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A Thesis for the Degree of Doctor of Philosophy

Application of Pulsed Ohmic Heating for Inactivation of Foodborne Pathogens

식중독 균 제어를 위한 펄스 옴 가열의 활용

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Abstract

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Pulsed ohmic heating has attracted attention as one of the novel thermal technologies enabling rapid and uniform heating. In pulsed ohmic heating, heat is generated inside of food by means of electric current passing through the food component different from conventional heating which heat transfer is implemented with conduction and convection. Even though foodborne pathogens can be inactivated effectively by pulsed ohmic heating compared to conventional heating, food sample still can be damaged by a high temperature of pulsed ohmic heating processing. In this regard, pulse ohmic heating should be optimized before applied in the food industry to the consumer's preference for minimum processing. Applicability of pulsed ohmic heating for inactivation of foodborne pathogens was identified in this thesis, and specific objectives of this study were, (i) to evaluate pulsed ohmic heating technology for inactivation of foodborne pathogens, (ii) to combine pulsed ohmic heating with other sanitizing technologies, (iii) to identify combined inhibitory effect of milk fat and lactose for inactivation of foodborne

pathogens by pulsed ohmic heating, (iv) to develop multiphysics model of pulsed ohmic heating for inactivation of foodborne pathogens in tomato juice, and (v) to develop and apply continuous-type pulsed ohmic heating system.

First, it was investigated whether foodborne pathogens can be inactivated effectively by pulsed ohmic heating without causing electrode corrosion and quality degradation of food sample. Buffered peptone water and tomato juice inoculated with pathogens (*Escherichia coli* O157:H7, *Salmonella enterica* serovar Typhimurium, *Listeria monocytogenes*) were treated with pulsed ohmic heating at different frequencies (0.06 – 1 kHz). Foodborne pathogens were inactivated effectively by pulsed ohmic heating. Moreover, electrode corrosion and quality degradation of tomato juice were not observed regardless of frequency. Therefore, pulse waveform was used in subsequent studies.

Because high temperature by individual treatment of pulsed ohmic heating can damage the food quality, developed pulsed ohmic heating was combined with various essential oil components or UV-C irradiation to reduce the heating temperature and time. Carvone, eugenol, thymol, and citral, which are registered for use as flavorings, were chosen to combine with pulsed ohmic heating. Combination treatment of pulsed ohmic heating with citral showed the most synergistic bactericidal effect against foodborne pathogens in buffered peptone water followed by thymol, eugenol, and carvone. Cell membrane destruction by combination treatment and the loss of cell membrane potential by essential oil

components were proposed as the bactericidal mechanism. When applied in salsa, inactivation of bacterial pathogens was the greatest for the combination treatment with thymol followed by with citral, eugenol, or carvone. Because color (b^* values) of salsa were improved by combination treatment with thymol compared to pulsed ohmic treated samples, the combination treatment can be used effectively to pasteurize salsa. Combination treatment of pulsed ohmic heating with UV-C irradiation also showed a synergistic effect for pathogen inactivation. Cell membrane damage increased in all three pathogens synergistically with the simultaneous treatment, while an additive effect was observed for lipid peroxidation values. Therefore, the proposed synergistic bactericidal mechanism of the simultaneous treatment consists of an acceleration of lipid peroxidation, which results in a synergistic effect on cell membrane pore formation. Sequential treatment of UV-C irradiation after pulsed ohmic heating showed a less bactericidal effect than the reversed sequential treatment or the simultaneous treatment in buffered peptone water. Heat shock proteins expressed after pulsed ohmic heating and recovery process after UV-C irradiation were supposed to contribute this phenomenon. On the other hand, the reductions in the levels of all three pathogens in tomato juice were not significantly different between the simultaneous and sequential treatments regardless of the treatment sequence ($p > 0.05$), and the color and lycopene content of tomato juice were not significantly deteriorated by the simultaneous treatment ($p > 0.05$). Therefore, not only

combination treatment of pulsed ohmic heating with thymol but also with UV-C irradiation can be used as an effective hurdle technology ensuring microbiological safety.

An empirical model was developed to predict the inactivation of foodborne pathogens by pulsed ohmic heating depending on the fat and lactose content (%) in milk. The combined effect of fat and lactose content was analyzed by response surface methodology with a central composite design. Both lactose and fat had an inhibitory effect on the inactivation of all three pathogens by pulsed ohmic heating. Inactivation of *E. coli* O157:H7 has a quadratic relationship with lactose and fat, whereas the cross product of treatment time with fat or lactose has a significant effect on the inactivation of *S.* Typhimurium and *L. monocytogenes* ($p < 0.05$). The developed model predicted the inactivation of all three pathogens well within the range of experimental conditions, and color change and lipid oxidation were not observed following pulsed ohmic heating. Even though pH values decreased slightly after treatment, the changes would not affect the product quality. Therefore, treatment conditions of pulsed ohmic heating should be decided carefully considering the nutrient content and type of pathogens when using this method to pasteurize milk.

Multiphysics modelling was conducted to analyze the pulsed ohmic heating more precisely. When pulsed ohmic heating processing of tomato juice was analyzed using multiphysics software, a cold spot was observed in the lower part

of the pulsed ohmic heating chamber, where some pathogens survived even though all pathogens were inactivated elsewhere of the ohmic heating chamber. The developed computational simulation model was verified for heating rate and pathogen inactivation. Furthermore, inactivation of acid-adapted foodborne pathogens, the heat resistance of which increased significantly, was predicted by the developed simulation model and validated with no significant differences between predicted and experimental results ($p > 0.05$). Therefore, juice processors can utilize a multiphysics model effectively to adjust processing time to achieve 5 log reductions of foodborne pathogens under the environmental conditions in which heat resistance of pathogens could be altered.

Even though the batch-type pulsed ohmic heating apparatus was used effectively to identify the characteristics of pulsed ohmic heating in the previous chapters, it is well known that continuous-type apparatus ohmic heating is more advantageous for bulk handling of juice products in the food industry. Therefore, continuous-type pulsed ohmic heating was developed, and effects of flow rate, voltage, and preheating on the heating rate and pathogen inactivation were identified. Both heating rate of samples and reduction rates of pathogens increased corresponding to decreased flow rate or increased treatment voltage. Preheating was used as an alternative way to prevent accelerated heating rate by increased voltage, and help inactivate pathogens in the early treatment stage without affecting the heating rate. Because preheating with additional equipment is

inconvenient and occupies valuable space, sequential three-cylinder type pulsed ohmic heating was developed. By applying the developed sequential pulsed ohmic heating, 5 log reductions were achieved for all three pathogens without preheating under the same treatment conditions. Therefore, sequential continuous-type pulsed ohmic heating can be effectively utilized to control foodborne pathogens in the juice industry.

In conclusion, the performance of pulsed ohmic heating for inactivation of foodborne pathogens varied significantly depending on the type of treatment to be combined, nutritional conditions of a food sample, and device type. Therefore, it is recommended to optimize pulsed ohmic heating to inactivate foodborne pathogens without quality degradation of food. Moreover, multiphysics modeling can be used effectively in optimizing the pulsed ohmic heating processing.

Keywords: pulsed ohmic heating, foodborne pathogen, food processing, electrode corrosion, food quality, combination treatment, empirical modeling, multiphysics modeling, computer simulation, continuous-type system

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Chapter I.

Evaluation of pulsed ohmic heating technology for inactivation of foodborne pathogens

I-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 3,000 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. Moreover, cross-contamination during processing commonly occurs from utensils/surfaces and infected food handlers (Sun, 2016).

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. Because pathogenic microorganisms can survive in the cold spots (Tewari and Juneja, 2008), novel thermal technologies such as radio-frequency heating, microwave heating, and ohmic heating have been proposed as alternatives to conventional heating,

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 has been 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 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 short pulse width was suggested as a solution to reduce electrode corrosion (Samaranayake and Sastry, 2005). In Samaranayake and Sastry's study, the application of pulsed ohmic heating was focused on the prevention of electrode corrosion. Because pathogenic bacteria and viruses are major biological hazards causing foodborne illness, pulsed ohmic heating was

applied to inactivate the foodborne pathogens without causing quality deterioration of food in the present study.

The aim of the present study was to evaluate pulsed ohmic heating technology for inactivation of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes*. Tomato juice was selected as a sample because several outbreaks have been reported in juice products despite its acidity. Color is a fundamental quality aspect influencing consumer's preference and lycopene not only affect the color but also has an antioxidant property (Min and Zhang, 2003; Shi and Maguer, 2000). Therefore, color and lycopene content changes were examined in the present study.

I-2. Materials and Methods

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^{o}C, a 1 ml 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.

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). 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 4,000 × 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 to 10^9 CFU/ml. Afterward, 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).

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^{o}C) and removed, 1 h prior to inoculation, and allowed to equilibrate to room temperature (22 ± 1^{o}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.

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 pulsed ohmic heating at various frequencies until reaching 75ºC. Untreated and ohmic treated BPW were centrifuged at 10,000 × g for 10 min at 4ºC. 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 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}.

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

Bactericidal treatment. 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 temperatures were 70°C and 80°C for BPW and tomato juice, respectively.

Bacterial enumeration. For microbial enumeration, each treated 25 ml sample was immediately transferred into a sterile stomacher bag (Labplas, Inc., Sainte-Julie, Quebec, Canada) containing 225 ml of sterile 0.2% PW 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% PW 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 to 48 h before counting colonies characteristic of the pathogens.

The overlay method was used to enumerate sub-lethally injured cells of *S.* Typhimurium and *L. monocytogenes*. After cells resuscitated on TSA at 37ºC for 2 h, plates were overlaid with 7 to 8 ml of selective medium (XLD or OAB). The plates were further incubated for 22 to 46 h at 37ºC 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.

Color and lycopene measurement. Color and lycopene content of treated and untreated tomato juice 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 the following equation. Absorbance was measured with a spectrofluorophotometer at 503 nm.

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

Statistical analysis. All experiments were replicated three times. 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 $p = 0.05$.

I-3. Results

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. I-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 the subsequent experiments.

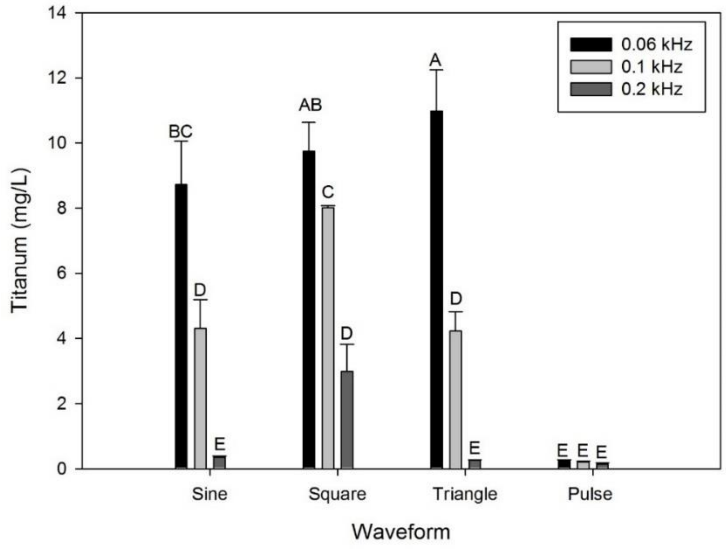


Fig. I-1. Concentration of titanium ions migrated into tomato juice subjected to ohmic heating at different waveforms and frequencies. The results are means from three replications, and error bars indicate standard deviations. Bars with different letters are significantly different ($p < 0.05$).

PI uptake ability. PI uptake values in BPW varied with frequency and type of pathogen (Table I-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.

Table I-1. PI uptake values of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* subjected to pulsed ohmic heating at different frequencies

Frequency (kHz)	*E. coli* O157:H7[a]	*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

Mean values ± standard deviation

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

Inactivation of bacterial pathogens. Reductions (log CFU/ml) of bacterial pathogens differed depending on frequency and type of sample (Table I-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 ($p < 0.05$). Resuscitated injured cell levels were calculated by subtracting the populations enumerated on selective media from those of media used for recovery (Table I-3). The level of resuscitation increased as frequency increased for all three pathogens and the values at 1 kHz were significantly larger those of lower frequencies ($p < 0.05$).

Table I-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

Mean values ± 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 I-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

Mean values ± 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) in Table I-2.

[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 ($p > 0.05$).

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 I-4). 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 ($p > 0.05$).

Table I-4. 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

Mean values ± standard deviation

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

I-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 food quality. The electrode corrosion rate of all waveforms, except pulse, increased as 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). 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.

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). Pore formation was also related to frequency in the present study. Decreased pore formation at high frequency was observed in previous studies, and insufficient time for charging the cell membrane at high frequency was presented as a reason similar to the previous researches (Kulshrestha and Sastry, 2003; Lima and Sastry, 1999; Somavat et al., 2012). Limited charging time at high frequency is assumed as a 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 the generation of injured pathogens have been reported including the near-infrared heating and lactic acid spray combination method (Ha and Kang, 2015). Even though the accelerated effect of electroporation at low frequency is not sufficient in

itself to 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.

The targeted temperature of BPW (70^{o}C) was lower than that of tomato juice (80^{o}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 as reported by other researchers (Espina et al., 2010; Pilavtepe-Çelik et al., 2009).

Color values and lycopene content of tomato juice subjected to every treatment were not significantly different from untreated samples in the present study ($p > 0.05$). Serious quality degradation of tomato juice can occur when holding time at high temperature is too long (Shi and Maguer, 2000). Even though it is indicated that the accelerated electroporation effect at low frequency was not enough to degrade quality in the present study, non-thermal effect of ohmic heating can affect quality aspects of food products. Therefore,

an acceptable frequency range should be determined for maintaining the food quality.

In conclusion, pulsed ohmic heating at low frequency effectively inactivated the 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, pulsed ohmic heating could be used effectively for tomato juice processing.

Chapter II.

Combination treatments of pulsed ohmic heating with other sanitizing technologies

II-1. Combination treatments of pulsed ohmic heating with various essential oil components

II-1. Introduction

Salsa is a piquant Mexican sauce, which usually consists of multiple ingredients such as fresh tomatoes, jalapeño peppers, onions, coriander leaves, and seasonings, and has become popular throughout the world (Sung and Kang, 2014). Acidic/acidified food products such as salsa and juice have been historically regarded as safe, but several foodborne illness outbreaks have been reported associated with low pH food products (Franco et al., 2010). Salsa was implicated in 70 foodborne outbreaks which resulted in 2,280 illness cases from 1990 to 2006, and *Salmonella*, *Campylobacter jejuni*, *Shigella*, *Staphylococcus aureus* and norovirus were considered to be the major causal agents (Franco and Simonne, 2009). Moreover, a multi-state outbreak of *Salmonella* Saintpaul associated with jalapeño and serrano peppers was reported in the United States in 2008 (CDC, 2008a). Considering the increasing popularity of Mexican restaurants in the United States, Mexican dishes that incorporate jalapeño and serrano peppers, such as salsa and guacamole, are potential vehicles of foodborne illness.

Consumer preference for natural, healthful and minimally processed foods has steadily increased for several years. Meanwhile, new challenges in food safety such as demographic changes, climate changes, and globalization of

trade have arisen (Doyle et al., 2015). The U. S. Centers for Disease Control and Prevention (CDC) reported that 3,000 people die because of foodborne outbreaks in the United States each year (CDC, 2010). Even though it is crucial to inactivate foodborne pathogens to ensure the microbiological safety of food, the quality of food may degrade during processing. Therefore, combining several mild preservation techniques simultaneously, namely hurdle technology, has been used to satisfy both microbiological safety and quality concerns of foods (Karatzas et al., 2000). Several recent research investigations have reported that hurdle technology can be used effectively as an alternative to individual treatments (Sagong et al., 2013; Sung et al., 2014).

Essential oil components have potential as a natural agent for food preservation by means of their antimicrobial properties (Solórzano-Santos and Miranda-Novales, 2012). Because the resistance of microorganisms increases in food matrices, a high concentration of essential oil components is required to ensure microbiological safety. However, such a high concentration of essential oil components results in undesirable flavor. Therefore, combination treatments of these components with other technologies have been reported to reduce the concentration of essential oil components needed (Friedman et al., 2009; Kim and Rhee, 2016). There are many essential oil components which are known to have antimicrobial properties such as carvone, carvacrol, cinnamaldehyde, citral, decanal, eugenol, and thymol. Among them, carvone,

eugenol, thymol, and citral were chosen to combine with pulsed ohmic heating, since they are registered for use as flavorings in foodstuffs by the European Commission (Di Pasqua et al., 2006).

In the present study, pulsed ohmic heating was combined with various essential oil components to inactivate *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* effectively. The bactericidal mechanism was identified by cell membrane damage with membrane potential. Quality aspects of salsa were determined by color and lycopene content.

II-1.2. Materials and Methods

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, South Korea). Stock and working cultures were prepared according to the method previously described in chapter I-2. Mixed culture cocktail contain approximately equal numbers of cells of each strain of *E. coli* O157:H7 (10^9 CFU/ml), *S.* Typhimurium (10^9 CFU/ml), and *L. monocytogenes* (10^8 CFU/ml).

Sample preparation and inoculation. Sterile BPW and pasteurized salsa (pH 3.7) were used in the present experiment. Each sample was stored in a refrigerator (4^{o}C) and removed 1 h prior to inoculation to equilibrate to room temperature (22 ± 1^{o}C). Pasteurized salsa was purchased at a local grocery store (Seoul, South Korea), and contained no chemical preservatives and included tomatoes, jalapeño peppers, onions, garlic, and distilled vinegar. Fifty ml (BPW) or g (salsa) of each sample were put into the pulsed ohmic chamber. A mixed culture cocktail (0.2 ml) was inoculated into each prepared

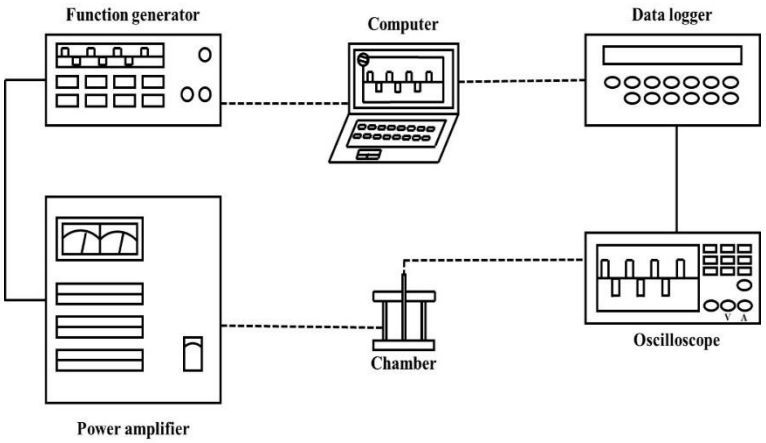
sample before treatment. The final bacterial populations were 10^6-10^7 CFU/g for *E. coli* O157:H7 and *S.* Typhimurium and 10^5-10^6 CFU/g for *L. monocytogenes*.

Essential oil components preparation. Carvone, eugenol, thymol, and citral were purchased from Sigma-Aldrich (St. Louis, MO). Each essential oil component was mixed with 99.5% ethanol (concentration of stock solution = 100 x of the working concentration) and used within 1 week after preparation (Kim and Rhee, 2016). The ethanol concentration in the final product was 0.99%.

Bactericidal treatment. Inoculated samples were treated with essential oil components, pulsed ohmic heating, or combination treatment of pulsed ohmic heating and essential oil components. Concentration of essential oil components (1 mM) and treatment time (60 s for BPW and 38 s for salsa) were selected based on preliminary experiments. The pulsed ohmic heating system (Fig. II-1A) consisted of a function generator, a precision power amplifier, a two-channel digital-storage oscilloscope, a data logger, and a chamber (Fig. II-1B). The function generator produced various waveforms at frequencies from 1 mHz to 10 MHz and a maximum output level of 5 V. The signals generated through the power amplifier were amplified up to a maximum

output of 141 V alternating current (AC). The signals expanded by the power amplifier were delivered to each of two titanium electrodes. The two-channel digital storage oscilloscope was used to measure signals, including waveform, frequency, voltage, and current. K-type thermocouples were inserted at the center of the ohmic heating chamber and temperatures were recorded at 0.6 s intervals by a data logger. The distance between the two electrodes was 4 cm, and the cross-sectional area was 60 cm^2. Samples without or with essential oil components were subjected to pulsed ohmic heating (0.05 duty ratio, 500 Hz). Electric field strengths were 13.3 V_{rms}/cm and 11.5 V_{rms}/cm for BPW and salsa, respectively. Addition of essential oil components did not significantly ($p > 0.05$) influence the temperature history (Fig. II-2). Samples were taken after each treatment, and populations of surviving microorganisms were enumerated.

(A)



(B)

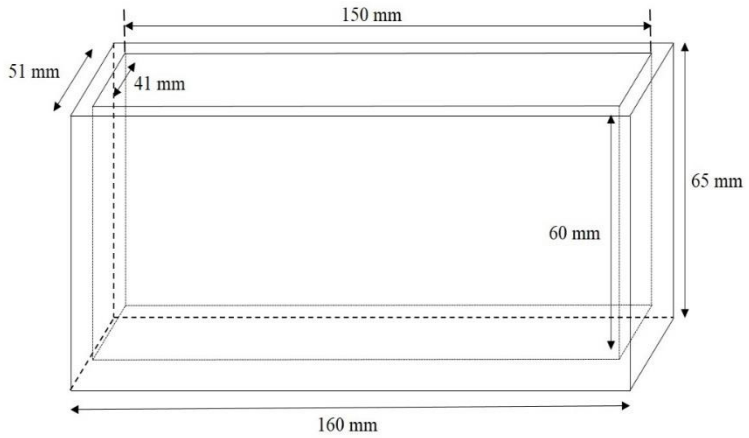


Fig. II-1. Schematic diagram of the pulsed ohmic heating system (A) and treatment chamber (B) at Seoul National University (Seoul, Korea).

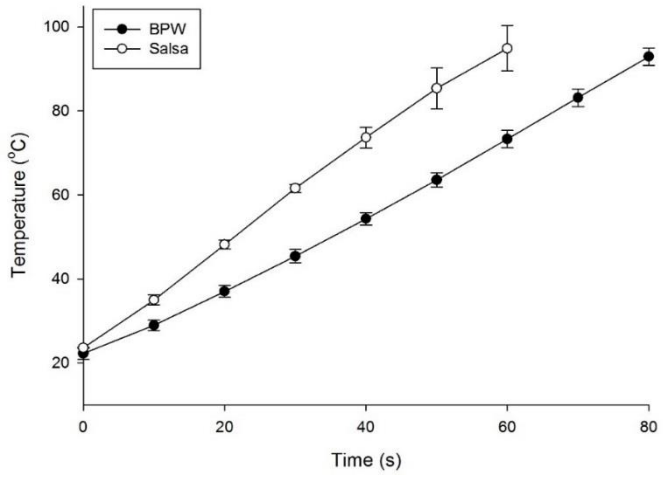


Fig. II-2. Temperature histories of buffered peptone water (BPW) and salsa subjected to pulsed ohmic heating (0.05 duty ratio, 500 Hz) without essential oil components. Electric field strengths were 13.3 V_{rms}/cm and 11.5 V_{rms}/cm for BPW and salsa, respectively. The temperature history was not significantly influenced by the addition of 1.0 mM essential oil components ($p > 0.05$).

