A THESIS FOR THE DEGREE OF MASTER OF SCIENCE

Effect of ohmic heating on inactivation of foodborne pathogens in liquid and solid-liquid food mixture

액체 및 고체상 혼합 식품 내의

병원성 미생물 저감화에 대한 옴가열의 효과

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Department of Agricultural Biotechnology

Seoul National University

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석사학위논문

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이 논문을 석사학위 논문으로 제출함

2012년 8월

서울대학교 대학원 농생명공학부

이　수　연

이수연의 석사 학위논문을 인준함

2012년 8월

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Abstract

Ohmic heating is a highly attractive technology by which internal heat as a result of electrical resistance occurs rapidly by passing an alternating current through a food product. However, ohmic heating has some technical limitation to ensure uniform commercial sterilization. In this study, continuous flow ohmic heating system and high frequency ohmic heating technology developed to provide the absence of thermal abuse in the food product and reduction of electrode corrosion problems. The efficacy of continuous ohmic heating for inactivating *Escherichia coli* O157:H7, *Salmonella* Typhimurium, and *Listeria monocytogenes* in orange and tomato juice was investigated with electric field strengths in the range of 25-40 V/cm for different treatment times. The temperature of the samples increased with increasing treatment time and electric field strength. The rate of temperature change for tomato juice was higher than for orange juice at all voltage gradients applied. Higher electric field strength or longer treatment time resulted in a greater reduction of pathogens. *E. coli* O157:H7 was reduced by more than 5 log after 60, 90, and 180 s treatment in orange juice with 40, 35, and 30 V/cm electric field strength,

respectively. In tomato juice, treatment with 25 V/cm for 30 s was sufficient to achieve a 5 log reduction of *E. coli* O157:H7. Similar results were observed in *S.* Typhimurium and *L. monocytogenes*. The concentration of vitamin C in continuous ohmic heated juice was significantly higher than in conventionally heated juice ($P < 0.05$). The effect of frequency and waveform of alternating current during ohmic heating on electrode corrosion, heating rate, inactivation of foodborne pathogens, and quality of salsa was also investigated. Salsa was treated with various frequencies (60 Hz to 20 kHz) and waveforms (sine, square, and sawtooth) at constant electric field strength of 12.5 V/cm. Electrode corrosion did not occur when the frequency exceeded 1 kHz. The heating rate of the sample was dependent on frequency up to 500 Hz, but there was no significant difference ($P > 0.05$) in the heating rate above 1 kHz. The electrical conductivity of the sample was increased with increasing frequency. At frequency of 60 Hz, square wave produced a lower heating rate than that of sine and sawthooth wave. The heating rate between waveforms was not significantly ($P > 0.05$) different as frequency increased. As frequency increased, the treatment time required to reduce *E. coli* O157:H7 and *S.* Typhimurium to below the detection limit (1 log CFU/g) decreased without affecting product quality. These results suggest that ohmic heating is useful for

inactivation of foodborne pathogens of liquid and solid-liquid food mixtures and have the potential for novel thermal process to control pathogens on food processing.

Keywords: **Continuous ohmic heating; High frequency ohmic heating; Electrode corrosion; Foodborne pathogens; Pasteurization**

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Contents

LIST OF TABLE

LIST OF FIGURES

I. INTRODUCTION

Ohmic heating is a highly attractive technology by which internal heat as a result of electrical resistance occurs rapidly by passing an alternating current through a food product (Zhao and Kolbe, 1998). This technology can provide a uniform temperature distribution with the absence of a cold spot in the product, because both liquid and solid phases can be heated simultaneously (Parrott, 1992). Therefore, it is an ideally suited to thermal processing of solid-liquid food mixture with minimal structural, nutritional, or organoleptic changes and as well as one that is microbiologically safe can be successfully manufactured in a short processing time (Rahman, 1999). The development of a continuous flow ohmic heating system can be open new possibilities for industries interested in continuous and instantaneous sterilization of liquid-particle mixtures (Sastry 1992). However, there is a little information concerning the inactivation of *Escherichia coli* O157:H7, *Salmonella* Typhimurium, and *Listeria monocytogenes* in food products. Therefore, it is required to examine the effect of continuous ohmic heating on inactivation of foodborne pathogens in food product to ensure uniform commercial sterilization.

Although viewed as a promising food processing technology, ohmic heating has also some technical limitation. Most of ohmic heating systems have been used at alternating current frequency of 50-60 Hz. One constraint of low alternating current frequency in ohmic heating is that electrolytic reactions can take place at the electrode surface, leading to product burning and corrosion of electrodes (Goullieux and Panin, 2005; Yonsawadigul et al., 1995). To prevent undesirable electrochemical reactions between electrodes and solid or viscous liquid products, increasing frequency or changing waveform of alternating current has been suggested (Renzik, 1996). There have been some efforts to clarify the impact of frequency and waveform of alternating current on the ohmic heating rate of solid or semi-solid food (Iimai et al., 1995; Lakkakula et al., 2004; Lima et al., 1999). However there is no comprehensive research on the effects of frequency on electrode corrosion, electrical conductivity and microbial inactivation in solid-liquid food mixture. Also, so far, the ohmic heating rate at different waveforms (sine, square, sawtooth wave) above 60 Hz was not studied. Therefore, it is required to examine the effect of frequency and waveform on the heating rate of food products to application of commercial ohmic sterilization.

Orange juice, tomato juice and salsa were selected as a model system to investigate the effects of continuous flow ohmic heating system and high frequency ohmic heating technology on food products, respectively. Fruit and vegetable juices were not recognized until recently as vehicles of foodborne illness because of their acidity (Mazzotta 2001). But recently, several outbreaks of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* have occurred in fruit and vegetable juices. In the United States 21 juice-associated outbreaks of human illness were reported to the CDC (Center for Disease Control and Prevention) between 1995 and 2005 (Vojdani et al. 2008). Salsa also presents a potential public health hazard because of the increased tolerance of foodborne pathogens to low pH food products (Raghubeer et al., 2000). A large multistate outbreak with more than 1,400 cases of *Salmonella* spp. infection associated with multiple raw produce items such as jalapeño peppers and tomatoes occurred in the United States, July 2008 (CDC, 2008).

The objective of this study was to evaluate the efficacy of continuous ohmic heating for inactivating *E. coli* O157:H7, *S.* Typhimurium, and *L.* monocytogenes in orange and tomato juice with various treatment times and electric field strengths. Additionally, we investigated the quality of orange juice and morphological changes in the cell after continuous ohmic and conventional heating treatment. Also, the effect of various frequencies and waveforms of alternating current on ohmic heating for inactivating *E. coli* O157:H7 and *S.* Typhimurium in salsa as well as electrode corrosion, heating rate, electrical conductivity, and quality of salsa were investigated.

II. MATERIALS AND METHODS

2.1. Effect of continuous ohmic heating to inactivate foodborne pathogens in orange and tomato juice

2.1.1. Bacterial strains

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 15315, ATCC 19114, ATCC 19115) were obtained from the Food Science and Human Nutrition culture collection at Seoul National University (Seoul, Korea). Stock cultures were stored in 0.7 ml of tryptic soy broth (TSB; Difco, Becton Dickinson, Sparks, MD, USA) with 0.3 ml of 50% glycerol at –80 °C prior to use. Working cultures were streaked onto tryptic soy agar (TSA; Difco), incubated at 37 °C for 24 h, and stored at 4 °C.

2.1.2. Preparation of cell suspension

All strains of *E. coli* O157:H7, *S.* Typhimurium, and *L.* monocytogenes were cultured individually in 5 ml TSB at 37 °C for 24 h, collected by centrifugation at 4000 × g for 20 min at 4 °C and washed three times with buffered peptone water (BPW; Difco, Sparks, MD). The final pellets were resuspended in BPW, corresponding to approximately 10^7-10^8CFU/ml. To inoculate orange juice, suspended pellets of the three strains of all species were combined to construct a culture cocktail. These cell suspensions were used in subsequent experiments.

2.1.3. Sample preparation and inoculation

Pasteurized single-strength orange juice (pH 3.94, 11.8 °Brix) and tomato juice (pH 4.14, 11.2 °Brix) were purchased at a local supermarket (Seoul, Korea). The sodium content of orange and tomato juice was 2.86 mg/100ml and 26.67 mg/100ml, respectively. Ten milliliters of the mixed culture cocktail (*E. coli* O157:H7, *S.* Typhimurium, and *L.* monocytogenes) were inoculated into three liters of juice at room temperature (22±2 °C) and mixed with a magnetic stir bar for 3 min. The final cell concentration was 106-107 CFU/ml. Inoculated juice samples were then immediately treated with continuous ohmic heating.

2.1.4. Continuous ohmic heating system

The experimental device (Fig. 1) consisted of a continuous ohmic heating chamber, a power supply, a data logger (34790A, Agilent Technologies, Palo Alto, CA), a product tank, and a volumetric pump (JWS600, Jeniewell, Seoul, Korea). The continuous ohmic heating chamber was composed of polyvinyl chloride (PVC) pipe of 0.51cm thickness, PVC caps, and two titanium plate electrodes. In order to reduce thermal loss and evaporation, both ends of the heating chamber were covered with PVC caps. The distance between the two electrodes was 2 cm, and the cross-sectional area was 120 cm^2. The product flows along the axis between the electrodes. Temperatures were monitored using a K-type thermocouple, placed at the exit and the geometric center of the chamber. The temperature of each sample was nearly uniform in the chamber, since the maximum difference among the measured temperatures at different locations was approximately within 1 °C. The supplied power in the chamber was alternating current at 60 Hz, and voltage was controlled by a variable transformer with a range of 100 to 200 V. Temperature, voltage, current, and time were recorded at 5 s intervals by a data logger linked to a computer.

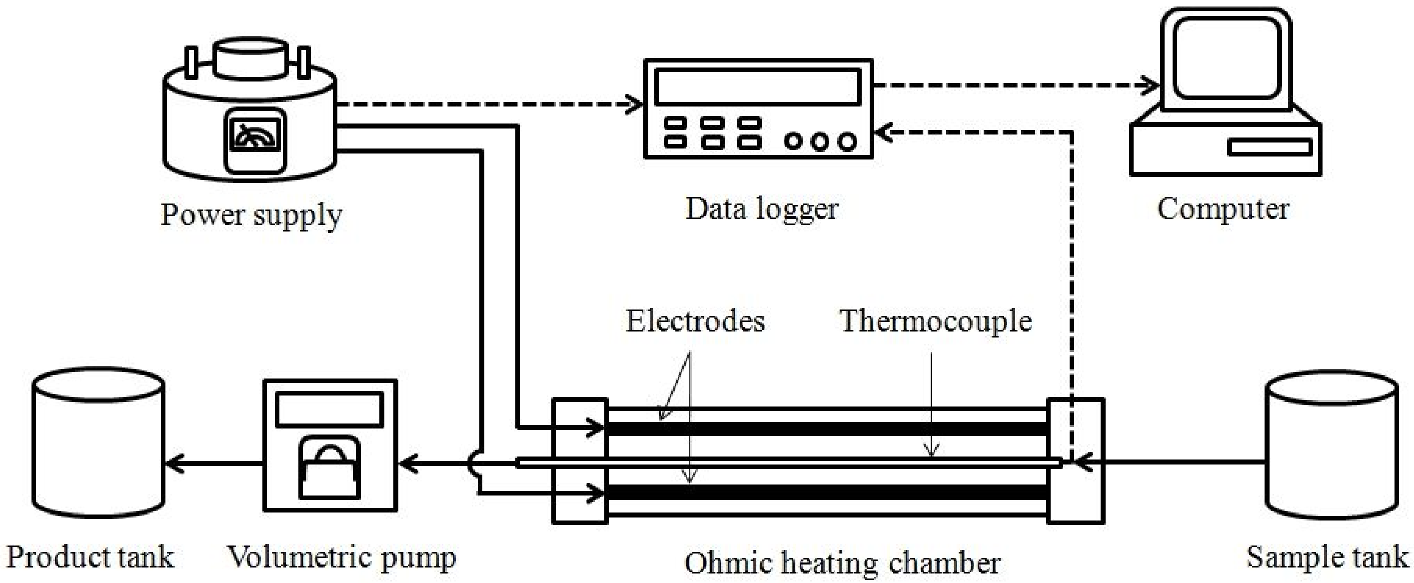


Fig. 1. Schematic diagram of the continuous ohmic heating system at Seoul National University (Seoul, Korea)

2.1.5. Continuous ohmic heating treatment

For the continuous ohmic heating treatment, 3 L of inoculated juice was placed in a 3 L product tank. The sample was pumped to the ohmic heating chamber by a volumetric pump and flow rate was maintained at 120 ml/min. When the sample passed through the exit of the chamber, power was then turned on and ohmic heating applied. Four different electric field strengths (25, 30, 35, and 40 V/cm) were applied to the sample with various treatment times. Following heat treatment, samples were taken at 30 to 60 s intervals for up to 300 s and immediately immersed in ice-water until the samples were cooled to 4 °C. To enumerate surviving pathogens, 10-fold serial dilutions were performed in buffered peptone water, and samples or diluents were spread plated onto selective medium. *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* were enumerated on Sorbitol MacConkey Agar (SMAC; Difco), Xylose Lysine Desoxycholate Agar (XLD; Difco), and Oxford Agar Base (OAB; Difco) with antimicrobic supplement (BactoTM Oxford Antimicrobic Supplement, Difco), respectively. When low levels of surviving cells were anticipated, 250 μl of sample were spread-plated onto each of four plates. All plates were incubated at 37 °C for 24-48 h before counting.

