A THESIS FOR THE DEGREE OF MASTER OF SCIENCE

Effect of ohmic heating for inactivation of food-borne pathogens in fruit juice

과일 주스 내에서의 병원성 미생물 저감화에 대한 옴가열의 효과

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석사학위논문

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지도교수 강동현

이 논문을 석사학위 논문으로 제출함

2014 년 2 월

서울대학교 대학원 농생명공학부

박 일 규

박일규의 석사 학위논문을 인준함

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ABSTRACT

The effect of electric field-induced ohmic heating for inactivation of *Escherichia coli* O157:H7, *Salmonella* Typhimurium and *Listeria monocytogenes* in buffered peptone water (BPW; pH 7.2) and apple juice (pH 3.5, 11.8 °Brix) was investigated in this study. BPW and apple juice were treated at temperatures (55, 58, and 60°C) and times (0, 10, 20, 25, and 30s) by ohmic heating and compared with conventional heating. The electric field strength was fixed at 30 V/cm and 60 V/cm in BPW and apple juice, respectively. Bacterial reduction resulting from ohmic heating was significantly different ($P < 0.05$) from that of conventional heating at 58 and 60°C in BPW and at 55, 58 and 60°C in apple juice for intervals of 0, 10, 20, 25, and 30s. These results show that electric field-induced ohmic heating led to additional bacterial inactivation at sublethal temperatures. Transmission electron microscopy (TEM) observations and the propidium iodide (PI) uptake test were conducted after treatment at 60°C for 0, 10, 20, 25 and 30s in BPW to observe the effects on cell permeability due to electroporation

caused cell damage. PI values when ohmic and conventional heating were compared were significantly different ($P < 0.05$) and these differences increased with increasing levels of inactivation of three food-borne pathogens. These results demonstrate that ohmic heating can more effectively reduce bacterial populations at reduced temperatures and shorter time intervals, especially in acidic fruit juices such as apple juice. Therefore, loss of quality can be minimized in a pasteurization process incorporating ohmic heating. Additionally, the optimization of ohmic heating conditions for inactivation of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* in 72, 48, 36, 24, and 18 °Brix apple juices was examined in this study. Voltage gradients of 30, 40, 50, and 60V/cm were used in all samples for recording temperatures. In all experiments, the heating rate was most rapid in 36 °Brix apple juice at 30, 40, 50, and 60V/cm. System performance coefficients (SPC) were obtained in 72, 48, 36, 24, and 18 °Brix apple juice at 30, 40, 50, and 60V/cm. For 24 °Brix apple juice, system performance at 30V/cm was the most efficient of all voltage gradients. Also, SPC values between 24 and 36 °Brix apple juice at 30V/cm were not significantly ($P >$

0.05) different, and 36 °Brix apple juice was more effective with respect to product yield. At 60V/cm, peak system efficiency occurred in 48 °Brix apple juice. Although system efficiency of 36 °Brix apple juice at 30V/cm and 48 °Brix apple juice at 60V/cm were significantly ($P < 0.05$) different, the disparity between those values was only 8%. In addition, 5-log reduction was accomplished in 36 °Brix apple juice at 30V/cm for 60s while it was achieved in 48 °Brix apple juice at 60V/cm within 20s. These results demonstrate that treatment of 48 °Brix apple juice at 60V/cm was the most effective combination with regard to economic and bactericidal considerations.

Keywords: **Electropermeabilization; Optimization; Ohmic heating; Food-borne pathogens; Electric field; Sterilization**

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I. INTRODUCTION

Since food-borne outbreaks involving Escherichia coli O157:H7 and Salmonella Typhimurium have been associated with fruit juices in recent years, juices have been recognized as vehicles of food-borne illness (1). E. coli O157:H7 has emerged as a significant food-borne pathogen, causing hemorrhagic colitis and hemolytic uremic syndrome (2, 3, 4). The initial outbreak involving E. coli O157:H7 contaminated apple juice occurred in the United States in 1991 (2). Then, the most serious outbreak to date E. coli O157:H7 linked to apple cider occurred in October 1996, when 70 people became sick and one person died (5). Salmonella Typhimurium is one of the most common Salmonella serotypes. The symptoms of salmonellosis include diarrhea, abdominal pain, mild fever, and chills (6, 7). In 1975, outbreaks of food-borne disease caused by S. Typhimurium were reported in the United States (8). Contamination of fruit juices with S. Typhimurium and E. coli O157:H7 results from their viability in low acid conditions under

refrigeration or even in their existence in food additives (2, 8, 9). Apple juice has a pH range of 3.5 to 4.0. E. coli O157:H7 has the ability to survive under low acidic conditions for 10 to 31 days at 8°C or 2 to 8 days at 25°C (10). Outbreaks related to L. monocytogenes in fruit juices have not been reported but the National Advisory Committee on Microbiological Criteria for Foods advised that the specific relationship between this pathogen and product is not known, therefore, E. coli O157:H7 and L. monocytogenes should be classified as target microorganisms (11). These pathogens have the potential to contaminate fruit juices such as apple juice or apple cider via improperly washed apples contaminated with food-borne pathogens from cow manure or soil (12, 13). The U.S. Food and Drug Administration (FDA) introduced new regulations to handle food-borne illness outbreaks in fruit juices. The FDA reported that a 5-log reduction target should be accomplished for the pertinent microorganism or the label warning statement should be attached to the bottle (14). In pasteurization processing to achieve a 5-log reduction, conventional heating is the method most commonly used in the fruit juice industry.

Fruit juice is typically pasteurized using the traditional hot-fill process in which juices are heated to 92-105°C for 15-30s (15). In recent years, thermal processing at 110°C for 30s has been followed by high temperature-short time pasteurization at 135°C for 3s (16). Although traditional heat treatment is performed in order to guarantee the microbiological safety of fruit juices, it is undesirable due to thermal damage to sensory quality, long processing times, high energy consumption and low heating efficiency (17, 18). To overcome these limitations, novel heating processes involving dielectric heating methods such as microwave heating, radio frequency heating, and ohmic heating have emerged as promising alternatives to conventional heating (19). These alternative technologies were developed for uniformly and rapidly heating food materials. While microwave heating is limited by penetration depth, and radio frequency heating is more appropriate for solid rather than liquid foods, ohmic heating is a particularly suitable system among innovative interventions for fruit juice processing (20, 21).

Ohmic heating is defined as a technology for uniformly heating food materials between electrodes by the passage of electric alternating-currents

(22). By using electricity, ohmic heating can not only circumvent the limitations of conventional heating but can also enhance inactivation of food-borne pathogens according to combination effects of heat and electricity. Though the electric field applied to generate ohmic heating is too mild in itself to inactivate food-borne pathogens such as E. coli O157:H7, S. Typhimurium and L. monocytogenes, the electric field at specific temperatures can result in tremendous bactericidal reduction. Such an excellent inactivation of pathogens is linked to the electroporation phenomenon in cells generated by an electric field. In previous studies, high voltage pulsed electric field (PEF) technology, which is applied only through a high voltage electric field and is classified as a nonthermal method, has been studied by a large number of scientists. However, efficacy of the moderate voltage electric field used in ohmic heating has been insufficiently studied.

Also, in ohmic heating, the heating rate is related to the electrical conductivity of a particle (23). Because of this characteristic, many food engineers have studied ohmic heating due to this aspect of electrical

conductivity. Castro et al. (24) studied the relationship between temperature and sugar content on the electrical conductivity of strawberry products during ohmic heating. Also, Icier et al. (25) investigated the electrical conductivity of apple and sourcherry juice concentrates subjected to ohmic heating. However, evaluationg the inactivation of food-borne pathogens based on the heating rates of juices of different solids content has not been well studied to date. In order to be apply an ohmic heating pasteurization system by the fruit juice industry, several factors such as the ratio of water to solids content, system performance efficiency, and reduction efficiency of food-borne pathogens should be considered.

The aim of the present study was to compare the inactivation of food-borne pathogens including E. coli O157:H7, S. Typhimurium and L. monocytogenes by ohmic heating and conventional heating in buffered peptone water (BPW) and apple juice. Also, the relationship between microbial inactivation and permeabilization of cell membranes by the electrical effect of ohmic heating will be elucidated in this study.

In addition, was to optimize the proportion of water to solids content of apple juice, system performance efficiency, and reduction efficiency of food-borne pathogens including *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* for attaining a high efficiency of sterilization against system performance in an ohmic heating system.

II. MATERIALS AND METHODS

2.1. Effect of Electropermeabilization by Ohmic heating for Inactivation of food-borne pathogens in Buffered Peptone Water and Apple juice

2.1.1. Bacterial cultures and cell suspension

Three strains each of *E. coli* O157:H7 (ATCC 35150, ATCC 43889, and ATCC 43890), *S.* Typhimurium (ATCC 19585, ATCC 43971, and DT 104) and *L. monocytogenes* (ATCC 19114, ATCC 19115, ATCC 15313) were provided by the School of Food Science bacterial culture collection of Seoul National University (Seoul, South Korea) for this study. Cultures for use in experiments were produced as follows: a single colony cultivated from frozen stocks on tryptic soy agar (TSA; Difco, Becton, Dickinson, Sparks, MD) was inoculated into 5ml of tryptic soy broth (TSB; Difco, Becton, Dickinson, Sparks, MD), incubated at 37°C for 24h, collected by

centrifugation at 4,000 × g for 20min at 4°C and washed three times with 0.2% peptone water (PW, Bacto, Becton, Dickinson, Sparks, MD). The final pellet were resuspended in 0.2% PW, corresponding to approximately 108 to 109 CFU/ml. Afterwards suspended pellets of the three pathogens were combined to comprise a mixed culture cocktail containing approximately equal number of cells of each strain of *E. coli* O157:H7 (107 CFU/ml), *S.* Typhimurium (107 CFU/ml), and *L. monocytogenes* (106 CFU/ml).

2.1.2. Treatment medium and inoculation

Sterile buffered peptone water (BPW; Difco, Sparks, MD, pH 7.2) was used in this experiment. Mixed culture cocktail (0.2 ml) was inoculated into 25ml of sterile BPW when BPW attained the treatment target temperature.

Pasteurized apple juice (pH 3.5, 11.8 °Brix) free of any preservatives was purchased from a local grocery store. A 0.2-ml aliquot of the mixed culture cocktail was inoculated into 25ml of apple juice at the treatment target temperature for each treatment.

2.1.3 Experimental apparatus

The ohmic heating system (Fig. 1) consisted of a function generator (33210A; Agilent Technologies, Palo Alto, CA), a precision power amplifier (4510; NF corp., Yokohama, Japan), a two-channel digital storage oscilloscope (TDS2001C; Tektronix, Inc., Beaverton, CO), a data acquisition instrument (34790A; Agilent Technologies), and an ohmic heating chamber. The ohmic heating chamber was made up of two titanium electrodes and teflon coated K-type thermocouples located in the middle of a rectangular container (2×15×6cm) consisting of component pyrex glass. The distance between the two titanium electrodes was 2cm, and the cross-sectional area was 60cm2. The function generator produced a diversity of waveforms such as sine, square, pulse, ramp, triangle, noise and custom waveforms in the frequency range of 1mHz to 10MHz and a maximum output signal of 5V. The signals generated through the power amplifier were amplified within 45Hz to 20kHz up to a maximum output of 141V AC. The signals expanded

by the power amplifier were delivered to each titanium electrode. The two-channel digital storage oscilloscope was used to measure signals including waveform, frequency, voltage and current. In this study, temperature was controlled by software (Labview; National Instrument). The function generator operated according to whether target temperature was reached or not. The function generator was activated when the sample temperature dropped to 0.01°C below the target temperature and cycled off when the target temperature was reached. Temperatures were acquired every second by the data acquisition instrument connected to a computer.