Bacterial enumeration. For microbial enumeration, each treated 50 ml (g) sample was immediately transferred into a sterile stomacher bag containing 100 ml of sterile 0.2% PW (4°C) and homogenized for 2 min using a stomacher. After homogenization, 1 ml samples were 10-fold serially diluted with 9 ml of sterile 0.2% PW and 0.1 ml of stomached or diluted samples were spread plated onto each selective or non-selective medium. SMAC agar, XLD agar, and OAB with antimicrobial supplement 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 to 48 h before counting colonies characteristic of the pathogens. The overlay method described in chapter I-2 was used to recover sub-lethally injured cells of *S.* Typhimurium and *L. monocytogenes*. SPRAB was used to recover injured cells of *E. coli* O157:H7.

PI uptake test. PI was used to determine cell membrane damage. The PI uptake test was conducted according to the method described in chapter I-2. BPW was subjected to each treatment under the same conditions as for bactericidal treatments.

$DiBAC_4(3)$ uptake test. The fluorescent dye Bis (1,3-dibutylbarbituric acid) trimethine oxonol ($DiBAC_4(3)$) was used to measure cell membrane

potential. BPW was subjected to each essential oil treatment under the same conditions as for antimicrobial treatments. Untreated and treated inoculated BPW was centrifuged at 10,000 × g for 10 min at 4°C. Supernatants were discarded, and the cell pellets were resuspended in 1 ml PBS. $DiBAC_4(3)$ was added to a final concentration of 2.5 μg/ml for *E. coli* O157:H7 and *S.* Typhimurium, and incubated for 15 min at 37°C. For *L. monocytogenes*, $DiBAC_4(3)$ was added to a final concentration of 0.5 μg/ml, and incubated for 2 min at 25°C. After incubation, samples were centrifuged two more times under the same conditions. The final cell pellets were resuspended in 1 ml PBS and fluorescence was measured with a spectrofluorophotometer at an excitation wavelength of 488 nm and an emission wavelength of 525 nm.

Color and lycopene measurement. The color and lycopene content of untreated and treated samples were measured. Treatment times for pulsed ohmic heating and each combination treatment were chosen based on preliminary experiments to ensure 5 log reductions of all three bacterial pathogens. All treated samples were cooled immediately in a crushed ice-water mixture. Color values were measured with a Minolta colorimeter. The values for L^*, a^*, and b^* were measured to evaluate color changes of salsa. Lycopene content in salsa was measured according to the method previously described in chapter I-2.

Statistical analysis. All experiments were replicated three times. All data were analyzed by the analysis of variance procedure of the Statistical Analysis System and mean values were separated using Duncan's multiple-range test. Significant differences in the processing treatments were determined at a significance level of $p = 0.05$.

II-1.3. Results

Bactericidal effect of essential oil components, pulsed ohmic heating, and combination treatments in BPW. The combination treatment of pulsed ohmic heating with citral or thymol exhibited a larger bactericidal effect than combination treatment with carvone or eugenol in BPW (Fig. II-3). Combination treatment with citral exhibited a synergistic effect for inactivation of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* on both selective and resuscitation media. Combination treatment with thymol exhibited a synergistic effect for inactivation of *E. coli* O157:H7 or *S.* Typhimurium, but not for *L. monocytogenes*. On the other hand, combination treatments with carvone or eugenol did not exhibit a synergistic effect for inactivation of any of the three pathogens. Reductions of all three pathogens by the combination treatments were less than 2.5 log CFU/ml and were not significantly different from the sum of essential oil and pulsed ohmic heating treatments ($p > 0.05$).

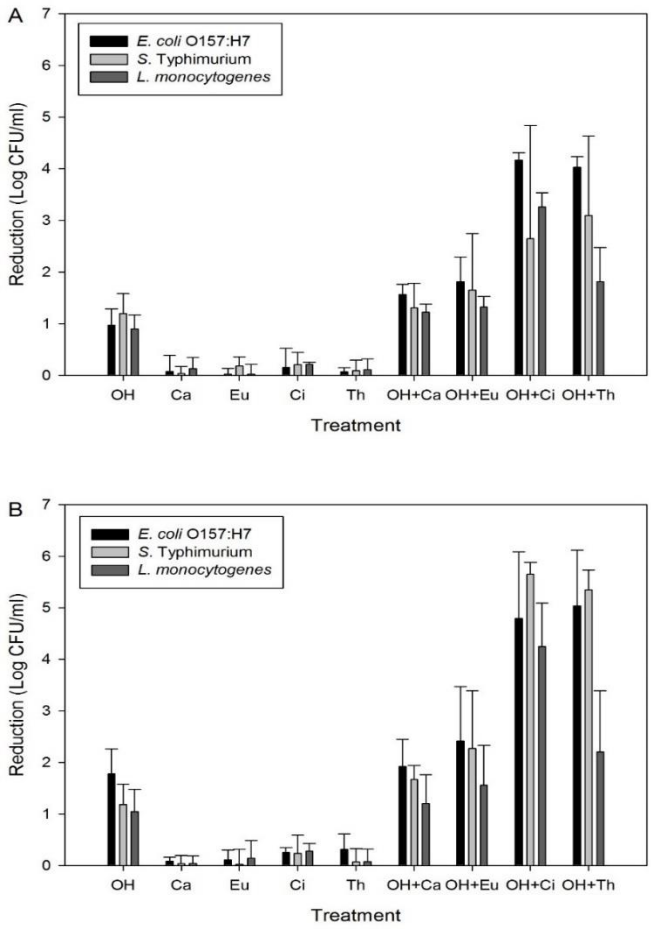


Fig. II-3. Reduction (log CFU/ml) of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* subjected to pulsed ohmic heating (OH), individual essential oil treatments, and combination treatments in buffered peptone water. Populations were enumerated using resuscitation media (A) and selective media (B).

Ca : carvone, Eu : eugenol, Ci : citral, Th : thymol

PI and $DiBAC_4(3)$ uptake ability. PI uptake values of all three pathogens were significantly larger for combination treatments of pulsed ohmic heating with citral or thymol ($p < 0.05$) than with carvone or eugenol (Table II-1). PI uptake values of *E. coli* O157:H7 subjected to combination treatment with citral and thymol were 31.02 and 26.20, respectively, which were significantly larger ($p < 0.05$) than with carvone (8.67), eugenol (8.30), and individual pulsed ohmic heating (1.47). PI uptake values of *S.* Typhimurium and *L. monocytogenes* showed the same trend as that of *E. coli* O157:H7. $DiBAC_4(3)$ uptake values of all three pathogens were significantly larger ($p < 0.05$) in thymol than in carvone or eugenol treated samples (Table II-2). $DiBAC_4(3)$ uptake value of *E. coli* O157:H7 subjected to thymol treatment was 842.73, which was significantly larger ($p < 0.05$) than that of carvone (7.36) or eugenol (-1.06). The same trend was observed for *S.* Typhimurium and *L. monocytogenes*.

Table II-1. PI uptake values of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* subjected to pulsed ohmic heating, essential oil and combination treatments[a]

	E. coli O157:H7	*S.* Typhimurium	*L. monocytogenes*
OH[b]	1.47 ± 0.62 A	-3.99 ± 3.70 A	30.27 ± 16.06 B
Carvone	1.52 ± 1.51 A	-1.49 ± 2.65 AB	-1.19 ± 0.64 A
Eugenol	0.78 ± 3.69 A	-1.26 ± 2.10 AB	5.35 ± 0.64 AB
Citral	1.01 ± 3.37 A	-3.69 ± 5.83 AB	4.52 ± 4.39 AB
Thymol	2.61 ± 0.68 A	1.92 ± 3.78 B	12.87 ± 4.76 AB
OH + Carvone	8.67 ± 2.88 A	2.73 ± 4.59 B	63.12 ± 23.29 C
OH + Eugenol	8.30 ± 4.19 A	2.77 ± 1.37 B	70.69 ± 12.19 C
OH + Citral	31.02 ± 13.0 B	16.47 ± 3.45 C	132.71 ± 19.73 D
OH +Thymol	26.20 ± 9.37 B	15.21 ± 5.86 C	112.63 ± 20.17 D

Mean values ± standard deviation

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

[b] OH: individual pulsed ohmic heating treatment
Carvone, Eugenol, Citral, Thymol: individual essential oil treatment
OH + Carvone, Eugenol, Citral, Thymol: combination treatment of ohmic and essential oil

Table II-2. DiBAC$_4$(3) values of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* subjected to individual essential oil treatments[a]

	E. coli O157:H7	*S.* Typhimurium	*L. monocytogenes*
Carvone	7.36 ± 0.82 A	-3.30 ± 35.92 A	55.44 ± 19.3 A
Eugenol	-1.06 ± 12.2 A	173.60 ± 142.1 A	108.45 ± 15.6 B
Citral	32.65 ± 5.97 A	1144.83 ± 291.8 B	132.78 ± 16.3 BC
Thymol	842.73 ± 84.3 B	1065.83 ± 238.0 B	154.41 ± 8.96 C

Mean values ± standard deviation

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

Application of essential oil components, pulsed ohmic heating, and combination treatments in salsa. The combination treatment of pulsed ohmic heating with thymol in salsa exhibited the largest bactericidal effect followed by combination treatments with citral, eugenol, and carvone (Fig. II-4). Combination treatment with thymol treatments exhibited a synergistic effect for inactivation of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* on both selective and resuscitation media. Combination treatment with citral treatments exhibited a synergistic effect for inactivation of *S.* Typhimurium, but not for *E. coli* O157:H7 or *L. monocytogenes*. Combination treatments of pulsed ohmic heating with carvone or eugenol treatment did not exhibit a synergistic effect for inactivation of any of three pathogens. Reductions of all three pathogens by the combination treatments were less than 2.8 log CFU/g and were not significantly different from the sum of the essential oil and pulsed ohmic heating treatments ($p > 0.05$).

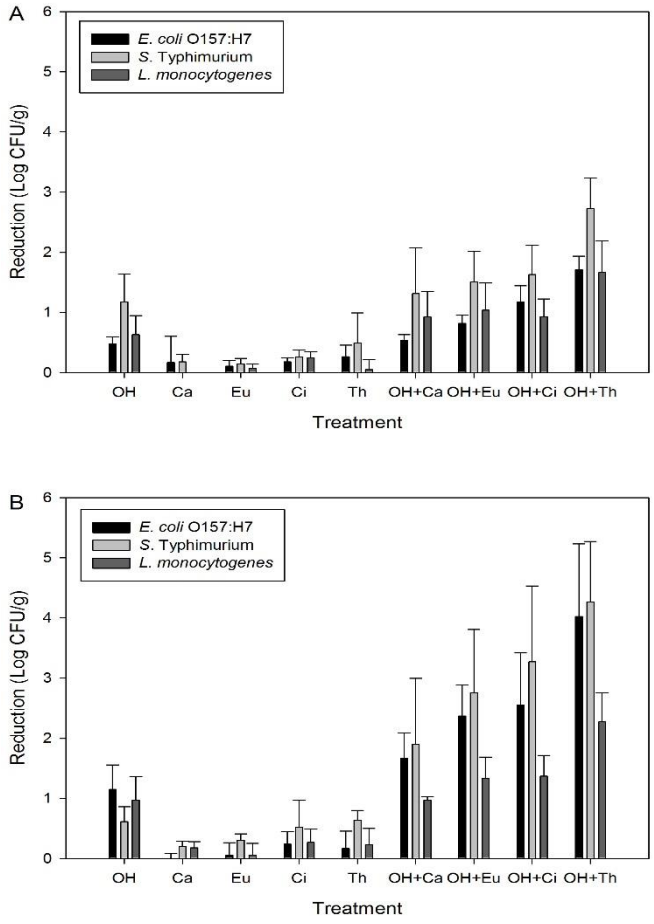


Fig. II-4. Reduction (log CFU/ml) of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* subjected to pulsed ohmic heating (OH), individual essential oil, and combination treatments in salsa. Populations were enumerated using resuscitation media (A) and selective media (B).
Ca : carvone, Eu : eugenol, Ci : citral, Th : thymol

Color and lycopene content of salsa. Color values of salsa were improved by the combination treatment of pulsed ohmic heating with thymol compared to combination treatment with carvone or individual pulsed ohmic treatment (Table II-3). Quality aspects (color value and lycopene content) of combination treatment with thymol treated samples were not significantly different from those of untreated samples ($p > 0.05$). On the other hand, the b^* value of pulsed ohmic treated samples and L^* value of combination treatment with carvone treated samples were significantly different from those of untreated samples ($p < 0.05$).

Table II-3. Color and lycopene content of salsa subjected to pulsed ohmic heating (OH) or combination treatments[a]

	Color			Lycopene content
	L^*	a^*	b^*	
Control	29.93 ± 1.18 A	8.55 ± 1.21 AB	8.49 ± 0.59 A	78.96 ± 7.17 A
OH	30.92 ± 1.81 A	10.12 ± 1.53 A	10.41 ± 0.67 B	76.10 ± 1.61 A
OH + Ca	26.96 ± 0.21 B	8.22 ± 0.73 AB	9.01 ± 0.49 AB	73.90 ± 6.38 A
OH + Th	28.32 ± 1.53 AB	7.60 ± 1.08 B	8.77 ± 1.61 AB	76.46 ± 13.3 A

Mean values ± standard deviation

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

Ca : carvone, Th : thymol

II-1.4. Discussion

In the present study, combined effect of pulsed ohmic heating and various essential oil components such as carvone, eugenol, thymol, and citral was investigated. The combination treatments of pulsed ohmic heating with citral and thymol exhibited a synergistic bactericidal effect against *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* whereas synergism was not observed in the combination treatments with carvone or eugenol. The cell membrane is postulated to be the primary target of the synergistic effect, and PI uptakes values were measured to quantify cell membrane damage. As expected, PI uptake values correlated with the bactericidal efficacy of treatments. Citral and thymol combined with pulsed ohmic heating exhibited a synergistic effect on membrane destruction, while carvone and eugenol combined with pulsed ohmic heating did not exhibit synergism. Ait-Ouazzou et al. (2011) also reported that the level of membrane destruction measured by PI uptake is dependent on the type of pathogen and essential oil. However, it was still wondered why only some essential oil components showed synergistic effects on cell membrane destruction even though the concentration of essential oil components is the same.

Chemical structure of the essential oil components can affect the antibacterial activity. Essential oil components containing a high percentage of phenolic compounds are known to have strong antibacterial properties (Burt, 2004). In the present study, eugenol and thymol have phenolic compounds in their structure whereas carvone and citral do not have phenolic compounds. Nevertheless, a synergistic antibacterial effect was observed when citral or thymol were combined with pulsed ohmic heating. Because the cell membrane is depolarized before destruction (Raafat et al., 2008), cell membrane potential of pathogens subjected to each essential oil component treatment was measured. Similar to trends in the PI uptake test, thymol has the largest $DiBAC_4(3)$ uptake value followed by citral, eugenol, and carvone. Therefore, it is concluded that essential oils, which cause severe depolarization of cell membranes, accelerated membrane destruction when combined with pulsed ohmic heating, and resulted in inactivation of bacterial pathogens. Because the antibacterial activity of essential oil components is not attributable to one specific mechanism, further study is needed to identify the several targets affected by different groups of essential oil components.

Because the combination treatments of pulsed ohmic heating with thymol or citral exhibited the synergistic bactericidal effect in BPW, it was investigated whether these same combination treatments have the same effect in salsa. Similar to BPW, the combination treatment with thymol exhibited the

largest bactericidal effect followed by with citral, eugenol, and carvone in salsa, which indicate that combination treatments with thymol or citral could be used to inactivate foodborne pathogens effectively in salsa. Even though the pH of salsa (3.7) is much lower than that of BPW (7.2), bacterial pathogens were inactivated more effectively in BPW than in salsa. Constituents of salsa which have lower electrical conductivity than the liquid phase, such as jalapeño peppers and onions, confer a protective effect on the foodborne pathogens (Kim and Kang, 2015). Because the difference of pathogen reductions between combination treatments of pulsed ohmic heating with thymol and with carvone was the largest among combination treatments, color and lycopene content were compared in samples of untreated, pulsed ohmic treated, combination treatment with carvone, and combination treatment with thymol. b^* value of pulsed ohmic treated samples and L^* value in samples of combination treatment with carvone were significantly different from untreated samples ($p < 0.05$) in the present study, which can adversely affect the consumer's preference. On the other hand, quality aspects in samples of combination treatment with thymol were not significantly different from those of untreated samples ($p > 0.05$). Therefore, the combination treatment with thymol could be used effectively to process salsa rather than individual pulsed ohmic heating treatment.

In conclusion, certain essential oils, which cause severe depolarization in cell membranes, accelerated membrane destruction when combined with pulsed ohmic heating. Combination treatments of pulsed ohmic heating with thymol or citral exhibited the synergistic bactericidal effect both in BPW and salsa. Moreover, quality indicators of salsa such as lycopene content and color values can be improved by combination treatment with thymol treatments rather than individual pulsed ohmic heating treatment. Therefore, the particular essential oil to be combined with pulsed ohmic heating processing should be determined carefully.

II-2. Combination treatment of pulsed ohmic heating with UV-C irradiation

II-2.1. Introduction

Non-thermal processing can be used effectively to inactivate foodborne pathogens while maintaining the nutritional and sensory characteristics of foods (Kim, Kim, et al., 2017). Among the many types of non-thermal treatments, UV-C irradiation still widely used in the food industry because the equipment is relatively inexpensive and easy to use (Bintsis et al., 2000). Many research investigations have reported that foodborne pathogens can be effectively inactivated by UV-C irradiation (Chun et al., 2009; Sommers et al., 2010). In particular, UV-C irradiation can be used to pasteurize juice products without causing quality deterioration. The mechanism of inactivation by UV-C irradiation is the cross-linking between adjacent pyrimidine nucleobases, which interrupts vital metabolic functions such as DNA replication (Wu et al., 2011). However, short penetration depth is a major limitation of UV-C irradiation and can be a critical obstacle to processing opaque liquid products. Recently, combination treatments of UV-C irradiation with other mild preservative techniques have shown promise to overcome this limitation. The different bactericidal targets of each treatment were generally identified as a major reason for the synergistic bactericidal effects of the hurdle technology.

From this perspective, the combination treatment of thermal and non-thermal processing, which have different bactericidal targets, is promising.

Thermal processing, which is still widely used in the food industry, can inactivate pathogenic and spoilage microorganisms. Disruption of the cell membrane, nucleic acids, and ribosomes is known as a major inactivation mechanism in thermal processing. Even though heat treatment is a secure way to ensure biological safety, the major limitation of the heat treatment is that the organoleptic properties of food can be affected by the severe thermal damage. The range of thermal damage to food depends on the treatment temperature and time, and it is well known that the heating rate and uniformity are very important to reduce the temperature and time. Pulsed ohmic heating is a novel technologies enabling uniform and rapid heating minimizing electrode corrosion as described in chapter I.

In the present study, pulsed ohmic heating was combined with UV-C irradiation to inactivate *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* effectively. The bactericidal mechanism was identified by the cell membrane damage with cell membrane lipid peroxidation values. The quality aspects of tomato juice were determined based on color and lycopene content.

II-2.2. Materials and Methods

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. Stock and working cultures were prepared according to the method previously described in chapter I-2. A mixed culture cocktail contains approximately equal numbers of cells of each strain of *E. coli* O157:H7 (10^9 CFU/ml), *S.* Typhimurium (10^9 CFU/ml), and *L. monocytogenes* (10^8 CFU/ml).

Sample preparation and inoculation. Sterile BPW and pasteurized tomato juice were used in this experiment. Each sample was stored in a refrigerator (4°C) and removed at least 12 h prior to inoculation to equilibrate to room temperature (22 ± 1°C). Fifty ml of each sample was put into the pulsed ohmic heating chamber. A mixed culture cocktail (0.2 ml) was inoculated into each prepared sample before treatment. The final bacterial populations were 10^6-10^7 CFU/g for *E. coli* O157:H7 and *S.* Typhimurium and 10^5-10^6 CFU/g for *L. monocytogenes*.

Bactericidal treatment. The inoculated samples were subjected to an individual or combination treatment of UV-C irradiation and pulsed ohmic heating (Fig. II-5). UV-C irradiation was carried out with a 254-nm germicidal low-pressure mercury lamp (LP lamp). Radiation intensities were measured using an UV fiber-optic spectrometer (AvaSpec-ULS2048; Avantes, Eerbeek, Netherlands). The lamp doses, which were calculated by multiplying the radiation intensity by the irradiation time, were 45.6 and 191.5 mJ/cm^2 for BPW and tomato juice, respectively. Pulsed ohmic heating treatments were carried out with a apparatus previously described in chapter II-1.2. The electric field strength was fixed at 13.4 V_{rms}/cm with a pulse waveform (0.05 duty ratio, 500 Hz). The treatment times were 50 s and 210 s for BPW and tomato juice, respectively. The target temperatures were 60^{o}C and 63^{o}C for BPW and tomato juice, respectively. The combination treatments were carried out by applying pulsed ohmic heating and UV-C irradiation simultaneously or sequentially. UV-C irradiation did not significantly influence the temperature history (p > 0.05) of pulsed ohmic heating (Fig. II-6). To compare the sequential and simultaneous treatments, all samples were cooled for the same time after pulsed ohmic heating. To assess food quality, the tomato juice was subjected to pulsed ohmic heating or simultaneous treatment for 190, 210, 230, and 250 s to identify the 5 log reduction conditions for *E. coli* O157:H7, *S.*

Typhimurium, and *L. monocytogenes*, and the pathogens were enumerated by resuscitation media.

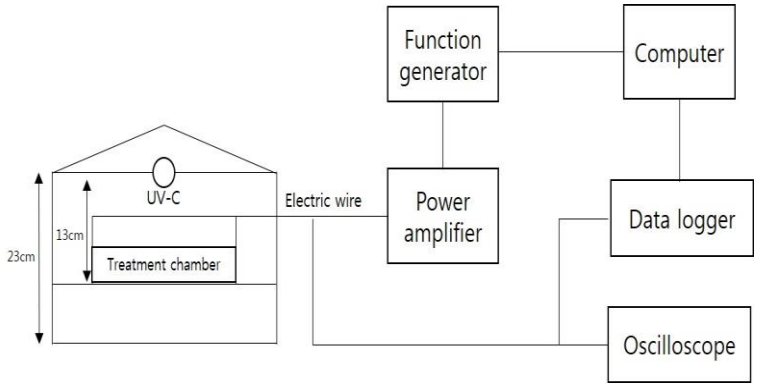


Fig. II-5. Schematic diagram of a combination treatment system of pulsed ohmic heating and UV-C irradiation at Seoul National University (Seoul, Korea).

