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Application of continuous-type pulsed ohmic heating system for inactivation of foodborne pathogens in buffered peptone water and tomato juice

Sang-Soon Kim[a], Sang-Hyun Park[a], Dong-Hyun Kang[a,b,*]

[a] Department of Agricultural Biotechnology, Center for Food and Bioconvergence, Research Institute for Agricultural and Life Sciences, Seoul National University, Seoul 08826, Republic of Korea,

[b] Institutes of Green Bio Science & Technology, Seoul National University, Pyeongchang-gun, Gangwon-do, 25354, Republic of Korea

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ABSTRACT

The purpose of this study was to inactivate *Escherichia coli* O157:H7, *Salmonella* Typhimurium, and *Listeria monocytogenes* by continuous-type pulsed ohmic heating in buffered peptone water (BPW) and tomato juice. First, BPW inoculated with the three pathogens were treated at different flow rates (0.2–0.4 LPM) and treatment voltages (9.43–12.14 V_{rms}/cm). Both heating rate of BPW and reduction rates of pathogens increased corresponding to decreased flow rate. Accordingly, higher numbers of pathogens survived at a higher flow rate (0.4 LPM). Increasing treatment voltage was an effective way to inactivate pathogens at 0.4 LPM, but the heating rate overly accelerated with increasing voltage adversely affecting food quality. Alternatively, increasing initial temperature by preheating can help inactivate pathogens in the early treatment stage without affecting heating rate. From the BPW experiments, we identified that treatment conditions such as flow rate, voltage, and initial temperature are important factors determining pathogen inactivation performance of continuous-type ohmic heating. When applied to tomato juice, 5 log reductions of all three pathogens were achieved by applying 12.14V_{rms}/cm ohmic heating with 0.2 LPM flow rate after preheating sample to 50 °C with a water bath. Quality aspects of color and lycopene content were observed, and a^* and b^* values decreased after treatment. Because preheating with additional equipment is inconvenient and occupies valuable space, we developed sequential three cylinder type ohmic heating. By applying the developed sequential ohmic heating, 5 log reductions were achieved for all three pathogens without preheating under the same treatment conditions. Therefore, we concluded that sequential continuous-type ohmic heating can be used utizied effectively to control foodborne pathogens by the juice industry.

1. Introduction

Fruit and vegetable juices have traditionally been considered safe because of their acidity, which is generally regarded to be too harsh for the survival of many microorganisms. However, several studies indicate that some pathogens can survive in acid environments. For example, *Escherichia coli* O157:H7, whose infections can result in death in young children and the elderly, is known to have various acid resistance systems based on the type of acidic envrionment encountered (Price, Wright, DeGraves, Castanie-Cornet, & Foster, 2004). The acid response mechanism of *E. coli* O157:H 7 is not fully known, but the stress response gene *rpoS* is known to be involved in the acid response (G. J. Leyer, Wang, & Johnson, 1995). On the other hand, Yuk and Marshall (2004) indicated that membrane fluidity change via adjusted membrane lipid composition also has an important role in acquisition of acid resistance. Other foodborne pathogens such as *Salmonella* Typhimurium and *Listeria monocytogenes* are also known to have acid resistance (Davis, Coote, & O'Byrne, 1996; Foster & Hall, 1990). These acid-adapted pathogens not only survive under acidic conditions but also have higher heat resistance than non-acid-adapted pathogens (G. Leyer & Johnson, 1993).

Consumption of apple cider contaminated with *E. coli* O157:H7 caused hemolytic uremic syndrome (HUS) in 17% of affected patients in 1991, which is the most severe manifestation of *E. coli* O157:H7 infection (Besser et al., 1993). Since then, juice-associated outbreaks of *E. coli* O157:H7 have been frequently reported. Vojdani, Beuchat, and Tauxe (2008) reported 5 outbreaks involving *E. coli* O157:H7 between 1995 and 2005 which resulted in 105 illnesses, 36 hospitalizations, and 1 death. Multistate outbreaks attributed to *Salmonella* Typhimurium infections also have been reported. Multistate outbreaks of *Salmonella* Typhimurium and Saintpaul infections associated with unpasteurized orange juice occurred in 2005 resulting in a 24% hospitalization rate

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

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(Jain et al., 2009). Even though outbreaks related to *Listeria monocytogenes* infection have not been reported, Conner, Brackett, and Beuchat (1986) identified that *L. monocytogenes* can survive for more than 63 days in cabbage juice at 5 °C. Therefore, it is very important for juice processors to eliminate *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* in juice products while seeking to minimize quality degradation.