2.1.6. Conventional heating treatment

In order to achieve identical thermal histories of ohmic heating and conventional heating, the conventional heating conditions were selected to be the same heating profile as in continuous ohmic heating treatment with 30 V/cm of electric field strength. For conventional heating treatment, pasteurized single-strength orange juice (250 ml) purchased at a local supermarket was transferred into a clean 500 ml conical flask (Duran, Schott) and was processed at 150 °C in an oil bath (OSB-2000, EYELA, NY, USA) for 180 s. To obtain a uniform temperature, the samples were stirred with glass rod by hand while heating treatment. A data logger was used to measure the juice temperature during the experiments.

2.1.7. Transmission electron microscopy

To obtain transmission electron microscopy (TEM) electron micrographs, *E. coli* O157:H7 cells in orange juice were rinsed three times with 0.1 M phosphate buffered saline (PBS) and collected by centrifugation at 4000×g for 10 min. The pellet was fixed in modified Karnovsky's (2% paraformaldehyde and 2% glutaraldehyde in 0.05M sodium cacodylate buffer) at 4 °C for 2-4 hr. After the primary fixation, cells were centrifuged and washed three times with 0.05M sodium cacodylate buffer. The cells were then fixed in 1% osmium tetroxide in 0.05M sodium cacodylate buffer (pH 7.2) at 4 °C for 2 hr and washed two times briefly with distilled water. The cells were then dehydrated using a graded ethanol series of 30, 50, 70, 80, 90, and three times at 100% for 10 min each. After dehydration, the cells were placed in 100% propylene oxide at 4 °C for 15 min and infiltrated using solutions of propylene oxide and Spurr's resin. A 1:1 solution of propylene oxide and Spurr's resin was placed on the cells for 2 hr and then placed in Spurr's resin overnight. Infiltrated samples were polymerized at 70 °C for 24

h. The samples were sectioned (slices 70 nm in thickness) using an ultramicrotome (MT-X; RMC, Tucson, AZ) and then stained with 2% uranyl acetate for 7 min and Reynold's lead citrate for 7 min. The sections were examined by TEM (LIBRA 120; Carl Zeiss, Heidenheim, Germany) and digitally photographed.

2.1.8. Color measurement

Color was measured using a Minolta colorimeter (model CR400, Minolta Co., Osaka, Japan). The values for *L*, *a*, and *b* were recorded to evaluate color changes of continuous ohmic heating and conventional heating treated juice. Pasteurized single-strength orange juice purchased at a local supermarket used as the control. A 7 ml sample was placed in the bottom half of a glass petri dish. The measuring head of the colorimeter was placed directly into the sample. The parameter *L* is a measure of lightness, *a* is an indicator of redness, and the parameter *b* is a measure of yellowness. All measurements were carried out in triplicate.

2.1.9. Vitamin C measurement

Ascorbic acid concentration in juice was determined using High-Performance Liquid Chromatography (HPLC) (Ultimate 3000, Dionex, Sunnyvale, CA) equipped with an autosampler and an UV detector set at 265 nm. A reversed-phase C18 column (5 μm particle size, 4.6 mm diameter, 250 mm length, Dionex) was used to separate the ascorbic acid using 50mM Potassium phosphate buffer and acetonitrile (95:5, v/v) as a mobile phase. The mobile phase was filtered using a 0.45 μm membrane filter (Micron separations Inc., Westboro, MA) and degassed via vacuum before being used on the column. A flow rate of 0.8 ml/min was employed, and retention time was 3.7 min. A standard calibration curve was obtained by using L-ascorbic acid (Sigma Chemical Co., St. Louis, MO) in concentrations ranging from to 5 to 80 mg/100 ml. The samples were centrifuged at 12500×g for 10 min in a Beckman Microfuge E (Beckman Instruments Inc., Palo Alto, CA) to remove pulp and coarse cloud particles. Ten microliters of supernatant was injected into the column using the HPLC autosampler.

2.1.10. Statistical analysis

All experiments were duplicate-plated and replicated three times. All data were analyzed by analysis of variance using the ANOVA procedure of Statistical Analysis System (SAS Institute, Cary, NC, USA) and means value were separated using Duncan`s multiple range test. Significant differences in the processing treatments were determined at a significant level of $P = 0.05$.

2.2. Effect of frequency and waveform on inactivation of foodborne pathogens in salsa by ohmic heating

2.2.1. Bacterial strains and cell suspension

Three strains each of *E. coli* O157:H7 (ATCC 35150, ATCC 43889, ATCC 43890) and *S.* Typhimurium (ATCC 19585, ATCC 43971, DT 104) were obtained from the Food Science and Human Nutrition culture collection at Seoul National University (Seoul, Korea). All strains of *E. coli* O157:H7 and

S. Typhimurium were cultured individually in 5 ml TSB at 37 °C for 24 h, collected by centrifugation at 4000 × g for 20 min at 4 °C and washed three times with buffered peptone water (BPW; Difco, Sparks, MD). The final pellets were resuspended in BPW, corresponding to approximately 10^8-10^9 CFU/ml. To inoculate salsa, suspended pellets of the three strains of all species were combined to construct a culture cocktail. These cell suspensions were used in subsequent experiments.

2.2.2. Sample preparation and inoculation

Pasteurized tomato-based salsa, pH 4.16, was purchased at a local supermarket (Seoul, Korea). The salsa contained no chemical preservatives and included tomatoes, jalapeño peppers, onions, garlic, and distilled vinegar. For inoculation, 0.2 ml of the mixed culture cocktail (*E. coli* O157:H7 and *S.* Typhimurium) was added to 25 g of salsa at 22±1 °C and mixed with a spatula for 2 min. The final cell concentration was 10^7-10^8 CFU/g. Inoculated salsa samples were then immediately subjected to ohmic heating.

2.2.3 High frequency ohmic heating system

The ohmic heating system (Fig. 2) consisted of a function generator (33210A, Agilent Technologies, Palo Alto, CA, USA), a precision power amplifier (4510, NF Corporation, Yokohama, Japan), a two channel digital storage oscilloscope (TDS2001C, Tektronix Inc., Beaverton, USA), a data logger (34790A, Agilent Technologies, Palo Alto, CA, USA) and a ohmic heating chamber. The function generator produced various wave forms at frequencies from 1 mHz to 10 MHz and a maximum output level of 5 V. The amplifier coupled with a function generator could deliver 1 kW of power and boost signals in the range 45 Hz to 20 kHz to a maximum output of 141 V AC. Amplified output was connected to each side of titanium electrodes in the ohmic heating chamber. When operating the function generator and the amplifier output signals (frequency, voltage and current at the sample) were measured with a two channel digital storage oscilloscope. The ohmic heating chamber was composed of two titanium plate electrodes in contact with the sample and K-type thermocouples inserted at the center of a 2 cm × 15 cm ×

6 cm rectangular Pyrex glass container of 0.5 cm thickness. The distance between the two electrodes was 2 cm, and the cross-sectional area was 60 cm^2. Temperatures were recorded at 1 s intervals by a data logger linked to a computer.

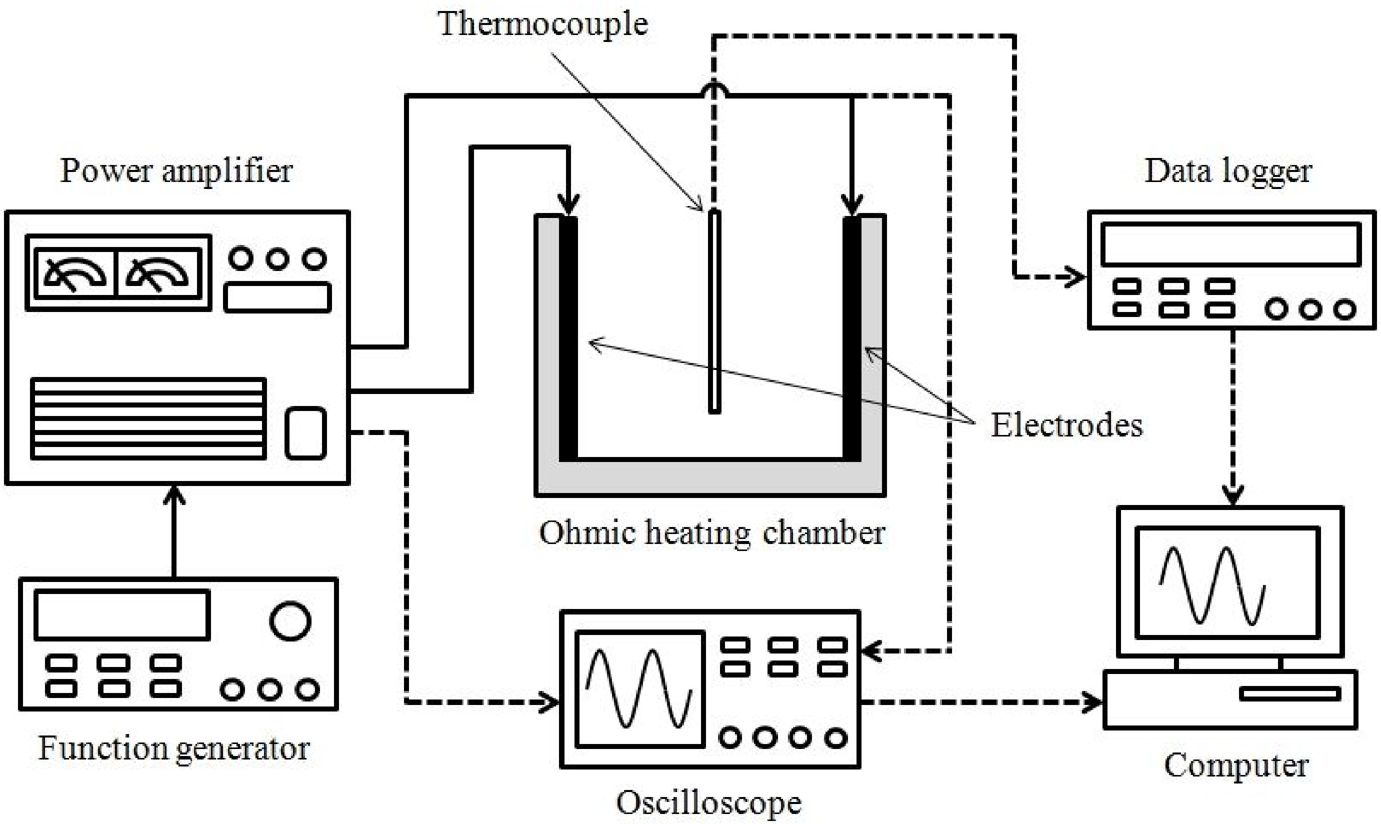


Fig. 2. Schematic diagram of high frequency ohmic heating system at Seoul National University (Seoul, Korea).