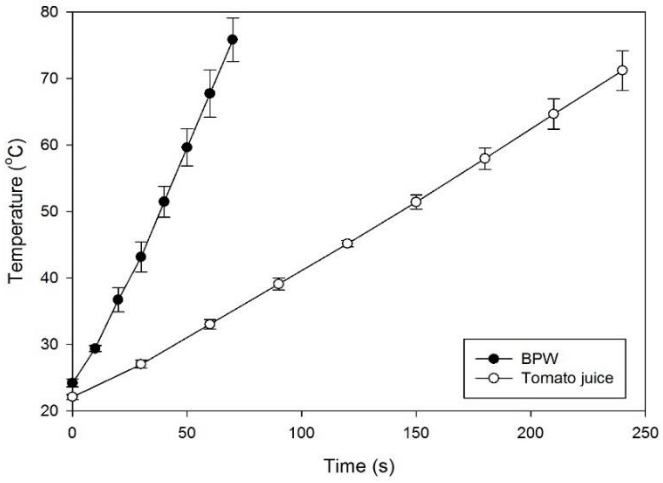


Fig. II-6. Temperature history of buffered peptone water (BPW) and tomato juice subjected to pulsed ohmic heating (0.05 duty ratio, 500 Hz, and 13.4 V_{rms}/cm). UV treatment did not significantly influence the temperature history of pulsed ohmic heating ($p > 0.05$).

Bacterial enumeration. For microbial enumeration, each 50 ml treated sample was transferred into a sterile stomacher bag containing 100 ml of sterile 0.2% PW (4ºC) and homogenized for 2 min using a stomacher. After homogenization, 1 ml samples were serially diluted 10-fold with 9 ml of sterile 0.2% PW, and 0.1 ml of stomached or diluted samples were spread plated onto each medium. Sorbitol MacConkey with Cefixime Tellurite selective supplement (CT-SMAC) agar (Difco), XLD, and OAB with antimicrobial supplement were used as selective media for the enumeration of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes*, respectively. All plates were incubated at 37°C for 24 to 48 h before counting colonies characteristic of the pathogens. The overlay method described in chapter I-2 was used to recover sub-lethally injured cells of *S.* Typhimurium and *L. monocytogenes*. SPRAB was used to recover injured cells of *E. coli* O157:H7.

GInaFit analysis. GInaFit analysis was used to identify the time required to achieve a 5 log reduction in pathogens (Geeraerd et al., 2005). The survial curves were analyzed by the log-linear + shoulder model.

The parameters of the log-linear + shoulder model are:

$$\log_{10}(N) = \log_{10}(N_0) - \frac{k_{max} \cdot t}{\ln(10)} + \log_{10}\left(\frac{e^{k_{max} \cdot S_l}}{1+(e^{k_{max} \cdot S_l}-1) \cdot e^{-k_{max} \cdot t}}\right)$$

where S_l is the shoulder length and k_{max} is the inactivation rate (min^{-1}).

The time required to achieve a 5 log reduction (t_5d) was calculated using following equation.

$$t_x d = S_l + (x) \cdot \frac{\ln(10)}{k_{max}}$$

PI uptake test. PI was used to determine cell membrane damage. The PI uptake test was conducted according to the method described in chapter I-2 with slight modifications. BPW was subjected to each treatment under the same conditions as those for bactericidal treatments. Untreated and treated BPW were centrifuged at 10,000 × g for 10 min at 4°C. The supernatants were discarded, and the cell pellets were resuspended in 3.5 ml of PBS to an optical density at 680 nm of approximately 0.35, 0.25, and 0.2 for *E. coli* O157:H7, *S*. Typhimurium, and *L. monocytogenes*, respectively. PI was added to a final concentration of 2.9 μM and incubated for 10 min. After incubation, the samples were resuspended in 1 ml of PBS, and centrifuged two times under the same conditions. The final cell pellets were resuspended in 1 ml of PBS and fluorescence was measured with a spectrofluorophotometer at an excitation wavelength of 493 nm and an emission wavelength of 630 nm.

DPPP test. Diphenyl-1-pyrenyl-phosphine (DPPP) was used to evaluate membrane lipid peroxidation (Rahman et al., 2010). DPPP was solubilized in di-methyl sulfoxide (DMSO) to prepare a 50 mM stock solution and was stored in the dark at -20^{o}C prior to use. Untreated and treated BPW were centrifuged at 10,000 × g for 10 min at 4^{o}C. The supernatants were discarded, and the cell pellets were resuspended in 3.5 ml PBS. DPPP was added to the untreated or treated samples to a final concentration of 50 μM and incubated for 60 min in the dark at room temperature. Fluorescence was measured with the spectrofluorophotometer at an excitation wavelength of 351 nm and an emission wavelength of 380 nm. Fluorescence data obtained for the untreated sample were subtracted from those obtained for all treated cells and were normalized to OD_{680}.

DPPP value = (fluorescence value of treated cells/OD_{680}) –

(fluorescence value of untreated cells/OD_{680})

SYBR green I test. SYBR green I was used to evaluate DNA damage (Han et al., 2016; Kang et al., 2018). Following UV-C irradiation, *E. coli* O157:H7 and *S.* Typhimurium samples were incubated with 100 μg/ml lysozyme at 37^{o}C for 4h while *L. monocytogenes* sample was incubated with not only 100 μg/ml lysozyme but also 10 μg/ml lysostaphin at 37^{o}C for 4h.

After incubation, SYBR green I (1:10,000 dilution; Molecular Probes, Eugene, OR, USA) at a working concentration (1:1) was applied for 15 min at 37°C. Fluorescence of the aliquot of each sample was measured with the spectrofluorophotometer (SpectraMax M2e; CA) at an excitation wavelength of 485 nm and an emission wavelength of 525 nm.

Color and lycopene measurement. The color and lycopene content of untreated and treated tomato juice samples were measured. All treated samples were cooled immediately in a crushed ice-water mixture. The color values were measured with a Minolta colorimeter. The values for L^*, a^*, and b^* were measured to evaluate color changes. The lycopene content in tomato juice was measured according to the method previously described in chapter I-2.

Statistical analysis. All inactivation experiments and quality measurements were replicated three times. The data were analyzed by the analysis of variance procedure of the Statistical Analysis System and mean values were separated using Duncan's multiple-range test. Significant differences were determined at a significance level of $p = 0.05$.

The fitness of the log-linear + shoulder model analyzed by GInaFit was evaluated by the root mean squared error (RMSE) and the regression coefficient (R^2).

$$\mathrm{RMSE} = \sqrt{\sum_{i=1}^{n_t} \frac{(y_{expi} - y_{pre})^2}{n_t - n_p}}$$

where y_{expi} refers to the experimental observations, y_{pre} refers to the model predictions, n_t refers to the number of data and n_p refers to the number of parameters.

II-2.3. Results

Inactivation of foodborne pathogens subjected to UV-C irradiation, pulsed ohmic heating, and simultaneous treatment. Simultaneous treatment of UV-C irradiation and pulsed ohmic heating exhibited a synergistic effect on the inactivation of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* in both BPW and tomato juice (Table II-4). In BPW, UV-C irradiation and pulsed ohmic heating exhibited an insignificant bactericidal effect (< 1 log reduction) for all three pathogens, but the reductions induced by the simultaneous treatment were higher than the sum of the reductions caused by the individual treatments. For example, the reductions for *E. coli* O157:H7 by UV-C irradiation, pulsed ohmic heating, and simultaneous treatment in BPW were 0.24, 0.80, and 2.54, respectively. The same trend was observed in tomato juice, and the reductions for *E. coli* O157:H7 by UV-C irradiation, pulsed ohmic heating, and simultaneous combination treatment were 0.48, 1.84, and 3.83, respectively.

Table II-4. Reduction levels of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* in buffered peptone water (BPW) and tomato juice following UV-C irradiation, pulsed ohmic heating, and simultaneous treatment (OH+UV)[a,b]

	Treatment	*E. coli* O157:H7	*S.* Typhimurium	*L. monocytogenes*
BPW	UV-C	0.24 ± 0.51 Aa	0.59 ± 0.61 Aa	0.38 ± 0.21 Aa
	Pulsed ohmic	0.80 ± 0.33 Aa	0.66 ± 0.39 Aa	0.21 ± 0.19 Aa
	OH+UV	2.54 ± 0.41 Ba	2.02 ± 0.74 Ba	1.82 ± 0.41 Ba
Tomato juice	UV-C	0.48 ± 0.73 Aa	0.70 ± 0.17 Aa	0.23 ± 0.11 Aa
	Pulsed ohmic	1.84 ± 0.13 Ba	0.43 ± 0.29 Bb	0.63 ± 0.23 Bb
	OH+UV	3.83 ± 0.84 Ca	2.19 ± 0.22 Bb	2.70 ± 0.38 Bb

Mean values ± standard deviation

[a] Values in the same column followed by the same upper-case letter are not significantly different for each sample ($p > 0.05$).

[b] Values in the same row followed by the same lower-case letter are not significantly different ($p > 0.05$).

PI uptake, DPPP, and SYBR green I test values of foodborne pathogens subjected to UV-C irradiation, pulsed ohmic heating, and simultaneous treatment. Cell membrane damage and lipid peroxidation, as determined by the PI uptake and the DPPP test, respectively, were the highest with simultaneous treatment for all three pathogens followed by pulsed ohmic heating and UV-C irradiation (Fig. II-7). For all three pathogens, the simultaneous treatment had a synergistic effect on the PI uptake, which was greater than the sum of the PI uptake values from the individual treatments. For example, the values for *S.* Typhimurium were 0.23, 2.46, and 7.2 after UV-C irradiation, pulsed ohmic heating, and simultaneous combination treatments, respectively. The PI uptake values for *L. monocytogenes* were significantly higher than those for *E. coli* O157:H7 or *S.* Typhimurium because gram-positive microorganisms such as *L. monocytogenes* and *S. aureus* have a thicker peptidoglycan layer (Peabody et al., 2015). The DPPP values after the simultaneous treatment exhibited an additive effect for all three pathogens, as the values for the simultaneous treatment were similar to the sums corresponding to the individual treatments. For example, the lipid peroxidation values for *S.* Typhimurium were 9.1, 20.2, and 31.6 for the UV-C irradiation, pulsed ohmic heating, and simultaneous combination treatments, respectively. On the other hand, DNA damage was not significant after UV-C irradiation in the present study (Table II-5)

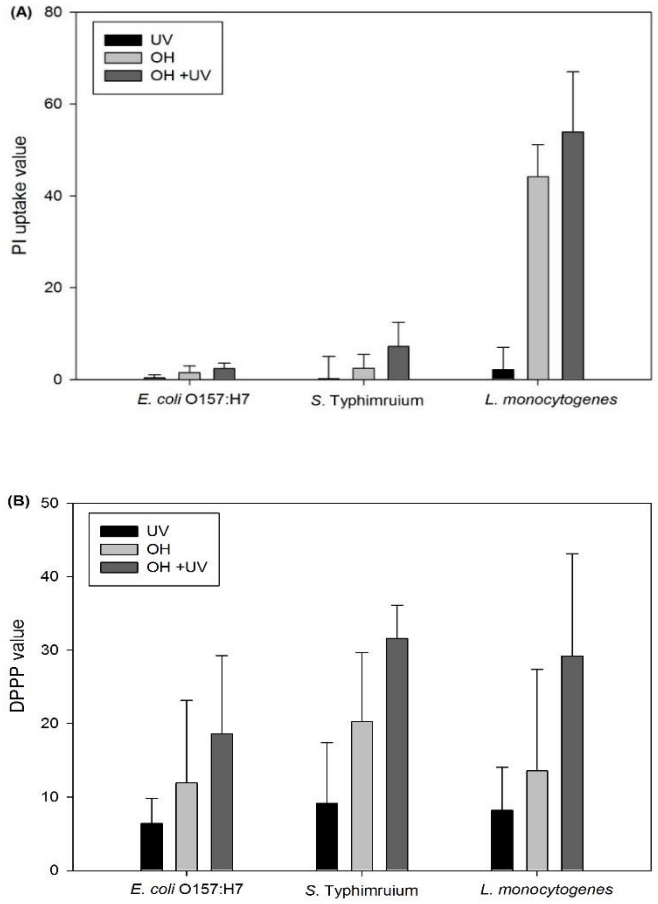


Fig. II-7. Cell membrane damage (A) and lipid peroxidation (B) values of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* subjected to UV-C irradiation (UV), pulsed ohmic heating (OH), and simultaneous treatment (OH+UV).

Table II-5. DNA damage values of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* following UV-C irradiation[a]

Treatment	*E. coli* O157:H7	*S.* Typhimurium	*L. monocytogenes*
Control	52.29 ± 0.85 A	53.58 ± 4.51 A	40.90 ± 2.78 A
UV-C	49.66 ± 11.5 A	45.88 ± 9.56 A	41.21 ± 6.91 A

Mean values ± standard deviation

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

Inactivation of foodborne pathogens subjected to pulsed ohmic heating and UV-C irradiation sequentially or simultaneously. Sequential treatments exhibited no significnat difference from simultaneous treatment in tomato juice ($p > 0.05$), but a significant difference ($p < 0.05$) was observed in BPW (Table II-6). The reduction in the levels of *E. coli* O157:H7 in BPW following the OH+UV treatment (3.30 log reduction) was significantly higher ($p < 0.05$) than that after the OH-UV treatment (1.76 log reduction). For *S.* Typhimurium and *L. monocytogenes* in BPW, the reduction levels after the OH+UV or UV-OH treatments were slightly higher than that after the OH-UV treatments, but a significant difference was not observed ($p > 0.05$).

Table II-6. **Reduction levels of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* in buffered peptone water (BPW) and tomato juice following sequential treatment of UV-C irradiation after pulsed ohmic heating (OH-UV), pulsed ohmic heating after UV-C irradiation (UV-OH) and simultaneous treatment (OH+UV)[a,b]**

	Treatment	*E. coli* O157:H7	*S.* Typhimurium	*L. monocytogenes*
BPW	OH-UV	1.76 ± 0.74 Aa	0.37 ± 0.44 Ab	0.60 ± 0.52 Aab
	UV-OH	2.85 ± 0.72 ABa	1.05 ± 0.18 Ab	1.62 ± 0.95 Aab
	OH+UV	3.30 ± 0.12 Ba	0.96 ± 0.43 Ab	1.39 ± 0.56 Ab
Tomato juice	OH-UV	4.78 ± 0.68 Aa	2.98 ± 0.28 Ab	5.31 ± 0.36 Aa
	UV-OH	4.78 ± 0.68 Aa	3.06 ± 0.43 Ab	5.21 ± 0.52 Aa
	OH+UV	5.12 ± 0.17 Aa	2.80 ± 0.72 Ab	5.51 ± 0.01 Aa

Mean values ± standard deviation

[a] Values in the same column followed by the same upper-case letter are not significantly different for each sample ($p > 0.05$).

[b] Values in the same row followed by the same lower-case letter are not significantly different ($p > 0.05$).

Color and lycopene content of tomato juice. For the tomato juice quality experiment, the treatment times were identified to ensure 5 log reductions for all three bacterial pathogens after pulsed ohmic heating and simultaneous treatment. When analyzed by GInaFit, the log-linear + shoulder models fitted well to the inactivation of pathogens by pulsed ohmic heating and simultaneous treatment with a high value of R^2 ($\geq$ 0.93) and a low value of RMSE ($\leq$ 0.84). For pulsed ohmic heating, the times (min) required to achieve 5 log reduction (t_5d) were 4.54, 4.06, and 3.96 for *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes*, respectively. On the other hand, these values were 4.16, 3.85 and 3.76 for *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes*, respectively, after the simultaneous treatments (Table II-7). Therefore, at least 4.54 and 4.16 min were required to achieve a 5 log reductions for all three pathogens by pulsed ohmic heating and by simultaneous treatment, respectively. After tomato juice was subjected to 4.54 min of pulsed ohmic heating or 4.15 min of simultaneous treatment, its color and lycopene content values were not significantly different ($p > 0.05$) from those of untreated sample (Table II-8).

Table II-7. Parameters[a] of the log-linear + shoulder model for the inactivation of *E. coli* O157:H7 (E), *S.* Typhimurium (S), and *L. monocytogenes* (L) in tomato juice subjected to pulsed ohmic heating and the combination treatment

	Heating method	S_l (min) ± SE	k_{max} ± SE	RMSE	R^2	t_5d (min)
E	Pulsed ohmic	2.98 ± 0.13	7.39 ± 0.87	0.25	0.99	4.54
	Combination	3.02 ± 0.04	10.14 ± 0.37	0.11	1.00	4.16
S	Pulsed ohmic	2.73 ± 0.20	8.67 ± 1.38	0.44	0.98	4.06
	Combination	2.72 ± 0.29	10.19 ± 2.32	0.75	0.96	3.85
L	Pulsed ohmic	2.11 ± 0.73	6.22 ± 2.56	0.83	0.93	3.96
	Combination	2.29 ± 0.52	7.83 ± 2.50	0.81	0.95	3.76

[a] SE: standard error, R^2: regression coefficient

Table II-8. Color and lycopene content of tomato juice subjected to pulsed ohmic heating (OH) or the combination treatment[a]

	Color			Lycopene content
	L^*	a^*	b^*	
Control	26.86 ± 0.49 A	5.28 ± 0.15 A	9.35 ± 0.04 A	61.54 ± 2.01 A
OH	26.87 ± 0.17 A	5.37 ± 0.22 A	9.37 ± 0.10 A	59.53 ± 3.43 A
Combination	26.62 ± 0.04 A	5.13 ± 0.06 A	9.27 ± 0.09 A	58.03 ± 1.66 A

Mean values ± standard deviation

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

II-2.4. Discussion

In the present study, combined effect of UV-C irradiation and pulsed ohmic heating for inactivation of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* was investigated. First, the simultaneous treatment of UV-C irradiation and pulsed ohmic heating exhibited a synergistic bactericidal effect. It was postulated that the difference in bactericidal targets between UV-C irradiation and pulsed ohmic heating is the reason for this synergistic bactericidal effect. However, in this study, DNA damage was not significant after UV-C irradiation (Table II-5). Otherwise, the cell membranes of all three pathogens were most significantly damaged by simultaneous treatment, followed by pulsed ohmic heating and UV-C irradiation. The PI uptake values after UV-C irradiation treatment by itself were very small (< 2.2), while the PI uptake values after the simultaneous treatment were much larger than the sum of the uptake values for the individual treatments. For example, the PI uptake values of *L. monocytogenes* were 2.16, 44.2, and 53.9 after UV-C irradiation, pulsed ohmic heating, and the simultaneous treatment, respectively. These results indicate that UV-C irradiation does not have a significant effect on the cell membrane itself but can accelerate cell membrane damage if pore formation is initiated by pulsed ohmic heating treatment.

Lipid peroxidation of the cell membrane is one possible mechanism of damaging foodborne pathogens by UV irradiation (Wu et al., 2011). Lipid peroxidation can increase membrane fluidity, reduce its integrity, disrupt cell osmotic balance and lead to cell wall rupture (Alwi and Ali, 2014). Because the PI uptake values after treatment with only UV-C irradiation were very small, it is presumed that lipid peroxidation by individual UV-C irradiation was not enough to form pores in all three pathogens used in this study. Because the lipid peroxidation values after pulsed ohmic heating were larger than those after UV-C irradiation for all three pathogens, following the same trend as the PI uptake values, it is proposed that a threshold lipid peroxidation value exists for cell membrane damage. Even though lipid peroxidation by UV-C irradiation cannot result in the threshold value by itself, the lipid peroxidation level can be increased by UV-C irradiation when combined with pulsed ohmic heating. Accelerated lipid peroxidation would cause severe damage to the cell membrane, and simultaneous treatment exhibited a synergistic effect on the PI uptake values for all three pathogens.

Simultaneous application of thermal and non-thermal technologies is ideal for inactivating pathogens without deteriorating food quality, but in many cases, it is difficult to apply thermal and non-thermal technologies simultaneously due to the limitations of industrial space and equipment size. Therefore, sequential treatments of UV-C irradiation and pulsed ohmic

heating was compared with simultaneous treatment on the inactivation of foodborne pathogens. In BPW, reductions in the levels of all three pathogens were lower after sequential treatment of UV-C irradiation after pulsed ohmic heating (OH-UV) than after the reverse treatment sequence (UV-OH) or simultaneous treatment (OH+UV). It is noteworthy that the treatment sequence has a significant effect on the inactivation of pathogens. Two factors could contribute to this phenomenon. First, heat shock proteins expressed after pulsed ohmic heating could play a significant role in the cross-protection against UV-C irradiation. Estilo and Gabriel (2017) also reported that the UV resistance of *Salmonella enterica* increased after exposure to heat stress. Second, the recovery process after UV-C irradiation could be inhibited by pulsed ohmic heating. In response to UV-C irradiation used for the inactivation of microorganisms, many pathogens have a mechanism of photoreactivation and dark repair (Escalona et al., 2010). The multienzyme repair process is involved in the dark repair mechanism and photolyase is needed for photoreactivation (Sanz et al., 2007). The enzymes related to the recovery mechanism can be immediately denatured by the high temperature of pulsed ohmic heating when UV-C irradiation is performed before or during pulsed ohmic heating. On the other hand, when UV-C irradiation is carried out after pulsed ohmic heating, enzymes related to the recovery mechanism would not be denatured completely. It is suggested that these two factors (heat shock

protein formation and UV recovery process) contributed to the inhibited reduction of pathogens by OH-UV treatment in BPW, but further study is needed. In contrast to BPW, reduction levels by the sequential treatment of UV-C irradiation and pulsed ohmic heating were not significantly different from that of the simultaneous treatment regardless of the treatment sequence in tomato juice ($p > 0.05$). Because the pH in tomato juice (3.6) is significantly lower than that in BPW (7.2), heat stress-related genes would be induced regardless of the treatment sequence because the bacterial heat shock response is activated not only by heat shock stress but also by many other unfavorable conditions (Ban et al., 2015). Moreover, recovery-related enzymes would be denatured because of the low pH. In this regard, reductions in levels of all three pathogens may not be significantly different according to treatment sequence in tomato juice ($p > 0.05$).

Juice processors should treat their juices to achieve a 5 log reduction in the number of targeted microorganisms (U. S. FDA., 2001). Treatment conditions to achieve 5 log reductions in *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* levels by pulsed ohmic heating and simultaneous treatment were identified with resuscitation medium in the present study because injured pathogens can recover into normal cells during storage (Wu, 2008). The times (min) required to achieve a 5 log reduction (t_5d) for all three pathogens were 4.54 and 4.16 for pulsed ohmic heating and simultaneous treatment,

respectively. The results indicate that treatment time of pulsed ohmic heating can be reduced by 8.37% by combining it with UV-C irradiation and that the reduced treatment time allowed a 6.77% decrease in treatment temperature. The quality improvement enabled by the reduced temperature was not observed in the present study because the treatment conditions of pulsed ohmic heating were not critical to the deterioration in quality, however, food can be deteriorated in many cases during pasteurization. In such a situation, combining pulsed ohmic heating with UV-C irradiation would not only minimize the quality degradation of juice products but also reduce treatment time, which brings an economic benefit.