Ohmic heating is a novel thermal technology facilitating rapid and uniform heating by means of electric current flowing through the food. Electrode corrosion has long been an obstacle for ohmic heating used in food processing because metal ions mitigrating into a food sample by electrode corrosion not only have a toxic potential but also oxidation ability (Tola, Rattan, & Ramaswamy, 2014). Several solutions have been suggested such as utilization of an inert electrode, high frequency, and a pulse waveform. Samaranayake and Sastry (2005) indicated that platinized-titanium electrodes are more inert than titanium, stainless steel, and graphite. The principle behind utilizing high frequency as well as a pulse waveform for preventing electrode corrosion is basically the same from the perspective of reducing the time of that electrons can transfer to the electrode surface (S.-Y. Lee, Ryu, & Kang, 2013; Samaranayake, Sastry, & Zhang, 2005). Using high frequency or pulsed ohmic heating, it is possible to inactivate foodborne pathogens effectively without causing electrode corrosion in salsa or tomato juice (S.-S. Kim, Choi, & Kang, 2017; S.-Y. Lee et al., 2013).

Ohmic heating is typically divided into batch and continuous-types of processing. In the field of research, batch-type ohmic heating apparatuses have usually been used to identify the characteristics of ohmic heating. For example, the effect of intrinsic factors of foods such as pH, fat, sugar and lactose content (S.-S. Kim, Jo, & Kang, 2017; I.-K. Park, Ha, & Kang, 2017) and extrinsic factors such as voltage and frequency on the performance of ohmic heating was identified (Murashita, Kawamura, & Koseki, 2016) by batch-type ohmic heating. In contrast to batch-type, continuous-type ohmic heating is more advantageous for bulk handling of juice products. Several studies characterizing inactivation efficacy of continuous-type ohmic heating on *E. coli* O157:H7, *S.* Typhimurium, *L. monocytogenes* (S. Y. Lee, Sagong, Ryu, & Kang, 2012), and spores of *Bacillus cereus*, *Alicyclobacillus acidoterrestris* (N. Kim, Ryang, Lee, Kim, & Rhee, 2017; Ryang, Kim, Lee, Kim, & Rhee, 2016) have been reported. However, to the best of our knowledge, systematic research including the effect of flow rate, treatment voltage, and initial temperature on the inactivation efficacy of continous-type pulsed ohmic heating has been limited.

In the present study, we investigated the efficacy of continuous-type pulsed ohmic heating for inactivation of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes*. First, the heating rate of BPW and pathogen inactivation was 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 ohmic heating apparatus was developed to reduce the preheating step.

2. Materials and methods

2.1. Bacterial cultures and cell suspension

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

2.2. 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), 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.

2.3. Continuous-type pulsed ohmic heating apparatus

Ohmic heating treatments were carried out in a previously described apparatus (S.-S. Kim, W. Choi et al., 2017) with a modified treatment chamber. The ohmic heating system (Fig. 1) 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 TDS 2001C; Tektronix, Inc. Beaverton, CO), a data logger (catalog number 34970A; Agilent Technologies), and continuous-type ohmic heating chamber. This ohmic heating chamber consisted of a sample tank, diaphragm pump (KNF Neuberger, Inc. New Jersey, USA), cylindrical treatment chamber, and product tank. 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. Additionally, sequential

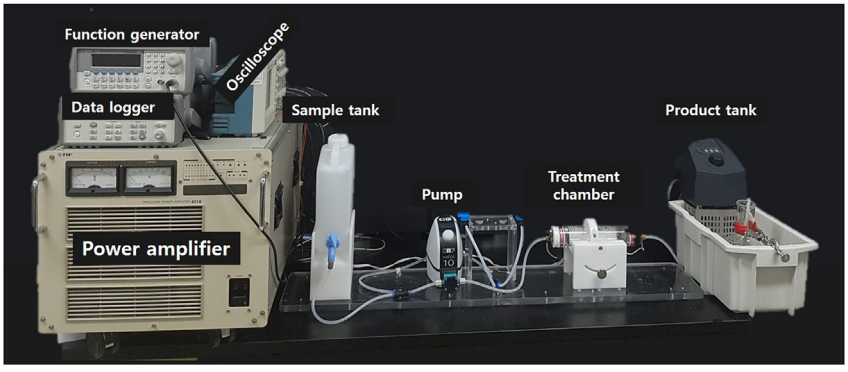

Fig. 1. Continuous type pulsed ohmic heating system.