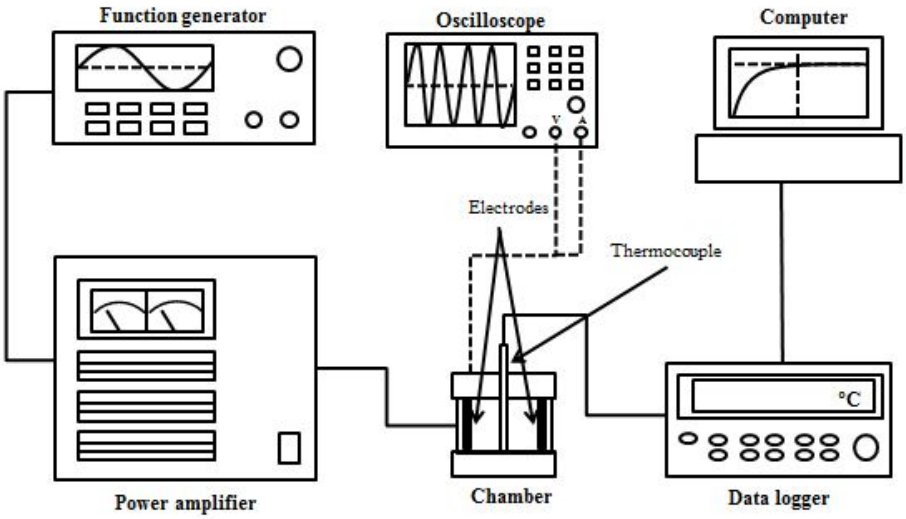


Fig. 1. Schematic diagram of the ohmic heating system used in this experiment.

2.1.4. Ohmic heating treatment and conventional heating treatment

For ohmic heating experiments, 25ml of sample was placed in the ohmic heating chamber. Experimental electric field strength conditions were 30V/cm in BPW and 60V/cm in apple juice; this was done in order to maintain constant temperature, because electrical conductivity of each product types was different. A 20kHz frequency and sine waveform were used in all ohmic heating experiments. Treatments were conducted at 55, 58, and 60°C for 0, 10, 20, 25, and 30s.

For conventional heating, a constant temperature water bath (BW-10G; Jeio Tech. Seoul, Korea) was used in this study. A 50ml conical centrifuge tube containing 25ml of sample was placed in the constant temperature water bath to maintain constant temperature. Sample temp- eratures were measured by means of a K-type thermocouple. All experiments involving conventional heating were carried out under the same time-temperature conditions described for ohmic heating treatment.

2.1.5. Microbial enumeration

For microbial enumeration, each treated 25ml sample of BPW and apple juice was immediately transferred into a sterile stomacher bag (Labplas Inc., Sainte-Julie, Quebec, Canada) containing 225ml of 0.2% PW and homogenized for 2min with a stomacher (EASY MIX, AES Chemunex, Rennes, France). After homogenization, 1ml aliquots of sample were 10-fold serially diluted in 9ml of 0.2% PW, and 0.1ml of sample or diluent was spread plated onto each selective medium. Sorbitol MacConkey agar (SMAC; Difco), Xylose lysine deoxycholate agar (XLD; Difco) and Oxford agar base(OAB; Difco) with antimicrobial supplement (Bacto Oxford Antimicrobic Supplement; Difco) were used as selective media for enumeration of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes*, respectively. Where low levels of surviving cells were expected, 1ml of undiluted stomacher bag contents was divided between four plates of each medium and spread-plated. After all plates were incubated at 37°C for 24-48h, colonies were counted.

2.1.6. Transmission Electron Microscopy

In order to evaluate the morphological differences in *E. coli* O157:H7 resulting from ohmic heating and conventional heating treatment, transmission electron microscopy (TEM) analysis was performed. BPW containing *E. coli* O157:H7 was treated by both methods, and then centrifuged at 3,000×g for 10min. The cells were fixed at 4°C for 2-4h in modified Karnovsky's fixative containing 2% paraformaldehyde and 2% glutaraldehyde in 0.05M sodium cacodylate buffer (pH 7.2). After primary fixation, each sample was centrifuged and rinsed three times with 0.05M sodium cacodylate buffer (pH 7.2) at 4°C for 10 min. Cells were then postfixed with 1% osmium tetroxide in 0.05M sodium cacodylate buffer (pH 7.2) at 4°C for 2h, briefly washed twice with distilled water at room temperature, and then stained overnight with 0.5% uranyl acetate at 4°C. Samples were dehydrated at room temperature through a graded ethanol series (10min each) of 30, 50, 70, 80, 90, and three times at 100%. Transition

was performed two times with 100% propylene oxide at room temperature for 15 min. The cells were then infiltrated for 2hr with a 1:1 solution of propylene oxide and Spurr's resin. Following that, samples were placed in Spurr's resin overnight. The cells were then immersed in Spurr's resin for 2h as a final step of infiltration and were polymerized at 70°C for 24h. Sections (70nm thick) were cut by means of an ultramicroto- me (MT-X, RMC, Tucson, AZ, USA), and then stained with 2% uranyl acetate for 7min followed by Reynolds' lead citrate for 7 min. Observations were carried out using a transmission electron microscope (LIBRA 120; Carl Zeiss,Oberkochen, Germany).

2.1.7. Determination of cell membrane damage by PI uptake

Cell membrane damage was evaluated by using the fluorescent dye propidium iodide (PI; Sigma-Aldrich, P4170). A stock solution of 1mg PI in 1ml sterile distilled water was prepared and stored at 4°C in the dark. Untreated, ohmic heated and conventionally heated cells were centrifuged at

10,000×g for 10 min at 4°C. Supernatants were discarded, and the cell pellets were washed and resuspended in Phosphate buffered saline (PBS; pH 7.2) to an optical density at 680nm of approximately 0.2 (SpectraMax M2e; Molecular Devices Korea, LLC.) for *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes*, corresponding to approximately 10^7CFU/ml. PI was added to a final concentration of 2.9μM. After incubation for 10 min, samples were washed two times with PBS at the same volume to remove excess dye. Final pellets were resuspended in PBS, and fluorescence was measured in a microplate reader (SpectraMax M2e; Molecular Devices Korea, LLC.) at an excitation wavelength of 493nm and an emission wavelength of 615nm. Fluorescence data for each sample were normalized with the optical density set at 680nm; values obtained for untreated cells were subtracted from all experimental values.

2.1.8. Statistical analysis

All experiments were done in triplicate with duplicate samples. Data were analyzed by analysis of variance (ANOVA) using the Statistical

Analysis System Program (SAS Institute, Cary, NC, USA), and mean values were separated using Duncan's multiple-range test. Significant differences were indicated at a value of $P < 0.05$.

2.2. Optimization of Ohmic heating for inactivation of food-borne pathogens in different concentrations of apple juice

2.2.1. Bacterial cultures and cell suspension

Three strains each of *E. coli* O157:H7 (ATCC 35150, ATCC 43889, and ATCC 43890), *S.* Typhimurium (ATCC 19585, ATCC 43971, and DT 104) and *L. monocytogenes* (ATCC 19114, ATCC 19115, ATCC 15313) were provided by the School of Food Science bacterial culture collection of Seoul National University (Seoul, South Korea) for this study. Cultures for use in experiments were produced as follows: single colonies cultivated from frozen stocks on tryptic soy agar (TSA; Difco, Becton, Dickinson, Sparks, MD) were inoculated into 5ml of tryptic soy broth (TSB; Difco, Becton,

Dickinson, Sparks, MD), incubated at 37°C for 24h, collected by centrifugation at 4,000 × *g* for 20min 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. Afterwards, suspended pellets of the three pathogens were combined to comprise a mixed culture cocktail containing approximately equal number of cells of each strain of *E. coli* O157:H7 (10^7 CFU/ml), *S.* Typhimurium (10^7 CFU/ml), and *L. monocytogenes* (10^6 CFU/ml).

2.2.2. Sample preparation and inoculation

Frozen apple juice concentrate (pH 3.5, 72 °Brix) was purchased a local grocery store (Incheon, Korea). Apple juice concentrate was diluted with sterile distilled water to 48, 36, 24, and 18 °Brix. Sugar concentration (°Brix) was measured by a digital refractometer (Atago co.,Ltd., Japan). Then a 0.2-ml aliquot of the mixed culture cocktail (*E. coli* O157:H7, *S.* Typhimurium,

and *L. monocytogenes*) was inoculated into each 25ml sample of apple juice of different solids content.

2.2.3. Experimental equipment

The experimental device (Fig. 1) consisted of a function generator (33210A; Agilent Technologies, Palo Alto, CA), a precision power amplifier (4510; NF corp., Yokohama, Japan), a two-channel digital storage oscilloscope (TDS2001C; Tektronix, Inc., Beaverton, CO), a data acquisition instrument (34790A; Agilent Technologies), and an ohmic heating chamber. Two titanium electrodes and teflon coated K-type thermocouples located in the middle of a rectangular container (2×15×6cm) consisting of component pyrex glass were used as an ohmic heating chamber. The distance between the two titanium electrodes and the cross-sectional area was 2cm and 60cm^2, respectively. Multiple waveforms such as sine, square, pulse, ramp, triangle, noise and custom waveforms could be produced by the function generator which permitted a frequency range of 1mHz to 10MHz and a maximum

output signal of 5V. These signals were expanded by the power amplifier from 45Hz to 20kHz and a maximum output of 141V AC. Each titanium electrode received signals amplified by the power amplifier. The signals, including waveform, frequency, voltage, and current, were measured using the two-channel digital storage oscilloscope. The data acquisition instrument was used to obtain temperature histories in this study.

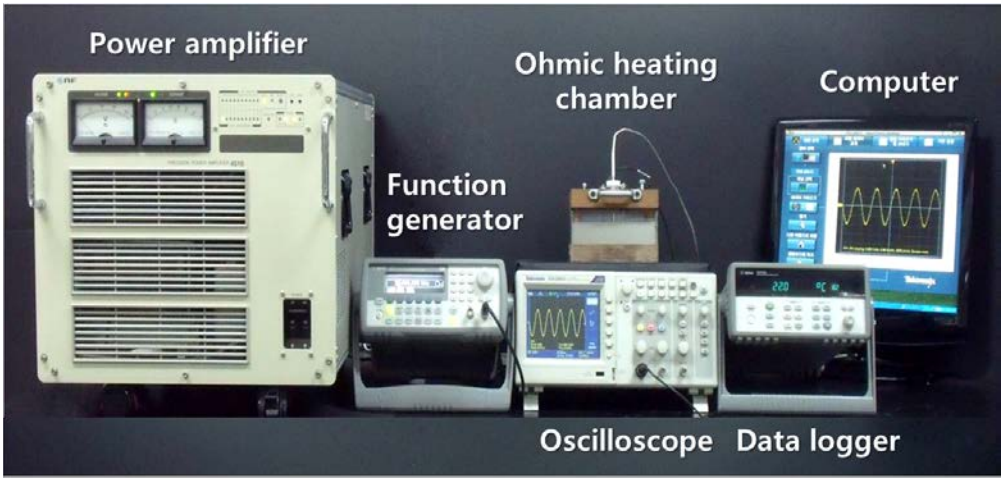


Fig. 2. Ohmic heating system at Seoul National University (Seoul, Korea)

2.2.4. Ohmic heating treatment

The ohmic heating chamber was filled with 25ml of sample for treatment. A 20kHz frequency and sine waveform were utilized in all experiments. For temperature and current data, treatments were conducted at 30, 40, 50, and 60V/cm in apple juice of 72, 48, 36, 24, and 18 °Brix apple juices for 90s. Temperature and current were recorded every 5s at a maximum of 90°C. Inoculated samples were treated at 30 and 60V/cm in 72, 48, 36, 24, and 18 °Brix apple juices for 0, 10, 20, 30, 40, 50, and 60s.