In conclusion, a synergistic bactericidal effect by simultaneous treatment of pulsed ohmic heating and UV-C irradiation was observed against *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes*. Based on the PI uptake and DPPP values, it was suggested that UV-C irradiation can accelerate lipid peroxidation when combined with pulsed ohmic heating, which results in the synergistic effect of the two methods on cell membrane damage. The synergistic effect on cell membrane damage in turn induces the synergistic bactericidal effect. When UV-C irradiation and pulsed ohmic heating was applied sequentially, the treatment order has a significant effect on pathogen inactivation in BPW ($p < 0.05$), but not in tomato juice. Heat shock protein induction after pulsed ohmic heating and recovery process after UV-C

irradiation were suggested as two contributing factors. Even though the quality degradation was not observed by pulsed ohmic heating in the present study, the time required to achieve a 5 log reduction (t_5d) in pathogens by pulsed ohmic heating was significantly decreased by combining it with UV-C irradiation. Therefore, the combination treatment of pulsed ohmic heating and UV-C irradiation can be used as an alternative hurdle technology to ensure microbiological safety in juice products.

Chapter III.

Combined inhibitory effect of milk fat and lactose for inactivation of foodborne pathogens by pulsed ohmic heating

III-1. Introduction

Consumer demand for foods of modified nutritional content has been increasing recently, and accordingly, such foods have appeared in the marketplace. Particularly, various milk products are being produced which have reduced fat and/or lactose content. The fat and lactose content of milk are 3-4 and 4-5%, respectively. Low fat (1-2%) or skimmed (0-0.5%) milk is preferred by some consumers who worry about obesity, and lactose-free milk is preferred by some consumers who have trouble digesting this sugar, which is known as lactose-intolerance.

Performance of pulsed ohmic heating is affected by intrinsic and extrinsic factors. Extrinsic factors such as voltage and frequency have a significant effect on the inactivation of pathogens. For example, accelerated heating rate by means of increased voltage and frequency results in rapid inactivation of foodborne pathogens (Baysal and İçier, 2010; Lee et al., 2013). Reduction of pathogens may also significantly affected by intrinsic factors. In particular, nutritive components such as fats, protein, and carbohydrates not only would change the electrical conductivity of food but also have a protective effect on the inactivation of pathogens. For example, inactivation levels of *S.* Typhimurium DT 104 and *Listeria innocua* decreased with increasing fat

content subjected to heat treatment (Bermúdez-Aguirre et al., 2008; Juneja and Eblen, 2000). Ramaswamy et al. (2009) also reported that casein and lactose are important factors affecting the baro-protection of *E. coli* in milk during high-pressure treatment. However, a combined effect of milk fat and lactose on the performance of the pulsed ohmic heating has not been investigated.

One of an important role of milk processing by pulsed ohmic heating is to inactivate harmful microorganisms such as *E. coli* O157:H7, *S*. Typhimurium, and *L. monocytogenes*. *E. coli* O157:H7 related outbreaks have been reported annually, which can cause bloody diarrhea and hemolytic uremic syndrome (Marder et al., 2014). Multistate outbreaks related to *S*. Typhimurium occurred in 2015, which can cause diarrhea, abdominal pain, mild fever and chills (Jain et al., 2009). *L. monocytogenes* can grow in foods at refrigerator temperatures and cause epidemic listeriosis, with a mortality rate of about 24% (Farber and Peterkin, 1991). These three pathogens have been detected in farm environmental samples and reported as common microorganisms related to dairy farm environmental outbreaks (Murinda et al., 2004). An outbreak of *E. coli* O157:H7 infections caused by commercially distributed raw milk was reported between 1992-1993 (Keene et al., 1997). The Centers for Disease Control and Prevention (CDC) reported pasteurized milk outbreaks involving *S*. Typhimurium in 2000 and 2002 and *L. monocytogenes* in 2007 resulting in 3 deaths (CDC, 2008b). Therefore, these three pathogens are used as target

microorganisms to identify the pasteurizing performance of pulsed ohmic heating.

The objective of the present study was to identify the combined effect of milk fat and lactose on the inactivation of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* by pulsed ohmic heating. Reductions of the three pathogens subjected to pulsed ohmic heating in the milk of various fat and lactose content were analyzed by response surface methodology (RSM). A predicted model developed by RSM was verified within the range used in the experiments, and quality aspects including pH, color, and lipid oxidation were assessed.

III-2. Materials and Methods

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. Stock and working cultures were prepared according to the method previously described in chapter I-2. A mixed culture cocktail contains approximately equal numbers of cells of each strain of *E. coli* O157:H7 (10^9 CFU/ml), *S.* Typhimurium (10^9 CFU/ml), and *L. monocytogenes* (10^8 CFU/ml).

Sample preparation and inoculation. Pasteurized lactose-free and low fat (1.5%) milk (pH 6.9) and sterilized cream containing 37% fat and emulsifier were purchased at a local grocery store (Seoul, South Korea). Milk and cream were stored under refrigeration (4.0^{o}C) until used for experiments. Cream and lactose (Difco) were added to milk to achieve fat contents of 1.5, 2.5, 3.5, 4.5, or 5.5% and lactose content of 0, 1, 2, 3, or 4%. Fat and lactose content were calculated on the basis of manufacturer declarations. Samples

were mixed using a magnetic stirrer and stir bar. Mixed-culture cocktail (0.2 ml) was inoculated into 50 ml of prepared sample.

Experimental design. The effects of lactose, fat, and treatment time on the inactivation of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* were identified using RSM. Lactose and fat levels ranged from 0 to 4% and 1.5 to 5.5%, respectively, depending on food products. Treatment time was determined to be 80 to 120 s based on the preliminary studies. A 3-factor Central Composite Design (CCD) was used and five levels for each factor were coded as -2, -1, 0, +1, +2, respectively (Table III-1). The 16 experiments were performed in random order.

Table III-1. **Variables and levels used for the central composite design**

X_i	Independent variables	Levels				
		-2	-1	0	+1	+2
X_1	Lactose content (%)	0	1	2	3	4
X_2	Fat content (%)	1.5	2.5	3.5	4.5	5.5
X_3	Time (s)	80	90	100	110	120

Bactericidal treatment. Pulsed ohmic heating treatments were carried out with the apparatus previously described in chapter II-2. Prepared and inoculated samples were subjected to pulsed ohmic heating (0.3 duty ratio, 10 kHz) with a fixed electric strength of 18.2 V_{rms}/cm. Samples were taken after each treatment and populations of surviving microorganisms were enumerated.

Bacterial enumeration. For microbial enumeration, each treated 50 ml sample was immediately transferred into a sterile stomacher bag containing 100 ml of sterile 0.2% PW (4°C) and homogenized for 2 min using a stomacher. After homogenization, 1 ml samples were 10-fold serially diluted with 9 ml of sterile 0.2% PW and 0.1 ml of stomached or diluted samples were spread-plated onto each selective medium. SMAC agar, XLD agar, and OAB with antimicrobial supplement 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 to 48 h before counting colonies characteristic of the pathogens.

Modeling and verification. The response was measured as the log reduction of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes*. Modeling was performed using the statistical analysis system. The following

second order polynomial equation was used to develop a predictive model for the inactivation of each pathogen by pulsed ohmic heating treatment.

$$\text{Log reduction} = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 + \beta_{12} X_1 X_2 + \beta_{23} X_2 X_3 + \beta_{13} X_1 X_3$$

where β_i are constant regression coefficients (β_0: constant term; β_1, β_2, β_3: linear effect; β_{11}, β_{22}, β_{33}: quadratic effect; β_{12}, β_{13}, β_{23}: interaction effect); X_1, X_2, and X_3 are lactose content (%), fat content (%), and treatment time (s), respectively.

A stepwise regression was performed using the PROC REG procedure with 'selection=backward' option to include only the significant ones ($p = 0.1$). Four treatment conditions were selected within the range of experimental conditions to verify the accuracy of the developed predictive models. The accuracy factor (A_f) and bias factor (B_f) were used to validate the predictive model. The A_f and B_f values were calculated with the following equations.

$$A_f = 10^{\frac{\sum|\log(predicted/observed)|}{n}}$$

$$B_f = 10^{\frac{\sum \log(predicted/observed)}{n}}$$

where n is the number of observations. A_f represents how absolutely close, on average, the predictions are to the observations. The larger the value,

the less accurate is the average estimate. B_f indicates by how much, on average, a model over-predicts or under-predicts the observed data. Perfect agreement between predictions and observations will lead to a bias factor of 1.

Color, pH, and lipid oxidation measurement. Each heat treatment time was calculated from the predicted model to achieve 5 log reductions for all three pathogens for color, pH, and lipid oxidation measurement experiments. The pH of treated and untreated samples was measured with a Seven Multi 8603 pH meter. The color of treated and untreated samples was measured using a Minolta colorimeter. Color values for L^*, a^*, and b^* were recorded to evaluate color changes of treated and untreated samples. Lipid oxidation was determined by measuring the levels of 2-thiobarbituric acid reactive substances (TBARS) values in the samples (Jung et al., 2016). Samples (3 ml) were vortexed with 9 ml of 7.5% TCA solution and 50 μl of butylated hydroxytoluene (7.2%). The homogenate was centrifuged at 2,090 × g for 15 min, and filtered through a Whatman No.4 filter paper (Whatman, Maidstone, UK). One ml of filtrate was transferred into a test tube, and 1 ml of a 20 mM 2-Thiobarbituric acid (TBA) was added. The tubes were heated in a water bath (BW-10G; Jeio Tech, Seoul, South Korea) at 90^{o}C for 30 min and cooled in tap water for 10 min. Absorbance was measured with spectrofluorophotometer

at 532 nm. The concentration of malondialdehyde (MDA) in a sample was expressed in milligrams of MDA per kilogram sample.

Statistical analysis. All experiments were replicated three times. All experimental data were analyzed by the ANOVA procedure and mean values were separated using Duncan's multiple-range test. Significant differences in the processing treatments were determined at a significance level of $p = 0.05$.

III-3. Results and discussion

Reductions of pathogens subjected to pulsed ohmic heating in milk of various lactose and fat content. Reductions of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* were significantly different corresponding to lactose content, fat content, and treatment time in the present study (Table III-2). In general, reductions of pathogens increased as lactose and fat content decreased and treatment time increased. At a fixed treatment time of 100 s, lactose and fat had a significant inhibitory effect on the inactivation of pathogens. Reductions (log CFU/ml) of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* increased by 1.19, 2.35, and 1.51, respectively as lactose content decreased from 4 to 0% (Trials 11 and 12). Similarly, reductions (log CFU/ml) of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* increased 2.14, 1.46, and 0.80, respectively as fat content decreased from 5.5 to 1.5% (Trials 13 and 14). Increased lactose or fat not only decreased the electrical conductivity of milk but also protected the foodborne pathogens from thermal damage. The contribution of food components such as salts, sugars, proteins, and fats on increased bacterial heat resistance was discussed previously (Espina et al., 2010). Juneja and Eblen (2000) also reported that increasing the sodium chloride concentration (0 – 6%)

protected *L. monocytogenes* against heat treatment (55 – 60^{o}C). It is concluded that more foodborne pathogens can survive in the milk of higher fat or lactose content when subjected to pulsed ohmic heating with fixed treatment time.

Table III-2. Reduction (log CFU/ml) of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* in milk of varying fat and lactose content subjected to pulsed ohmic heating of varying time intervals[a,b]

Trial	Lactose (%)	Fat (%)	Time (s)	Reduction (log CFU/ml)		
				E. coli O157:H7	*S.* Typhimurium	*L. monocytogenes*
1	1.0	2.5	90	0.49 ± 0.77 ABa	0.80 ± 0.05 ABa	0.65 ± 0.41 ABa
2	1.0	2.5	110	2.48 ± 0.36 DEa	3.30 ± 0.28 Fa	2.73 ± 0.83 Fa
3	1.0	4.5	90	0.41 ± 0.20 ABa	0.70 ± 0.37 ABa	0.37 ± 0.19 Aa
4	1.0	4.5	110	2.27 ± 0.35 DEa	2.59 ± 0.80 EFa	1.99 ± 0.18 CDEFa
5	3.0	2.5	90	0.72 ± 0.13 ABa	0.87 ± 0.22 ABa	0.70 ± 0.20 ABa
6	3.0	2.5	110	2.31 ± 0.22 DEa	2.72 ± 0.37 EFa	2.24 ± 1.05 DEFa
7	3.0	4.5	90	0.25 ± 0.42 Aa	0.47 ± 0.50 Aa	0.31 ± 0.24 Aa
8	3.0	4.5	110	2.13 ± 0.09 DEa	2.48 ± 0.47 DEa	1.65 ± 0.54 BCDEa
9	2.0	3.5	100	1.82 ± 0.38 CDa	1.43 ± 0.14 BCa	1.29 ± 0.47 ABCDa
10	2.0	3.5	100	2.06 ± 0.15 Da	1.80 ± 0.67 CDa	1.42 ± 0.49 ABCDa
11	0.0	3.5	100	2.34 ± 0.41 DEa	3.33 ± 0.33 Fa	2.57 ± 0.81 EFa
12	4.0	3.5	100	1.15 ± 0.71 BCa	0.98 ± 0.34 ABa	1.06 ± 0.52 ABCa
13	2.0	1.5	100	2.87 ± 0.41 Ea	2.48 ± 0.98 DEa	1.88 ± 0.48 CDEFa
14	2.0	5.5	100	0.73 ± 0.54 ABa	1.02 ± 0.30 ABa	1.08 ± 0.24 ABCa
15	2.0	3.5	80	0.20 ± 0.50 Aa	0.65 ± 0.14 ABa	0.75 ± 0.48 ABa
16	2.0	3.5	120	3.58 ± 0.18 Fa	5.45 ± 0.05 Gb	4.69 ± 0.71 Gb

Mean values ± standard deviation

[a] Values in the same column that are followed by the same uppercase letter are not significantly different ($p > 0.05$).

[b] Values in the same row that are followed by the same lowercase letter are not significantly different ($p > 0.05$).

Predictive modeling based on response surface methodology. RSM is a useful tool for analyzing and predicting the effect of several factors on the inactivation of foodborne pathogens (Kwak et al., 2011). In the present study, regression analysis of the experimental data produced the following predictive equations for *E. coli* O157:H7 [1], *S.* Typhimurium [2], and *L. monocytogenes* [3], respectively.

[1] $Y = -6.655 + 0.3838X_1 - 0.4413X_2 + 0.1035X_3 - 0.0488X_1^2 - 0.0350X_2^2 - 0.0001X_3^2 - 0.0250X_1X_2 + 0.0040X_2X_3 - 0.0028X_1X_3$

[2] $Y = 25.59 - 0.2988X_1 + 0.0106X_2 - 0.5730X_3 + 0.0213X_1^2 - 0.0066X_2^2 + 0.0036X_3^2 - 0.0056X_1X_2 + 0.0338X_2X_3 + 0.1350X_1X_3$

[3] $Y = 23.40 + 0.3063X_1 + 0.3713X_2 - 0.5428X_3 + 0.1150X_1^2 + 0.0313X_2^2 + 0.0034X_3^2 + 0.0050X_1X_2 - 0.0083X_2X_3 - 0.0103X_1X_3$

The analysis of variance indicated that the developed models were significant for all three pathogens (Table III-3). Response surface 3D contour plot was described to identify the combined inhibitory effect of lactose and fat on the inactivation of pathogens when the treatment time is fixed at the coded 0 level (Fig. III-1). The contour plot of *E. coli* O157:H7 has a convex

upward surface, while those of *S.* Typhimurium and *L. monocytogenes* have a convex downward surface. The inhibitory effect of fat was more significant than that of lactose for inactivation of *E. coli* O157:H7 (Fig. III-1A). On the other hand, the inhibitory effect of lactose was similar or more significant than that of fat for inactivation of *S.* Typhimurium and *L. monocytogenes* (Fig. III-1B and Fig. III-1C). It seems that the protective effect of fat on the inactivation of *E. coli* O157:H7 is more significant than for *S.* Typhimurium and *L. monocytogenes* as described previously (Kim and Kang, 2015). The region including high fat and lactose may heat more slowly than its surroundings due to lower electrical conductivity. Therefore, pathogens present within the fat or lactose phase receive less thermal damage than surroundings. Further study is needed to identify why the protective effect of fat is more significant for *E. coli* O157:H7 than *S.* Typhimurium and *L. monocytogenes*.

Table III-3. Analysis of variance of inactivation models of pathogens subjected to pulsed ohmic heating

Pathogen	Analysis of variance				
	DF	*Sum of squares*	*F-value*	*p-value*	R^2
E. coli O157:H7					
Total model	9	14.47	5.67**	0.0235**	0.8947
Linear	3	14.40	16.91***	0.0025***	0.8904
Quadratic	3	0.05	0.05	0.9818	0.0029
Cross product	3	0.02	0.03	0.9930	0.0015
Lack of Fit	5	1.67	11.63	0.2189	
S. Typhimurium					
Total model	9	25.61	8.70***	0.0080***	0.9288
Linear	3	23.03	23.48***	0.0010***	0.8354
Quadratic	3	2.51	2.56	0.1508	0.0911
Cross product	3	0.06	0.07	0.9763	0.0023
Lack of Fit	5	1.89	5.53	0.3116	
L. monocytogenes					
Total model	9	17.23	7.76**	0.0107**	0.9209
Linear	3	14.81	20.01***	0.0016***	0.7916
Quadratic	3	2.28	3.08	0.1119	0.1219
Cross product	3	0.14	0.19	0.9012	0.0074
Lack of Fit	5	1.47	34.83	0.1279	

***1% and **5% probability level

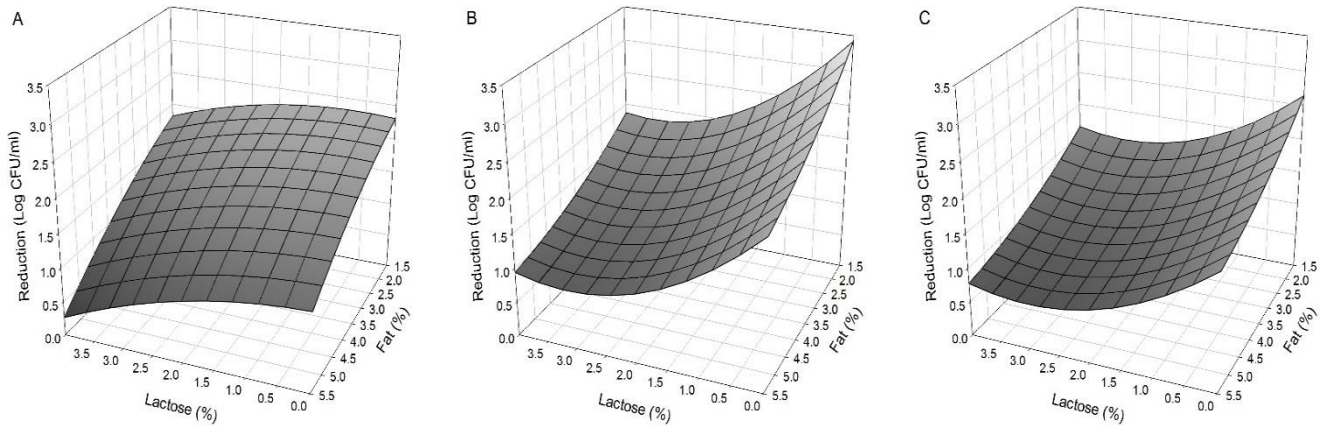


Fig. III-1. Response surface 3D contour plot indicating the effect of lactose and fat content on inactivation of *E. coli* O157:H7 (A), *S.* Typhimurium (B), and *L. monocytogenes* (C) subjected to pulsed ohmic heating for 100 s.

Validation of the predictive model. The predictive model equations (1-3) were reduced with stepwise backward selection (Table III-4). A stepwise regression of the experimental data produced the following predictive equations including only the significant factors for *E. coli* O157:H7 [4], *S.* Typhimurium [5], and *L. monocytogenes* [6], respectively ($p < 0.1$).

[4] $Y = -6.228 + 0.0870X_3 - 0.0439X_1^2 - 0.0475X_2^2$

[5] $Y = 20.73 - 0.4769X_3 + 0.0030X_3^2 - 0.0028X_2X_3 - 0.0035X_1X_3$

[6] $Y = 21.51 - 0.4818X_3 + 0.0029X_3^2 - 0.0023X_2X_3 - 0.0025X_1X_3$

Inactivation of *E. coli* O157:H7 has a quadratic relationship with lactose (X_1) and fat (X_2), whereas the cross product (X_1X_3 and X_2X_3) has a significant effect on the inactivation of *S.* Typhimurium and *L. monocytogenes* ($p = 0.1$). These results indicate that the effect of fat and lactose on the reduction of *E. coli* O157:H7 is more significant at higher than at lower levels, and the effects on *S.* Typhimurium and *L. monocytogenes* become more significant as treatment time increases. A similar tendency was observed in the previous experiment, which indicated that inactivation of *E. coli* O157:H7 has a quadratic relationship with fat whereas that of *S.* Typhimurium or *L.*

monocytogenes has a linear relationship with fat (data not shown). The reduced model including only the significant factors was verified under four treatment conditions which are within the range of experimental conditions. The observed reductions of pathogens were not significantly different from predicted levels (Table III-5). The accuracy factor (A_f) and bias factor (B_f) were used to validate the predictive model. For all three pathogens, A_f values were not over than 1.35, and B_f values were between 0.83 and 1.17 (Table III-6). Because accuracy factor (A_f) and bias factor (B_f) were close to 1, it is concluded that the developed model reliably predicted the inactivation of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes*. Previous research investigation also reported that models developed by RSM predicted inactivation of pathogens well (Song et al., 2016).