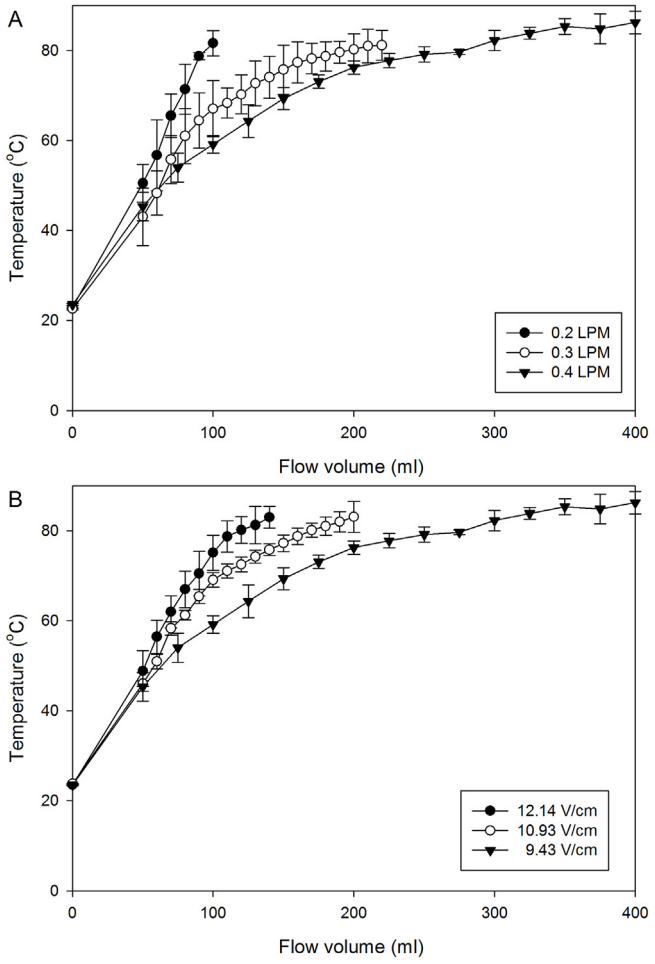

Fig. 2. Temperature history of ohmic treated buffered peptone water at fixed voltage (9.43 V_{rms}/cm) and varying flow rate (A) and fixed flow rate (0.4 LPM) and varying voltage (B).

three cylinder type ohmic heating was developed with a downsized treatment chamber (Fig. 7). The distance between electrodes in sequential three cylinder type ohmic heating was 5 cm and other conditions were the same as described above.

2.4. 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 (1 kHz) 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 °C by a constant-temperature water bath (BW-10G; Jeio Tech, Seoul, South Korea), 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 °C with a water bath, and subsequently subjected to 12.14 Vrms/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.

2.5. Bacterial enumeration

For microbial enumeration, each treated 1 ml sample was immediately transferred into 9 ml of sterile 0.2% peptone water and 10-fold serially diluted. Diluted samples were spread-plated onto each selective medium. Sorbitol MacConkey (SMAC) agar (Difco), xylose lysine deoxycholate (XLD) agar (Difco), and Oxford agar base (OAB; Difco) with antimicrobial supplement (Bacto Oxford antimicrobial supplement; Difco) were used as selective media for enumeration of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes*, respectively. All plates were incubated at 37 °C for 24–48 h before counting colonies characteristic of the pathogens.