2.2.5. Microbial enumeration

For enumeration of bacteria, each treated 25ml sample was instantly transferred into a sterile stomacher bag (Labplas Inc., Sainte-Julie, Quebec, Canada) containing 225ml of 0.2% PW and homogenized for 2min with a stomacher (Easy Mix, AES Chemunex, Rennes, France). After homogenization, 1-ml aliquots of sample were 10-fold serially diluted in 9ml

of 0.2% PW, and 0.1ml of sample or diluent was spread plated onto each selective medium. Sorbitol MacConkey agar (SMAC; Difco), Xylose lysine deoxycholate agar (XLD; Difco) and Oxford agar base (OAB; Difco) with antimicrobial supplement (Bacto Oxford Antimicrobic Supplement; Difco) were used as selective media for enumeration of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes*, respectively. Where low levels of surviving cells were anticipated, 1ml of undiluted stomacher bag contents was separated into four plates of each medium and spread-plated. All plates were incubated at 37°C for 24-48h, and colonies were counted.

2.2.6. System performance coefficient measurement

The system performance coefficient (SPC) of ohmic heating was determined from temperature, voltage, and current data (24) and calculated as follows (equation 1):

$$\mathbf{SPC} = \frac{\boldsymbol{mCp\Delta T}}{\sum \boldsymbol{\Delta VIt}} \qquad (1)$$

Where SPC is system performance coefficient, m is mass (g), Cp is specific heat capacity (J/g K), ΔT is difference between final temperature and initial temperature (K), ΔV is voltage applied (V), I is current (A), and t is time (s). $\sum \Delta \boldsymbol{VIt}$ is the energy given to the system $\boldsymbol{mCp\Delta T}$ is energy given to the system minus energy loss during ohmic heating. The ratio of $\boldsymbol{mCp\Delta T}$ to $\sum \Delta \boldsymbol{VIt}$ indicated the system performance coefficient (26).

2.2.7. Color and pH measurement

To assess color changes of treated apple juice, a Minolta colorimeter (CR400; Minolta Co., Osaka, Japan) was used in this study. Qualities of color were expressed by values of L*, a*, and b* of apple juice. L*, a*, and b* values signify color lightness, redness, and yellowness, respectively. pH was measured with a pH meter (Seven Multi 8603; Mettler Toledo, Greifensee, Switzerland).

2.2.8. Statistical analysis

All experiments were conducted three times with duplicate samples. Data were analyzed by analysis of variance (ANOVA) using the Statistical Analysis System Program (SAS Institute, Cary, NC, USA), and mean values were separated using Duncan's multiple-range test. Significant differences were indicated at a value of $P < 0.05$.

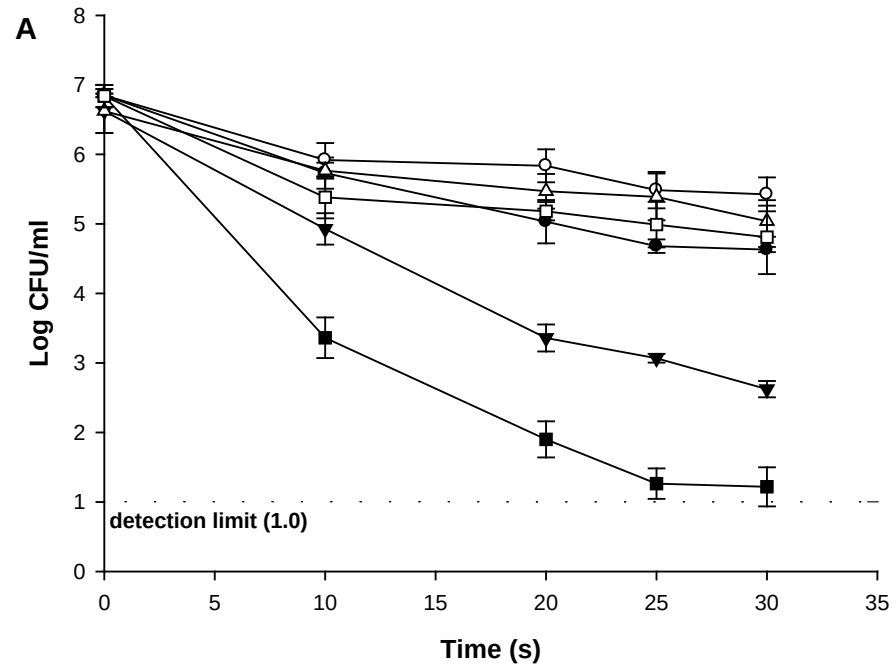
III. RESULTS

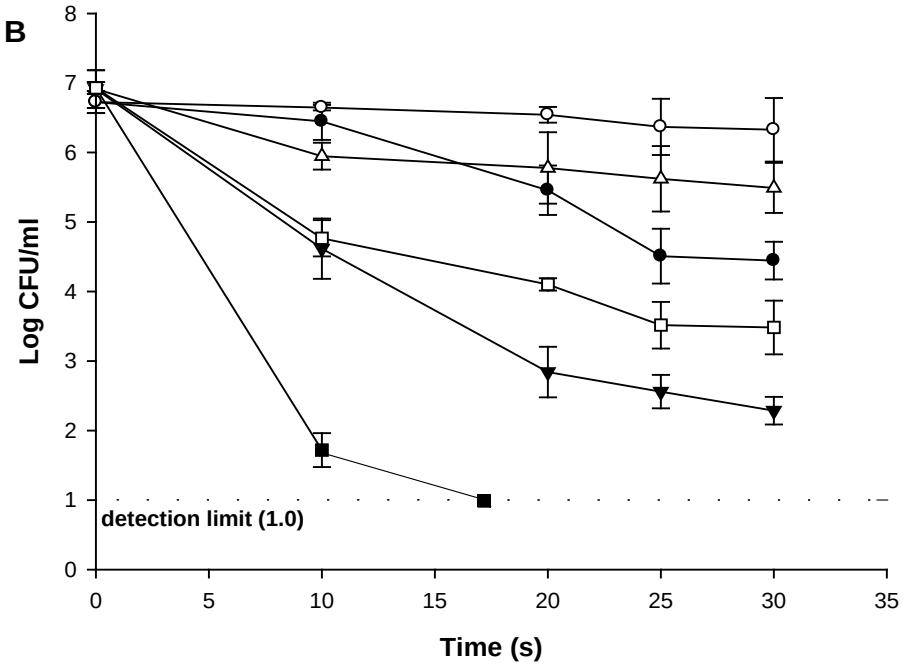
3.1. Effect of Electropermeabilization by Ohmic heating for Inactivation of food-borne pathogens in Buffered Peptone Water and Apple juice

3.1.1. Comparison of ohmic heating and conventional heating on inactivation of food-borne pathogens in BPW

The survival of *E. coli* O157:H7, *S.* Typhimurium and *L. monocytogenes* in BPW (pH 7.2) following ohmic heating and conventional heating is shown in Fig. 3. In general, the reduction of *E. coli* O157:H7, *S.* Typhimurium and *L. monocytogenes* increased with increasing temperature and time. Differences of reduction between ohmic heating and conventional heating for these pathogens increased with processing time. The disparity in log reductions between the two methods was significantly different ($P < 0.05$) after ohmic heating and conventional heating at both 58 and 60°C. Ohmic

heating for 30s at 58°C accomplished 4.00-, 4.63- and 1.11-log reductions in *E. coli* O157:H7, *S.* Typhimurium and *L. monocytogenes*, respectively. Conventional heating under the same conditions resulted in 1.58, 1.42 and 0.41 log reductions, which was less than that obtained by ohmic heating, for all three pathogens. The difference between ohmic and conventional heating for reducing food-borne pathogens was greatest at 60°C for 30s. Log reductions of 5.62, 6.93 and 4.37 were observed in *E. coli* O157:H7, *S.* Typhimurium and *L. monocytogenes*, respectively, after ohmic heating at 60°C for 30s, whereas conventional heating at 60°C for 30s achieved 2.3-, 3.45- and 0.97-log reductions in those pathogens. The degree of reduction was similar for *E. coli* O157:H7 and *S.* Typhimurium. However, gram-positive *L. monocytogenes* was more resistant to inactivation.


A
Log CFU/ml
detection limit (1.0)
Time (s)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Log CFU/ml
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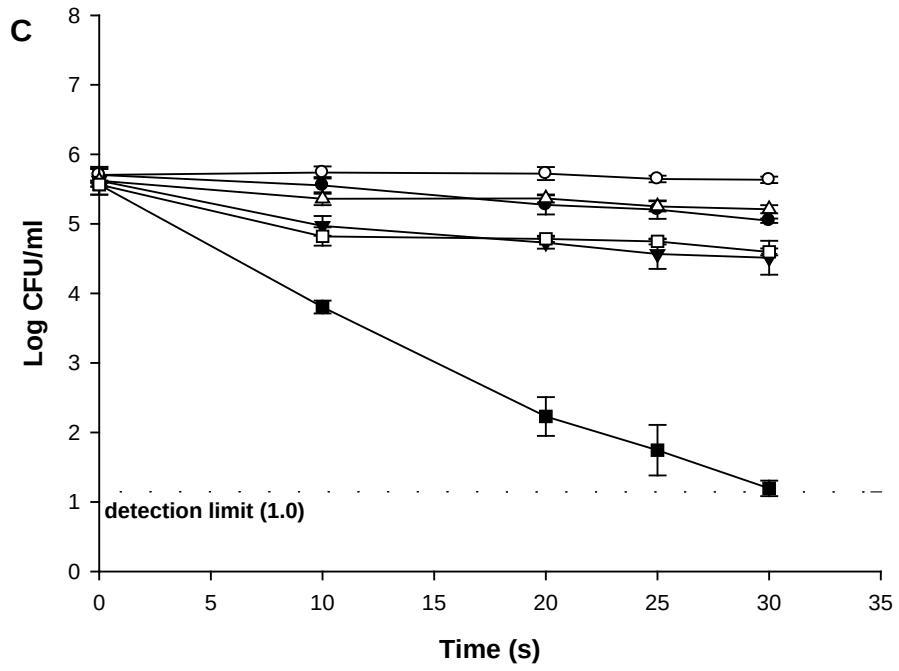
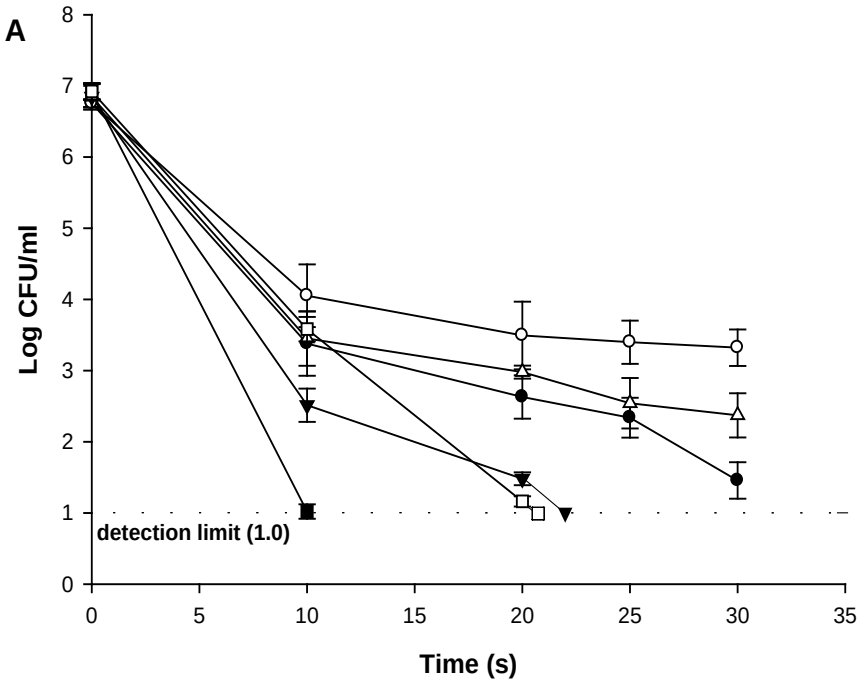