Table III-4. Estimated regression coefficients of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* with significant values ($p < 0.1$)

	E. coli O157:H7		*S.* Typhimurium		*L. monocytogenes*	
	Estimate	p-value	Estimate	p-value	Estimate	p-value
Intercept	-6.228	<0.0001	20.73	0.0477	21.51	0.0237
X_3	0.0870	<0.0001	-0.4769	0.0269	-0.4818	0.0138
X_1X_3	-	-	-0.0035	0.0109	-0.0025	0.0328
X_2X_3	-	-	-0.0028	0.0346	-0.0023	0.0443
X_1^2	-0.0439	0.0776	-	-	-	-
X_2^2	-0.0475	0.0070	-	-	-	-
X_3^2	-	-	0.0030	0.0078	0.0029	0.0045

Table III-5. Predicted and observed reduction of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* subjected to pulsed ohmic heating

		Reduction (Log CFU/ml)					
		E. coli O157:H7		*S.* Typhimurium		*L. monocytogenes*	
Lactose (%)	Fat (%)	Pred[a]	Observ	Pred	Observ	Pred	Observ
0	1.5	2.36	2.77 ± 0.55	2.93	2.45 ± 0.46	2.19	1.63 ± 0.33
0	5.5	1.03	1.57 ± 0.25	1.83	2.06 ± 0.40	1.28	1.23 ± 0.35
4	1.5	1.66	1.33 ± 0.57	1.54	1.38 ± 0.10	1.52	0.99 ± 0.68
4	5.5	0.33	0.49 ± 0.03	0.44	0.49 ± 0.51	0.30	0.25 ± 0.24

[a] Pred : Predicted value, Observ : Observed value

Table III-6. **Accuracy factors (A_f) and bias factors (B_f) of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes***

	Verification value	
	A_f	B_f
E. coli O157:H7	1.35	0.83
S. Typhimurium	1.14	1.02
L. monocytogenes	1.27	1.27

Color, pH, and lipid oxidation values. Changes in color, pH, and lipid oxidation values in samples of low (0% lactose and 1.5% fat) and high (4% lactose and 5.5% fat) levels of nutrients were observed in the present study. Color and TBARS values were not significantly degraded ($p > 0.05$) in samples of both low and high nutrient levels, whereas pH values decreased significantly ($p < 0.05$) in both types of samples (Table III-7). However, the degree of pH change was not great for either type of samples. pH values of samples having low levels of nutrients decreased from 6.99 to 6.91 after pulsed ohmic heating, and those containing high levels decreased from 6.96 to 6.89. Even though color and TBARS values of ohmic heated samples were not significantly degraded ($p > 0.05$) in the present study, more treatment time is needed to satisfy the pasteurization standards for milk. These standards are based on the destruction of *Coxiella burnetii*, which is the most heat-resistant milk-borne pathogen (Claeys et al., 2013). Therefore, further study is needed to identify quality changes occurring in milk containing high and low levels of nutrients subjected to treatment conditions necessary to inactivate *C. burnetii.*

Table III-7. Color, pH, and lipid oxidation (TBARS) values of untreated or treated samples of different lactose and fat content[a]

Lactose (%)	Fat (%)	Treatment	pH	Color			TBARS
				L^*	a^*	b^*	
0	1.5	Untreated	6.99 ± 0.04 A	68.86 ± 0.91 A	-3.04 ± 0.09 A	1.24 ± 0.21 A	0.74 ± 0.26 A
		Treated	6.91 ± 0.01 B	68.01 ± 2.15 A	-3.01 ± 0.14 A	1.25 ± 0.08 A	0.86 ± 0.31 A
4	5.5	Untreated	6.96 ± 0.02 A	69.57 ± 1.27 A	-2.93 ± 0.13 A	1.78 ± 0.30 B	0.71 ± 0.27 A
		Treated	6.89 ± 0.02 B	68.85 ± 1.61 A	-2.90 ± 0.06 A	1.73 ± 0.33 B	0.76 ± 0.20 A

Mean values ± standard deviation

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

In conclusion, combined inhibitory effect of lactose and milk fat on the inactivation of foodborne pathogens by pulsed ohmic heating was identified in the present study. The inhibitory effect of fat was more significant in *E. coli* O157:H7 than for *S*. Typhimurium and *L. monocytogenes*. Predictive models developed using stepwise backward selection showed that inactivation of *E. coli* O157:H7 has a quadratic relationship with lactose (X_1) and fat (X_2) content, whereas the cross products (X_1X_3 and X_2X_3) have a significant effect on the inactivation of *S*. Typhimurium, and *L. monocytogenes*. Developed predictive models were verified under four treatment conditions, and observed values were not significantly different from those predicted. Color change and lipid oxidation were not observed, while pH values slightly decreased following pulsed ohmic heating treatment. Therefore, treatment conditions for pulsed ohmic heating pasteurization should be decided considering lactose and fat content, and models developed by RSM could be used effectively to predict inactivation of pathogens.

IV. Multiphysics modeling of pulsed ohmic heating for inactivation of foodborne pathogens in tomato juice

IV-1. Introduction

Predicting microbial destruction in thermal processing of foods has been topic of intense investigation. First, the resistance of each pathogen to different lethal temperatures should be identified to predict the microbial destruction by thermal processing. Because most microorganisms are known to be destructed exponentially at lethal temperature, D-value, the time required to reduce the population of a microbe by 90%, is widely used as an indicator of thermal resistance of pathogens. D-values are usually calculated empirically because the values differ significantly according to the type of pathogen, nutritional components, pH, and viscosity of food samples (Juneja, 2003; Juneja and Eblen, 1999). After D-values at various temperatures are determined, z-value, another heat resistance indicator, is calculated from the D-values. Subsequently, lethality (F value) is determined from the heating rate and the z-value to predict microbial destruction in thermal processing. Recently, researchers insisted that microbial destruction (log reduction) doesn't follow a linear relationship with temperature (Geeraerd et al., 2005). Several non-log-linear models such as Weibull and log-linear + shoulder model have been proposed, and predictive modeling based on Weibull model has been reported.

Modeling of food processing has undergone a significant shift from empirical to a physics-based modeling approach (Saguy, 2016). Empirical modeling has long been used to analyze, predict, and optimize food processing. For example, response surface methodology has been used to optimize treatment conditions in food processing as indicated in chpater III. However, empirical modeling provides no insight into the underlying mechanisms and is unrelated to the physics-based model (Saguy, 2016). On the other hand, physics-based modeling including computational simulation enables food engineers to predict precisely temperature distribution and microbial destruction at a specific point in time. Several researchers analyzed the temperature distribution and microbial inactivation of novel thermal technologies such as microwave, radio-frequency, and ohmic heating by physics-based modeling.

Tomato-based foods such as tomato juice, tomato paste, and salsa can be processed effectively by pulsed ohmic heating because electrical conductivity, which is an important factor in determining the ohmic heating rate of foods, of tomato-based products is relatively high as indicated in chapter I and II. Tomato juice has traditionally been regarded as safe because of its acidity, but several outbreaks have been reported in fruit juices (Enache and Chen, 2007). Moreover, foodborne pathogens such as *Escherichia coli* O157:H7, *Salmonella enterica* serovar Typhimurium, and *Listeria monocytogenes* are

known to be adapt to acidic environments (Lee et al., 2015). The heat resistance of these pathogens increased by acid-adaptation, which induces cross-protection against thermal treatments (Álvarez-Ordóñez et al., 2008; Mazzotta, 2001). Even though eliminating these acid-adapted pathogens during juice processing is very important, inactivation of acid-adapted pathogens in tomato juice has not been analyzed by the computer simulation. The objective of the present study was to predict inactivation of acid-adapted foodborne pathogens by pulsed ohmic heating in tomato juice using computer simulation.

IV-2. Materials and Methods

Model parameters. Electrical and thermo-physical properties of tomato juice, electrodes, and surrounding materials are essential in modeling the pulsed ohmic heating system. Processed tomato juice is composed of water (88.7%), protein (0.5%), fat (0.3%), and carbohydrates (10.5%). The following equation was utilized to predict the density (ρ), specific heat (c_p), and thermal conductivity (k) of tomato juice;

$$\rho = \sum_{i=1}^{n} \rho_i X_i \qquad (1)$$

$$c_p = \sum_{i=1}^{n} c_{pi} X_i \qquad (2)$$

$$k = \sum_{i=1}^{n} k_i Y_i \qquad (3)$$

where a food material has n components, ρ_i, c_{pi}, and k_i are the density, specific heat, and thermal conductivity of the i th component, respectively, X_i is the weight fraction.

Y_i is the volume fraction of the i th component, obtained as follows:

$$Y_i = \frac{X_i/\rho_i}{\sum_{i=1}^{n}(\frac{X_i}{\rho_i})} \qquad (4)$$

The electrical conductivity of tomato juice was identified from voltage and current data and calculated as follows:

$$\sigma = \frac{LI}{AV} \qquad (5)$$

where σ is the electrical conductivity (S/m), L is the distance between electrodes (m), I is the current (A), A is the cross-sectional area of the electrodes (m^2), and V is the voltage (V).

The electrode and surrounding materials were made of titanium and acrylic plastic, respectively, the properties of which were imported from embedded COMSOL material library, V4.3. Electrical and thermo-physical properties of tomato juice, titanium electrodes, and the acrylic plastic treatment chamber are listed in Table IV-1.

Notation

Symbol	Parameter	Units	Note
ρ	Density	kg/m^3	
c_p	Specific heat	J/kg·K	
k	Thermal conductivity	W/m·K	
σ	Electrical conductivity	S/m	Experimental results
D_T	Decimal reduction time	min	Experimental results
z-value	Death rate change	^oC	Experimental results
Q	Heat generation	J	
v	Kinematic viscosity	m^2/s	
V_0	Applied voltage	V	100
T_0	Initial temperature	K	294.15
C_0	Initial cell concentration	CFU/ml	10^7

Table IV-1. Electrical and thermo-physical properties of materials used for computer simulation

Material properties	Unit	Tomato juice	Titanium[a]	Acrylic plastic[a]
Density (ρ)	kg/m^3	-0.0003T^2 -0.0337T+1061.8	4,940	1,760
Thermal conductivity (k)	W/(m·K)	-0.000006T^2+0.0017T+0.5428	7.5	0.1
Specific heat (c_p)	J/(kg·K)	0.0042T^2+0.1359T+3882.9	710	1,000
Electrical conductivity (σ)	S/m	0.0176T-4.7043	74,070	0

[a] Imported from COMSOL material library, V4.3.

Cell suspension preparation and inoculation. Three strains each of *E. coli* O157:H7 (ATCC 43890, ATCC 35150, ATCC 43889), *S.* Typhimurium (ATCC 19585, ATCC 43971, DT 104), and *L. monocytogenes* (ATCC 19111, ATCC 19115, ATCC 15313) were obtained from the bacterial culture collection of the School of Food Science, Seoul National University (Seoul, Korea) and prepared as a method described in chapter I-2. A mixed culture cocktail contains approximately equal numbers of cells of each strain of *E. coli* O157:H7 (10^8 CFU/ml), *S.* Typhimurium (10^7 CFU/ml), and *L. monocytogenes* (10^7 CFU/ml). The mixed-strain cocktail (0.2 ml) was inoculated into 50 ml samples of processed tomato juice, purchased from a local grocery store (Seoul, Korea) and stored at room temperature (22 ± 1°C) before treatment. Acid-adapted cultures were grown in TSB adjusted to pH 5 with 1 N HCl and prepared as same with non-acid-adapated pathogens.

D- and z- value calculation. For D-value experiments, acid- or non-acid-adapted pathogens were inoculated into 5 ml of tomato juice in test tubes, equilibrated to 45, 50, 55, or 60°C by immersion in a constant-temperature water bath. Treatment time was adjusted separately for each temperature. The number of surviving pathogens was plotted on a logarithmic scale as a function of time (min). D_T, the time needed to decrease the pathogen population by 90% (1 log) at temperature T (°C), was calculated by plotting surviving

microorganisms against time on semi-log coordinates and by the following equation.

$$\log\left(\frac{N}{N_0}\right) = -\frac{t}{D_T} \qquad (6)$$

where N_0 = initial pathogen population (CFU/ml), N = pathogen population after treatment (CFU/ml), t = time (min).

The z-values (°C) were calculated as the negative inverse slope of the linear regression line for the log D-values over the range of heating temperatures tested.

Bactericidal treatment. The pulsed ohmic heating system described in chapter II-1.2 was used. Each 50 ml tomato juice sample, inoculated with a mixed-culture cocktail, was subjected to 25 V_{rms}/cm pulsed ohmic heating for 0, 30, 50, 60, 65, 70, or 75 s.

Microbial enumeration. For microbial enumeration, each treated 50 ml sample was immediately transferred into a sterile stomacher bag containing 100 ml of sterile 0.2% PW and homogenized for 2 min using a stomacher. For the D-value experiment, 0.1 ml of treated sample was transferred to 9.9 ml sterile 0.2% PW. After homogenization, 1 ml samples were 10-fold serially diluted with 9 ml of sterile 0.2% PW and 0.1 ml of stomached samples or

diluents were spread plated onto each selective medium. SMAC agar, XLD agar, and OAB with antimicrobial supplement 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 to 48 h before counting colonies characteristic of the pathogens. Experiments for pathogen inactivation were replicated three times.

Equations for modeling. Following equations for the electric field, heat transfer, incompressible laminar-flow, and microbial destruction were used.

Governing equation for the electric field. The Laplace equation defining the electric field distribution in an pulsed ohmic heating was given by De Alwis and Fryer (1990):

$$\nabla \cdot (\sigma(T)\nabla V) = 0 \qquad (7)$$

where $\sigma(T)$ is the electrical conductivity (S/m) at temperature T (K), and V is the applied voltage (V).

Boundary and initial conditions for ohmic conductor were given by:

Electrode with a ground: $V = 0$

Electrode with an electric potential: $V = V_0$

Electrical insulation at the walls: $n \cdot (\sigma(T)\nabla V) = 0$

where n is the unit vector perpendicular to the boundary.

Governing equation for heat transfer. The governing equation including heat generation and transfer is defined as follows (Geedipalli et al., 2008):

$$\rho c_p \frac{\partial T}{\partial t} = \nabla \cdot (k \nabla T) + Q - \rho c_p u \nabla T \qquad (8)$$

where *t* is the time (s), *T* is the temperature (K), u is the velocity (m/s), ρ is the density (kg/m^3), *k* is the thermal conductivity (W/m·K), c_p is the specific heat (J/kg·K) at constant pressure and Q is the heat source (J) generated by pulsed ohmic heating. The heat sources produced by pulsed ohmic heating can be calculated by the following equation.

$$Q = \sigma(T) \cdot |\nabla V|^2 \qquad (9)$$

The boundary condition for convective cooling at the wall of the chamber was given by:

$$-n \cdot (-k \nabla T) = h \cdot (T_{ext} - T) \qquad (10)$$

where *h* is the dimensionless form of the convective heat transfer coefficient, and T_{ext} is the external environment temperature.

Governing equation for incompressible laminar flow. If the flow condition is in an incompressible state, the density of fluid can be assumed to be constant with respect to time and space. The governing equations expressed using Cartesian coordinates can be described by the following laminar flow model.

$$\rho\frac{\partial u}{\partial t}+\rho(u\cdot\nabla)u=\nabla\cdot[-p\mathrm{I}+\nu(\nabla u+(\nabla u)^{\mathrm{T}})]+F \quad (11)$$

$$\rho\nabla\cdot u=0 \quad (12)$$

where t is the time (s), u is the velocity (m/s), p is the pressure (Pa), ν is the kinematic viscosity (m^2/s), I is the identity tensor, F is the volumetric force incurred by the buoyancy-driven force, and superscript T is the transpose of a matrix. Initial conditions of velocity and pressure were set at zero ($u=0,\ p=0$).

Equations for microbial destruction. In this study, the following first-order reaction equation was assumed to be an appropriate model to predict the inactivation of foodborne pathogens based on experimental D-value results:

$$r=k_T c \quad (13)$$

The relationship between the reaction rate constant and the decimal reduction time is defined as follows (Sun, 2007):

$$k_T=\frac{2.303}{D_T} \quad (14)$$

where r is the reaction rate, c is the concentration, and k_T is the reaction rate constant.

Computer simulation setup. Pulsed ohmic heating chamber described in chapter II-1.2 was analyzed using multiphysics software (V4.3, COMSOL Inc., Palo Alto, CA) based on the finite element method (FEM). AutoCAD 2016 (Autodesk, Inc., San Rafael, Cal.), used to create the 3D geometry of the ohmic heating chamber, was imported into COMSOL. The partial differential equations (PDEs) were solved, and a parallel direct sparse solver (PARDISO) was used to reduce the computational time. The mesh sizes were controlled resulting in a total number of 54,034 tetrahedral mesh elements. The software was run using a personal computer (Intel Core i7-4790 CPU @ 4.00GHz (2 Processors), 32 GB RAM on a Windows 7 64-bit operating system). Simulated results were compared with experimental results at points 1, 2, and 3 (Fig. IV-1) for validation.

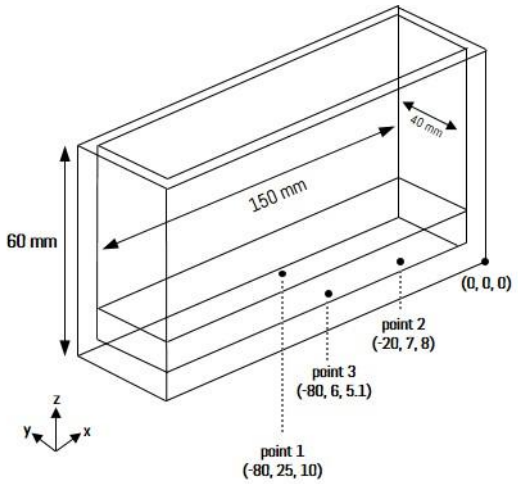


Fig. IV-1. Dimension (mm) and position of point 1, 2 and 3 of pulsed ohmic heating chamber.

Statistical analysis. Simulated and experimental populations of pathogens were analyzed by the t-test of the Statistical Analysis System. Significant differences between experimental and simulated results were determined at a significance level of $p = 0.05$.

IV-3. Results and discussion

The thermal resistance of non-acid-adapted foodborne pathogens. The thermal resistance of foodborne pathogens at constant temperature in tomato juice differed for each pathogen (Table IV-2). For every isothermal temperature investigated in the present study, D-values of *E. coli* O157:H7 were higher than those of *S.* Typhimurium or *L. monocytogenes*, which indicates that *E. coli* O157:H7 had the highest resistance to pulsed ohmic heating under the treatment conditions in the present study. D-values of *S.* Typhimurium were higher than those of *L. monocytogenes* for 45-55^{o}C, but z-value of *S.* Typhimurium was lower than that of *L. monocytogenes*. Thus, it is supposed that D-values of *S.* Typhimurium under higher temperature conditions (> 60^{o}C) would be lower than those of *L. monocytogenes*. Similar results were reported by Mazzotta (2001), who investigated heat resistance of *E. coli* O157:H7, *Salmonella*, and *L. monocytogenes* in fruit juices, and identified that *Salmonella* had lower heat resistance than *E. coli* O157:H7. On the other hand, Murphy et al. (2004) reported that D-values of *E. coli* O157:H7 were lower than those of *Salmonella* or *L. monocytogenes* in ground pork, and surmised that heat resistance of pathogens would differ depending on the type of sample, pH, nutritional components, and other environmental factors. In this regard, the heat resistance of pathogens should be identified in the same

conditions for those of computational simulation. Utilizing heat resistance data from experiments in a buffer to the food processing simulation would result in the significant difference because the resistances in the buffer and in food matrix are considerably different due to acidity, nutritional content, and so on.

Table IV-2. D-values (min) and z-values (ºC) of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* in tomato juice

Bacterium	D-value (min)				z-value
	45	50	55	60	(ºC)
E[a]	14.90 ± 2.58	8.60 ± 0.83	1.55 ± 0.75	0.26 ± 0.16	8.13 ± 1.21
S	12.87 ± 1.47	4.09 ± 0.87	1.02 ± 0.19	0.14 ± 0.07	7.68 ± 0.91
L	11.56 ± 1.54	1.78 ± 0.61	0.60 ± 0.11	0.15 ± 0.06	8.13 ± 0.50

Mean values ± standard deviation

[a] E: *E. coli* O157:H7, S: *S.* Typhimurium, L: *L. monocytogenes*

Computational analysis of pulsed ohmic heating. A computational simulation in the present study was conducted by simultaneously combining physical-based numerical approaches using COMSOL multiphysics with experimental models for D-values and electrical conductivity. When velocity distributions, temperature profiles, and pathogen concentrations in tomato juice subjected to 45 s ohmic heating were analyzed by the combined computational simulation (Fig. IV-2), upward-moving streamlines were observed at the center of the ohmic heating chamber (Fig. IV-2A). Because heat losses by natural convection to the outside occurred at the bottom and the side of the heating chamber, the temperature at the center of the chamber is relatively higher than that at side or bottom. Convection causes relatively hot fluids at the center to rise, and this phenomenon contributes to temperature distribution of ohmic heating as shown in Fig. IV-2B. Most of the upper part of tomato juice samples showed a red color indicating a temperature of around 50^{o}C while the lower part of tomato juice displayed a yellow color corresponding to temperatures around 40^{o}C after 45 s treatment. Marra et al. (2009) also reported that more cold areas were observed at the sample surface in a closed cylindrical ohmic heating cell, and Varghese et al. (2014) indicated that these colder external shells were critical areas to be monitored. Fryer et al. (1993) indicated that for low-viscosity systems, where fluid viscosity is comparable to that of water, it is possible to assume that the liquid temperature

is uniform. Considering tomato juice was much more viscous than water in the present study and the processing time was much shorter than that in Fryer et al's study, it is suggested that convection inside of the chamber in the present study was not enough to ensure heating uniformity of tomato juice. Because the cold point was observed in the lower part of the ohmic heating chamber, it is predicted that some pathogens still can survive in these areas of the chamber after 45 s treatment (Fig. IV-2C), which conditions all pathogens were inactivated elsewhere.

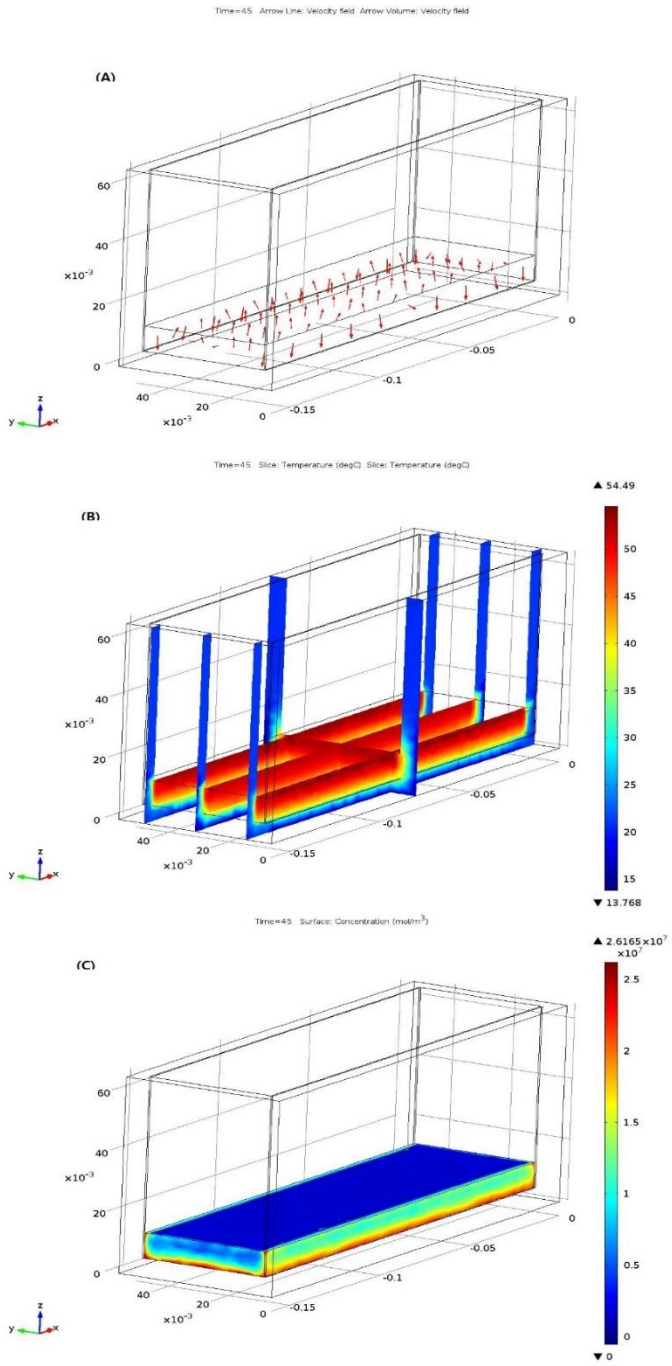


Fig. IV-2. Simulated velocity distribution (A), temperature distribution (B), and concentration of *E. coli* O157:H7 (C) following 45 s pulsed ohmic heating treatment.

