA spray combination method suggested by Ha and Kang (2015). We assumed that the accelerated electroporation effect at low frequency is also related to sub-lethal injury of bacterial pathogens. Even though the accelerated effect of electroporation at low frequency is not sufficient in itself inactivate bacterial pathogens, the level of resuscitation of bacterial pathogens significantly decreased at low frequencies. Therefore, low frequency pulsed ohmic heating could be used effectively to inactivate bacterial pathogens without producing sub-lethal injury. Reductions of MS-2 bacteriophage also increased at low frequency, but the mechanism was not identified in the present study.

The targeted temperature of BPW (70 °C) was lower than that of tomato juice (80 °C), but reductions of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* were larger in BPW than in tomato juice in the present study. There are two possible reasons for this phenomenon. First, *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* have strong acid resistance and can survive under conditions of low pH, typical of many juice products (Lin et al., 1996; Phan-Thanh et al., 2000). Secondly, it is harder to inactivate pathogens in food rather than in buffer due to the protective effect of food. The protective effect of juice on the inactivation of *E. coli* O157:H7 was reported by other researchers (Espina et al., 2010; Pilavtepe-Çelik et al., 2009). Contrary to bacterial pathogens, the reductions of MS-2 bacteriophage were larger in tomato juice than in BPW. The sensitivity of MS-2 bacteriophage to the low pH of juice products was reported previously (Horm and D'Souza, 2011; Su

et al., 2010), and we concluded that MS-2 bacteriophage is more sensitive to acid than are *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes*.

Color values and lycopene content of tomato juice subjected to every treatment were not significantly different from untreated samples in the present study ($p > 0.05$). Shi and Maguer (2000) reported that serious quality degradation of tomato juice can occur when holding time at high temperature is too long. Because ohmic heating has the advantage of rapid and uniform heating, quality degradation of juice products can be minimized by ohmic heating as reported previously (Leizerson and Shimoni, 2005; Vikram et al., 2005). For further study, we examined the effect of frequency on the quality of tomato juice in the present investigation. We indicated that the accelerated electroporation effect at low frequency was not enough to degrade quality. On the other hand, Lee et al. (2013) reported that ascorbic acid content significantly decreased at 0.06–0.1 kHz whereas color and lycopene content were not significantly affected by frequency in salsa. These results suggest that the non-thermal effect of ohmic heating can affect quality aspects of food products. Therefore, an acceptable frequency range should be determined for maintaining the quality of food products.

In conclusion, pulsed ohmic heating at low frequency effectively inactivated the bacteriophage surrogate as well as pathogenic bacteria without producing sub-lethal injury. The increased electroporation effect at low frequencies was suggested as a reason for the reduced resuscitation level. Moreover, quality of tomato juice was not degraded and electrode corrosion was not observed regardless of frequency. Therefore, we recommend using low frequency pulsed ohmic heating for tomato juice processing rather than higher frequency. Duty ratio is also an important factor of pulsed ohmic heating, and further study about the effect of duty ratio on the inactivation of pathogenic microorganisms is required.

Acknowledgements

This research was supported by Agriculture, Food and Rural Affairs Research Center Support Program, Ministry of Agriculture, Food and Rural Affairs, Republic of Korea. This research was also supported by a grant (14162MFDS973) from Ministry of Food and Drug Safety in 2015.

References

Awuah, G.B., Ramaswamy, H.S., Tang, J., 2014. Radio-frequency Heating in Food Processing: Principles and Applications. CRC Press.

Centers for Disease Control and Prevention (CDC), 2010. CDC Report 1 in 6 Get Sick from Foodborne Illnesses Each Year. New Estimates More Precise, available at: http://www.cdc.gov/media/pressrel/2010/r101215.html (Accessed 6 November 2015).

Centers for Disease Control and Prevention (CDC), 2011. CDC 2011 Estimates: Findings. available at: http://www.cdc.gov/foodborneburden/2011-foodborne-estimates.html (Accessed 6 November 2015).