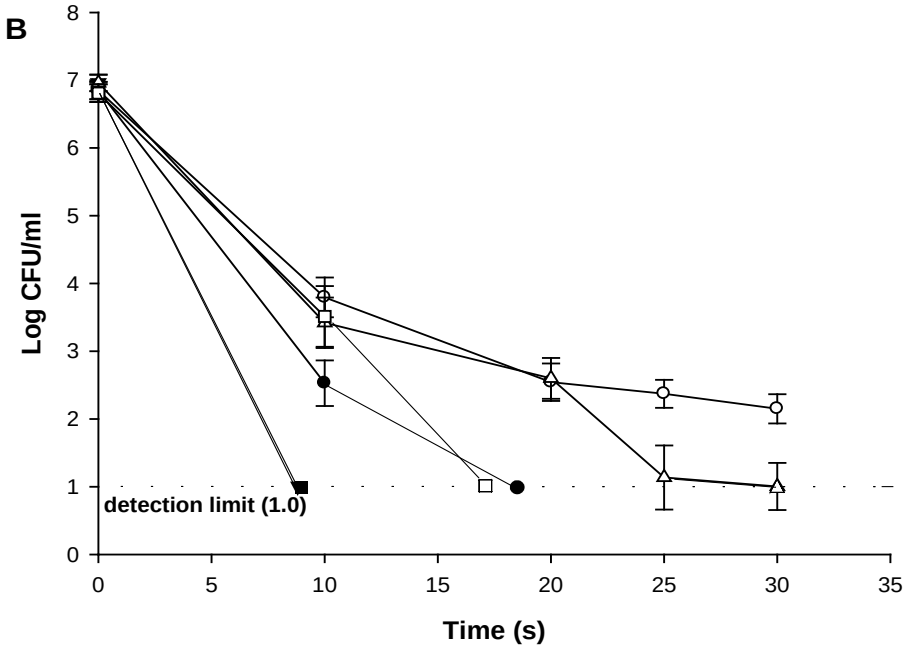
Fig. 3. Survival curves corresponding to microbial inactivation by ohmic heating and conventional heating treatment in BPW (pH 7.2) at different temperatures for *E. coli* O157:H7 (A), *S.* Typhimurium (B) and *L. monocyto-genes* (C) for 0, 10, 20, 25 and 30s. Symbols: ●, 55°C - Ohmic heating; ○, 55°C – Conventional heating; ▼, 58°C – Ohmic heating; ▵, 58°C – Conventional heating; ■, 60°C – Ohmic heating; □, 60°C – Conventional heating.

3.1.2. Comparison of ohmic heating and conventional heating on inactivation of food-borne pathogens in apple juice

Populations (LogCFU/ml) of *E. coli* O157:H7, *S.* Typhimurium and *L. monocytogenes* in apple juice (pH 3.5, 11.8 °Brix) are depicted in Fig. 4. Levels of surviving cells subjected to both ohmic and conventional heating in apple juice decreased more than in BPW owing to the low pH of apple juice. Log reductions of 5.30, 6.85 and 3.06 were observed in *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes*, respectively, after ohmic heating at 55°C for 30s. For conventional heating, reduction levels of 3.43, 4.70 and 1.20 log were observed after heating at the same time and temperature. Survival curve patterns for ohmic and conventional heating in apple juice at 55°C and in BPW at 60°C were similar with respect to the different effects of both treatments. When treated with ohmic heating at 58 and 60°C in apple juice, levels of surviving cells of the three food-borne pathogens decreased to below the detection limit (1 log CFU/ml) within 30s. With conventional heating, populations of the three pathogens at 58°C did

not drop below the detection limit within 30s and populations of *L. monocytogenes* were not reduced to below the detection limit within 30s. Although populations of *E. coli* O157:H7 and *S.* Typhimurium at 60°C were reduced to below the detection limit within 30s, reduction time is much longer than ohmic heatinng. Based on overall microbial reduction patterns in BPW and apple juice, ohmic heating resulted in 2-3 -log greater reductions of food-borne pathogens than conventional heating.


A
Log CFU/ml
detection limit (1.0)
Time (s)
0
1
2
3
4
5
6
7
8
0
5
10
15
20
25
30
35


B
Log CFU/ml
detection limit (1.0)
Time (s)
0
1
2
3
4
5
6
7
8
0
5
10
15
20
25
30
35

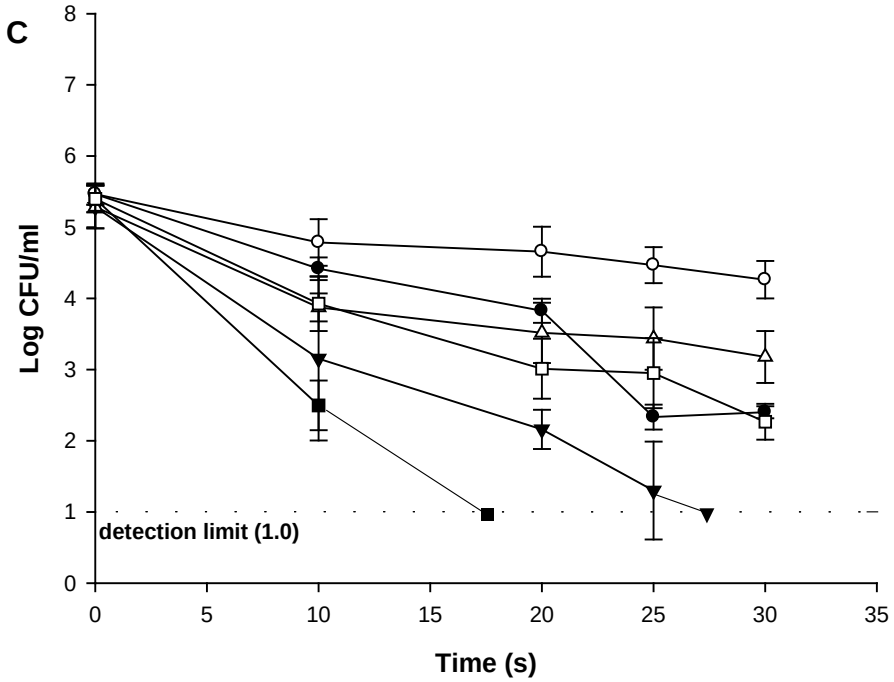


Fig. 4. Survival curves corresponding to microbial inactivation by ohmic heating and conventional heating treatment in apple juice (pH 3.5, 11.8 °Brix) at different temperatures for *E. coli* O157:H7 (A), *S.* Typhimurium (B) and *L. monocytogenes* (C) for 0, 10, 20, 25 and 30s. Symbols: Symbols: ●, 55°C - Ohmic heating; ○, 55°C – Conventional heating; ▼, 58°C – Ohm- ic heating; ∆, 58°C – Conventional heating; ■, 60°C – Ohmic heating; □, 60°C – Conventional heating.

3.1.3. Transmission electron microscopy analysis

Ohmic heating- and conventional heating-induced morphological cell damage was characterized by using TEM (micrographs shown in Fig. 5). The appearance of *E. coli* O157:H7, which was not exposed to either ohmic or conventional heating, is visible in Fig. 5A and B. Structural differences between conventional (Fig. 5C and D) and ohmic heat-treated (Fig. 5E and F) cells are clearly shown in the photographs. Images of conventional heat-treated cells disclosed slight decomposition of cellular structures such as the cell wall and cytoplasmic membrane, and eruption of internal cellular substances. In the case of ohmic heat-treatment, these phenomena were much more pronounced, consisting of severe rupture of cytoplasmic membranes and leakage of intracellular contents. These occurrences ultimately led to cell death.

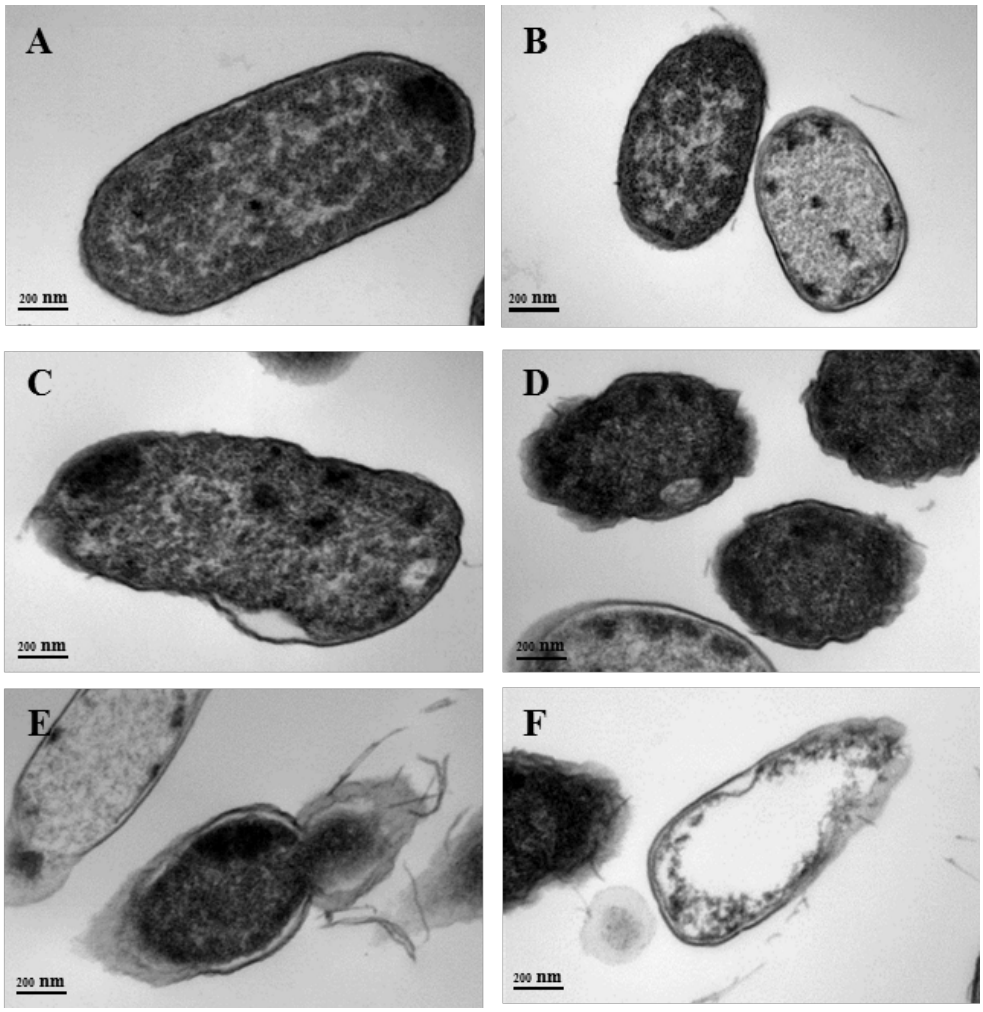


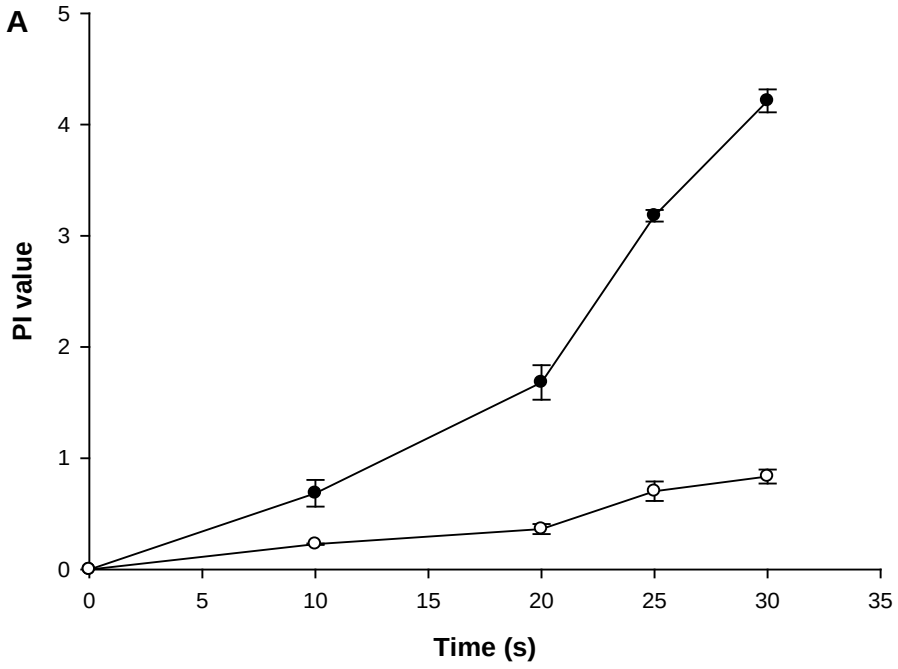
Fig. 5. TEM micrographs of non-treated, conventional heat-treated and ohmic heat-treated *E. coli* O157:H7. Conventional heating and ohmic heating treatments were conducted in BPW (pH 7.2) at 60°C for 30s. (A) and (B). non-treated cells; cell membrane was intact. (C) and (D). conventional heat- treated cells; this morphological image showed shrinkage of intracellular substances or slight membrane damage. (E) and (F). ohmic heat-treated cells; release of intracellular substances was much more pronounced than for conventional heat-treated cells.