Verification of computer simulation. After executing computational simulation, it is needed to verify that model values accurately predict observed values. Heating rate and inactivation of pathogens by computational simulation were compared with experimental results for verification. Even though heating rate trend of computational simulation had more linear shape than experimental results, the heating rates of simulation at points 1, 2, and 3 were very similar to experimental results within an error range of 4.5^{o}C (Fig. IV-3). In the cases of pathogen inactivation trends, experimental results were much closer to simulated results at point 2 than points 1 or 3 (Fig. IV-4). These results indicate that inactivation of pathogens at point 2 followed an overall inactivation pattern by ohmic heating rather than at points 1 or 3, in which foodborne pathogens would be extinguished. Because heat resistances were differed for the type of pathogen, the times needed to inactivate pathogens by ohmic heating were different for each pathogen. When inactivation trend was simulated at point 2, 76.3, 70.9, and 68.3 s would be needed to inactivate *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes*, respectively, to below the detection limit. These simulated treatment intervals were very similar to experimental results, which indicated that 75, 70, and 70 s were needed to inactivate *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes*, respectively, to below the detection limit. From the results above, it is verified that developed simulation model can predict the temperature increase and

microbial inactivation without significant difference with experimental results. The results are consistent with previous research investigations reporting that physics-based modeling can predict the temperature distribution and microbial inactivation precisely.

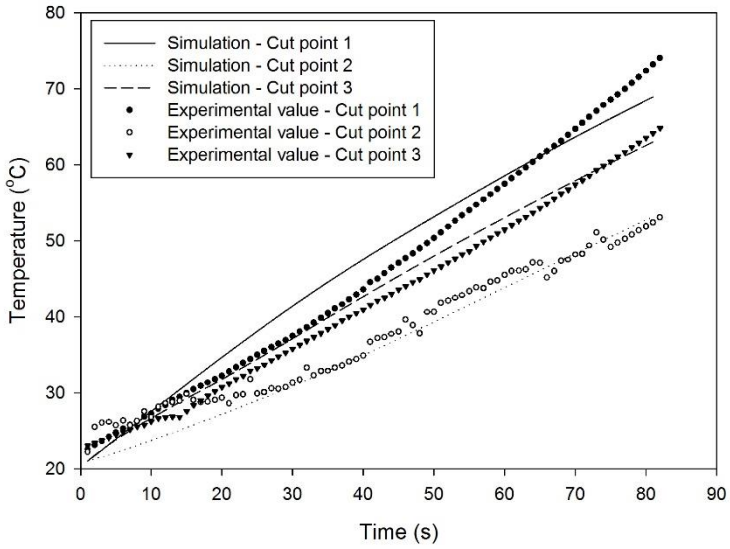


Fig. IV-3. Simulated (line plot) and experimental (scatter plot) temperatures of tomato juice at points 1, 2, and 3.

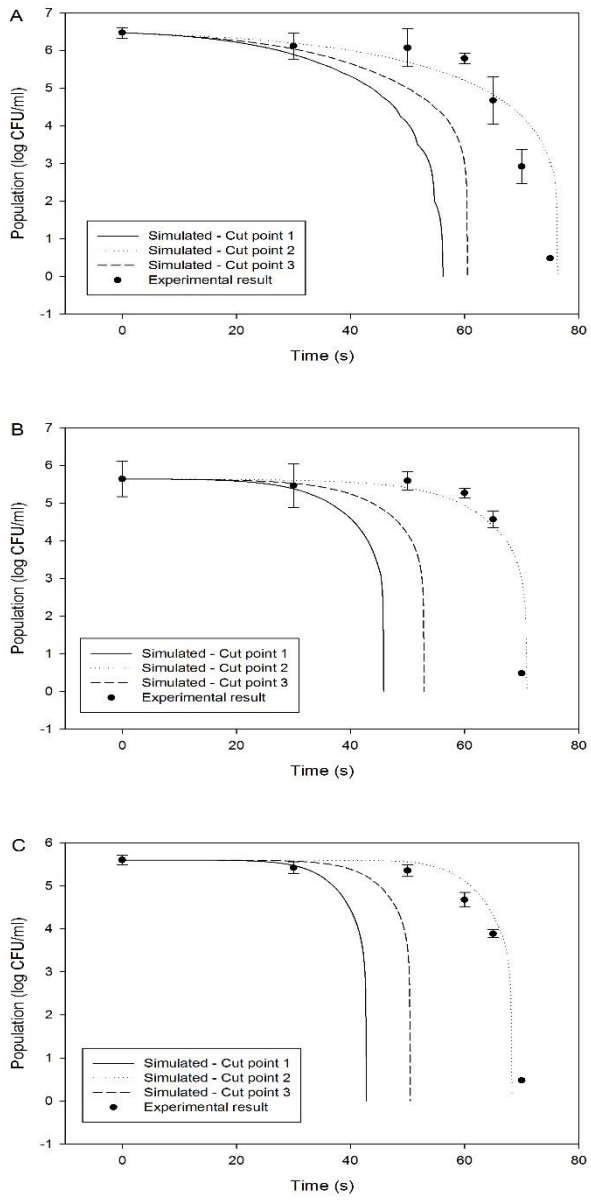


Fig. IV-4. Simulated (line plot) and experimental (scatter plot with standard deviation) inactivation curves of *E. coli* O157:H7 (A), *S.* Typhimurium (B), and *L. monocytogenes* (C).

Prediction and verification of acid-adapted pathogens. Heat resistance increased for acid-adapted pathogens compared to non-acid-adapted pathogens (Table IV-2 and IV-3). D-values increased considerably at 55^{o}C and 60^{o}C for acid-adapted *E. coli* O157:H7, whereas for acid-adapted *S.* Typhimurium and *L. monocytogenes*, D-values significantly increased at 45^{o}C and 50^{o}C compared to non-acid-adapted cells. In this regard, the z-value of acid-adapted *E. coli* O157:H7 was higher than that of non-acid-adapted cells. Conversely, z-values of acid-adapted *S.* Typhimurium and *L. monocytogenes* were lower than those of non-acid-adapted *S.* Typhimurium and *L. monocytogenes*, respectively. Mazzotta (2001) also reported that D-values of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* increased after acid-adaptation in apple, orange, and white grape juices, and z-values increased or decreased depending on the type of pathogen and juice. Altered D-values by acid-adaptation were adopted to computational simulation to predict inactivation trend of acid-adapted pathogens by pulsed ohmic heating, and point 2 was used to predict the inactivation trend considering the results of verification experiment. When simulated results were compared with experimental results, experimental log reductions were higher than computationally predicted values, which is consistent with previous studies (Liu et al., 2018; Xu et al., 2018). Xu et al. (2018) reported that experimental log reductions by radio-frequency pasteurization of *Enterococcus faecium*

were higher than those of predicted model, and pointed out that an unavoidable delay may have occurred removing treated samples from the processing container. It is postulated that not only the delay but also the mixing effect during the sampling stage would contribute to increased experimental log reductions. Despite these unavoidable experimental limitations, the differences between simulated and experimental data were not significant under any treatment conditions ($p > 0.05$) when analyzed by the Satterthwaite t-test considering inequality of variance (Table IV-4). The results indicate that computational simulation can reflect the increased heat resistance by acid-adaptation effectively. Moreover, it is suggested that the computational model can be utilized to predict the inactivation trend of pathogens which heat resistance increased abnormally due to the climate change and cross-protection.

Table IV-3. Altered D-values (min) and z-values (ºC) of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* by acid-adaptation in tomato juice

Bacterium	D-value (min)				z-value (ºC)
	45	50	55	60	
E[a]	18.89 ± 5.33	10.18 ± 2.78	4.73 ± 2.49	0.81 ± 0.31	11.34 ± 2.17
S	23.04 ± 7.28	6.52 ± 2.01	1.04 ± 0.21	0.18 ± 0.07	7.01 ± 0.52
L	21.50 ± 4.75	4.02 ± 1.61	0.79 ± 0.32	0.22 ± 0.07	7.42 ± 0.20

Mean values ± standard deviation

[a] E: *E. coli* O157:H7, S: *S.* Typhimurium, L: *L. monocytogenes*

Table IV-4. Simulated and experimental inactivation of acid-adapted *E. coli* O157:H7 (E), S. Typhimurium (S), and *L. monocytogenes* (L) subjected to pulsed ohmic heating

Treatment time	E		S		L	
	Sim[a,b]	Exp	Sim	Exp	Sim	Exp
0	5.85^{A}	5.85 ± 0.53^{A}	5.46^{A}	5.46 ± 0.19^{A}	6.04^{A}	6.04 ± 0.12^{A}
30	5.82^{A}	5.71 ± 0.67^{A}	5.46^{A}	5.55 ± 0.14^{A}	6.04^{A}	5.98 ± 0.05^{A}
50	5.69^{A}	5.65 ± 0.88^{A}	5.43^{A}	5.68 ± 0.13^{A}	5.98^{A}	5.90 ± 0.16^{A}
60	5.43^{A}	4.54 ± 0.87^{A}	5.26^{A}	4.95 ± 0.17^{A}	5.72^{A}	5.64 ± 0.09^{A}
65	5.17^{A}	4.68 ± 0.61^{A}	4.94^{A}	3.98 ± 0.46^{A}	5.30^{A}	5.00 ± 0.30^{A}
70	4.73^{A}	3.55 ± 0.81^{A}	4.26^{A}	2.97 ± 0.88^{A}	4.39^{A}	3.46 ± 1.04^{A}
75	3.90^{A}	1.70 ± 1.08^{A}	$<0.48^{A}$	$<0.48^{A}$	$<0.48^{A}$	0.91 ± 0.75^{A}

[a] Sim: Simulated results, Exp: Experimental results

[b] Values in the same column that are followed by the same letter are not significantly different ($p > 0.05$).

In conclusion, inactivation of foodborne pathogens by pulsed ohmic heating in tomato juice was analyzed using computational simulation in the present study. Pulsed ohmic heating was analyzed using electrical and thermo-physical properties of materials and the thermal resistance values of foodborne pathogens. When 45 s ohmic heated tomato juice was represented, upward-moving streamlines were observed at the center of the chamber which contributed to temperature distribution of pulsed ohmic heating. Because simulated temperature at the lower part of the ohmic heating chamber was lower than that of the higher part, it was predicted that some pathogens can survive at the bottom of the treatment chamber. Simulated heating rates were very similar to experimental values within an error range of 4.5^{o}C, and experimental inactivation rates were similar to simulated values at point 2. Even though experimental acid-adapted pathogen reductions were higher than those of simulated, significant differences were not observed between experimental and simulated results ($p > 0.05$). Therefore, it was demonstrated that a computational simulation model can be utilized effectively to predict inactivation trends of acid-adapted foodborne pathogens, and this model could be helpful for juice processors desiring 5-log reductions of target organisms because processing conditions should be adjusted for the environmental conditions affecting heat resistance of pathogens.

V. Development and application of continuous-type pulsed ohmic heating system

V-1. Introduction

Pulsed ohmic heating is a novel thermal technology facilitating rapid and uniform heating by means of electric current flowing through the food as indicated in the previous chapters. It was possible to inactivate foodborne pathogens effectively without causing electrode corrosion in salsa, tomato juice, and milk using batch-type pulsed ohmic heating as indicated in chapter I, II, and III. Even though the batch-type pulsed ohmic heating apparatus was used effectively to identify the characteristics of pulsed ohmic heating in the previous chapters, it is well known that continuous-type apparatus ohmic heating is more advantageous for bulk handling of juice products in the food industry. Several studies characterizing inactivation efficacy of continuous-type ohmic heating on *E. coli* O157:H7, *S.* Typhimurium, *L. monocytogenes* (Lee et al., 2012), and spores of *Bacillus cereus*, *Alicyclobacillus acidoterrestris* (Kim, Ryang, et al., 2017; Ryang et al., 2016) have been reported. However, systematic research including the effect of flow rate, treatment voltage, and initial temperature on the inactivation efficacy of continuous-type pulsed ohmic heating has been limited.

In the present study, an efficacy of continuous-type pulsed ohmic heating for inactivation of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes*

was investigated. First, the heating rate and pathogen inactivation were identified at various flow rates, voltage, and initial temperature. Secondly, treatment condition achieving 5 log reductions for all three pathogens in tomato juice were identified and quality aspect changes under that condition were observed by analyzing color and lycopene content. Finally, a sequential three-cylinder type pulsed ohmic heating apparatus was developed to reduce the preheating step.

V-2. Materials and Methods

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. Stock and working cultures were prepared according to the method previously described in chapter I-2. A mixed culture cocktail containing approximately equal numbers of cells of each strain of *E. coli* O157:H7 (10^9 CFU/ml), *S.* Typhimurium (10^8 CFU/ml), and *L. monocytogenes* (10^9 CFU/ml).

Sample preparation and inoculation. Sterile BPW and pasteurized tomato juice, stored at room temperature were used in this experiment. A mixed culture cocktail (4 ml) was inoculated into each 500 ml sample before treatment. The final bacterial populations were 10^6-10^7 CFU/g for *E. coli* O157:H7 and *L. monocytogenes* and 10^5-10^6 CFU/g for *S.* Typhimurium.

Continuous-type pulsed ohmic heating apparatus. Pulsed ohmic heating treatments were carried out with the apparatus previously described in chapter II-1.2 with a modification . The pulsed ohmic heating system (Fig. V-1) consisted of a function generator, a precision power amplifier, a two-channel digital-storage oscilloscope, a data logger, sample tank, diaphragm pump (KNF Neuberger, Inc., New Jersey, USA), product tank, and continuous-type pulsed ohmic heating chamber. Two rounded titanium electrodes of 4 cm diameter were adjoined at each edge of the treatment chamber. The distance between the two electrodes was 14 cm. Sample temperature was measured at the treatment chamber outlet with a K-type thermocouple.

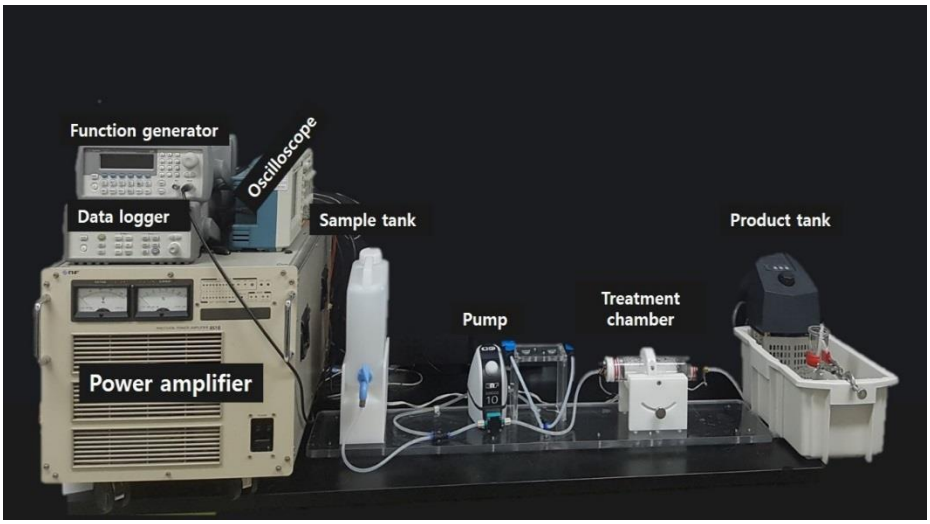


Fig. V-1. Continuous-type pulsed ohmic heating system at Seoul National University.

Bactericidal treatment. Inoculated BPW samples were treated with continuous-type pulsed ohmic heating with differing flow rate, treatment voltage, and initial sample temperature. For the flow rate experiment, prepared BPW was subjected to 9.43 V_{rms}/cm pulsed ohmic heating at varying flow rates (0.2, 0.3, and 0.4 LPM). For the voltage experiment, prepared BPW was subjected to 9.43, 10.93, and 12.14 V_{rms}/cm pulsed ohmic heating at 0.4 LPM. For the initial sample temperature experiment, temperatures of BPW were adjusted to 25, 30, 35, and 40^{o}C by a constant-temperature water bath, and subsequently subjected to 10.93 V_{rms}/cm pulsed ohmic heating at 0.4 LPM. For food application, initial temperatures of tomato juice were adjusted to 40, 45, and 50^{o}C with a water bath, and subsequently subjected to 12.14 V_{rms}/cm pulsed ohmic heating at 0.2 LPM. Samples were taken after treatment volume of 150, 250, and 350 ml, and populations of surviving microorganisms were enumerated.

Bacterial enumeration. For microbial enumeration, each treated 1 ml sample was immediately transferred into 9 ml of sterile 0.2% PW and 10-fold serially diluted. Diluted samples were spread-plated onto each selective medium. SMAC agar, XLD agar, and OAB with antimicrobial supplement 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 to 48 h before counting colonies characteristic of the pathogens.

Color and lycopene content measurement. The color and lycopene content of untreated and treated tomato juice were evaluated. Tomato juice preheated to 50°C was subjected to 12.14 V_{rms}/cm pulsed ohmic heating at 0.2 LPM and measured after a treatment volume of 150 ml. Color values were measured with a Minolta colorimeter. L^*, a^*, and b^* values were measured to evaluate color changes of tomato juice. The color difference ($\triangle E$) is calculated by the following equation.

$$\triangle E = \sqrt{(L^*{}_t - L^*{}_c)^2 + (a^*{}_t - a^*{}_c)^2 + (b^*{}_t - b^*{}_c)^2}$$

where $L^*{}_t$, $a^*{}_t$, $b^*{}_t$ are the colorimetric values of treated tomato juice and $L^*{}_c$, $a^*{}_c$, $b^*{}_c$ are the colorimetric values of untreated tomato juice.

Lycopene content in tomato juice was measured according to the method previously described in chapter I-2. The concentrations of lycopene in tomato juice were determined using absorbance and sample weight.

Statistical analysis. All experiments were replicated three times. Data were analyzed by the analysis of variance procedure of the Statistical Analysis System and mean values were separated using Duncan's multiple-range test. Significant differences were determined at a significance level of $p = 0.05$.

V-3. Results and discussion

Effect of flow rate and treatment voltage on heating rate and pathogen inactivation. The heating rate of BPW was inversely proportional to flow rate (Fig. V-2A) in the present study. At a fixed voltage of 9.43 V_{rms}/cm, tomato juice samples were heated to 80^{o}C as 100, 200, and 275 ml flow volumes were subjected to flow rates of 0.2, 0.3, and 0.4 LPM, respectively. Because BPW was subjected to pulsed ohmic heating for a longer time interval at a lower flow rate, temperatures increased more rapidly at the lower flow rate. Temperatures of BPW also increased more rapidly corresponding to increased treatment voltage (Fig. V-2B). At a fixed flow rate of 0.4 LPM, BPW reached 80^{o}C after 120, 170, and 275 ml flow volumes were treated with treatment voltages of 12.14, 10.93, and 9.43 V_{rms}/cm, respectively. Because heat generation (Q) by ohmic heating is proportional to electrical conductivity (k) and the square of electric field strength (E), temperatures at each flow volume increased more rapidly with higher voltage, which generates a higher electric field strength. Reductions of all three pathogens increased as flow rate decreased from 0.4 to 0.2 LPM because the thermal effect is the major principle of microorganism inactivation by ohmic heating (Fig. V-3). For example, surviving populations of *E. coli* O157:H7 were 5.97, 4.61, and 2.24

log CFU/ml for 0.4, 0.3, and 0.2 LPM flow rates, respectively, at a flow volume of 150 ml (Fig. V-3A). It is noteworthy that populations of all three pathogens were greater than 3.5 log CFU/ml at a flow rate of 0.4 LPM when collected at 350 ml flow volume. Therefore, the ohmic heating voltage was increased from 9.43 to 10.93 and 12.14 V_{rms}/cm to inactivate pathogens at 0.4 LPM. Reductions of all three pathogens increased proportionally to increased treatment voltage (Fig. V-4). For example, populations of *E. coli* O157:H7 were 5.97, 2.87, and 2.47 log CFU/ml for 9.43, 10.93, and 12.14 V_{rms}/cm voltages, respectively, at 150 ml flow volume (Fig. V-4A). Lee et al. (2012) also reported that electric field strength is an important factor for inactivation of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* in orange and tomato juice by continuous pulsed ohmic heating. Even though Baysal and İçier (2010) reported that an additional non-thermal effect associated with increased voltage can affect the inactivation of *Alicyclobacillus acidoterrestris* spores in orange juice by ohmic heating at 70°C, the voltage differential in the present study (2.71 V_{rms}/cm) was too small to expect this additional non-thermal effect. From the results in the present study, it is concluded that flow rate and voltage in pulsed ohmic heating are fundamental factors affecting the heating rate and inactivation of pathogens.