2.6. Color, lycopene content measurement

The color and lycopene content of untreated (control) and treated tomato juice were evaluated. Tomato juice preheated to 50 °C was subjected to 12.14 Vrms/cm pulsed ohmic heating (1 kHz) at 0.2 LPM and measured after a treatment volume of 150 ml. Color values were measured with a Minolta colorimeter (model CR400; Minolta Co. Osaka, Japan). L^*, a^*, and b^* values (parameters of lightness, redness, and yellowness, respectively) were measured to evaluate color changes of tomato juice. The color difference (△E) is calculated by the following equation.

$$\Delta E = \sqrt{(L_t^* - L_c^*)^2 + (a_t^* - a_c^*)^2 + (b_t^* - b_c^*)^2}$$

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 previously described method (S.-Y. Lee et al., 2013). The concentrations of lycopene in tomato juice were determined using absorbance and sample weight with equation (1). Absorbance was measured with a spectrofluorophotometer (Spectramax M2e; Molecular Devices, Sunnyvale, CA) at 503 nm.

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

2.7. Statistical analysis

All experiments were replicated three times. 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 were determined at a significance level of $p = 0.05$.

3. Results and discussion

3.1. Effect of flow rate and treatment voltage on heating rate of BPW and pathogen inactivation

The heating rate of BPW was inversely proportional to flow rate (Fig. 2A) in the present study. At a fixed voltage of 9.43 V/cm, tomato juice samples were heated to 80 °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 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. 2B). At a fixed flow rate of 0.4 LPM, BPW reached 80 °C after 120, 170, and 275 ml flow volumes were treated with treatment voltages of 12.14, 10.93, and 9.43 V/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. Because thermal effect is the major principle of microorganism inactivation by ohmic heating (S.-S. Kim & Kang, 2015), reductions of all three pathogens increased as flow rate decreased from 0.4 to 0.2 LPM (Fig. 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