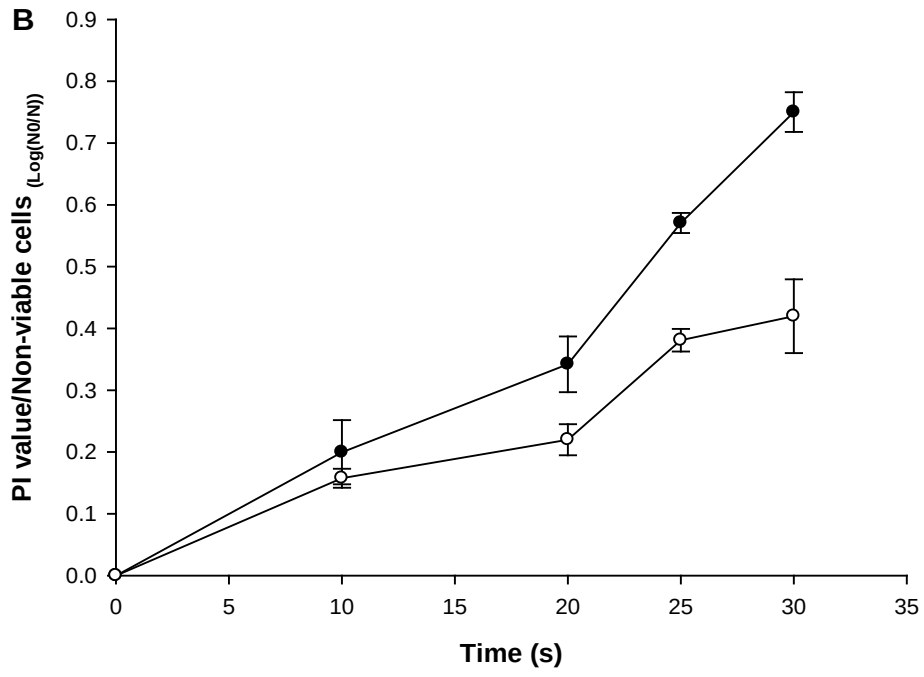
3.1.4. Relationship between loss of viability and PI uptake after ohmic and conventional heating

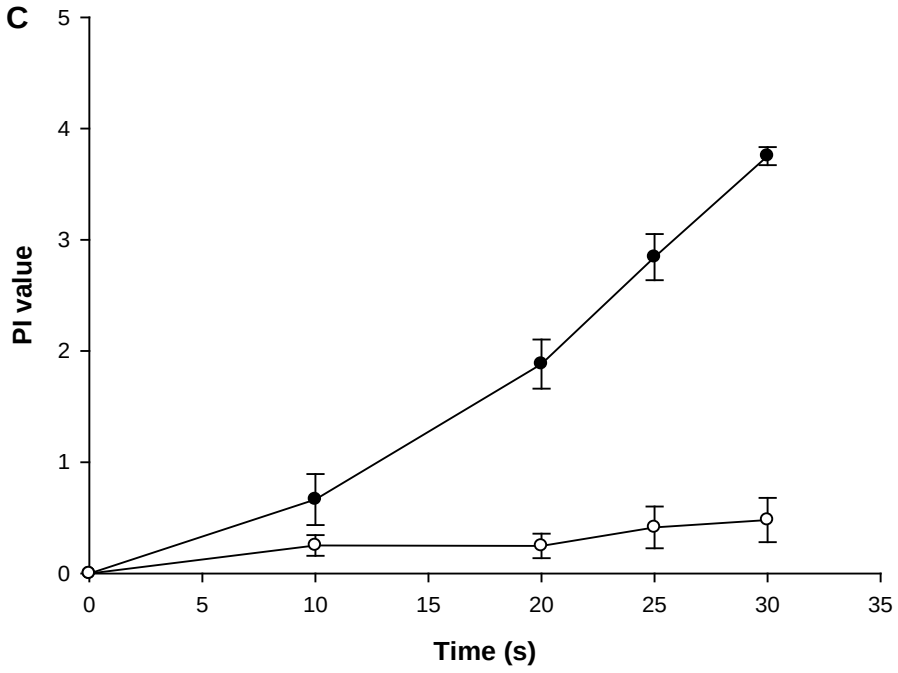
As a further study of membrane damage, *E. coli* O157:H7, *S.* Typhimurium and *L. monocytogenes* were stained with the fluorescent dye PI, which passed through damaged cell membranes after ohmic heating and conventional heating. To investigate the relationship between inactivation and membrane permeability of these pathogens, PI value and PI value per non-viable cells (Log N_0/N) at 60°C for 0, 10, 20, 25, and 30s were plotted, as shown in Fig. 6. PI uptake in cells gradually increased depending on time duration of ohmic and conventional heating.

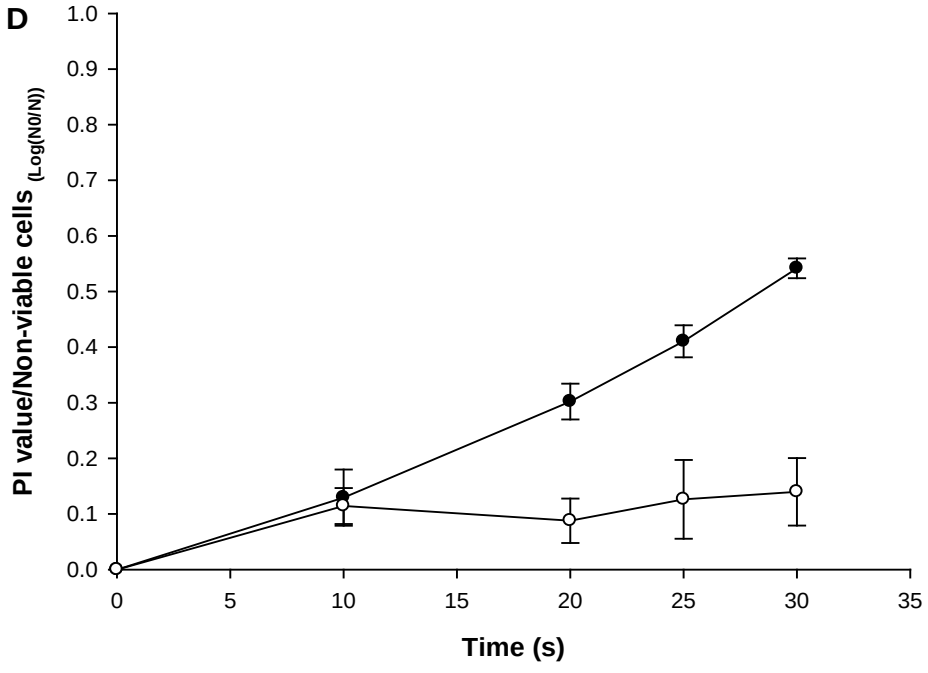
Similar patterns of PI uptake were observed in *E. coli* O157:H7, *S.* Typhimurium *and L. monocytogenes*. PI uptake induced by ohmic heating was significantly higher at 60°C compared with conventional heating. Also, PI for non-viable cells of *E. coli* O157:H7 and *S.* Typhimurium treated with ohmic heating at 60°C was much higher compared to conventional heating at the same temperature. However, incase of *L. monocytogenes*, PI value for

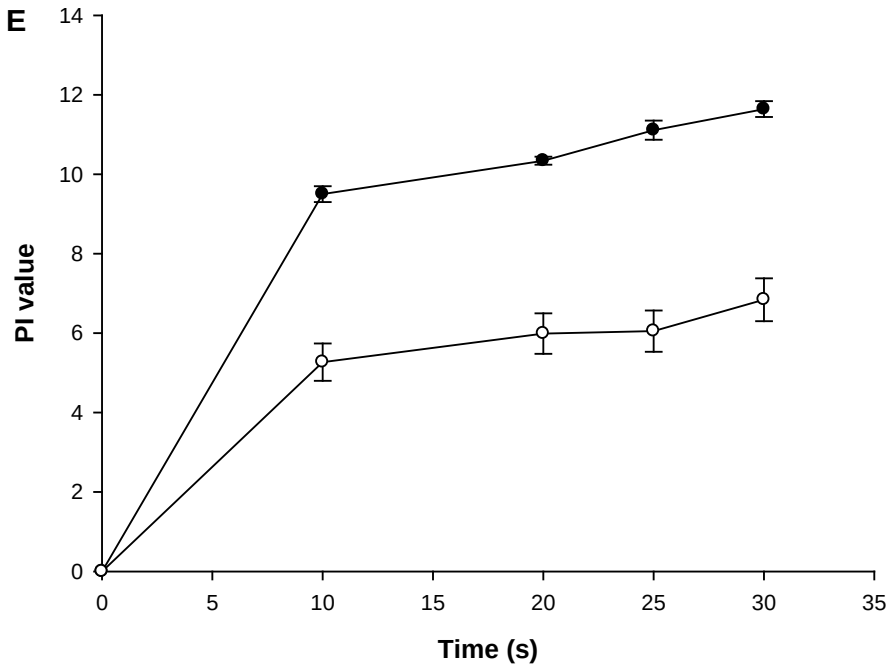
non-viable cells treated with ohmic heating were lower than when subjected conventional heating, although inactivation resulting from ohmic heating was much higher than for conventional heating. Based on the relationship between PI uptake and cell death, the effect of cell permeabilization by ohmic heating and conventional heating on cell death was identified.