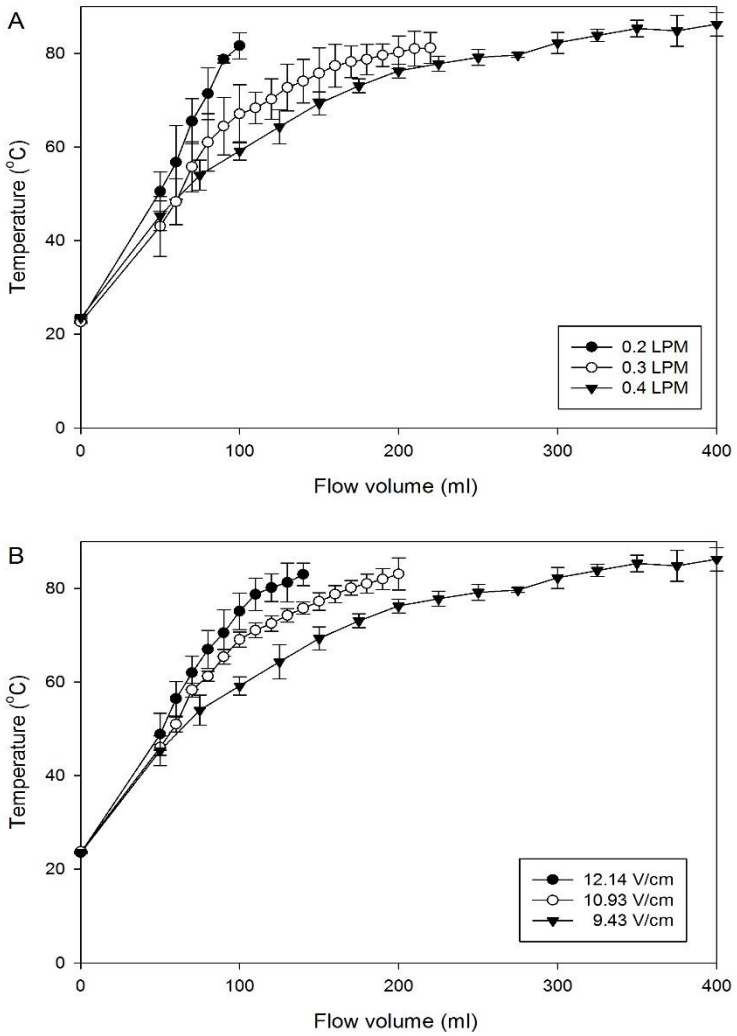


Fig. V-2. Temperature histories of pulsed ohmic treated buffered peptone water at fixed voltage (9.43 V_{rms}/cm) and varying flow rate (A) and fixed flow rate (0.4 LPM) and varying voltage (B).

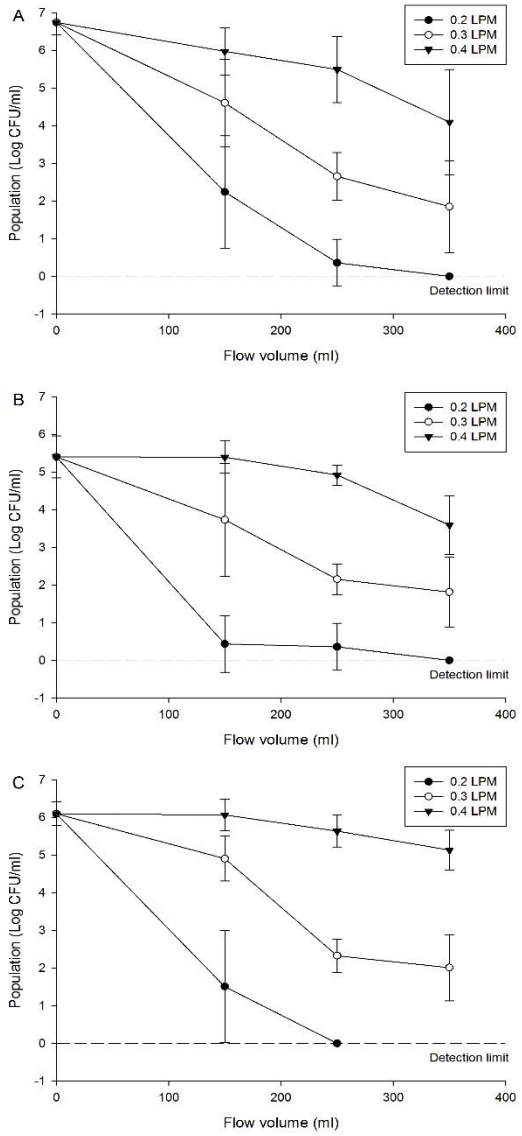


Fig. V-3. Populations of *E. coli* O157:H7 (A), *S.* Typhimurium (B), and *L. monocytogenes* (C) in buffered peptone water subjected to pulsed ohmic heating at varying flow rate and treatment volume. The voltage of pulsed ohmic heating was fixed at 9.43 V_{rms}/cm.

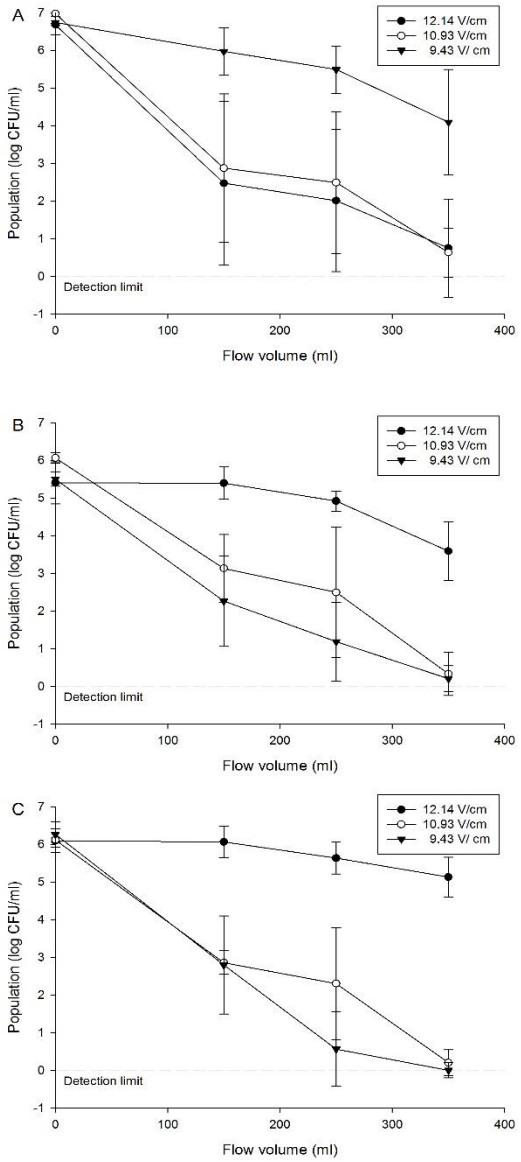


Fig. V-4. Populations of *E. coli* O157:H7 (A), *S.* Typhimurium (B), and *L. monocytogenes* (C) in buffered peptone water subjected to pulsed ohmic heating with varying voltage and treatment volume. Sample flow rate was fixed at 0.4 LPM.

Effect of preheating on the pathogen inactivation. In the present study, increasing voltage was an effective way to inactivate pathogens when using a high flow rate (0.4 LPM), but some pathogens can survive at the early treatment stage (150 ml treatment volume). Although less than 0.8 log CFU/ml survived with 12.14 V_{rms}/cm pulsed ohmic heating treatment at a 350 ml flow volume, surviving populations of all three pathogens were > 2 log CFU/ml with both 10.93 and 12.14 V_{rms}/cm treatments at 150 ml flow volume (Fig. V-4). Because treated BPW samples were collected in the product tank after 150 ml flow volume, surviving pathogens at this early stage represent a severe microbiological hazard. Increasing more voltage can solve this problem, but the heating rate would be excessively accelerated by the increased voltage which can cause quality degradation in foods. Therefore, BPW was preheated to inactivate pathogens at the early sampling stage (150 ml flow volume) with 10.93 V_{rms}/cm treatment. Reductions of all three pathogens increased relative to the increased initial temperature of BPW (Fig. V-5). For example, surviving populations of *S.* Typhimurium were 3.08, 1.93, 0.34, or 0.30 log CFU/ml for BPW subjected to pulsed ohmic heating after preheating to 25, 30, 35, or 40°C, respectively. It is noteworthy that populations of all three pathogens were less than 0.6 log CFU/ml (> 5.0 log reduction) when BPW was preheated to over than 35°C and then subjected to 10.93 V_{rms}/cm with 0.4 LPM flow rate, the treatment condition in which >2 log CFU/ml of all three pathogens survived

without preheating. From these results, it is identified that preheating before pulsed ohmic heating can be used not only to increase the electrical conductivity of the sample (Varghese et al., 2014) but also to ensure safety in the early treatment stage while minimizing quality degradation of food.

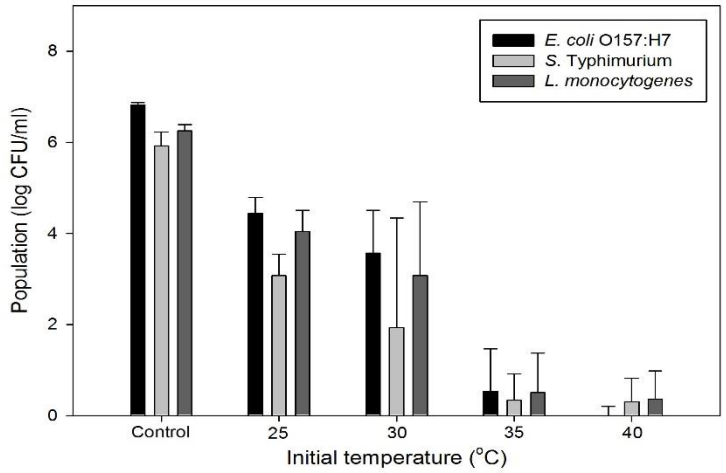


Fig. V-5. Populations of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* in buffered peptone water subjected to pulsed ohmic heating with varying initial temperature and treatment volume of 150 ml. Sample flow rate and the pulsed ohmic heating voltage were fixed at 0.4 LPM and 10.93 V_{rms}/cm, respectively.

Application in tomato juice processing. From BPW experiment results, it was identified that flow rate, treatment voltage, and initial sample temperature are important factors in continuous-type pulsed ohmic heating. Subsequently, continuous-type pulsed ohmic heating system was applied to inactivate foodborne pathogens in tomato juice. Similar to BPW, reduction of all three pathogens increased relative to the increased initial temperature of tomato juice (Fig. V-6). Because processors should treat juice to achieve more than 5 log reduction of the number of microorganisms (U. S. FDA., 2001), treatment conditions needed to ensure 5 log reduction were identified. More than 5 log reductions of all three pathogens were achieved by applying 12.14 V_{rms}/cm pulsed ohmic heating to 0.2 LPM tomato juice when preheated to 50^{o}C in the present study. However, treatment conditions of pulsed ohmic heating should be adjusted considering the electrical conductivity of juice. For instance, Lee et al. (2012) and Sagong et al. (2011) reported that the time required to achieve a minimum 5 log reduction is shorter for tomato juice than for orange juice due to higher electrical conductivity. Becuase color is considered as one of the major quality attributes of foods influencing the consumer's choice (Min and Zhang, 2003) and lycopene is the pigment responsible for the color of tomato juice and has a natural antioxidant property (Shi and Maguer, 2000), color and lycopene content were selected as representative quality parameters in the present study. Color values a^* and b^*

significantly decreased ($p < 0.05$) after pulsed ohmic heating treatment while L^* values and lycopene content were not significantly different from those of untreated tomato juice (Table V-1). The color difference ($\triangle E$) of pulsed ohmic heated samples was 2.05 ± 0.98. Even though a^* and b^* values decreased significantly with pulsed ohmic heating ($p < 0.05$), it is well known that quality degradation of juice is less with ohmic heating compared to conventional heating (Leizerson and Shimoni, 2005a, b). Lee et al. (2012) also reported that color values of orange juice changed after continuous ohmic heating, but were nevertheless much closer to those of untreated samples than were their conventionally heated counterparts. Therefore, it is postulated that ohmic treated tomato juice quality would be better than that of conventionally treated juice even though further study is needed.

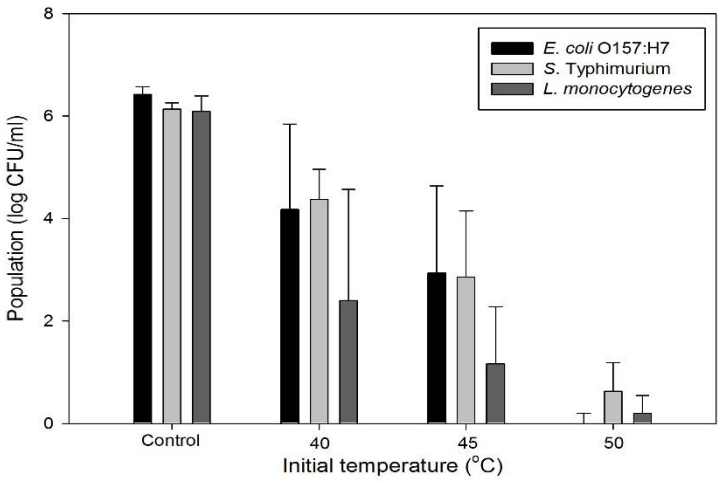


Fig. V-6. Populations of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* in tomato juice subjected to pulsed ohmic heating with varying initial temperature and treatment volume of 150 ml. Sample flow rate and pulsed ohmic heating voltage was fixed at 0.2 LPM and 12.14 V_{rms}/cm, respectively.

Table V-1. Quality aspects of tomato juice (50ºC initial temperature) untreated or treated with 12.14 V_{rms}/cm pulsed ohmic heating[a]

Treatment	Color				Lycopene content (mg/ kg tissue)
	L^*	a^*	b^*	$\triangle E$	
Untreated	27.69 ± 0.39A	4.87 ± 0.04A	9.40 ± 0.12A	-	17.02 ± 3.32A
Pulsed Ohmic	28.19 ± 1.09A	4.20 ± 0.16B	7.67 ± 1.01B	2.05 ± 0.98	17.37 ± 0.78A

Mean values ± standard deviation

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

Development and application of three sequential cylinder type treatment chamber. Based on the inactivation rates of pathogens in BPW and tomato juice, it is identified that preheating is a crucial step for inactivating foodborne pathogens in the initial sampling step to prevent accelerated overheating. Roux et al. (2016) also included a preheating section with a plate heat exchanger (hot water) in a continuous ohmic heating pilot plant to sterilize liquid products. However, preheating of food samples with additional equipment such as a water bath or plate heat exchanger is inconvenient and space consuming. Combining pressure with ohmic heating is an effective way to reduce the preheating step (Park et al., 2014), but it is difficult to combine pressure with a continuous ohmic heating process. Therefore, sequential three-cylinder type pulsed ohmic heating was developed with a downsized treatment chamber (Fig. V-7). The distance between electrodes in sequential three-cylinder type pulsed ohmic heating was 5 cm and other conditions were the same. In sequential pulsed ohmic heating, the total electrical resistance decreased to one third of original resistance because three treatment chamber was paralleled connected, and total electrical current increased threefold. Therefore, not only the first and/or second chamber itself can function as a preheating chamber but also temperature increased more rapidly by the increased electrical current. When the developed system was applied for inactivating pathogens in BPW under

the same conditions without preheating, reductions of all three pathogens were > 5 log (data not shown). Increasing electric field strength by utilizing sequential electrodes compared to the conventional two electrodes may additionally enhance inactivation efficiency of ohmic heating as pointed out by Ryang et al. (2016). Based on the results in the present study, it is suggested that sequential type pulsed ohmic heating can reduce the preheating step needed for inactivation of foodborne pathogens.

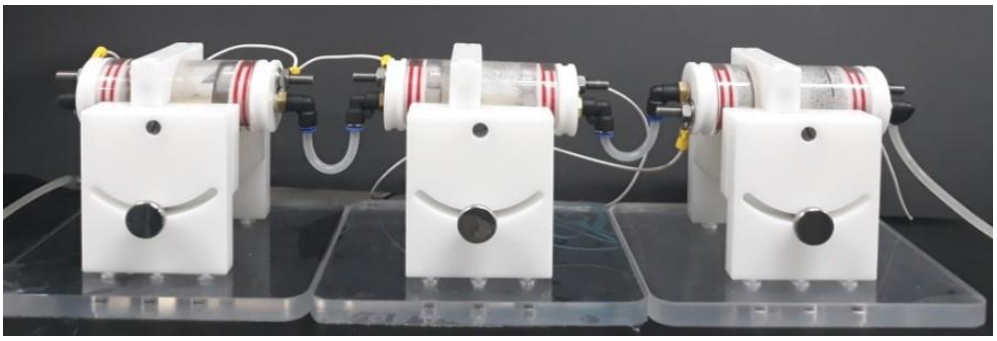


Fig. V-7. Sequential three-cylinder type pulsed ohmic heating chamber.

In conclusion, continuous-type pulsed ohmic heating was applied to inactivate foodborne pathogens in BPW and tomato juice in the present study. The flow rate and treatment voltage were fundamental factors affecting the heating rate and inactivation of foodborne pathogens in BPW, and preheating of samples was suggested as a way to control pathogens at the early treatment stage to prevent overheating. For application in tomato juice processing, optimization of flow rate, voltage, and initial sample temperature was found to be necessary for achieving more than 5 log reductions of all three pathogens. Quality aspects of color and lycopene content were measured under these conditions indicating that a^* and b^* values decreased compared to those of untreated tomato juice. Additionally, a sequential three-cylinder type chamber was developed which achieved more than 5 log reductions without preheating. Therefore, continuous-type pulsed ohmic heating treatment including a sequential chamber could be utilized effectively to control foodborne pathogens by the juice industry as an alternative to conventional heating. Further investigation is needed to identify optimum conditions for controlling pathogens without incurring quality degradation of juice products.

References

Ait-Ouazzou, A., Cherrat, L., Espina, L., Lorán, S., Rota, C., Pagán, R., 2011. The antimicrobial activity of hydrophobic essential oil constituents acting alone or in combined processes of food preservation. Innovative Food Science & Emerging Technologies 12, 320-329.

Álvarez-Ordóñez, A., Fernández, A., López, M., Arenas, R., Bernardo, A., 2008. Modifications in membrane fatty acid composition of *Salmonella* Typhimurium in response to growth conditions and their effect on heat resistance. International Journal of Food Microbiology 123, 212-219.

Alwi, N.A., Ali, A., 2014. Reduction of *Escherichia coli* O157, *Listeria monocytogenes* and *Salmonella enterica* sv. Typhimurium populations on fresh-cut bell pepper using gaseous ozone. Food Control 46, 304-311.

Ban, G.-H., Kang, D.-H., Yoon, H., 2015. Transcriptional response of selected genes of *Salmonella enterica* serovar Typhimurium biofilm cells during inactivation by superheated steam. International Journal of Food Microbiology 192, 117-123.

Baysal, A.H., İçier, F., 2010. Inactivation kinetics of *Alicyclobacillus acidoterrestris* spores in orange juice by ohmic heating: effects of voltage gradient and temperature on inactivation. Journal of Food Protection 73, 299-304.

Bermúdez-Aguirre, D., Mawson, R., Barbosa-Cánovas, G., 2008. Microstructure of fat globules in whole milk after thermosonication treatment. Journal of Food Science 73.

Bintsis, T., Litopoulou-Tzanetaki, E., Robinson, R.K., 2000. Existing and potential applications of ultraviolet light in the food industry–a critical review. Journal of the Science of Food and Agriculture 80, 637-645.

Burt, S., 2004. Essential oils: their antibacterial properties and potential applications in foods—a review. International Journal of Food Microbiology 94, 223-253.

Centers for Disease Control and Prevention (CDC), 2008a. Outbreak of *Salmonella* serotype Saintpaul infections associated with multiple raw produce items--United States, 2008. MMWR. Morbidity and Mortality Weekly Report, 57(34), 929.

Centers for Disease Control and Prevention (CDC), 2008b. Outbreak of *Listeria monocytogenes* infections associated with pasteurized milk from a local dairy--Massachusetts, 2007. MMWR. Morbidity and mortality weekly report, 57(40), 1097.

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

(last accessed at 2018-04-02)

Centers for Disease Control and Prevention (CDC), 2011. CDC 2011 Estimates: Findings, available at http://www.cdc.gov/foodborneburden/2011-foodborne-estimates.html (last accessed at 2018-04-02)

Chun, H., Kim, J., Chung, K., Won, M., Song, K.B., 2009. Inactivation kinetics of *Listeria monocytogenes*, *Salmonella enterica* serovar Typhimurium, and *Campylobacter jejuni* in ready-to-eat sliced ham using UV-C irradiation. Meat Science 83, 599-603.

Claeys, W.L., Cardoen, S., Daube, G., De Block, J., Dewettinck, K., Dierick, K., De Zutter, L., Huyghebaert, A., Imberechts, H., Thiange, P., 2013. Raw or heated cow milk consumption: Review of risks and benefits. Food Control 31, 251-262.

De Alwis, A., Fryer, P., 1990. A finite-element analysis of heat generation and transfer during ohmic heating of food. Chemical Engineering Science 45, 1547-1559.

Di Pasqua, R., Hoskins, N., Betts, G., Mauriello, G., 2006. Changes in membrane fatty acids composition of microbial cells induced by addiction of thymol, carvacrol, limonene, cinnamaldehyde, and eugenol in the growing media. Journal of Agricultural and Food Chemistry 54, 2745-2749.

Doyle, M.P., Erickson, M.C., Alali, W., Cannon, J., Deng, X., Ortega, Y., Smith, M.A., Zhao, T., 2015. The food industry's current and future role in preventing microbial foodborne illness within the United States. Clinical Infectious Diseases 61, 252-259.

Enache, E., Chen, Y., 2007. Survival of *Escherichia coli* O157: H7, *Salmonella*, and *Listeria monocytogenes* in cranberry juice concentrates at different Brix levels. Journal of Food Protection 70, 2072-2077.

Escalona, V.H., Aguayo, E., Martínez-Hernández, G.B., Artés, F., 2010. UV-C doses to reduce pathogen and spoilage bacterial growth in vitro and in baby spinach. Postharvest Biology and Technology 56, 223-231.

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. Journal of Food Protection 73, 2189-2196.

Estilo, E.E.C., Gabriel, A.A., 2017. Previous stress exposures influence subsequent UV-C resistance of *Salmonella enterica* in coconut liquid endosperm. LWT-Food Science and Technology 86, 139-147.

Farber, J., Peterkin, P., 1991. *Listeria monocytogenes*, a food-borne pathogen. Microbiological Reviews 55, 476-511.

Franco, W., Hsu, W.-Y., Simonne, A.H., 2010. Survival of *Salmonella* and *Staphylococcus aureus* in Mexican red salsa in a food service setting. Journal of Food Protection 73, 1116-1120.

Franco, W., Simonne, A.H., 2009. Mexican food safety trends: examining the CDC data in the United States from 1990 to 2006. Food Protection Trends 29, 204-210.

Friedman, M., Zhu, L., Feinstein, Y., Ravishankar, S., 2009. Carvacrol facilitates heat-induced inactivation of *Escherichia coli* O157: H7 and inhibits formation of heterocyclic amines in grilled ground beef patties. Journal of Agricultural and Food Chemistry 57, 1848-1853.

Fryer, P., De Alwis, A., Koury, E., Stapley, A., Zhang, L., 1993. Ohmic processing of solid-liquid mixtures: heat generation and convection effects. Journal of Food Engineering 18, 101-125.

Geedipalli, S., Datta, A., Rakesh, V., 2008. Heat transfer in a combination microwave–jet impingement oven. Food and Bioproducts Processing 86, 53-63.