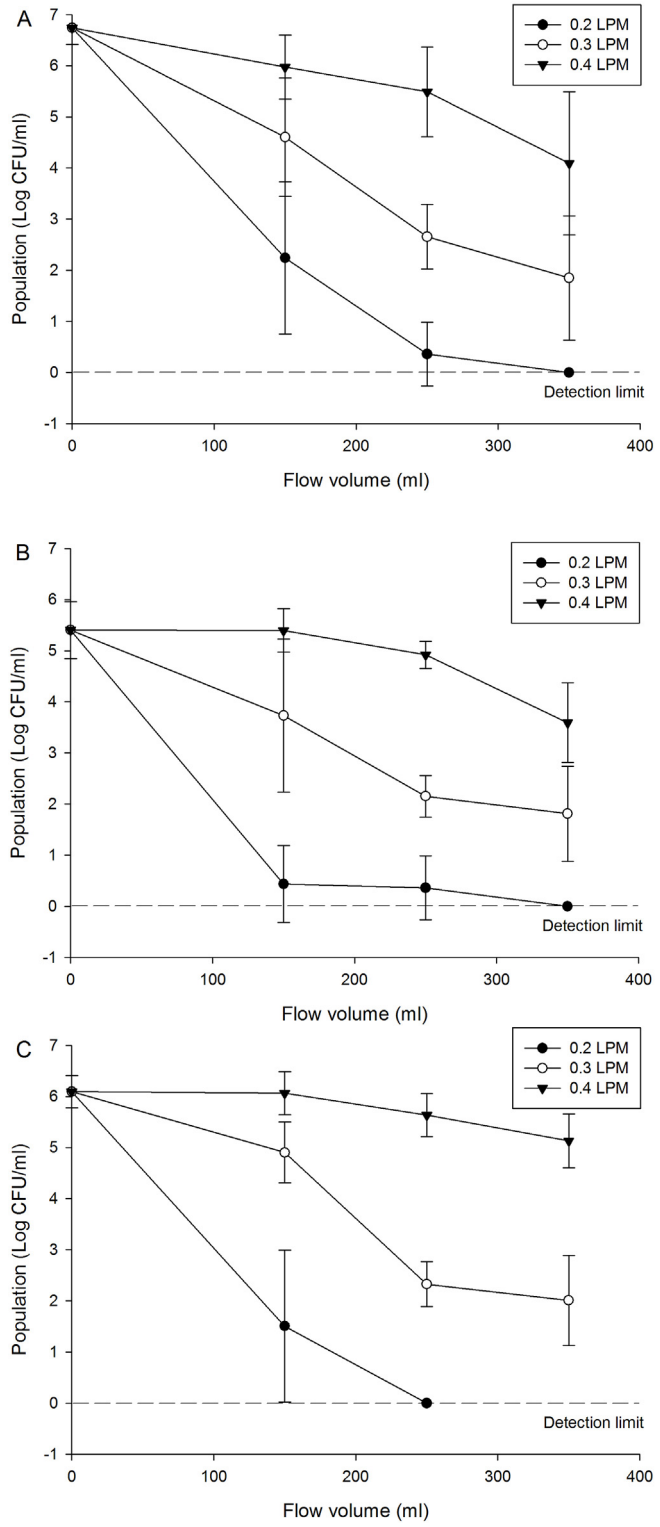

Fig. 3. Populations of *E. coli* O157:H7 (A), *S.* Typhimurium (B), and *L. monocytogenes* (C) in buffered peptone water subjected to ohmic heaitng at varying flow rate and treatment volume. Ohmic heating voltage was fixed at 9.43 V_{rms}/cm.

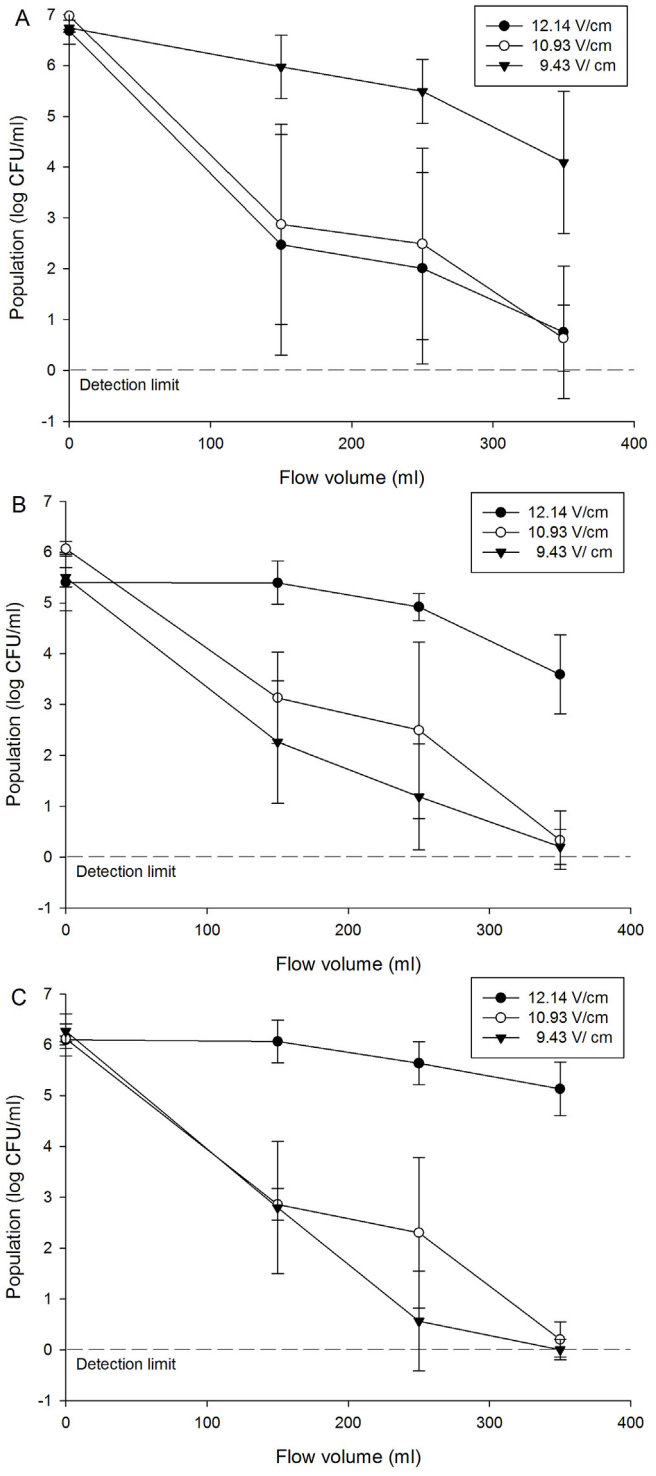

Fig. 4. Populations of *E. coli* O157:H7 (A), *S.* Typhimurium (B), and *L. monocytogenes* (C) in buffered peptone water subjected to ohmic heating with varying voltage and treatment volume. Sample flow rate was fixed at 0.4 LPM.