A
PI value
Time (s)
0
1
2
3
4
5
0
5
10
15
20
25
30
35


B
PI value/Non-viable cells (Log(N0/N))
Time (s)
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
0
5
10
15
20
25
30
35


C
PI value
Time (s)
0
1
2
3
4
5
0
5
10
15
20
25
30
35


D
PI value/Non-viable cells (Log(N0/N))
Time (s)
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
0
5
10
15
20
25
30
35


E
PI value
Time (s)
0
2
4
6
8
10
12
14
0
5
10
15
20
25
30
35

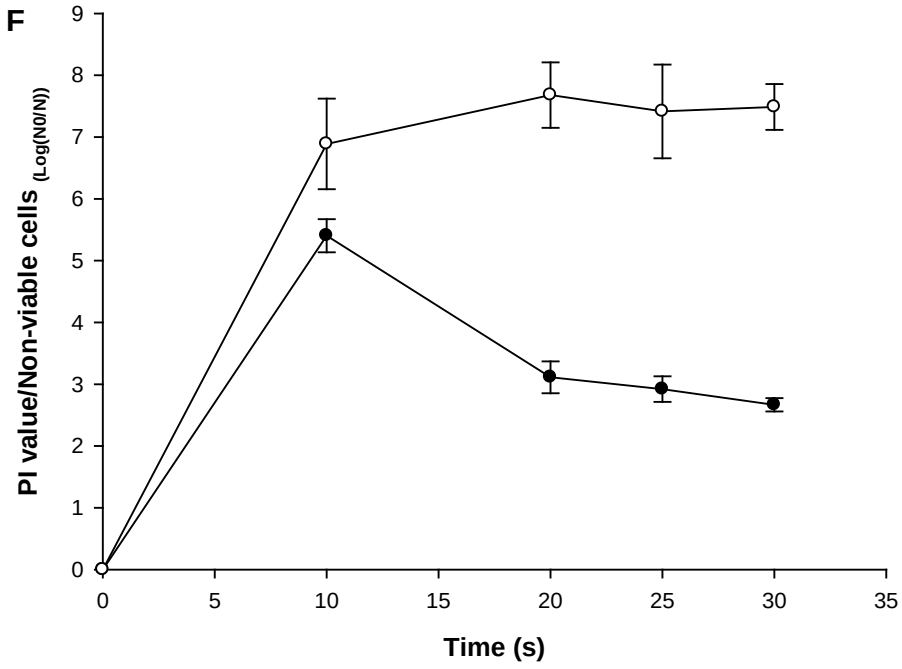

F
PI value/Non-viable cells (Log(N0/N))
Time (s)
0
1
2
3
4
5
6
7
8
9
0
5
10
15
20
25
30
35

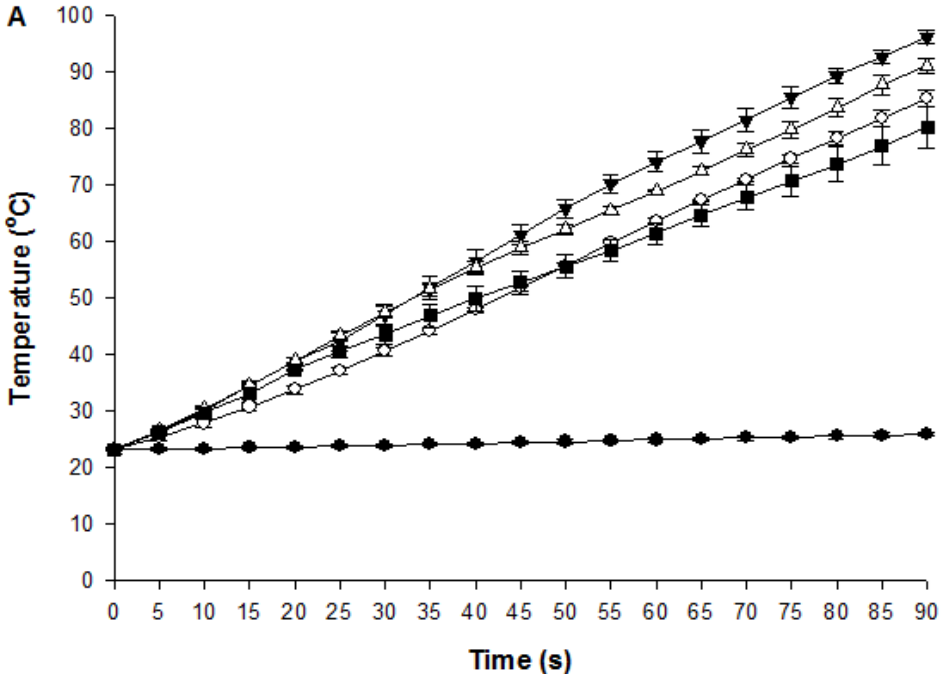
Fig. 6. Relationship between loss of viability and PI uptake after ohmic heating and conventional heating treatment at 60°C for 0, 10, 20, 25 and 30s. (A) *E. coli* O157:H7: PI value, (B) *E. coli* O157:H7: PI value / Non viable cells, (C) *S.* Typhimurium: PI value, (D) *S.* Typhimurium: PI value / Non viable cells, (E) *L. monocytogenes*: PI value, (F) *L. monocytogenes*: PI value / Non viable cells, Symbols: ●, Ohmic heating; ○, Conventional heating.

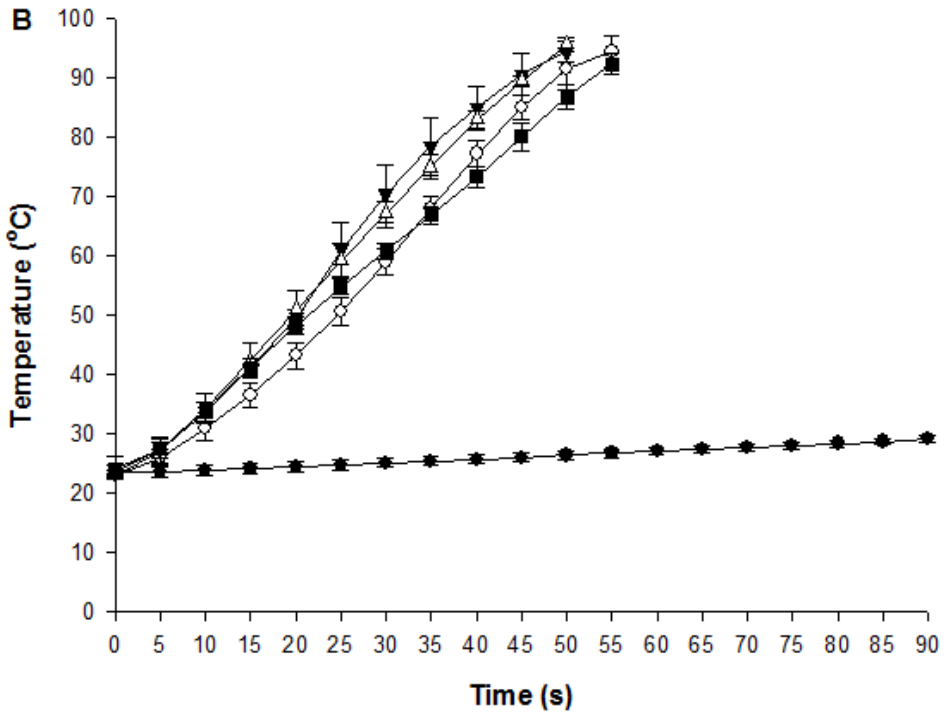
3.2. Optimization of Ohmic heating for inactivation of food-borne pathogens in different concentration of apple juice

3.2.1. Temperature profiles at different concentrations of apple juice

The heating rates of various concentrations of apple juice during ohmic heating at different voltage gradients are shown Fig. 7. Temperature rise in higher concentrations is quicker than in lower concentrations of juice up to 36 °Brix. However, when approaching 48 °Brix, the rate of temperature increase began to decline. Higher applied voltage reduced the difference in temperature rise throughout the range of apple juice concentration.

In all experiments, temperature rise was most rapid in apple juice of 36 °Brix at 30, 40, 50, and 60V/cm. The temperature of 36 °Brix apple juice reached 90°C after 90s, 60s, 30s, and 20s, respectively. For 72 °Brix juice, temperature increase was very slight compared to other concentrations at all voltage gradients.


A
Temperature (°C)
Time (s)
0
10
20
30
40
50
60
70
80
90
100
0
5
10
15
20
25
30
35
40
45
50
55
60
65
70
75
80
85
90


B
Temperature (°C)
Time (s)
0
10
20
30
40
50
60
70
80
90
100
0
5
10
15
20
25
30
35
40
45
50
55
60
65
70
75
80
85
90

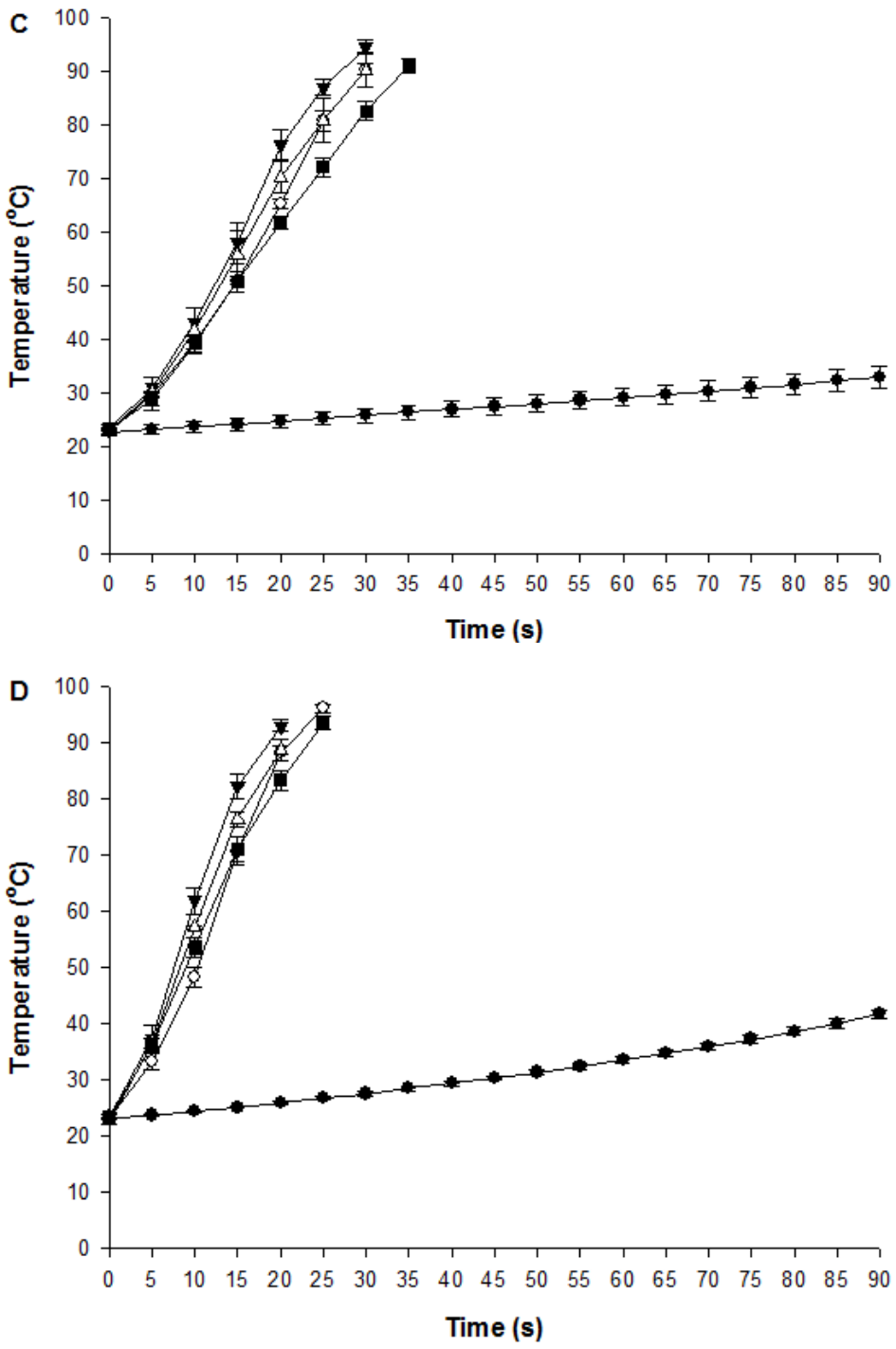


Fig. 7. Temperature-time curves of apple juice of 18 (■), 24 (△), 36 (▼), 48

(○), and 72(●) °Brix during ohmic heating at voltage gradients of 30V/cm (A), 40V/cm (B), 50V/cm (C), and 60V/cm (D).

3.2.2. System performance efficiency at different concentrations of apple juice and voltage gradients

Fig. 8 shows system performance efficiency of ohmic heating at different sample concentrations and voltage gradients. All SPC values at 40, 50, and 60V/cm were not as high as that of 30V/cm except for 72 °Brix apple juice. The efficiency of ohmic heating sharply decreased at 40V/cm in 48, 36, 24, and 18 °Brix apple juice. As applied voltage increased, overall SPC gradually increased in the range of 40 to 60V/cm. Ohmic heating was the most efficient along with each SPC value of 0.75, 0.46, 0.58, and 0.65 in 24, 24, 36, and 48 °Brix apple juice at 30, 40, 50, and 60V/cm, respectively. Following to higher voltage gradients, system efficiency increased in higher soluble solids concentration of samples. SPC values at 30V/cm were 0.52, 0.73, 0.75, and 0.71 in 48, 36, 24, and 18 °Brix juice, respectively. System efficiency at 60V/cm was 0.65, 0.64, 0.56, and 0.55 in 48, 36, 24, and 18 °Brix juice, respectively. In the case of 72 °Brix apple juice, SPC values were absolutely lower than in any other concentration of apple juice.