2.2.4. Ohmic heating treatment

For the ohmic heating treatment, 25 g of inoculated salsa was placed in the ohmic heating chamber. In all experiments, the electric field strength was fixed at 12.5 V/cm. Seven frequencies (60, 100, 300, 500, 1 k, 10 k, and 20 k Hz) and three wave forms (sine, square, and sawtooth) were applied to each sample and heated to 90 °C. At selected time intervals, the 25 g treated sample were immediately transferred into sterile stomacher bags (Labphas Inc., Sainte-Juilie, Quebec, Canada) containing 225 ml of BPW and homogenized for 2 min with a stomacher (EASY MIX, AES Chemunex, Rennes, France). After homogenization, 1 ml aliquots of sample were serially diluted in 9 ml of BPW, and 0.1 ml of sample or diluent was spread-plated onto each selective medium. *E. coli* O157:H7 and *S.* Typhimurium were enumerated on Sorbitol MacConkey Agar (SMAC; Difco) and Xylose Lysine Desoxycholate Agar (XLD; Difco), respectively. All plates were incubated at 37 °C for 24-48 h before counting.

2.2.5. Electrical conductivity measurement

Electrical conductivity of samples was determined from voltage and current data (Palaniappan and Sastry, 1991) and calculated as follows:

$$\sigma = \frac{L}{AR} \tag{1}$$

where σ is electrical conductivity (S/m), L is distance between electrodes (m), A is area of cross-section of the electrodes (m^2) and R is resistance of the sample (Ω).

2.2.6. Analysis of electrode corrosion

Scanning electron microscopy (SEM) was performed to obtain information regarding changes of electrode surfaces due to electrode corrosion. Surfaces of the electrodes after ohmic heating treatment were examined using a Field-Emission SEM (SUPRA 55VP; Carl Zeiss, Jena, Germany). Concentrations of Ti (from the titanium electrodes) migrating into the salsa sample were taken as measures of electrode corrosion. In each experimental run, once ohmic heating was completed, a 1 g sample was collected into a polypropylene sample bottle, and then stabilized by adding 30 ml of concentrated nitric acid (60%, v/v). An unheated sample in the ohmic heating chamber was used as a blank. Quantitative analyses of the metal ions were performed by an inductively coupled plasma–mass spectrometer (ICP–MS; 820-MS, Varian, Australia).

2.2.7. Color and pH measurement

Color was measured using a Minolta colorimeter (model CR400, Minolta Co., Osaka, Japan). Color values for L*, a*, and b* were recorded to evaluate color changes of salsa subjected to ohmic heating at different frequencies. Measurements were taken from treated and untreated uninoculated samples taken at three different locations and averaged. L*, a*, and b* values indicate color lightness, redness and yellowness of the sample, respectively. The pH was measured with a pH meter (Seven multi 8603;

Mettler Toledo, Greifensee, Switzerland).

2.2.8. Lycopene and Vitamin C measurement

The total lycopene content was measured spectrophotometrically following the method performed by Davis et al. (2003). This method determines the content of lycopene and other derivates such as hydroxy lycopene and lycopene epoxides. Salsa samples (0.6 g) were extracted with a mixture of 5 mL 95% ethanol, 5 mL 0.05% BHT in acetone, and 10 mL hexane on ice for 15 min. After 3 mL of distilled water was added to the sample extract, the mixture was shaken and separated into two layers. The absorbance of the upper, hexane layer was measured spectrophotometrically using a Beckman DU@ series 68 spectrophotometer (Beckman Instruments, Inc., Fullerton, California, USA) with 1 cm square cuvettes at 503 nm against a hexane blank. The amounts of lycopene in salsa were then estimated using the absorbance at 503 nm and the sample weight (Beerh and Siddappa, 1959; Fish et al., 2002).

Ascorbic acid concentration in salsa was determined using High-Performance Liquid Chromatography (HPLC) (Ultimate 3000, Dionex, Sunnyvale, CA) equipped with an autosampler and an UV detector set at 265 nm. A reversed-phase C18 column (5 µm particle size, 4.6 mm diameter, 250 mm length, Dionex) was used to separate the ascorbic acid using 50 mM Potassium phosphate buffer (pH 7.2) and acetonitrile (95:5, v/v) as a mobile phase. The mobile phase was filtered using a 0.45 µm membrane filter (Micron separations Inc., Westboro, MA) and degassed via vacuum before being applied to the column. A flow rate of 0.5 ml/min was employed, and retention time was 3.7 min. A standard calibration curve was obtained by using L-ascorbic acid (Sigma Chemical Co., St. Louis, MO) in concentrations ranging from to 5 to 80 mg/100 ml. The samples (5 g) were homogenized with 10 mL of 5% (62.5 mM) metaphosphoric acid with a stomacher at a medium speed for 1 min. The homogenate was centrifuged at 12,000g for 10 min at 5 °C. The supernatant was vacuum-filtered through Whatman No. 1 paper and then passed through a Millipore 0.45 µm membrane. Ten microliters of supernatant was injected into the column using the HPLC autosampler.

2.2.9 Statistical analysis

All experiments were duplicate-plated and replicated three times. All data were analyzed by analysis of variance using the ANOVA procedure of the Statistical Analysis System (SAS Institute, Cary, NC, USA) and means 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. RESULTS

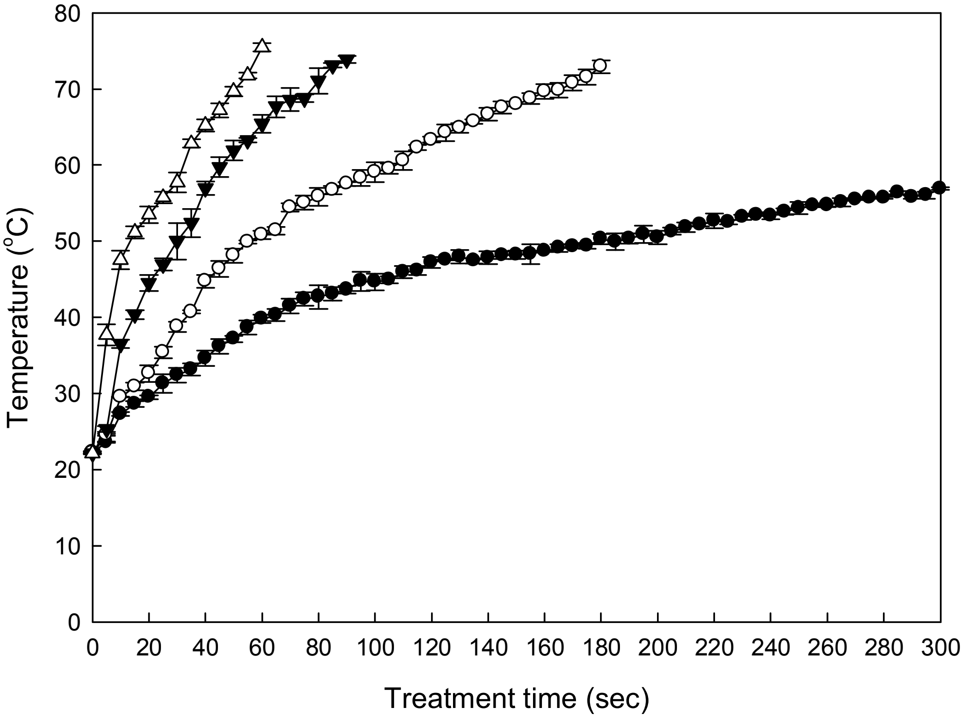
3.1. Effect of continuous ohmic heating to inactivate foodborne pathogens in orange and tomato juice

3.1.1. Temperature curves of orange and tomato juice

Temperatures of orange and tomato juice during continuous ohmic heating treatment with voltage gradients of 25, 30, 35 and 40 V/cm are shown in Fig. 3. The maximum treatment time of orange juice, at which the 5log reduction started, was determined as 60, 90, and 180 s for 30, 35, and 40 V/cm electric field strengths, respectively (Fig. 3a). At the same electric field strength, temperature increased with increasing treatment time. Heating times decreased as a result of higher heating rates resulting from higher voltage gradients applied. At 25 V/cm electric field strength, the temperature of orange juice did not exceeded 60 °C until 300 s had elapsed. At 40 V/cm, orange juice was heated from room temperature to 75.5 °C in 60 s. For the same voltage gradients, the maximum treatment time of tomato juice was determined as 30, 55, 65, and 100 s (Fig. 3b). The rate of temperature change for tomato juice was higher than for orange juice at all voltage gradients applied. Unlike orange juice, the temperature of tomato juice reached 76 °C at both 25 V/cm and 40 V/cm electric field strengths after 100 s and 30 s, respectively.

A comparison of the thermal histories of orange juice subjected to continuous ohmic heating with 30 V/cm of electric field strength and conventional heating is shown in Fig. 4. For both conventionally and continuous ohmic heated samples had a similar temperature profiles. Continuous ohmic heating has more rapid and uniform temperature profile than conventional heating. Conventional heating had a little longer lag period and time to reach targeted temperature.

(a)


Temperature (°C)
Treatment time (sec)
0
10
20
30
40
50
60
70
80
0
20
40
60
80
100
120
140
160
180
200
220
240
260
280
300

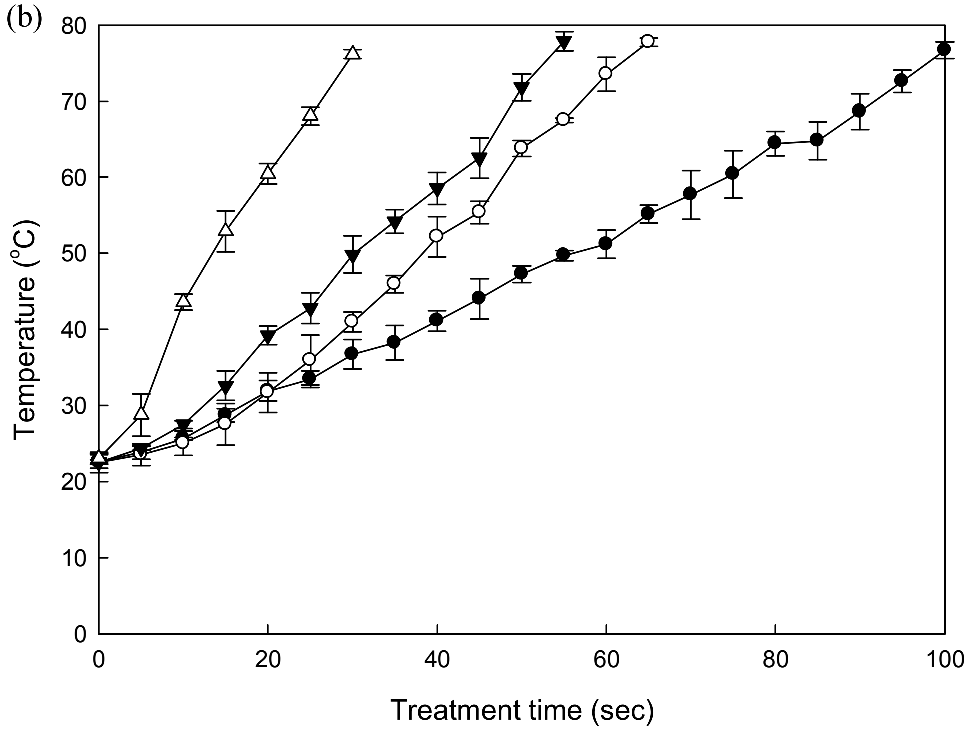


Fig. 3. Temperature curves of orange juice (a) and tomato juice (b) during continuous ohmic heating at different electric field strengths. ●, 25V/cm, ○, 30V/cm, ▼, 35V/cm, △, 40V/cm

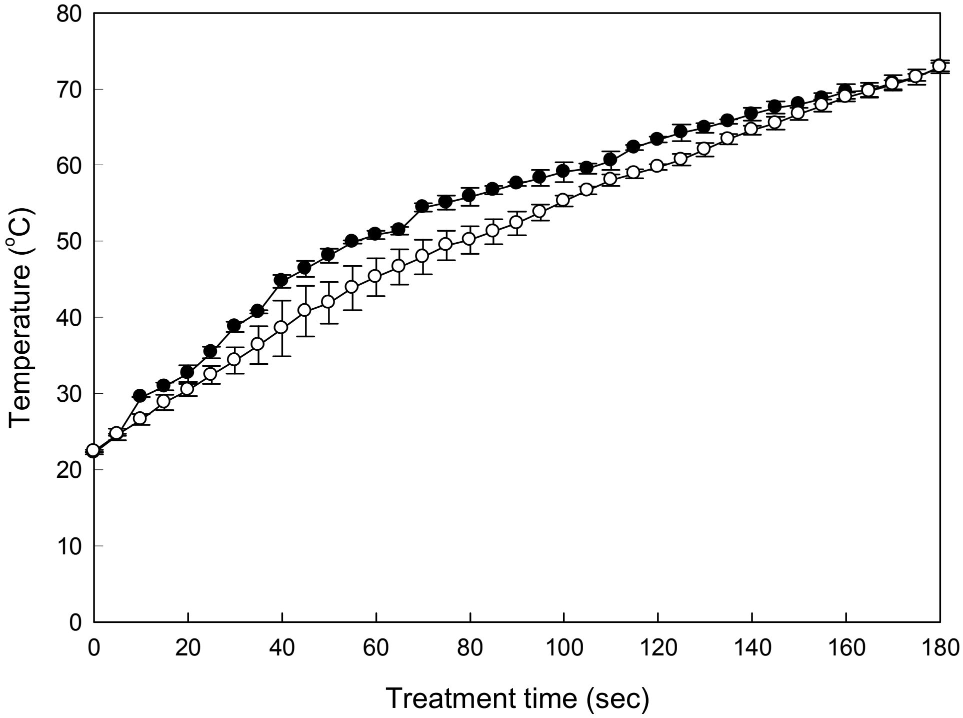


Fig. 4. Temperature curves of orange juice processed by continuous ohmic heating at 30 V/cm (●) and conventional heating (○).