LPM flow rates, respectively, at a flow volume of 150 ml (Fig. 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, we increased the ohmic heating voltage 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 proportional to increased treatment voltage (Fig. 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. 4A). S. Y. 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 ohmic heating. Even though Baysal and İçier (2010) reported that an additional non-thermal effect associated with increased voltage (20 V/cm) 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/cm) was too small to expect this additional non-thermal effect. From our results, we concluded that flow rate and voltage in ohmic heating are fundamental factors affecting the heating rate and inactivation of pathogens.

3.2. 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 nevertheless 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 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. 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 voltage can solve this problem, but the heating rate would be excessively accelerated by increased voltage which can cause quality degradation in foods (S.-S. Kim et al., 2016). Therefore, we preheated BPW 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 increased initial temperature of BPW (Fig. 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, we identified that preheating before ohmic heating can be used not only to increase electrical conductivity of the sample (Varghese, Pandey, Radhakrishna, & Bawa, 2014), but also to ensure safety in the early treatment stage while minimizing quality degradation of food.

3.3. Application in tomato juice processing

From BPW experiment results, we identified that flow rate, treatment voltage, and initial sample temperature are important factors in continuous-type pulsed ohmic heating. We then applied continous-type ohmic heating system to inactivate foodborne pathogens in tomato

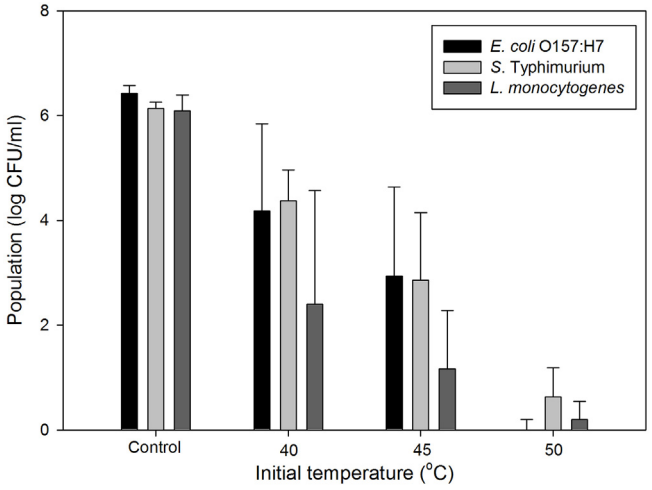

Fig. 5. Populations of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* in buffered peptone water subjected to ohmic heating with varying initial temperature and treatment volume of 150 ml. Sample flow rate and ohmic heating voltage was fixed at 0.4 LPM and 10.93V_{rms}/cm, respectively.

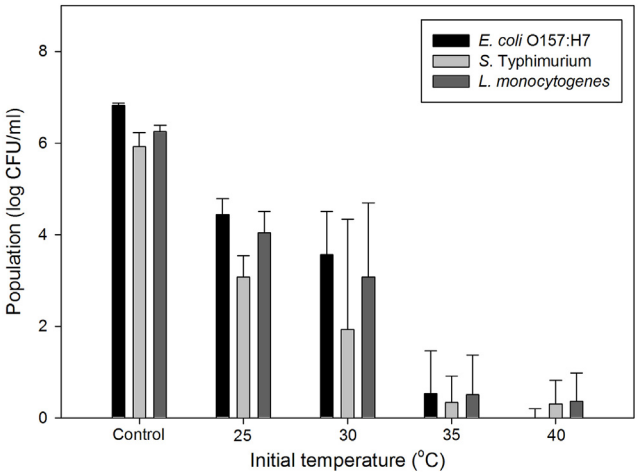

Fig. 6. Populations of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* in tomato juice subjected to ohmic heating with varying initial temperature and treatment volume of 150 ml. Sample flow rate and ohmic heating voltage was fixed at 0.2 LPM and 12.14 V_{rms}/cm, respectively.