Cho, M., Cates, E.L., Kim, J.-H., 2011. Inactivation and surface interactions of MS-2 bacteriophage in a TiO 2 photoelectrocatalytic reactor. Water Res. 45, 2104–2110.

Espina, L., Somolinos, M., Pagán, R., García-Gonzalo, D., 2010. Effect of citral on the thermal inactivation of *Escherichia coli* O157: H7 in citrate phosphate buffer and apple juice. J. Food Prot. 73, 2189–2196.

Ha, J.-W., Kang, D.-H., 2013. Simultaneous near-infrared radiant heating and UV radiation for inactivating *Escherichia coli* O157: H7 and *Salmonella enterica* serovar Typhimurium in powdered red pepper (Capsicum annuum L.). Appl. Environ. Microb. 79, 6568–6575.

Ha, J.-W., Kang, D.-H., 2015. Combining lactic acid spray with NIR radiant heating to inactivate *Salmonella enterica* serovar Enteritidis on almond and pine nut kernels. Appl. Environ. Microb. 81, 4517–4524.

Hartman, P.A., Hartman, P.S., Lanz, W.W., 1975. Violet red bile 2 agar for stressed coliforms. Appl. Environ. Microb. 29, 537–539.

Horm, K.M., D'Souza, D.H., 2011. Survival of human norovirus surrogates in milk, orange, and pomegranate juice, and juice blends at refrigeration (4 C). Food Microbiol. 28, 1054–1061.

Kalchayanand, N., Sikes, A., Dunne, C., Ray, B., 1998. Factors influencing death and injury of foodborne pathogens by hydrostatic pressure-pasteurization. Food Microbiol. 15, 207–214.

Kim, S.-S., Kang, D.-H., 2015a. Comparative effects of ohmic and conventional heating for inactivation of *Escherichia coli* O157: H7, *Salmonella enterica* serovar Typhimurium, and *Listeria monocytogenes* in skim milk and cream. J. Food Prot. 78, 1208–1214.

Kim, S.-S., Kang, D.-H., 2015b. Comparison of pH effects on ohmic heating and conventional heating for inactivation of *Escherichia coli* O157: H7, *Salmonella enterica* Serovar Typhimurium and *Listeria monocytogenes* in orange juice. LWT-Food Sci. Technol. 64, 860–866.

Kim, S.S., Kang, D.H., 2015c. Effect of milk fat content on the performance of ohmic heating for inactivation of *Escherichia coli* O157: H7, *Salmonella enterica* Serovar Typhimurium and *Listeria monocytogenes*. J. Appl. Microbiol. 119, 475–486.

Kulshrestha, S., Sastry, S., 2003. Frequency and voltage effects on enhanced diffusion during moderate electric field (MEF) treatment. Innov. Food Sci. Emerg. 4, 189–194.

Lee, S.-Y., Ryu, S., Kang, D.-H., 2013. Effect of frequency and waveform on inactivation of *Escherichia coli* O157: H7 and *Salmonella enterica* serovar Typhimurium in salsa by ohmic heating. Appl. Environ. Microb. 79, 10–17.

Leizerson, S., Shimoni, E., 2005. Stability and sensory shelf life of orange juice pasteurized by continuous ohmic heating. J. Agric. Food Chem. 53, 4012–4018.

Lima, M., Sastry, S.K., 1999. The effects of ohmic heating frequency on hot-air drying rate and juice yield. J. Food Eng. 41, 115–119.

Lin, J., Smith, M.P., Chapin, K.C., Baik, H.S., Bennett, G.N., Foster, J.W., 1996. Mechanisms of acid resistance in enterohemorrhagic *Escherichia coli*. Appl. Environ. Microb. 62, 3094–3100.