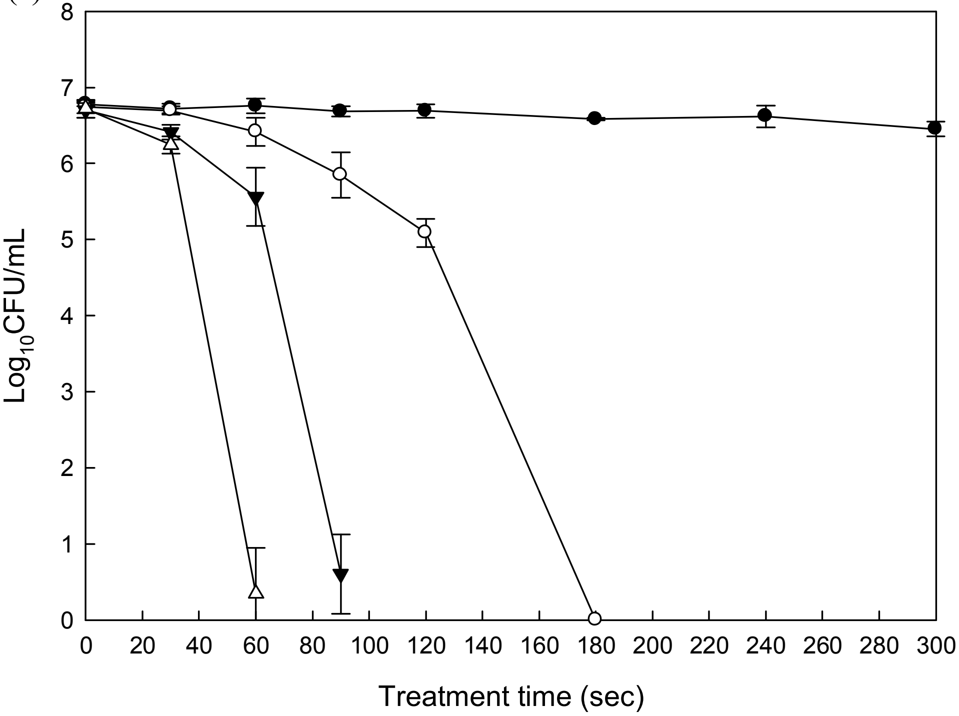
3.1.2. Inactivation of microorganisms in orange and tomato juice after continuous ohmic heating treatment

The survival of *E. coli* O157:H7, *S.* Typhimurium and *L. monocytogenes* in orange juice during continuous ohmic heating is shown in Fig. 5. As electric field strength increased from 25 to 40 V/cm, surviving populations of the three pathogens decreased more effectively. The levels of surviving cells of the three pathogens were reduced by > 5 log within 60 s when treated with 40 V/cm electric field strength. At 35 V/cm, levels of *E. coli* O157:H7 were reduced by 1.14 and 6.1 log CFU/ml after 60 s and 90 s, respectively. Cell numbers of *S.* Typhimurium experienced a significant reduction of 1.32 log CFU/ml after 60 s and > 6.52 log reduction to below the detection limit (<1 CFU/ml) after 90 s treatment. For *L. monocytogenes*, the reduction was 1.23 log CFU/ml after 60 s and 5.1 log CFU/ml after 150 s. At 30 V/cm, the numbers of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* were reduced to below the detection limit after 180 s treatment. However, at 25 V/cm, there were no appreciable differences in microbial levels between controls.

Fig. 6 shows surviving populations of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* from tomato juice treated with the same electric field strengths. Ohmic heating with 40 V/cm electric field strength reduced the three pathogens to below the detection limit (< 1 CFU/ml) after 30 s treatment. At 35 V/cm, *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* were reduced to below undetectable levels after 55 s treatment and 30 V/cm reduced these pathogens to below the detection limit after 65 s. Unlike orange juice, levels of *S.* Typhimurium in tomato juice were greatly reduced to undetectable levels after 100 s treatment at 25 V/cm. Populations of *E. coli* O157:H7 and *L. monocytogenes* decreased by 6.19 and 5.41 log CFU/ml after 100 s, respectively.

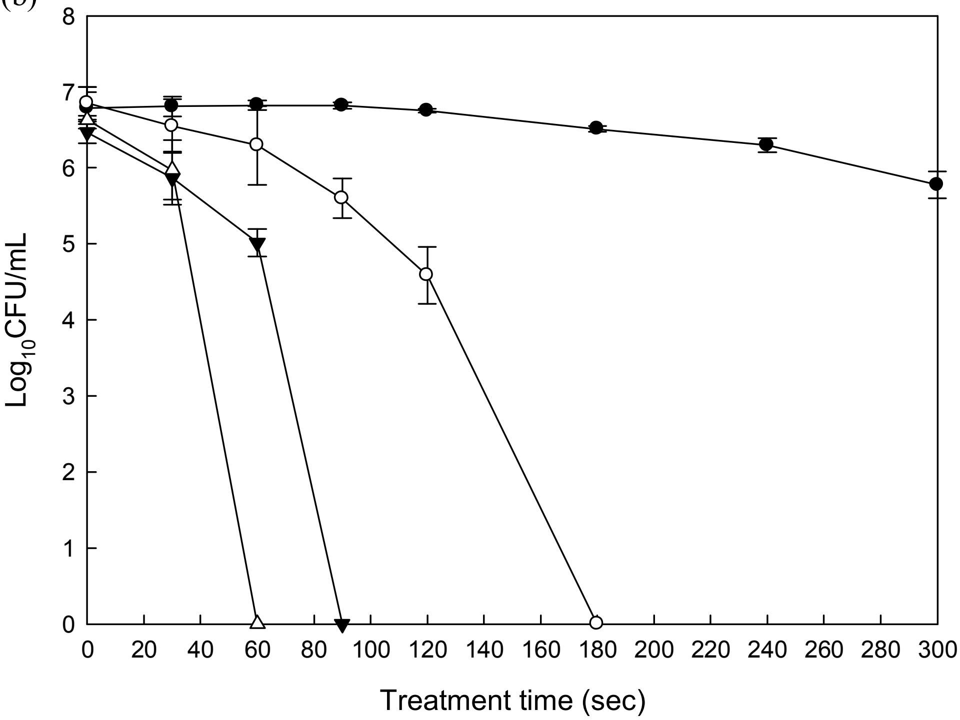
These results indicate that higher electric field strength increases the inactivation of ohmic heating in orange juice and tomato juice. Tomato juice required shorter treatment times to reduce pathogens to below the detection limit compared to orange juice.

(a)



Log10CFU/mL
Treatment time (sec)
0
20
40
60
80
100
120
140
160
180
200
220
240
260
280
300
0
1
2
3
4
5
6
7
8

(b)



Log10CFU/mL
0
1
2
3
4
5
6
7
8
0
20
40
60
80
100
120
140
160
180
200
220
240
260
280
300
Treatment time (sec)

(c)

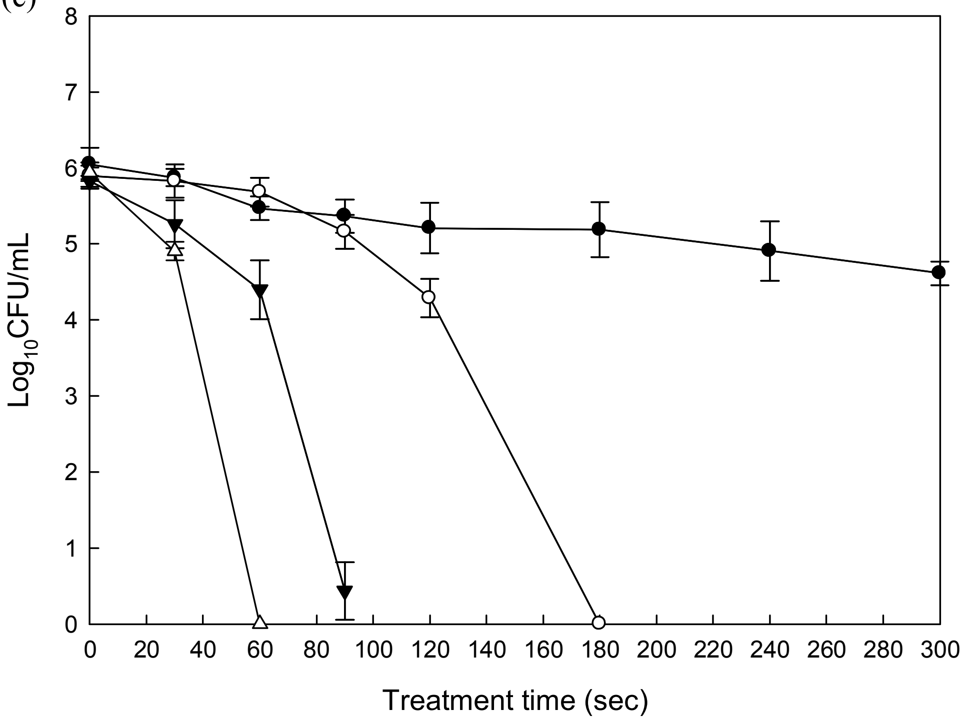
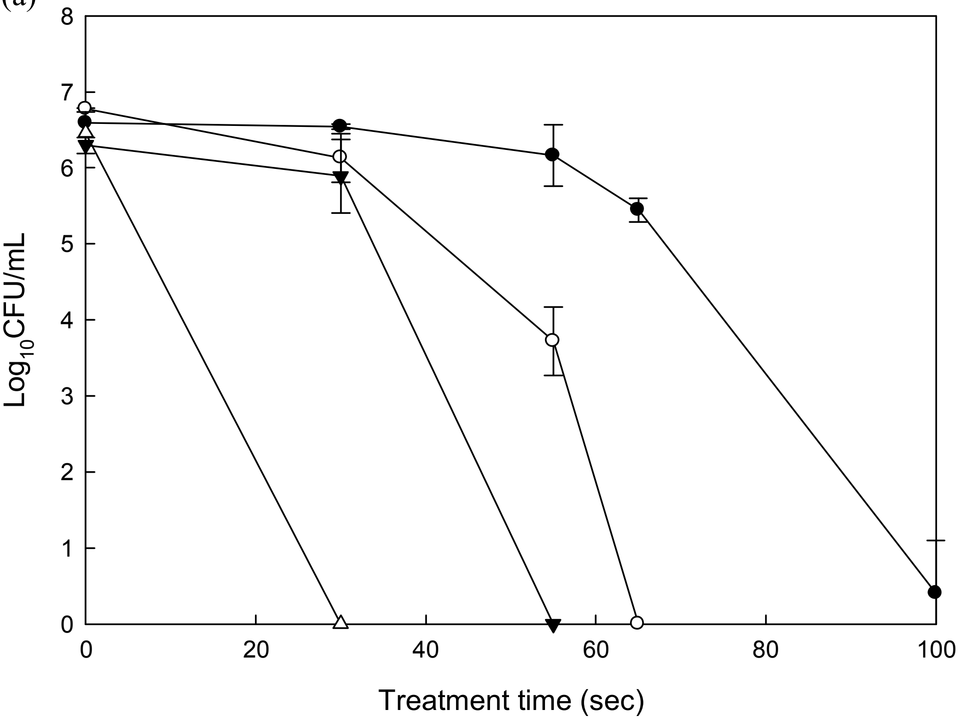
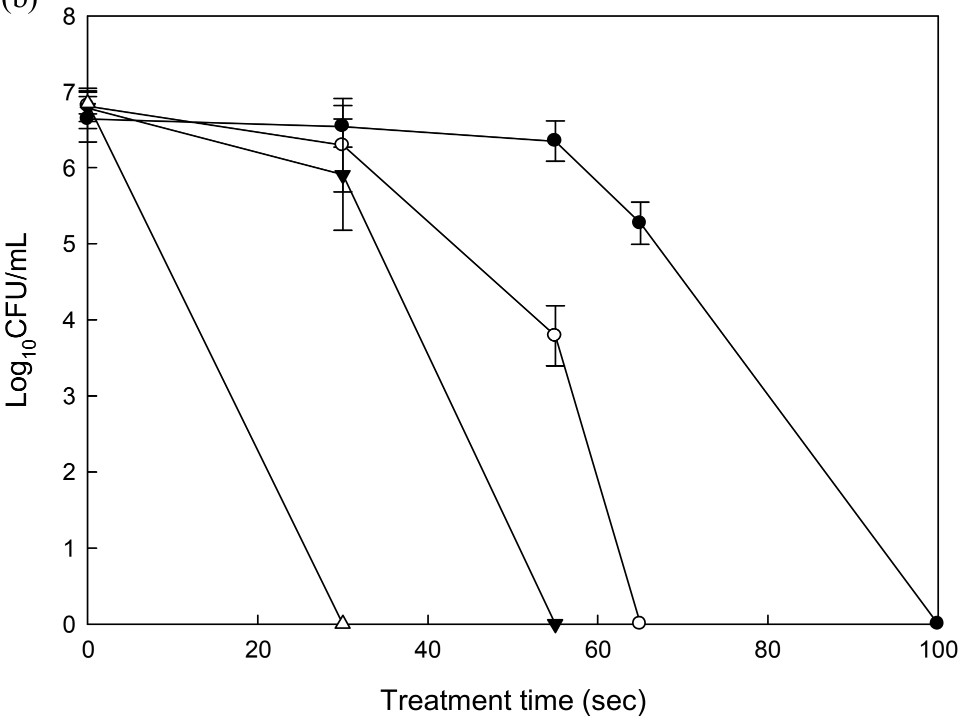