juice. Similar to BPW, reduction of all three pathogens increased relative to increased initial temperature of tomato juice (Fig. 6). Because processors should treat juice to achieve more than 5 log reduction of the number of microorganisms (U. S. FDA. 2001), we identified treatment conditions needed to ensure 5 log reduction. Even though more than 5 log reductions of all three pathogens were achieved by applying 12.14 V/cm ohmic heating to 0.2 LPM tomato juice when preheated to 50 °C in the present study, ohmic heating treatment conditions should be adjusted considering the electrical conductivity of juice. For instance, S. Y. Lee et al. (2012) and Sagong, Park, Choi, Ryu, and Kang (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. Color is considered as one of major quality attributes of foods influencing the consumer's choice (Min & Zhang, 2003). Lycopene is the pigment responsible for the color of tomato juice and has a natural antioxidant property (Shi & Maguer, 2000). Therefore, we selected color and lycopene content as a representative quality parameters in the present study when ohmic heating was applied under treatment conditions found to result in > 5 log reductions of all three pathogens. A^* and b^* color values significantly decreased after ohmic heating treatment while L^* values and lycopene content were not significantly different from those of untreated tomato juice (Table 1). The color difference ($\triangle E$) of ohmic heated samples was 2.05 ± 0.98. Even though a^* and b^* values decreased significantly with ohmic heating, it is well known that quality degradation of juice is less with ohmic heating compared to conventional heating (Leizerson & Shimoni, 2005a, 2005b). S. Y. Lee et al. (2012) also reported that a and b 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, we postulated that ohmic treated tomato juice quality would be better than that of conventionally treated juice even though further study is needed.

3.4. Development and application of three sequential cylinder type treatment chamber

Based on the inactivation rates of pathogens in BPW and tomato juice, we identified that preheating is a crucial step for inactivating foodborne pathogens in the initial sampling step to prevent accelerated overheating. Roux et al. (2016) 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

Table 1
Quality aspects of tomato juice (50 °C initial temperature) untreated or treated with 12.14 V_{rms}/cm ohmic heating.

Treatment	Color				Lycopene content (mg/kg tissue)
	L*	a*	b*	△E	
Untreated	27.69 ± 0.39A	4.87 ± 0.04A	9.40 ± 0.12A	–	17.02 ± 3.32A
Ohmic heating	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 ($p > 0.05$).

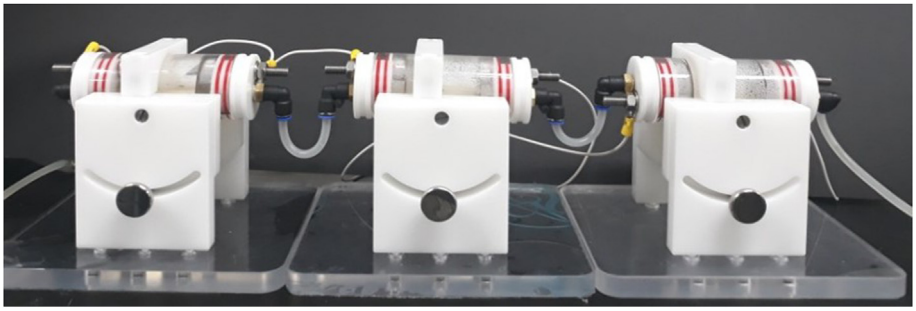

Fig. 7. Sequential three cylinder type pulsed ohmic heating system.

is inconvenient and space consuming. Even though combining pressure with ohmic heating is an effective way to reduce the preheating step (S. H. Park, Balasubramaniam, & Sastry, 2014), it is difficult to combine pressure with a continuous ohmic heating process. Therefore, we developed a sequential three cylinder type ohmic heating system (Fig. 7). In sequential ohmic heating, total electrical resistance decreased to one third of original resistance because three treatment chamber was paralled 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 we applied our developed system for inactivating pathogens in BPW under the same conditions as described in section 3.2 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 additionaly enhance inactivation efficiency of ohmic heating as pointed out by Ryang, Kim, Lee, Kim, Lee, et al. (2016). Based on our result, we suggest that sequential type ohmic heating can reduce the preheating step needed for inactivation of foodborne pathogens.

4. Conclusion

We applied continuous-type pulsed ohmic heaitng to inactivate foodborne pathogens in BPW and tomato juice in the present study. Flow rate and treatment voltage were fundamental factors affecting 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 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.

Acknowledgments

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