However, system performance efficiency in 72 °Brix apple juice still increased, along with higher voltage gradients.

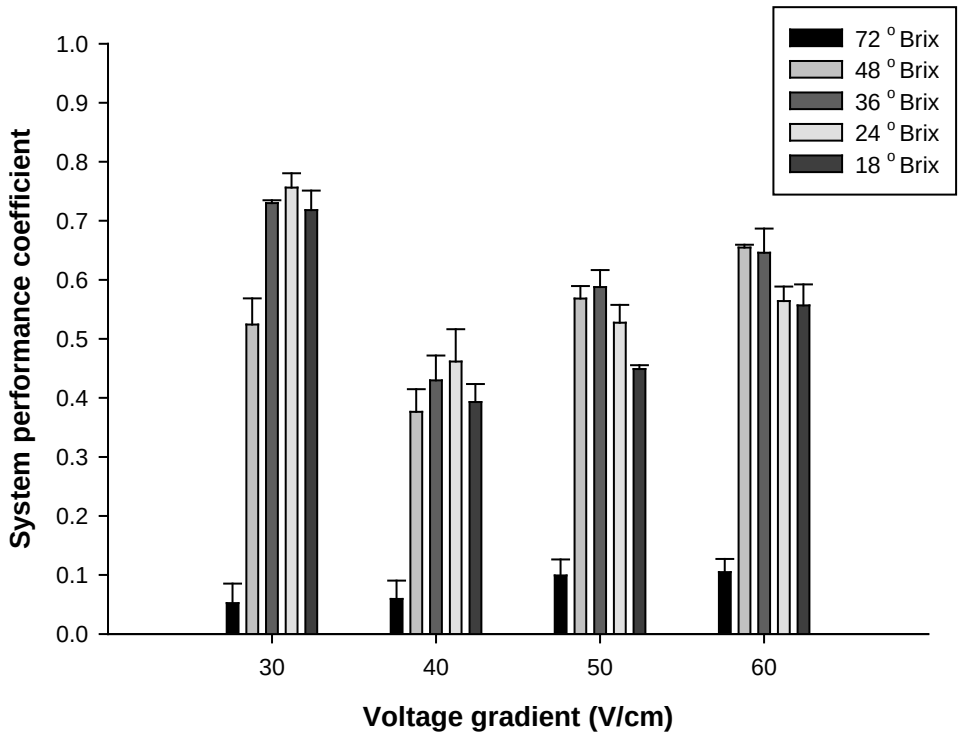
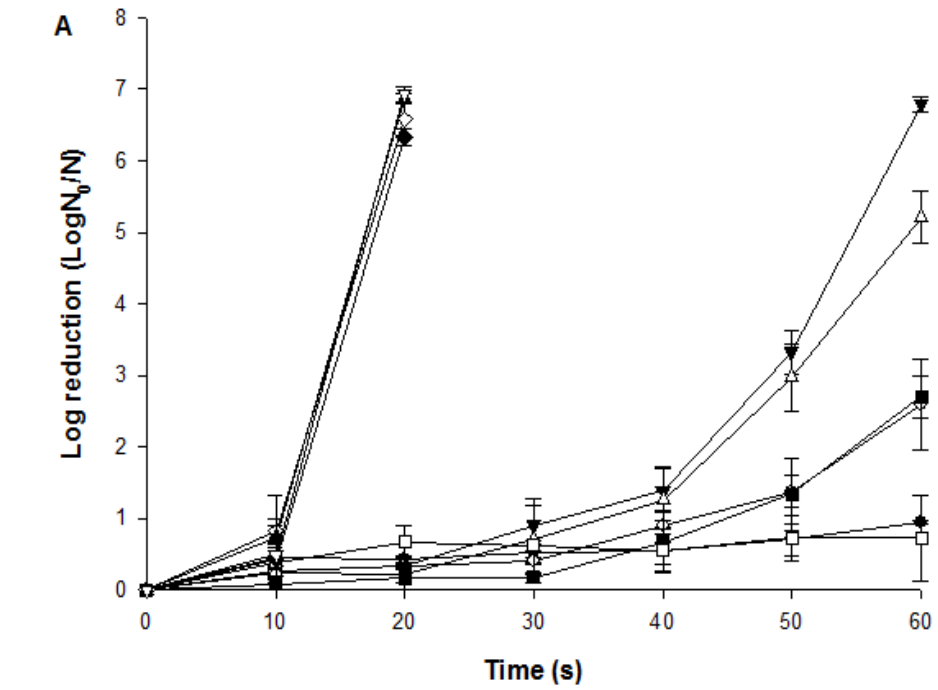


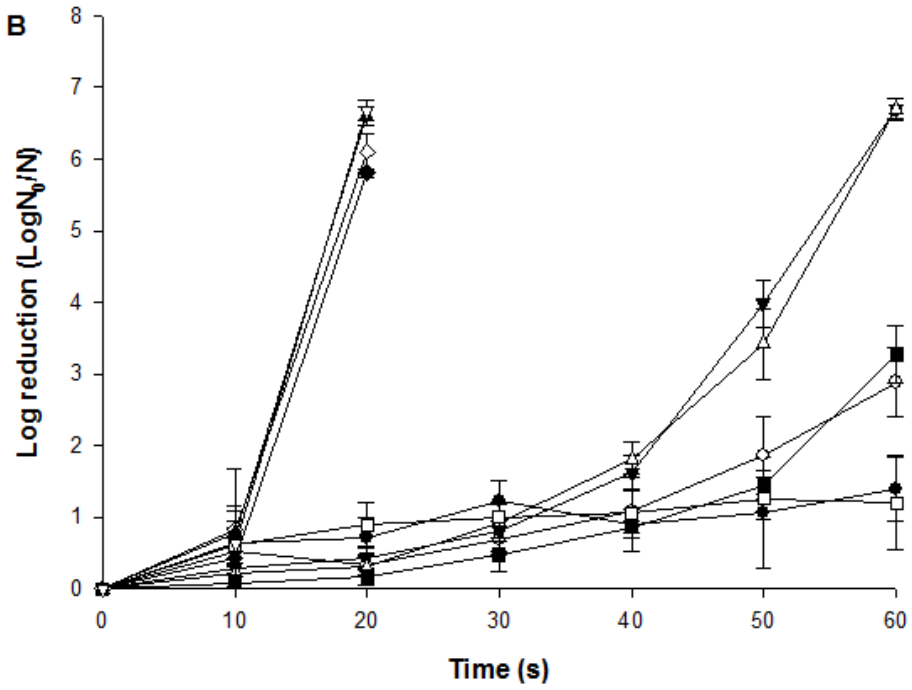
Fig. 8. System performance coefficient bars for heating apple juices of 18, 24, 36, 48, and 72 °Brix during ohmic heating at voltage gradients of 30V/cm, 40V/cm, 50V/cm, and 60V/cm.

3.2.3. Effect of ohmic heating for inactivation of food-borne pathogens at different voltage gradient

Fig. 9 shows the reduction of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocyto- genes* in 72, 48, 36, 24, and 18 °Brix apple juice during ohmic heating. Inactivation of the three food-borne pathogens generally increased with increasing temperature and time. For destruction of *E. coli* O157:H7, ohmic heating for 60s at 30V/cm achieved 0.94, 2.59, 6.78, 5.20, and 2.70 log reductions in 72, 48, 36, 24, and 18 °Brix apple juice, respectively. Also, reductions of 1.39, 2.88, 6.70, 6.69, and 3.26 log CFU/g in concentrations of 72, 48, 36, 24, and 18 °Brix, respectively, were observed in *S.* Typhimurium after ohmic heating. In the case of *L. monocytogenes*, levels of log reduction following ohmic heating were 0.47, 1.73, 5.00, 3.90, and 1.13, respectively, in juice concentrations of 72, 48, 36, 24, and 18 °Brix. From these results at 30V/cm, maximum log reductions of three food-borne pathogens were observed in 36 °Brix apple juice. At 60V/cm, dramatic reduction curves were discovered in apple juice during ohmic heating. Reductions of *E. coli*

O157:H7 were 6.32, 6.58, 6.88, and 6.93 log in 48, 36, 24, and 18 °Brix juice, respectively, after ohmic heating for 20s. Similarly, ohmic heating for 20s accomplished 5.80, 6.10, 6.60, and 6.68 log reductions of *S*. Typhimurium in 48, 36, 24, and 18 °Brix juice, respectively. Log reductions of 5.71, 5.70, 5.82, and 5.93 in 48, 36, 24, and 18 °Brix apple juice, respectively, were observed for *L. monocytogenes*. These levels of survival were below the detection limit (l log CFU/ml) for all three food-borne pathogens. However, these same pathogens were only slightly inactivated in 72 °Brix apple juice at 30 or 60V/cm.


A
Log reduction (LogN0/N)
Time (s)
0
1
2
3
4
5
6
7
8
0
10
20
30
40
50
60


B
Log reduction (LogN0/N)
Time (s)
0
1
2
3
4
5
6
7
8
0
10
20
30
40
50
60

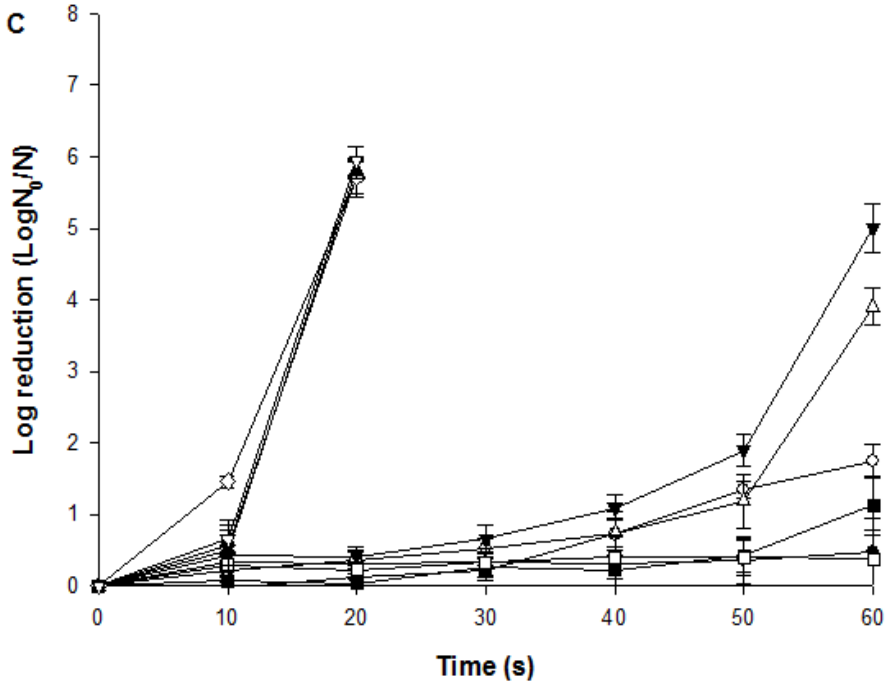


Fig. 9. Inactivation of *E. coli* O157:H7 (A), *S.* Typhimurium (B), and *L. monocytogenes* (C) in 18 (■), 24 (△), 36 (▼), 48 (○), and 72 °Brix (●) apple juice at 30V/cm and 18 (▽), 24 (▲), 36 (◇), 48 (◆), and 72 °Brix (□) apple juice at 60V/cm treated with ohmic heating for 0, 10, 20, 30, 40, 50, and 60s.