Fig. 5. Survival curves for *E. coli* O157:H7 (a), *Salmonella* Typhimurium (b), and *L. monocytogenes* (c) in orange juice subjected to ohmic heating at 25 V/cm (●), 30 V/cm (○), 35 V/cm (▼), and 40 V/cm (△). The results are means from three experiments, and error bars indicate standard errors.

(a)



(b)



(c)

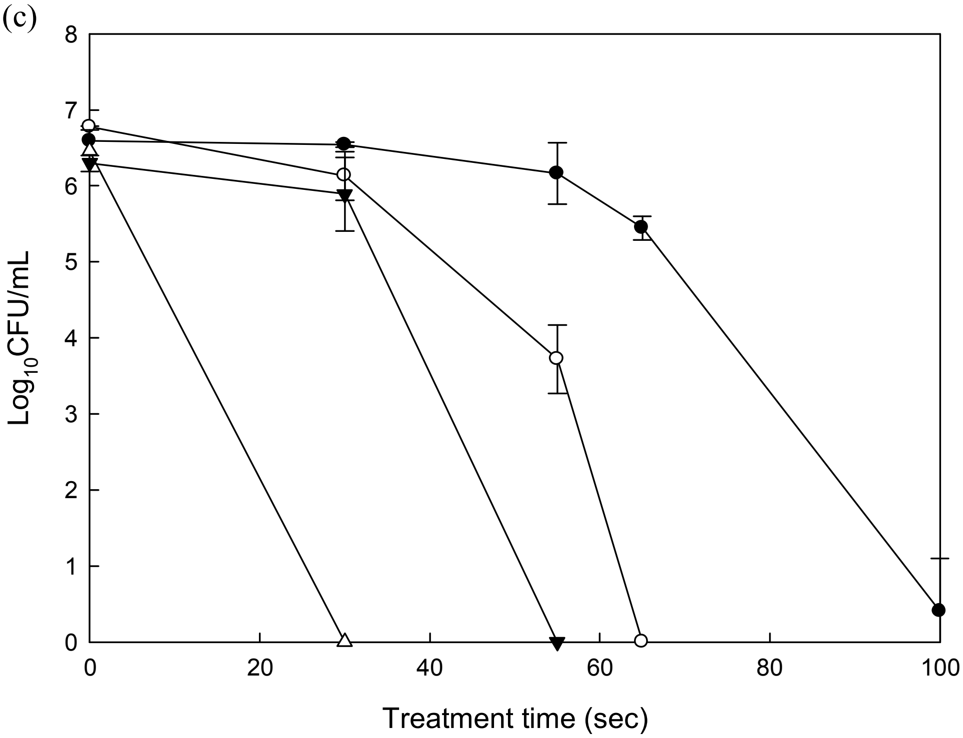


Fig. 6. Survival curves for *E. coli* O157:H7 (a), *Salmonella* Typhimurium (b), and *L. monocytogenes* (c) in tomato juice subjected to ohmic heating at 25 V/cm (●), 30 V/cm (○), 35 V/cm (▼), and 40 V/cm (△). The results are means from three experiments, and error bars indicate standard errors.

3.1.3. TEM examination of inactivated bacterial cell

TEM was used to examine the ultrastructural changes of continuous ohmic heating on *E. coli* O157:H7 cells in comparison with conventional heating. The aggregation of cytoplasm was observed in both continuous ohmic heating and conventional heating treated cells as compared to the untreated cells (Fig. 7). However, *E. coli* O157:H7 cells treated with continuous ohmic heating had undergone significant changes compared to conventionally heated cells. An enlarged periplasmic space and uneven cell wall were observed only in continuous ohmic heating treated cells.

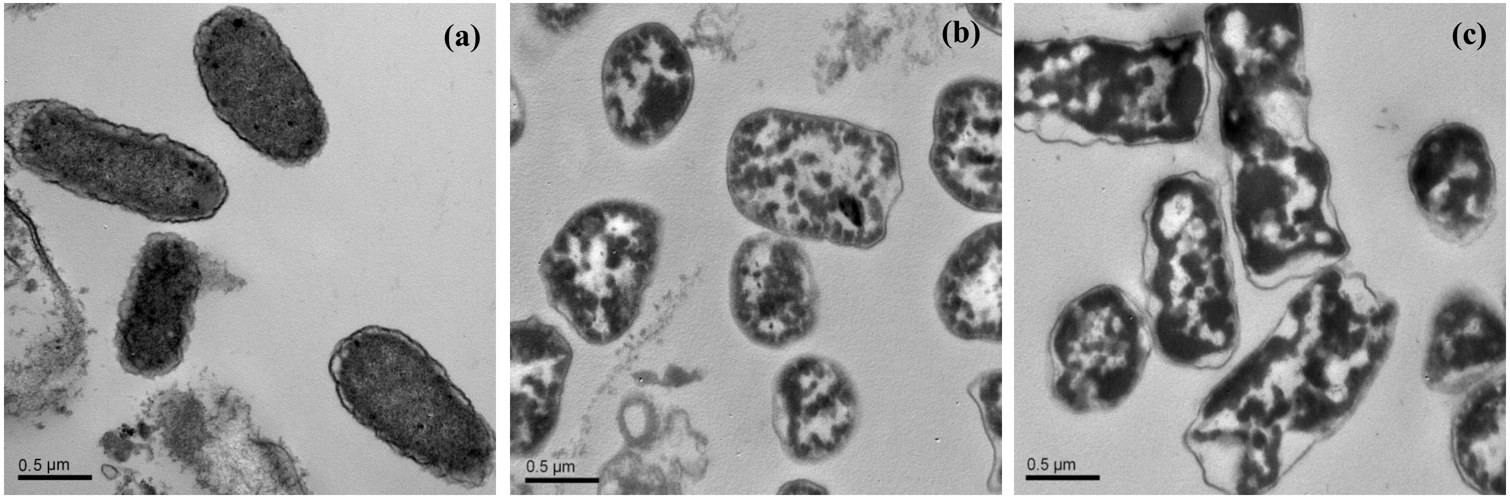


Fig. 7. TEM electron micrographs of *E. coli* O157:H7 in orange juice, untreated (a), conventional heated for 180 sec (b), and continuous ohmic heated at 30 V/cm for 180sec (c).

3.1.4. Effects of continuous ohmic heating on color in orange juice

Color measurements of orange juice after continuous ohmic and conventional heating treatment are shown in Table 1. The results are expressed in L, a, and b values. No significant differences ($P > 0.05$) in L value change were found among orange juice samples subject to any heating treatment. The a value of continuous ohmic heated orange juice was not significantly different ($P > 0.05$) from that of non-treated samples. However, conventional heating was significantly different ($P < 0.05$) and also showed a greater reduction in b value than in orange juice treated with continuous ohmic heating. The b value of orange juice treated with continuous ohmic heating was 29.06, much higher than the value of 26.34 for orange juice heated conventionally. The b value of continuous ohmic heating treated orange juice was much closer to that of the control than was the conventionally heated counterpart.

3.1.5. Influence of continuous ohmic and conventional heating on vitamin

C concentration

Effects of continuous ohmic and conventional thermal treatments of ascorbic acid in orange juice are presented in Table 1. The initial ascorbic acid concentration of orange juice was about 56.88 mg/100ml. Degradation of ascorbic acid was observed in both continuous ohmic and conventionally treated orange juices. However, the concentration of vitamin C in continuous ohmic heated orange juice was significantly higher than in conventionally heated orange juice ($P < 0.05$). These results showed that the destruction of vitamin C was influenced by the method of heating.

Table 1. Color values†, ascorbic acid content of ohmic and conventional heating treated orange juice

Heating method	Color			Ascorbic acid (mg/100ml)
	L	*a*	*b*	
Control	57.0±0.5[a]	-6.6±0.0[a]	32.3±0.2[a]	56.9±0.4[a]
Ohmic	56.1±0.3[a]	-8.2±0.4[ab]	29.1±0.2[b]	42.0±0.8[b]
Conventional	57.5±0.4[a]	-9.9±0.1[b]	26.3±0.1[c]	38.6±0.8[c]

Mean values±standard deviation.

[a-c] Means with same columns followed by different superscript are significantly different (*P<0.05*)

† Color values are *L* (lightness), *a* (redness), and *b* (yellowness).

3.2. Effect of frequency and waveform on inactivation of foodborne pathogens in salsa by ohmic heating

3.2. 1. Analysis of electrode corrosion related to applied frequencies

SEM micrographs of electrode surfaces treated with frequencies ranging from 60 Hz to 20 kHz of sine wave are shown in Fig. 8. Electrode-fouling, the formation of film of food particles on the electrode, occurred in the low frequency range (60 Hz and 100 Hz). As frequency exceeded 300 Hz, electrode fouling diminished. When conducting experiments on the samples at 1 kHz and higher, corrosion on the electrode surface was no longer visible.

The concentration of titanium ions in the sample following ohmic heating at different frequencies was measured (Table 2). When 60 Hz was applied, the migrated concentration of titanium was highest (4.91 mg/kg) among all the treatments. Increasing frequency resulted in lower concentrations of titanium in the sample. The concentration of titanium fell below the detection limit (1 ppb) at higher frequency (above 300 Hz) and in the blank sample.