Park, I.-K., Kang, D.-H., 2013. Effect of electropermeabilization by ohmic heating for inactivation of *Escherichia coli* O157: H7, *Salmonella enterica* serovar Typhimurium, and *Listeria monocytogenes* in buffered peptone water and apple juice. Appl. Environ. Microb. 79, 7122–7129.

Phan-Thanh, L., Mahouin, F., Aligé, S., 2000. Acid responses of *Listeria monocytogenes*. Int. J. Food Microbiol. 55, 121–126.

Pilavtepe-Çelik, M., Buzrul, S., Alpas, H., Bozoğlu, F., 2009. Development of a new mathematical model for inactivation of *Escherichia coli* O157: H7 and *Staphylococcus aureus* by high hydrostatic pressure in carrot juice and peptone water. J. Food Eng. 90, 388–394.

Samaranayake, C.P., Sastry, S.K., 2014. Electrochemical Reactions during Ohmic Heating and Moderate Electric Field Processing. In: Ohmic Heating in Food Processing, 119.

Samaranayake, C.P., Sastry, S.K., Zhang, H., 2005. Pulsed ohmic heating–a novel technique for minimization of electrochemical reactions during processing. J. Food Sci. 70, e460–e465.

Sastry, S.K., Barach, J.T., 2000. Ohmic and inductive heating. J. Food Sci. 65, 42–46.

Sastry, S.K., Heskitt, B.F., Sarang, S.S., Somavat, R., Ayotte, K., 2014. Why Ohmic Heating? Advantages, Applications, Technology, and Limitations. In: Ohmic Heating in Food Processing, 7.

Scallan, E., Hoekstra, R.M., Angulo, F.J., Tauxe, R.V., Widdowson, M.-A., Roy, S.L., Jones, J.L., Griffin, P.M., 2011. Foodborne illness acquired in the United States—major pathogens. Emerg. Infect. Dis. 17.

Shi, J., Maguer, M.L., 2000. Lycopene in tomatoes: chemical and physical properties affected by food processing. Crit. Rev. Food Sci. 40, 1–42.

Somavat, R., Mohamed, H.M., Chung, Y.-K., Yousef, A.E., Sastry, S.K., 2012. Accelerated inactivation of *Geobacillus stearothermophilus* spores by ohmic heating. J. Food Eng. 108, 69–76.

Su, X., Howell, A.B., D'Souza, D.H., 2010. The effect of cranberry juice and cranberry proanthocyanidins on the infectivity of human enteric viral surrogates. Food Microbiol. 27, 535–540.

Sun, D.-W., 2011. Handbook of Frozen Food Processing and Packaging. CRC Press.

Tewari, G., Juneja, V., 2008. Advances in Thermal and Non-thermal Food Preservation. John Wiley & Sons.

The National Advisory Committee on Microbiological Criteria for Foods (NACMCF), 1992. Hazard analysis and critical control point system. Int. J. Food Microbiol. 16, 1–23.

Tola, Y.B., Rattan, N.S., Ramaswamy, H.S., 2014. 11 Electrodes in Ohmic Heating. In: Ohmic Heating in Food Processing, 141.

Tsong, T.Y., 1990. On electroporation of cell membranes and some related phenomena. Bioelectrochem. Bioenerg. 24, 271–295.

Tzedakis, T., Basseguy, R., Comtat, M., 1999. Voltammetric and coulometric techniques to estimate the electrochemical reaction rate during ohmic sterilization. J. Appl. Electrochem 29, 819–826.

Vikram, V., Ramesh, M., Prapulla, S., 2005. Thermal degradation kinetics of nutrients in orange juice heated by electromagnetic and conventional methods. J. Food Eng. 69, 31–40.

Wu, V., 2008. A review of microbial injury and recovery methods in food. Food Microbiol. 25, 735–744.

Wu, V., Fung, D., 2001. Evaluation of T.hin agar layer method for recovery of heat-injured foodborne pathogens. J. Food Sci. 66, 580–583.