Geeraerd, A., Valdramidis, V., Van Impe, J., 2005. GInaFiT, a freeware tool to assess non-log-linear microbial survivor curves. International Journal of Food Microbiology 102, 95-105.

Ha, J.-W., Kang, D.-H., 2015. Combining Lactic Acid Spray with Near-Infrared Radiation Heating To Inactivate *Salmonella enterica* Serovar

Enteritidis on Almond and Pine Nut Kernels. Applied and Environmental Microbiology 81, 4517-4524.

Han, L., Patil, S., Boehm, D., Milosavljević, V., Cullen, P., Bourke, P., 2016. Mechanisms of inactivation by high-voltage atmospheric cold plasma differ for *Escherichia coli* and *Staphylococcus aureus*. Applied and Environmental Microbiology 82, 450-458.

Jain, S., Bidol, S.A., Austin, J.L., Berl, E., Elson, F., Williams, M.L., Deasy III, M., Moll, M.E., Rea, V., Vojdani, J.D., 2009. Multistate outbreak of *Salmonella* Typhimurium and Saintpaul infections associated with unpasteurized orange juice—United States, 2005. Clinical Infectious Diseases 48, 1065-1071.

Juneja, V., Eblen, B., 1999. Predictive thermal inactivation model for *Listeria monocytogenes* with temperature, pH, NaCl, and sodium pyrophosphate as controlling factors. Journal of Food Protection 62, 986-993.

Juneja, V., Eblen, B., 2000. Heat inactivation of *Salmonella* Typhimurium DT104 in beef as affected by fat content. Letters in Applied Microbiology 30, 461-467.

Juneja, V.K., 2003. Predictive model for the combined effect of temperature, sodium lactate, and sodium diacetate on the heat resistance of *Listeria monocytogenes* in beef. Journal of Food Protection 66, 804-811.

Jung, S., Nam, K.C., Jo, C., 2016. Detection of malondialdehyde in processed meat products without interference from the ingredients. Food Chemistry 209, 90-94.

Kang, J.-W., Kim, S.-S., Kang, D.-H., 2018. Inactivation dynamics of 222 nm krypton-chlorine excilamp irradiation on Gram-positive and Gram-negative foodborne pathogenic bacteria. Food Research International 109, 325-333.

Karatzas, A., Bennik, M., Smid, E., Kets, E., 2000. Combined action of S-carvone and mild heat treatment on *Listeria monocytogenes* Scott A. Journal of Applied Microbiology 89, 296-301.

Keene, W.E., Hedberg, K., Herriott, D.E., Hancock, D.D., McKay, R.W., Barrett, T.J., Fleming, D.W., 1997. A prolonged outbreak of *Escherichia coli* O157: H7 infections caused by commercially distributed raw milk. Journal of Infectious Diseases 176, 815-818.

Kim, D.-K., Kim, S.-J., Kang, D.-H., 2017. Bactericidal effect of 266 to 279 nm wavelength UVC-LEDs for inactivation of Gram positive and Gram negative foodborne pathogenic bacteria and yeasts. Food Research International 97, 280-287.

Kim, N., Ryang, J., Lee, B., Kim, C., Rhee, M., 2017. Continuous ohmic heating of commercially processed apple juice using five sequential electric fields results in rapid inactivation of *Alicyclobacillus*

acidoterrestris spores. International Journal of Food Microbiology 246, 80-84.

Kim, S., Rhee, M., 2016. Highly enhanced bactericidal effects of medium chain fatty acids (caprylic, capric, and lauric acid) combined with edible plant essential oils (carvacrol, eugenol, β-resorcylic acid, trans-cinnamaldehyde, thymol, and vanillin) against *Escherichia coli* O157: H7. Food Control 60, 447-454.

Kim, S.S., Kang, D.H., 2015. 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*. Journal of Applied Microbiology 119, 475-486.

Kulshrestha, S., Sastry, S., 2003. Frequency and voltage effects on enhanced diffusion during moderate electric field (MEF) treatment. Innovative Food Science & Emerging Technologies 4, 189-194.

Kwak, T.Y., Kim, N.H., Rhee, M.S., 2011. Response surface methodology-based optimization of decontamination conditions for *Escherichia coli* O157: H7 and *Salmonella* Typhimurium on fresh-cut celery using thermoultrasound and calcium propionate. International Journal of Food Microbiology 150, 128-135.

Lee, J.-Y., Kim, S.-S., Kang, D.-H., 2015. Effect of pH for inactivation of *Escherichia coli* O157: H7, *Salmonella* Typhimurium and *Listeria*

monocytogenes in orange juice by ohmic heating. LWT-Food Science and Technology 62, 83-88.

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. Applied and Environmental Microbiology 79, 10-17.

Lee, S.Y., Sagong, H.G., Ryu, S., Kang, D.H., 2012. Effect of continuous ohmic heating to inactivate *Escherichia coli* O157: H7, *Salmonella* Typhimurium and *Listeria monocytogenes* in orange juice and tomato juice. Journal of Applied Microbiology 112, 723-731.

Leizerson, S., Shimoni, E., 2005a. Effect of ultrahigh-temperature continuous ohmic heating treatment on fresh orange juice. Journal of Agricultural and Food Chemistry 53, 3519-3524.

Leizerson, S., Shimoni, E., 2005b. Stability and sensory shelf life of orange juice pasteurized by continuous ohmic heating. Journal of Agricultural and Food Chemistry 53, 4012-4018.

Lima, M., Sastry, S.K., 1999. The effects of ohmic heating frequency on hot-air drying rate and juice yield. Journal of Food Engineering 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*. Applied and Environmental Microbiology 62, 3094-3100.

Liu, S., Ozturk, S., Xu, J., Kong, F., Gray, P., Zhu, M.-J., Sablani, S.S., Tang, J., 2018. Microbial validation of radio frequency pasteurization of wheat flour by inoculated pack studies. Journal of Food Engineering 217, 68-74.

Marder, E.P., Garman, K.N., Ingram, L.A., Dunn, J.R., 2014. Multistate outbreak of *Escherichia coli* O157: H7 associated with bagged salad. Foodborne Pathogens and Disease 11, 593-595.

Marra, F., Zell, M., Lyng, J., Morgan, D., Cronin, D., 2009. Analysis of heat transfer during ohmic processing of a solid food. Journal of Food Engineering 91, 56-63.

Mazzotta, A.S., 2001. Thermal inactivation of stationary-phase and acid-adapted *Escherichia coli* O157: H7, *Salmonella*, and *Listeria monocytogenes* in fruit juices. Journal of Food Protection 64, 315-320.

Min, S., Zhang, Q., 2003. Effects of Commercial-scale pulsed electric field processing on flavor and color of tomato juice. Journal of Food Science 68, 1600-1606.

Murinda, S., Nguyen, L., Nam, H., Almeida, R., Headrick, S., Oliver, S., 2004. Detection of sorbitol-negative and sorbitol-positive Shiga toxin-producing *Escherichia coli*, *Listeria monocytogenes*, *Campylobacter jejuni*, and *Salmonella* spp. in dairy farm environmental samples. Foodborne Pathogens & Disease 1, 97-104.

Murphy, R., Beard, B., Martin, E., Duncan, L., Marcy, J., 2004. Comparative study of thermal inactivation of *Escherichia coli* O157: H7, *Salmonella*, and *Listeria monocytogenes* in ground pork. Journal of Food Science 69.

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. Applied and Environmental Microbiology 79, 7122-7129.

Park, S.H., Balasubramaniam, V., Sastry, S.K., 2014. Quality of shelf-stable low-acid vegetables processed using pressure–ohmic–thermal sterilization. LWT-Food Science and Technology 57, 243-252.

Peabody, M.A., Laird, M.R., Vlasschaert, C., Lo, R., Brinkman, F.S., 2015. PSORTdb: expanding the bacteria and archaea protein subcellular localization database to better reflect diversity in cell envelope structures. Nucleic Acids Research 44, D663-D668.

Phan-Thanh, L., Mahouin, F., Aligé, S., 2000. Acid responses of *Listeria monocytogenes*. International Journal of Food Microbiology 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. Journal of Food Engineering 90, 388-394.

Raafat, D., Von Bargen, K., Haas, A., Sahl, H.-G., 2008. Insights into the mode of action of chitosan as an antibacterial compound. Applied and Environmental Microbiology 74, 3764-3773.

Rahman, M.M., Ninomiya, K., Ogino, C., Shimizu, N., 2010. Ultrasound-induced membrane lipid peroxidation and cell damage of *Escherichia coli* in the presence of non-woven TiO_2 fabrics. Ultrasonics Sonochemistry 17, 738-743.

Ramaswamy, H.S., Jin, H., Zhu, S., 2009. Effects of fat, casein and lactose on high-pressure destruction of *Escherichia coli* K12 (ATCC-29055) in milk. Food and Bioproducts Processing 87, 1-6.

Roux, S., Courel, M., Birlouez-Aragon, I., Municino, F., Massa, M., Pain, J.-P., 2016. Comparative thermal impact of two UHT technologies, continuous ohmic heating and direct steam injection, on the nutritional properties of liquid infant formula. Journal of Food Engineering 179, 36-43.

Ryang, J., Kim, N., Lee, B., Kim, C., Lee, S., Hwang, I., Rhee, M., 2016. Inactivation of *Bacillus cereus* spores in a tsuyu sauce using continuous ohmic heating with five sequential elbow-type electrodes. Journal of Applied Microbiology 120, 175-184.

Sagong, H.-G., Cheon, H.-L., Kim, S.-O., Lee, S.-Y., Park, K.-H., Chung, M.-S., Choi, Y.-J., Kang, D.-H., 2013. Combined effects of ultrasound and surfactants to reduce *Bacillus cereus* spores on lettuce and carrots. International Journal of Food Microbiology 160, 367-372.

Sagong, H.-G., Park, S.-H., Choi, Y.-J., Ryu, S., Kang, D.-H., 2011. Inactivation of *Escherichia coli* O157: H7, *Salmonella* Typhimurium, and *Listeria monocytogenes* in orange and tomato juice using ohmic heating. Journal of Food Protection 74, 899-904.

Saguy, I.S., 2016. Challenges and opportunities in food engineering: Modeling, virtualization, open innovation and social responsibility. Journal of Food Engineering 176, 2-8.

Samaranayake, C.P., Sastry, S.K., 2005. Electrode and pH effects on electrochemical reactions during ohmic heating. Journal of Electroanalytical Chemistry 577, 125-135.

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

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

Sanz, E.N., Davila, I.S., Balao, J.A., Alonso, J.Q., 2007. Modelling of reactivation after UV disinfection: effect of UV-C dose on subsequent photoreactivation and dark repair. Water Research 41, 3141-3151.

Sastry, S.K., Barach, J.T., 2000. Ohmic and inductive heating. Journal of Food Science 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. Ohmic Heating in Food Processing, 7-14.

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. Emerging Infectious Diseases 17.

Shi, J., Maguer, M.L., 2000. Lycopene in tomatoes: chemical and physical properties affected by food processing. Critical Reviews in Food Science and Nutrition 40, 1-42.

Solórzano-Santos, F., Miranda-Novales, M.G., 2012. Essential oils from aromatic herbs as antimicrobial agents. Current Opinion in Biotechnology 23, 136-141.

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

Sommers, C.H., Sites, J.E., Musgrove, M., 2010. Ultraviolet light (254 nm) inactivation of pathogens on foods and stainless steel surfaces. Journal of Food Safety 30, 470-479.

Song, M., Kim, H., Rhee, M., 2016. Optimization of heat and relative humidity conditions to reduce *Escherichia coli* O157: H7 contamination and maximize the germination of radish seeds. Food Microbiology 56, 14-20.

Sun, D.-W., 2007. Computational fluid dynamics in food processing. CRC Press.

Sun, D.-W., 2016. Handbook of frozen food processing and packaging. CRC Press.

Sung, H.-J., Kang, D.-H., 2014. Effect of a 915 MHz microwave system on inactivation of *Escherichia coli* O157: H7, *Salmonella* Typhimurium, and *Listeria monocytogenes* in salsa. LWT-Food Science and Technology 59, 754-759.

Sung, H.-J., Song, W.-J., Kim, K.-P., Ryu, S., Kang, D.-H., 2014. Combination effect of ozone and heat treatments for the inactivation of *Escherichia coli* O157: H7, *Salmonella* Typhimurium, and *Listeria monocytogenes* in apple juice. International Journal of Food Microbiology 171, 147-153.

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. International Journal Food Microbiology 16, 1-23.

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

Tsong, T.Y., 1990. On electroporation of cell membranes and some related phenomena. Journal of Electroanalytical Chemistry and Interfacial Electrochemistry 299, 271-295.

Tzedakis, T., Basseguy, R., Comtat, M., 1999. Voltammetric and coulometric techniques to estimate the electrochemical reaction rate during ohmic sterilization. Journal of Applied Electrochemistry 29, 819-826.

U. S. Food and Drug Administration (U. S. FDA)., 2001. Guidance for Industry: The Juice HACCP Regulation – Questions & Answers. Available from https://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/Juice/ucm072981.htm#F (last accessed at 2018-04-02)

Varghese, K.S., Pandey, M., Radhakrishna, K., Bawa, A., 2014. Technology, applications and modelling of ohmic heating: a review. Journal of Food Science and Technology 51, 2304-2317.

Wu, D., You, H., Jin, D., Li, X., 2011. Enhanced inactivation of *Escherichia coli* with Ag-coated TiO_2 thin film under UV-C irradiation. Journal of Photochemistry and Photobiology A: Chemistry 217, 177-183.

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

Xu, J., Liu, S., Tang, J., Ozturk, S., Kong, F., Shah, D.H., 2018. Application of freeze-dried *Enterococcus faecium* NRRL B-2354 in radio-frequency pasteurization of wheat flour. LWT 90, 124-131.

국문 초록

펄스 옴 가열은 신속하고 균일한 가열이 가능한 신 가열 기술 중 하나로 주목을 받고 있다. 열 전달이 전도 및 대류를 통해 이루어지는 기존 가열 방식과는 다르게 펄스 옴 가열은 식품 성분을 통과하는 전류에 의해 식품 내부에서 열이 발생하기 때문에 기존 가열에 비해 펄스 옴 가열을 이용해 식중독 균을 효과적으로 저감 (inactivation)할 수 있지만 고온에 의해서 식품의 품질이 변질될 가능성이 있다. 따라서, 펄스 옴 가열 장치를 식품 산업에 적용하기에 앞서 최소한의 가공을 선호하는 소비자의 기호에 맞추어 최적화해야 한다. 본 연구에서는 식중독 균의 저감을 위한 펄스 옴 가열 장치의 적용 가능성을 확인하였으며, 세부적인 목표는 1) 펄스 옴 가열 장치의 식중독 균 제어 효과 규명, 2) 다른 위생 처리들과 조합 처리 연구, 3) 우유에서 펄스 옴 가열을 통한 식중독 균 저감 예측을 위한 경험적 모델 개발, 4) 펄스 옴 가열을 통한 토마토주스 내 식중독 균 저감에 대한 멀티피직스 (Multiphysics) 모델 개발, 5) 연속식 펄스 옴 가열 장치의 개발 및 적용이다.

첫째로, 펄스 옴 가열에 의해 전극 부식 및 품질 변화 없이 식중독 균이 효과적으로 저감되는지를 확인하였다. 식중독 균 (*Escherichia coli* O157:H7, *Salmonella enterica* serovar Typhimurium, *Listeria monocytogenes*)을 접종 시킨 완충 펩톤수 (Buffered peptone water; BPW)와 토마토주스를 여러 주파수 (0.06-1 kHz)에서 펄스 옴 가열 처리하였다. 펄스 옴 가열에 의해 식중독 균이 효과적으로 제어되었다. 또한 주파수에 관계 없이 전극 부식 및 토마토 주스의 품질 변화가 관찰되지 않았다 ($p > 0.05$). 따라서, 추후 연구에서 펄스 옴 가열을 활용하였다.

펄스 옴 가열 단독 처리에 의한 높은 온도는 식품의 품질을 손상시킬 수 있기 때문에 개발된 펄스 옴 가열을 여러 에센셜 오일 성분 또는 자외선과 조합 처리하여 가열 온도와 시간을 단축하였다. 에센셜 오일 성분 중 식품 향료로 쓰일 수 있는 카르본 (carvone), 유제놀 (eugenol), 티몰 (thymol), 시트랄 (citral)을 선정하여 펄스 옴 가열과 조합하였다. BPW 에서는 펄스 옴 가열과 시트랄의 조합 처리가 식중독 균에 대한 저감 효과가 가장 좋았으며 이어서 티몰, 유제놀, 카르본과의 조합 처리 순으로 그 효과가 좋았다. 에센셜 오일 성분에 의한 세포막 전위 상실과 병행

처리에 의한 세포막 파괴가 저감 메커니즘으로 제안되었다. 살사에서는 펄스 옴 가열 처리와 티몰의 조합 처리가 식중독 균 저감 효과가 가장 좋았으며 이어서 시트랄, 유제놀, 카르본과의 조합 처리 순으로 저감 효과가 좋았다. 펄스 옴 가열 단독 처리에 비해서 티몰과의 조합 처리를 통해 살사의 색 (b^*)을 잘 보존할 수 있었기 때문에 이 조합 처리를 살사를 살균하는데 있어 효과적으로 이용할 수 있을 것이다. 펄스 옴 가열과 자외선 병행 처리 또한 식중독 균 저감에 대한 시너지 효과를 나타냈다. 식중독 균의 세포막 손상 정도는 병행 처리에 의해서 시너지 효과를 나타낸 반면 세포막 지질의 산화 정도에 대해서는 상가 효과 (additive effect)가 관찰되었다. 따라서, 동시 처리에 의해서 지질 산화가 촉진되어 세포막 손상에 대해 시너지 효과를 나타냄을 메커니즘으로 제안할 수 있다. BPW 에서 펄스 옴 가열 후 자외선을 순차적으로 처리한 경우 역순 처리 또는 동시 처리보다 그 살균효과가 적게 나타났다. 자외선 조사 후 회복 과정 및 열 충격 단백질의 발현이 이러한 현상에 기여한다고 추정된다. 반면, 토마토주스에서는 순차적 처리와 동시 처리 간의 유의적인 차이가 없었으며 ($p > 0.05$), 동시 처리에 의한 토마토 주스의 색 및 리코펜

함량 변화는 관찰되지 않았다. 따라서, 펄스 옴 가열과 티몰의 조합 처리 뿐 아니라 자외선과의 병행 처리를 미생물학적인 안전성을 확보하기 위한 효과적인 기술로 이용할 수 있다.

우유의 지방 및 유당 함량에 따라 펄스 옴 가열에 의한 식중독균의 저감 양상을 예측하기 위한 경험적인 모델을 개발되었다. 지방 및 유당 함량의 복합적인 효과를 반응 표면 방법론 (Response-surface methodology, RSM)의 중심합성계획 (Central composite design, CCD)을 이용하여 분석하였다. 유당과 지방 모두 펄스 옴 가열에 의한 식중독 균의 저감에 대해 억제 효과 (inhibitory effect)를 나타냈다. *E. coli* O157:H7 의 저감 정도는 유당 및 지방 함량에 대해서 2 차 관계 (quadratic relationship)를 따르는 반면 *S.* Typhimurium 과 *L. monocytogenes* 의 저감은 처리 시간과 지방 또는 유당 함량의 교차곱 (cross product)이 유의적인 영향을 미쳤다 ($p < 0.1$). RSM 으로 개발된 모델은 실험 조건의 범위 내에서 세 식중독 균의 저감 정도를 잘 예측했으며, 펄스 옴 가열 후 색 변화 또는 지질 산화가 관찰되지 않았다. 처리 후 pH 가 약간 감소했지만 제품의 품질에 영향을 미칠 정도는 아니였다. 따라서 펄스 옴 가열을 이용하여 우유를 살균할 때 영양 함량과 식중독

균의 종류를 고려하여 처리 조건을 신중하게 결정해야 한다.

펄스 옴 가열을 보다 정확하게 분석하기 위해 멀티피직스 모델링을 수행했다. 펄스 옴 가열을 통한 토마토 주스 가공을 멀티피직스 소프트웨어를 이용하여 분석했을 때, 챔버의 다른 모든 곳에서 식중독 균이 저감되는 조건에서도 균이 살아 있는 냉점 (cold spot)이 챔버의 하부에서 관찰되었다. 개발된 컴퓨터 시뮬레이션 모델을 가열 속도와 식중독 균 저감에 대해서 검증하였다. 또한 열 저항성이 증가한 산 적응 (acid-adapted) 식중독 균의 저감을 개발된 시뮬레이션 모델을 통해 예측하였을 때 시뮬레이션 결과와 실험 결과 값 사이의 유의적인 차이가 관찰되지 않았다 ($p > 0.05$). 따라서 식중독 균의 열 저항성이 변화될 수 있는 환경에서 식중독 균을 5 log 이상의 저감하기 위해 처리 시간을 조절할 때 멀티피직스 모델을 효과적으로 활용할 수 있다.

지금까지 이용된 회분식 (batch-type) 시스템은 펄스 옴 가열의 특성을 확인하는 데는 효과적이었지만 연속식 (continuous-type) 시스템이 식품 산업에서 주스 제품을 대량으로 처리하는 데 보다 유리하다고 알려져 있다. 따라서, 연속식 펄스 옴 가열 장치를

구축하고 유속, 전압 및 예열이 가열 속도와 식중독 균 저감에 미치는 영향을 확인하였다. 유속의 감소 또는 전압의 증가에 따라서 가열 속도 및 식중독 균의 저감 정도가 증대되었다. 또한 추가적으로 전압을 증가시키는 대신 예열 (preheating)을 통해 가열 속도의 변화 없이 초기 처리 단계에서 식중독 균 저감 시킬 수 있었다. 추가적인 장비를 통해 예열을 하는 것은 불편하고 공간을 차지하기 때문에 순차적인 (sequential) 펄스 옴 가열 챔버를 구축하였다. 구축된 순차적인 펄스 옴 가열을 통해 이전과 동일한 처리 조건에서 예열 없이 세 종류의 식중독 균을 5 log 이상 저감할 수 있었다. 따라서 주스 산업에서 식중독 균을 제어하는데 순차적인 챔버를 포함하는 연속식 펄스 옴 가열을 효과적으로 이용할 수 있을 것이다.

결론적으로, 식중독 균의 저감을 위한 펄스 옴 가열의 성능은 조합될 처리의 종류, 식품의 영양 조건, 장치의 유형에 따라 크게 변하였다. 따라서 식품의 품질 변화 없이 식중독 균을 저감화하기 위해 펄스 옴 가열을 최적화하는 것을 추천한다. 또한 펄스 옴 가열 처리를 최적화하는데 멀티피직스 모델링을 효과적으로 이용할 수 있을 것이다.

주제어 : 펄스 옴 가열, 식중독 균, 식품 가공, 전극 부식, 식품 품질, 조합 처리, 경험적 모델링, 멀티피직스 모델링, 컴퓨터 시뮬레이션, 연속식 장치

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