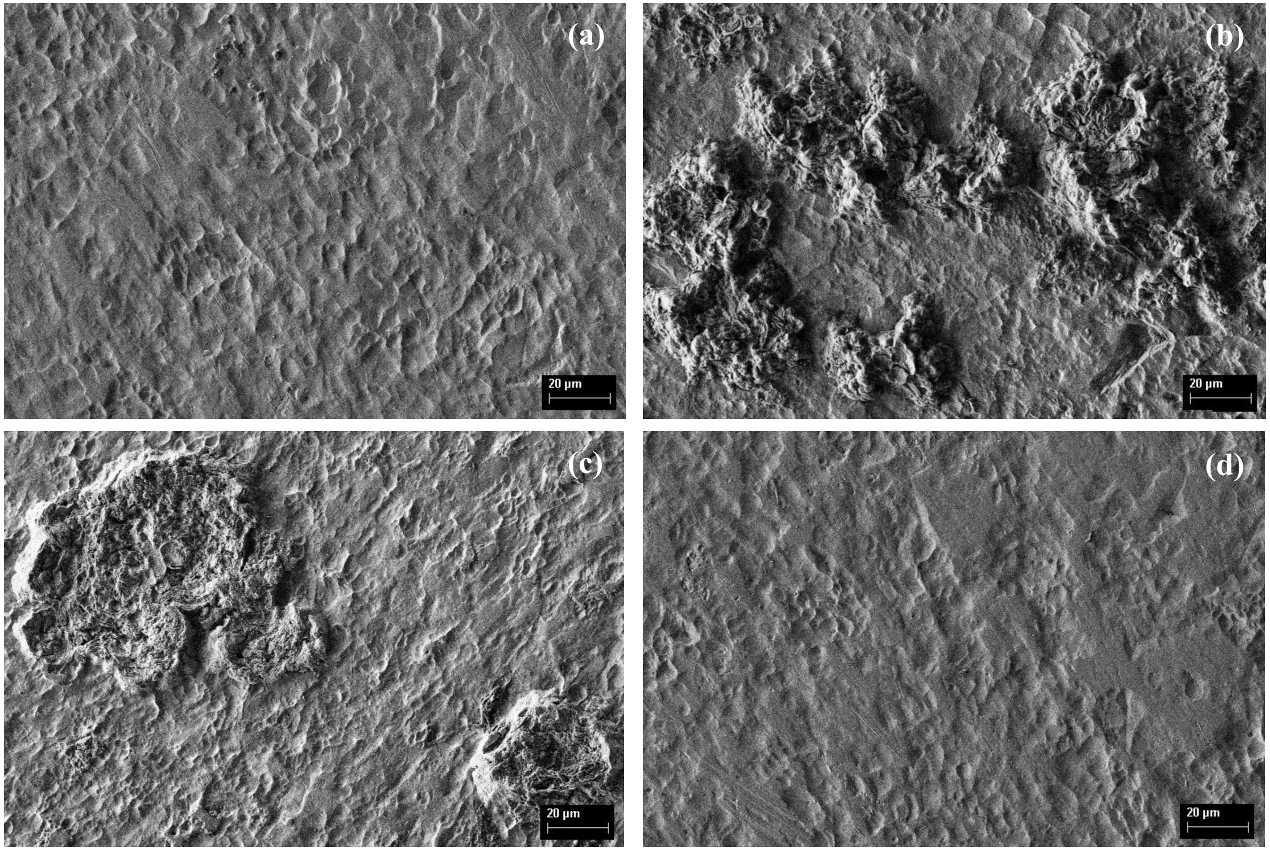


Fig. 8. SEM micrographs of titanium electrode. (a) Untreated electrode. Electrode after ohmic heating treatment with frequency of (b) 60 Hz, (c) 100 kHz, and (d) 1 kHz.

Table 2. The concentration of titanium ions migrated into salsa after ohmic heating treatment at different frequencies[a].

Frequency (Hz)	Titanium (mg/kg)
0	ND[b]
60	4.91±0.88
100	0.16±0.15
300	ND
500	ND
1k	ND
10k	ND
20k	ND

[a] Results were expressed as mean±SD.

[b] ND, below detection limit (1 ppb).

3.2.2. Temperature curves of salsa at different frequencies

The heating rate of salsa during ohmic heating at various frequencies from 60 Hz to 20 kHz of sine wave and a constant voltage gradient of 12.5 V/cm is shown in Fig. 9. At the same frequency, temperature increased with increasing treatment time. Heating times decreased as a result of higher heating rates resulting from higher applied frequencies. The heating rate of salsa was dependent on frequency up to 500 Hz, but there was no significant effect on the heating rate in the range from 1 kHz to 20 kHz ($P > 0.05$). Salsa increased from room temperature (20.0 °C) to 90.9 °C when exposed to frequencies ranging from 1 kHz to 20 kHz for 50 s. At 60 Hz, the temperature of salsa did not exceed 60 °C until 50 s had elapsed.

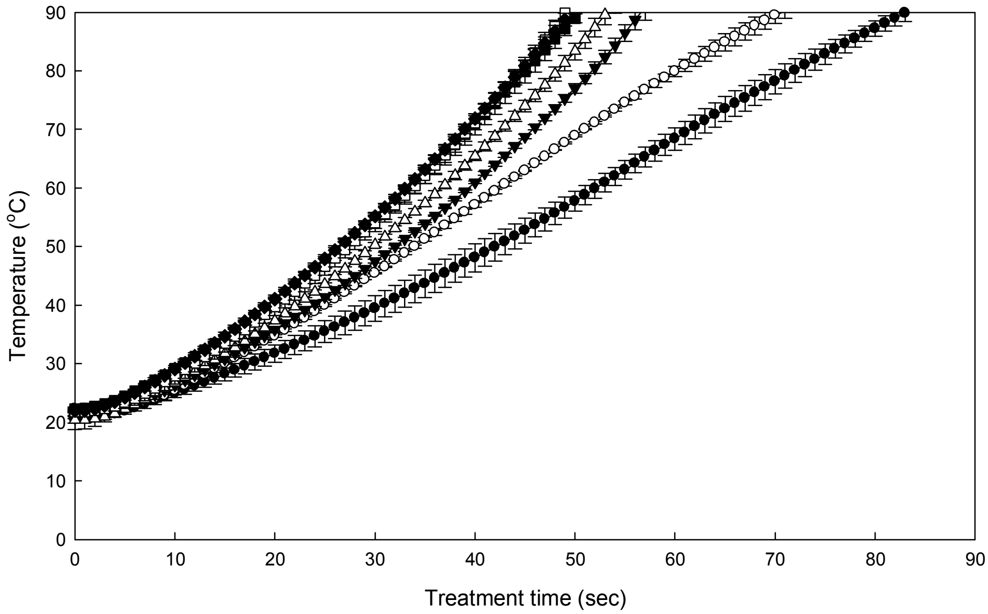


Fig. 9. Temperature curves of salsa during ohmic heating at different frequencies. Frequency levels were 60 Hz (●), 100 Hz (○), 300 Hz (▼), 500 Hz (△), 1 kHz (■), 10 kHz (□), and 20 kHz (◆).

3.2.3. Effect of frequency on electrical properties of salsa

The electrical conductivity curves for salsa subjected to various frequencies from 60 Hz to 20 kHz of sine wave are presented in Fig 10. At the same frequency, electrical conductivity of the sample increased linearly with temperature. As frequency increased, the electrical conductivity of the sample increased. However, no significant difference in electrical conductivity of the sample was observed in the range from 1 kHz to 20 kHz ($P > 0.05$). The electrical conductivity of the sample varied slightly at 60 Hz and 100 Hz although temperature of the sample increased to 90 °C. In the high frequency range (1 kHz to 20 kHz), electrical conductivity of the sample was higher than at the low frequency range (below 500 Hz).

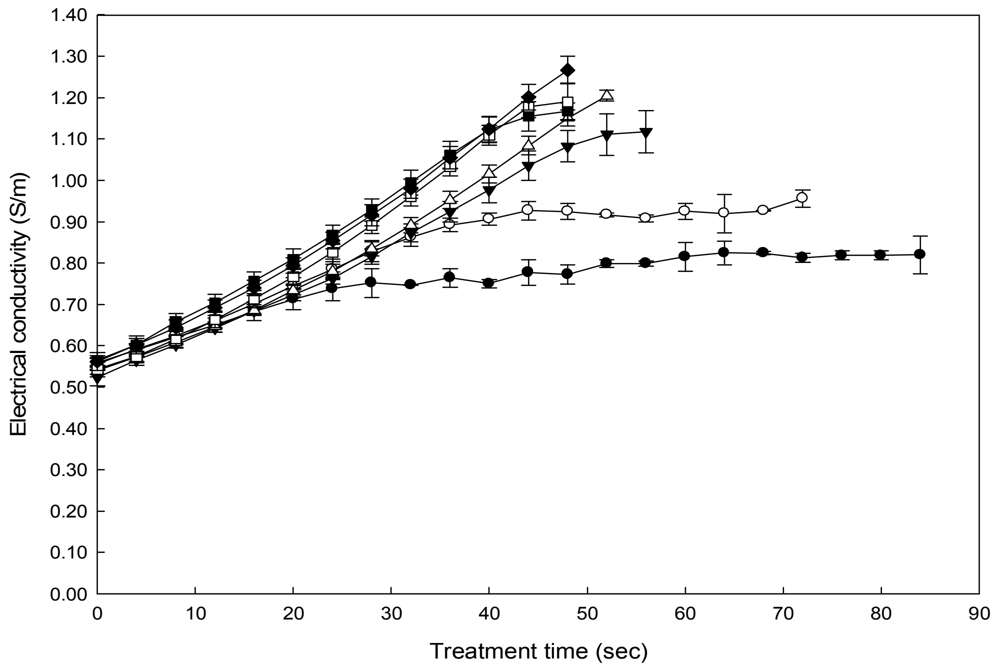


Fig. 10. Electrical conductivity of salsa during ohmic heating at different frequencies. Frequency levels were 60 Hz (●), 100 Hz (○), 300 Hz (▼), 500 Hz (△), 1 kHz (■), 10 kHz (□), and 20 kHz (◆).

3.2.4. Temperature curves of salsa at different waveforms

The comparison of the thermal histories of salsa subjected to ohmic heating with sine, square and sawtooth waves at 60 Hz, 500 Hz, and 20 kHz is shown in Table 3. The heating rate for the square wave was significantly lower than for sine and sawtooth waves at 60 Hz. A significant difference in temperature of the sample was observed starting at 20 s. The mean time taken to reach 90 °C was 95 s for the 60 Hz square wave, while the 60 Hz sine wave and 60 Hz sawtooth wave took 82 s and 75 s, respectively. As frequency increased, the differences in heating rates between waveforms decreased. There was no significant difference in heating rates between the waveforms from 500 Hz to 20 kHz. The sine and sawtooth waveforms had similar heating rates at all frequencies except for 60 Hz.

Table 3. Temperatures of salsa following ohmic heating treatments with different frequency and waveform[a].

Treatment time (s)	Temperature (°C)								
	60 Hz			500 Hz			20 kHz		
	Sine wavefrom	Squre waveform	Sawtooth waveform	Sine wavefrom	Squre waveform	Sawtooth waveform	Sine wavefrom	Squre waveform	Sawtooth waveform
0	20.69±0.56 A	20.66±0.30 A	19.89±0.26 A	19.48±0.30 A	19.21±0.20 A	19.47±0.22 A	21.91±0.23 A	21.76±0.61 A	22.47±0.62 A
5	22.47±0.48 A	22.30±0.62 A	22.31±0.78 A	21.50±0.69 A	20.99±0.23 A	21.28±0.29 A	24.23±0.50 A	24.16±0.02 A	23.58±1.05 A
10	26.02±0.31 A	25.36±0.91 A	26.17±1.40 A	25.15±0.73 A	24.20±0.59 A	25.08±0.32 A	29.09±0.64 A	29.18±0.04 A	28.24±0.75 A
15	30.29±0.23 AB	28.66±0.98 A	30.79±1.41 B	30.01±0.83 A	29.22±0.61 A	29.96±0.30 A	34.68±0.64 A	34.72±0.06 A	34.02±0.67 A
20	34.86±0.10 B	32.28±1.02 A	35.76±1.39 B	35.40±0.48 A	34.82±0.56 A	35.43±0.38 A	41.03±0.66 A	40.82±0.20 A	40.35±0.66 A
25	39.77±0.17 B	36.12±1.11 A	41.06±1.38 B	42.66±0.30 A	41.16±0.54 A	41.48±0.44 A	47.93±0.70 A	47.41±0.43 A	47.31±0.68 A
30	44.88±0.37 B	40.24±1.21 A	46.52±1.26 B	49.31±0.38 A	48.11±0.51 A	48.15±0.64 A	55.17±0.68 A	54.41±0.73 A	54.75±0.52 A
35	49.91±0.60 B	44.41±1.28 A	52.01±1.27 C	56.37±0.55 A	55.52±0.66 A	55.30±0.86 A	63.16±0.79 A	62.44±0.78 A	62.73±0.57 A
40	54.58±0.84 B	48.62±1.30 A	57.16±1.22 C	64.33±0.76 A	63.77±0.92 A	63.37±1.12 A	71.84±0.84 A	71.29±0.87 A	71.45±0.60 A
45	59.16±0.94 B	52.72±1.36 A	62.37±1.09 C	72.81±0.97 A	72.81±1.24 A	72.05±1.46 A	80.85±0.95 A	80.45±1.05 A	80.67±0.77 A
50	63.89±1.11 B	56.66±1.46 A	67.76±1.07 C	82.10±1.42 A	82.67±1.60 A	82.14±1.88 A	90.89±1.10 A	91.01±1.43 A	90.45±0.25 A
55	68.43±1.18 B	60.44±1.30 A	73.21±1.07 C	92.05±1.49 A	92.94±1.10 A	92.16±0.75 A	-	-	-
60	72.90±1.32 B	64.25±1.20 A	79.32±1.04 C	-	-	-	-	-	-
65	77.01±1.38 B	68.02±1.03 A	84.47±1.06 C	-	-	-	-	-	-
70	81.11±1.05 B	71.95±1.15 A	88.80±0.31 C	-	-	-	-	-	-
75	85.09±0.69 B	75.69±1.03 A	92.54±0.73 C	-	-	-	-	-	-
80	89.14±0.50 B	79.23±1.56 A	-	-	-	-	-	-	-
85	92.40±0.13 B	82.61±1.10 A	-	-	-	-	-	-	-
90	-	85.89±0.52 A	-	-	-	-	-	-	-
95	-	90.26±1.05 A	-	-	-	-	-	-	-

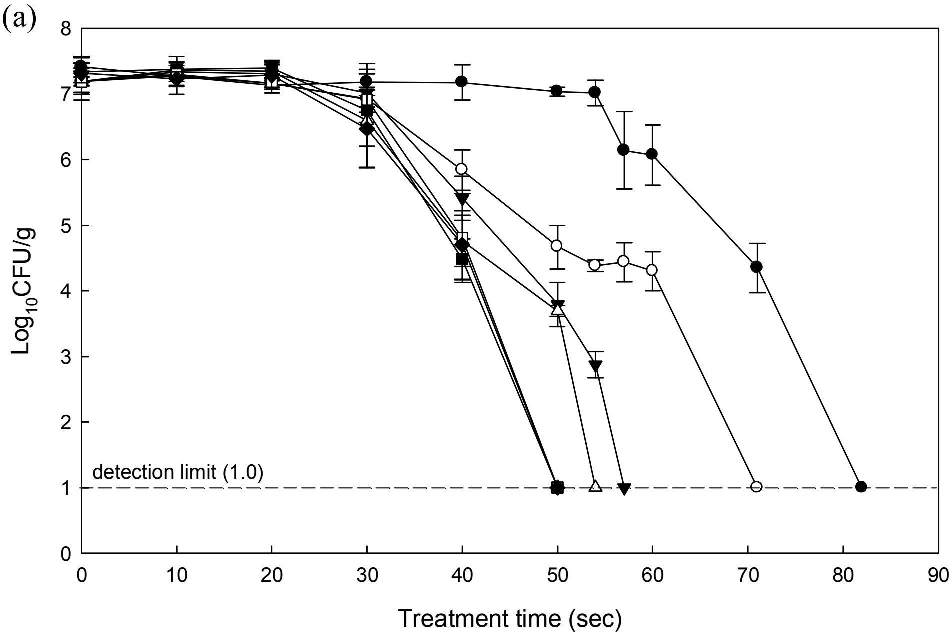
[a] The results were expressed as mean±SD. Values in the same row for the same frequency that are followed by the same uppercase letter are not significantly different ($P > 0.05$).

[b] -, temperature of salsa above 93°C.

3.2.5. Effect of frequency on inactivation of microorganisms in salsa

The survival of *E. coli* O157:H7 and *S.* Typhimurium in salsa during ohmic heating is shown in Fig. 11. As sine wave frequency increased from 60 Hz to 20 kHz, surviving populations of both pathogens decreased more effectively. The levels of surviving cells of both pathogens were reduced to below the detection limit (1 log CFU/g) within 50 s when treated at frequencies of 1 kHz, 10 kHz, and 20 kHz. At 500 Hz, survival of *E. coli* O157:H7 were reduced by 3.50 log CFU/ml after 50 s and to below the detection limit after 54 s treatment. Cell numbers of *S.* Typhimurium experienced a significant reduction of 3.85 log CFU/ml after 50 s and > 6.47 log reduction to below the detection limit after 54 s treatment. At 300 Hz, the numbers of *E. coli* O157:H7 and *S.* Typhimurium were reduced to below the detection limit after 57 s treatment. The levels of *E. coli* O157:H7 and *S.* Typhimurium in the sample were greatly reduced to undetectable levels when treated by 100 Hz and 60 Hz after 71 s and 82 s, respectively.

(a)



Log10CFU/g
Treatment time (sec)
detection limit (1.0)
0
1
2
3
4
5
6
7
8
0
10
20
30
40
50
60
70
80
90

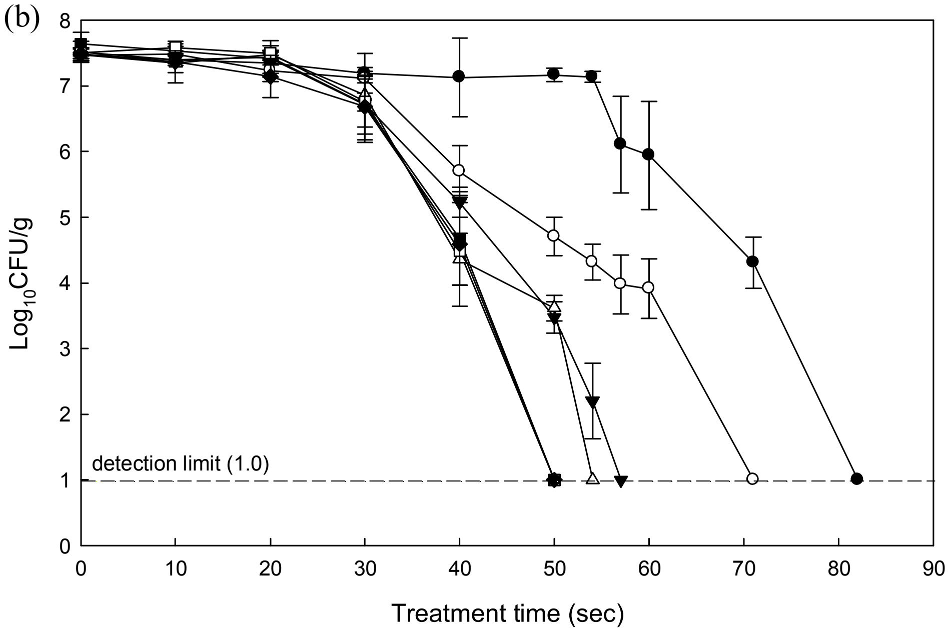


Fig. 11. Survival curves for *E. coli* O157:H7 (a), and *S.* Typhimurium (b), in salsa treated with 60 Hz (●), 100 Hz (○), 300 Hz (▼), 500 Hz (△), 1 kHz (■), 10 kHz (□), and 20 kHz (◆).

3.2.6. Influence of frequency during ohmic heating on color and pH of salsa

Color and pH values of salsa after ohmic heating treatment with sine wave frequencies ranging from 60 Hz to 20 kHz are summarized in Table 4. L*, a*, and b* values of ohmic heating treated samples were not significantly different ($P > 0.05$) from that of non treated samples. There was no significant pH difference between untreated and treated salsa. The pH value of untreated salsa was 4.16±0.01 while the pH of treated salsa was ca. 4.15±0.01. Thus, ohmic heating at various frequencies did not affect the color and pH value of salsa ($P > 0.05$).

3.2.7. Effect of frequency during ohmic heating on lycopene and vitamin C of salsa

The lycopene content of salsa without ohmic heating was 105.04±0.85 mg/kg. The lycopene content of salsa treated at 60 Hz to 20 kHz ranged from

106.43±1.61 to 107.86±1.21 mg/kg. (Table 4). There were no statistically significant differences in lycopene content between untreated and treated salsa ($P > 0.05$). The ascorbic acid content of salsa without ohmic heating treatment was 15% higher than that of the salsa treated at a frequency of 60 Hz, and 19% higher than that of salsa treated at a frequency of 100 Hz ($P < 0.05$), respectively. However, there were no significant differences of ascorbic acid content between salsa treated at frequencies above 300 Hz and that of non-treated salsa ($P > 0.05$).

Table 4. Color values, pH and nutritional content of treated and untreated salsa by ohmic heating at different frequencies [a].

Frequency (Hz)	pH	Color[b] L*	a*	b*	Ascorbic acid (mg/100g)	Lycopene (mg/kg)
0	4.16±0.01 a	31.39±0.46 a	16.85±0.89 a	25.01±0.83 a	16.53±1.26 a	105.04±0.85 a
60	4.16±0.01 a	31.85±0.59 a	17.12±1.16 a	25.00±0.75 a	14.08±0.99 b	107.77±3.06 a
100	4.15±0.01 a	31.61±0.63 a	17.07±0.69 a	24.42±0.49 a	13.42±0.22 b	107.86±1.21 a
300	4.17±0.00 a	31.42±0.35 a	17.03±0.05 a	24.69±0.44 a	14.62±1.69 ab	107.15±1.16 a
500	4.16±0.01 a	31.36±0.50 a	16.74±0.46 a	24.52±0.62 a	14.60±0.41 ab	106.43±1.61 a
1k	4.15±0.01 a	31.43±0.57 a	17.19±0.45 a	24.61±0.14 a	15.04±0.80 ab	106.93±1.32 a
10k	4.15±0.00 a	31.46±0.14 a	16.84±0.07 a	24.71±0.55 a	14.98±1.41 ab	106.79±0.64 a
20k	4.15±0.01 a	31.98±0.33 a	17.23±0.37 a	25.29±0.17 a	15.43±1.85 ab	107.51±2.27 a

[a] The results were expressed as mean±SD. Values in the same column that are followed by the same letters are not significantly different ($P > 0.05$).

[b] Color values are L* (lightness), a* (redness), and b* (yellowness).

IV. Discussion

All three foodborne pathogens in orange and tomato juice were significantly reduced by continuous ohmic heating treatment. Inactivation of microorganisms by ohmic heating is mainly due to the thermal effect and heat is generated instantly inside the food. There was an additional killing effect caused by electrical current itself; several studies have been conducted on this mechanism of inactivation (Sun et al., 2008). Bacterial death due to chemical effects during low voltage electric current treatment might be due to the presence of chloride containing compounds in the treatment medium (Parellieux and Sicard, 1970) or due to the formation of hydrogen peroxide (Shimada and Shimahara, 1982). Palaniappan et al. (1990) reported membrane damage that caused permeability modification and leakage of cellular contents by combination of these chemical factors. Kulshrestha and Sastry (2003) assumed that the electric breakdown or electroporation mechanism of cell membranes by electric current is the predominant nonthermal effect of ohmic heating. In the present study, we examined the morphological change of microorganisms influenced by continuous ohmic and conventional heating using TEM. Although the shrinkage of intracellular materials was observed both continuous ohmic and conventionally heated cells, continuous ohmic heated cell show the development of space between cell wall and the membrane and irregular changes of the cell wall. Yoon et al. (2002) observed that the electroporation caused by the electric field with ohmic heating increased the permeability of the yeast cell wall. The enlarged periplasmic space and the severe loss of cytoplasm in continuous ohmic heated cells could be due to removal of the cell membrane caused by electropermeabilization. Therefore, the TEM electron micrographs verify the destruction of the vegetative cell and suggest that continuous ohmic heating has not only a thermal-lethal effect but also a nonthermal-lethal effect due to the effect of electric current on microorganisms.

The ohmic heating rates of orange and tomato juice increased with increasing electric field strength. This results is in agreement with the heat generation equation $Q = kE^2$. The rate of heating is directly proportional to the square of the electric field strength, E, and electrical conductivity. In addition, the heating rates of tomato juice were observed to be higher than those of orange juice at all electric field strengths. This is thought to result from differences in electrical conductivity and ionic content (e.g. salt) of the juices. Palaniappan and Sastry (1991) concluded that tomato juice had higher heating rates than orange juice at all temperatures since the electrical conductivities of tomato juice were higher than those of orange juice at any given temperature. Wang and Sastry (1993) reasoned that salt concentration provided a strong effect on conductivity and the ohmic heating rate of samples. The effects might be highly dependent on the salt concentration of brine infusion. Since electrical conductivity is influenced by ionic content (Ruan et al., 2000), tomato juice, which has higher a sodium content (26.67 mg/100ml) than orange juice (2.86 mg/100ml) heated faster at all voltage gradients.

Because of different heating rates between juices, the treatment time needed to achieve a 5-log reduction was also different. At 25 V/cm electric field strength, there was no appreciable inactivation of *E. coli* O157:H7 in orange juice after a 300 s treatments, but tomato juice experienced a more than 6-log reduction. Sagong et al. (2011) reported that the ohmic heating treatment time required to achieve a minimum 5-log reduction in tomato juice was from 30 to 60 s faster than in orange juice. Similar results were observed for *S.* Typhimurium and *L. monocytogenes* in our studies. As the electric field strengths increased, the treatment time needed to achieve a 5-log reduction was reduced for all three foodborne pathogens. Baysal (2010) suggested that moderate increases in the voltage gradient seemed to enhance the inactivation effect of *Alicyclobacillus acidoterrestris* spores suspended in orange juice during ohmic heating treatment. In the present study, the most effective treatment condition for pasteurization in both orange and tomato juice was 40 V/cm of electric field strength.

Although there has been no research on inactivation of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* in fruit and vegetable juice by continuous ohmic heating, other researchers have compared conventional and ohmic heating. The effects of continuous ohmic heating and conventional pasteurization on the browning index in orange juice were investigated (Leizerson et al., 2005b). In the present study, we used the Hunter color parameters (*L*, *a*, and *b*) to describe color changes after thermal processing. The lightness (*L*) of orange juice exposed to both heating methods was not significantly different from the control ($P > 0.05$). Small changes in *a* and *b* color coordinates were observed after both heating treatments. However, *a* and *b* color values of continuous ohmic heating treated samples were much closer to that of the control than were the conventionally heated counterparts. In the study performed by Vikram et al. (2005), the ohmic heating method had a higher vitamin C retention compared to other methods, followed by infrared and conventional heating. This result was similar to our data which showed that ascorbic acid concentration decreased by 26% after continuous ohmic heating treatment compared to fresh orange juice and this result is higher to the conventional heating results (32%). These results indicated that the heating method had a definite influence on the color and the contents of vitamin C. The most important aspect of the continuous ohmic heating treatment is the lack of overheating due to rapid and uniform heat transfer aspects and these are may be affecting the quality of orange juice.

The effect of frequency of alternating current during ohmic heating on electrode corrosion, heating rate, inactivation of foodborne pathogens, and quality of salsa was examined. The impact of waveforms of alternating current on the ohmic heating rate of salsa was also investigated. As frequency increased, electrode fouling and migration of titanium ion decreased. At low frequencies (60-100 Hz), formation of adherent surface films on electrodes some blue and violet coloration was observed. Simultaneously, migration of titanium ions from electrodes into the surrounding salsa occurred. These results imply that electrochemical reactions occurred at the electrode surface, leading to product burning and corrosion of electrodes. The electrolytic reactions and electrode corrosion during ohmic heating can be described based on theory in electrical circuit analysis. When voltage is applied to a pair of electrodes, the electrons in the electrolyte transferred to the electrode surface resulting in an increase in thickness of ions (Sawyer et al., 1995). This layer is often called the electrical double layer capacitor as it behaves as a capacitor (Perez, 2004).

The current is utilized to charge the double layer until the threshold voltage equals that of the charging current, which does not produce any chemical reactions or charge transfer at this stage (Rubinstein, 1995). Once the capacitor is fully charged above the threshold voltage, Faradaic current flows and electrochemical reactions occur at the electrode surface (Goullieux and Pain, 2005). Under conditions such as low alternating frequencies, a chain of chemical reactions involving mass transport of electroactive species to the electrode occur and accelerate electrode corrosion (Wang, 2006). On the other hand, with high alternating current frequencies (above 1 kHz) the rapid movement of electric charge periodically reverses direction supplied to the electrode. Thus, the capacitor cannot reach anymore to the threshold voltage because there was no time to fully charge the double layer capacitor (Sawyer et al., 1995). Only the charging current will flow at the electrode surface and electrochemical Faradaic reactions will not start (Perez, 2004). Based on these basic theory it has been shown that corrosion of the electrodes can be limited by applying high frequencies.

Increasing the frequency of alternating current not only reduces electrode corrosion, but also increases the ohmic heating rate of salsa. Frequency can be one of the key parameters for rapid heating by increasing electrical conductivity of salsa. As mentioned earlier, the heat generation of ohmic heating is given by the equation $Q = kE^2$. Thus, the heat generated is increased by increasing electrical conductivity and electric field strength. In this study, electric field strength was fixed at 12.5 V/cm to only examine the impact of frequency during ohmic heating. Our results agree with the equation: more heat was generated as a result of higher electrical conductivity of salsa resulting from higher applied frequencies. In contrast to our results, there have been some studies reported that heating rate increased with decreasing frequency due to the resulting reduction of impedance (Iimai et al., 1998; Lakkakula et al., 2004; Lima et al., 1999). However, such a reduction of impedance which acts as the resistance of several factors in the alternating current circuit can result in more rapid heat generation by increasing the electrical conductivity shown in equation 1. Therefore, ohmic heating at high frequencies is very effective for rapid heating of food products. The waveform of alternating current also has an influence on the heat generation of salsa during ohmic heating. We observed that the heating rate of salsa for a 60 Hz square wave was significantly ($P < 0.05$) lower than for 60 Hz sine and sawtooth waves. This result was in agreement with other researches which showed that the square wave was less efficient than the sine wave at 60 Hz (Lima et al., 1999; Lima and Sastry, 1999). These investigators mentioned that changing of the waveform affected electrical conductivity, and this effect increased as frequency decreased (Lima et al., 1999). For this reason, no significant ($P > 0.05$) difference in the heating rate between the waveforms was observed as frequency increased above 500 Hz. Also, ohmic heating at frequencies above 1 kHz is desirable to prevent electrode corrosion. Therefore, the impact of different waveforms at low frequency range cannot be an important factor in ohmic heating.

The treatment time required to reduce *E. coli* O157:H7 and *S.* Typhimurium to below the detection limit (1 log CFU/g) were decreased as the frequency increased. At a frequency of 60 Hz, 82 s was required to reduce the levels of *E. coli* O157:H7 and *S.* Typhimurium in salsa to undetectable levels, but only 50 s was required at 1 kHz. In addition, there were no significant (P > 0.05) differences in pH, color, and nutritional content between salsa treated at frequencies above 300 Hz and that of non-treated salsa. The significant differences in ascorbic acid content observed in salsa treated at low frequencies (60 and 100 Hz), might be related to severe electrode corrosion as a result of electrochemical reactions. Based on our results, there was no significant (P > 0.05) difference in treatment time to inactivate both pathogens in salsa, and no significant quality differences between untreated salsa and salsa treated at frequencies ranging from 1 kHz to 20 kHz. Therefore, frequencies above 1 kHz offer the most effective treatment condition for inactivating pathogens in salsa.

Our results indicate that ohmic heating treatment can be effectively used to inactivate *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* in orange and tomato juice and control *E. coli* O157:H7, and S. Typhimurium in salsa as well as preventing undesirable electrode corrosion and quality deterioration.

V. Conclusion

This study indicated that continuous ohmic heating treatment can be effectively used to inactivate *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* in orange and tomato juice with higher quality than conventional heating. Also, application of high-frequency (above 1 kHz) ohmic heating leads to effective inactivation of *E. coli* O157:H7, and S. Typhimurium in salsa, as well as preventing undesirable electrode corrosion and quality deterioration. The effect of inactivation depends on applied electric field strength, electrical conductivity, frequency, and treatment time rather than waveform. Therefore, ohmic heating is a very promising alternative technology to control of foodborne pathogens in food products, allowing the processor to obtain products with superior microbiological and organoleptic quality.

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Ⅵ. 국문초록

옴가열 기술은 식품에 교류전류를 통과시켜 식품 자체가 가지고 있는 전기적 저항성에 의해 내부에서 급속하게 열을 발생시키는 기술이다. 그러나 옴가열 기술을 상업적 살균시스템에 적용하는 데는 몇 가지 한계점이 있다. 옴가열 기술의 살균효과에 대한 연구가 미비하였으며, 고체 및 액체상이 혼합되어 있는 식품에 적용할 경우 전류를 공급하는 전극판의 부식문제가 심각하여 균일한 살균이 불가능하다. 본 연구에서는 식품의 열 손상 및 전극판의 부식 문제를 개선하고자 연속식 옴 가열 시스템과 고 주파수 옴가열 시스템을 구축하였다. 25-40 V/cm 의 전기장 세기에 따른 오렌지와 토마토 주스 내 *Escherichia coli* O157:H7, *Salmonella* Typhimurium 과 *Listeria monocytogenes* 의 저감화 효과를 연구하였다. 전기장의 세기가 증가함에 따라 샘플의 가열 속도가 증가하였고, 전기전도도가 높은 토마토 주스가 오렌지

주스보다 빠른 가열속도를 보였다. 전기장의 세기 또는 처리 시간이 증가함에 따라 병원성균의 사멸도 크게 증가하였다. *E. coli* O157:H7 은 40, 35, 30 V/cm 전기장 세기에서 처리한 결과, 각각 60, 90, 180 초의 처리시간 후에 5 log 이상 감소하였다. *S.* Typhimurium 과 *L. monocytogenes* 도 비슷한 결과를 보였다. 연속식 옴가열 처리를 한 주스가 종래의 가열 방법을 이용한 주스에 비해 유의적으로 더 높은 비타민 C 함량을 나타냈다. 또한 옴가열 처리에 있어 교류전류의 주파수와 파형이 전극 부식, 가열속도, 식품유래 병원성균의 저감화 및 고체 식품의 품질에 미치는 효과에 대한 연구를 수행하였다. 전기장 세기는 12.5 v/cm 로 고정한 상태에서, 다양한 주파수 (60-20kHz) 및 파형 (정현파, 구형파, 톱니파)을 살사소스에 처리하였다. 주파수가 증가함에 따라 전극판의 부식이 감소하여 1 kHz 이상에서는 더 이상 전극 부식이 나타나지 않았다. 500 Hz 까지는 주파수가 증가함에 따라 살사의 가열속도가 증가하였으나, 1 kHz

이상에서는 가열속도에 유의적인 차이가 보이지 않았다. 옴 가열의 열 발생에 있어 중요한 요소인 살사의 전기전도도는 주파수가 증가함에 따라 증가하였다. 60 Hz 에서 구형파가 정현파 및 톱니파에 비해 낮은 가열속도를 나타냈다. 하지만 주파수가 증가함에 따라 파형에 따른 가열속도의 유의적인 변화가 나타나지 않았다. 주파수가 증가함에 따라 *E. coli* O157:H7 과 *S.* Typhimurium 을 품질의 손상 없이 검출한계 (1 log CFU/g)이하로 저감화하는데 필요로 하는 시간이 감소하였다. 이러한 결과는 새롭게 구축된 옴 가열 시스템이 식품의 열 손상과 전극판의 부식을 감소시킴으로써, 액체 및 반고체 식품의 병원성균을 저감화 하는데 효과적으로 이용될 수 있음을 보여준다.

주요어: 연속식 옴 가열, 고주파수 옴 가열, 전극부식, 식중독균, 살균

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