3.2.4. The influence of ohmic heating on color and pH of apple juice

Color and pH values of 18, 24, 36, 48, and 72 °Brix apple juice following ohmic heating at 30 and 60V/cm are shown in Table 1. All experiments were limited to a maximum treatment time of 60s. In case of 60V/cm, treatment time was restricted to 20s in 18, 24, 36, and 48 °Brix apple juice because microbial survival dropped to below the detection limit at this point of time. L*, a*, and b* values between non-treated and samples treated with ohmic heating were not significantly ($P > 0.05$) different. The pH values of treated samples did not significantly differ from those of non-treated samples.

Table 1. Color values[b] and pH of treated and untreated apple juices of 18, 24, 36, 48, and 72 °Brix at 30 and 60V/cm during ohmic heating.

Voltage gradient (V/cm)	Mean ± SD[a]					
	Solids content (°Brix)	Treatment time (s)	pH	Color[b]		
				L*	a*	b*
30V/cm	72	0s	3.42 ± 0.00^{A}	26.47 ± 0.06^{A}	0.38 ± 0.01^{A}	4.11 ± 0.01^{A}
		60s	3.42 ± 0.01^{A}	26.44 ± 0.08^{A}	0.38 ± 0.02^{A}	4.09 ± 0.05^{A}
	48	0s	3.51 ± 0.00^{A}	25.43 ± 0.29^{A}	0.49 ± 0.03^{A}	4.66 ± 0.09^{A}
		60s	3.51 ± 0.01^{A}	25.47 ± 0.72^{A}	0.47 ± 0.11^{A}	4.56 ± 0.22^{A}
	36	0s	3.54 ± 0.00^{A}	24.85 ± 0.10^{A}	0.49 ± 0.04^{A}	5.16 ± 0.05^{A}
		60s	3.54 ± 0.01^{A}	24.76 ± 0.19^{A}	0.54 ± 0.02^{A}	5.05 ± 0.19^{A}
	24	0s	3.57 ± 0.01^{A}	24.72 ± 0.65^{A}	0.32 ± 0.04^{A}	5.02 ± 0.35^{A}
		60s	3.57 ± 0.01^{A}	24.55 ± 0.08^{A}	0.38 ± 0.01^{A}	5.46 ± 0.09^{A}
	18	0s	3.59 ± 0.00^{A}	24.23 ± 0.23^{A}	0.25 ± 0.02^{A}	5.30 ± 0.43^{A}
		60s	3.60 ± 0.00^{A}	24.51 ± 0.19^{A}	0.27 ± 0.02^{A}	5.58 ± 0.12^{A}
60V/cm	72	0s	3.45 ± 0.01^{A}	26.01 ± 0.05^{A}	0.37 ± 0.03^{A}	4.10 ± 0.11^{A}
		60s	3.44 ± 0.00^{A}	26.03 ± 0.02^{A}	0.36 ± 0.07^{A}	4.19 ± 0.08^{A}
	48	0s	3.52 ± 0.01^{A}	25.36 ± 0.23^{A}	0.47 ± 0.02^{A}	4.26 ± 0.06^{A}
		20s	3.53 ± 0.00^{A}	25.32 ± 0.39^{A}	0.46 ± 0.09^{A}	4.38 ± 0.15^{A}
	36	0s	3.54 ± 0.01^{A}	24.56 ± 0.21^{A}	0.49 ± 0.01^{A}	5.28 ± 0.08^{A}
		20s	3.54 ± 0.01^{A}	24.55 ± 0.11^{A}	0.52 ± 0.09^{A}	5.17 ± 0.02^{A}
	24	0s	3.56 ± 0.00^{A}	24.45 ± 0.42^{A}	0.36 ± 0.01^{A}	5.39 ± 0.31^{A}
		20s	3.55 ± 0.00^{A}	24.55 ± 0.18^{A}	0.37 ± 0.04^{A}	5.41 ± 0.19^{A}
	18	0s	3.58 ± 0.01^{A}	24.43 ± 0.43^{A}	0.28 ± 0.07^{A}	5.35 ± 0.03^{A}
		20s	3.57 ± 0.01^{A}	24.33 ± 0.12^{A}	0.27 ± 0.01^{A}	5.42 ± 0.10^{A}

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

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

IV. DISCUSSION

Novel thermal processing interventions for inactivation of food-borne pathogens by the fruit juice industry involves the utilization of sophisticated systems, which enable reduced processing temperatures and times to prevent loss of nutritional and sensory quality, while still offering outstanding bactericidal efficacy. Ohmic heating is one of the most promising thermal processing systems for effectively inactivating food-borne pathogens and controlling processing conditions in this respect. Ohmic heating is an appropriate technology for minimizing degradation of juice quality due to the fundamental property of ohmic heating, which generates internal heat in food materials (26). Furthermore, an additional effect, induced electroporation in the cell membrane by electric fields, confers an advantage for maintaining fruit juice quality. To achieve the target reduction for food-borne pathogens, treatment temperatures and times for ohmic heating are lower and shorter than for conventional heat processing because of the electrical effect. Such

an effect for microbial inactivation is demonstrated by electroporation of cell membranes. Electroporation is related to charge transport resulting from differences between the internal potential and external potential of cells subjected to electric fields (27). This phenomenon causes formation of pores in the lipid bilayer and proteins of cell membranes (28). In previous studies, the effect of high voltage electric fields accompanying PEF, has been sufficiently studied by other scientists. However, the effect of moderate electric fields associated with ohmic heating has not been adequately investigated.

The purposes of this study were to investigate differences of microbial inactivation in BPW (pH 7.2) and apple juice (pH 3.5, 11.8 °Brix) between ohmic and conventional heat treatment, and changes of cell membrane permeability based on electroporation theory under ohmic heating. Electric field strength, which is applied in ohmic heating, is too weak to inactivate food-borne pathogens by electroporation alone. Palaniappan and Sastry reported that the lethal effect of electricity was inadequate for reduction of microorganisms by ohmic heating (29). However, the lethal effect of cell

electroporation is an important factor for inactivating food-borne pathogens when combined with heating. Moderate electric fields associated with ohmic heating induce reversible electroporation in microbial cell membranes (30). Although reversible electroporation is a transient phenomenon, it causes a tremendous bactericidal effect at certain temperatures (Fig. 2). In the present study, sample pH was fixed at 7.2 using BPW to evaluate the effect of moderate electric fields to reduce food-borne pathogens without altering other conditions. Moreover, since electrochemical reactions, which produce chemicals affecting incativation of food-borne pathogens, can occur at standard line voltage frequency (60Hz) during ohmic heating, 20kHz high frequency that does not cause electrochemical reactions, was used in this study (31, 32). At 58°C treatment for 30s, 2.42-, 3.21- and 0.70-log reductions and at 60°C for 30s, 3.59-, 3.48, and 3.40-log reductions in E. coli O157:H7, S. Typhimurium and L. monocytogenes, respectively, were induced by ohmic heating electric fields. Reduction levels of the three food-borne pathogens at 58 and 60°C in combination with the electric effect were 2-3 times higher than the thermal effect resulting from conventional heating.

This is explained through increased cell membrane permeability by formation of pores in cell membranes owing to electric fields, which results in accelerated cell death when combined with heating.

In order to identify increased levels of cell membrane permeability in electric fields at sublethal temperatures, TEM analysis and PI uptake experiments were conducted. TEM showed visual changes in morphology of cell membranes but could not indicate quantitative values. Therefore, PI uptake was performed to generate quantitative data. PI, which emits fluorescence when binding to nucleic acids, does not pass through intact cell membranes (33). PI was adopted as an indicator to assess membrane permeability or membrane damage. The fact that enhanced membrane permeability resulting from electric fields plays an important role for inactivating microorganisms, is demonstrated in Fig. 5. However, PI values indicate different patterns depending on species of food-borne pathogen. For E. coli O157:H7 and S. Typhimurium, Fig. 5 show a linear relationship between the number of non-viable cells and cells with permeated membranes (PI value). In the case of L. monocytogenes, levels of PI value per non-viable

cells for conventional heating was much higher than for ohmic heating, although PI uptake following ohmic heating was higher than for conventional heating. In Fig. 5, PI values for 30s exposure at 60°C were 11.64 and 6.84 in corresponding to log reductions of 4.37 and 0.97 (Fig. 2) for ohmic heating and conventional heating, respectively. Low levels of cell inactivation in comparison with PI uptake resulted in the PI value per non-viable cells for conventional heating to be higher than for ohmic heating. This result implies that cells of L. monocytogenes had not been deprived of their ability to proliferate when subjected to heat treatment despite permeabilization of their cell membranes, but ohmic heating-induced electric fields inactivated cells of L. monocytogenes without greatly reducing their viability (34, 35). As shown in Fig. 3, the ohmic heating process for inactivation of food-borne pathogens, was more effective in apple juice due to its higher acidity (pH 3.5), compared with conventional heating.

These overall results demonstrate that the electric effect of ohmic heating is a very important factor for reducing process times and temperatures by enhancing levels of inactivation of E. coli O157:H7, S. Typhimurium and L.

monocytogenes. Especially, ohmic heating is a particularly effective technology for processing acidic fruit juices such as apple juice, because it has a dramatic pasteurization effect on food-borne pathogens resulting from the combination of low pH, thermal treatment, and the electric effect. This allows for a noticeable reduction in treatment time and temperature. Therefore, ohmic heating is one of the most promising new technologies available to the fruit juice industry. In order to incorporate ohmic heat processing by the fruit juice industry, it is necessary to optimize conditions.

Processing conditions should be optimized for application by the fruit juice industry based on the electrical property of ohmic heating (21). Such an electrical characteristic, along with juice concentration could have an influence on temperature rise and microbial inactivation (36, 37). These aspects, along with system performance efficiency which affects processing cost were considered in this study.

There are various factors affecting on electrical conductivity of liquids. One researcher reported that electrical conductivity is relies on chemical components, ion activity, and viscosity of liquids (38). A study by

Palaniappan et al. (39) stated that the relationship between electrical conductivity and temperature was linear but conductivity decreased with soluble solids content increased in tomato and orange juices. However, there was an optimum concentration (36 °Brix) which facilitated the fastest rate of temperature rise in apple juice (Fig. 7). Although there was no significant ($P > 0.05$) difference as voltage increased. The levels of temperature rise in 24 and 36 °Brix juice were scarcely different at 40, 50, and 60V/cm except for 30V/cm. The rate of temperature increase was the major factor for inactivation food-borne pathogens including *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* within short time intervals.

Icier and Ilicali reported that SPC values varied according to the voltage gradient applied (36). The energy loss for a voltage gradient of 30V/cm was the lowest for all experiments when 24 °Brix apple juice was subjected to ohmic heating (Fig. 8). There were no significantly ($P > 0.05$) different SPC values between 24 and 36 °Brix apple juice, which indicated that 75 and 73% of the electrical energy applied to the system, respectively, was utilized for heating. Since commercial processing of higher concentration apple juice has

the advantage of greater production yield (of 18 °Brix), pasteurization of 36 °Brix apple juice was more efficient than for 24 °Brix apple juice. At 60V/cm, the SPC value for 48 °Brix apple juice, which is the actual electrical energy used to heat the samples, was 65%, the highest among all concentrations. SPC values of 36 °Brix at 30V/cm and 48 °Brix at 60V/cm were significantly ($P > 0.05$) different but this difference was relatively small.

Splittstoesser et al. (40) found that D-values at 52, 55, and 58°C for *E. coli* O157:H7 in apple juice were 12, 5, and 1min, respectively. In case of *S.* Typhimurium, D-values reported in raw milk were at 21.1min at 51.8°C and 1.7min at 57.2°C (41). Also, a study by Doyle demonstrated that a pasteurization protocol for *L. monocytogenes* required heating to 76.4 to 77.8°C and holding for 15.4s (42). Based on previous studies, it is important to reach the thermal death point for inactivation of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes*. When a voltage gradient of 30V/cm was applied to 36 °Brix samples for 60s, levels of inactivation of all three food-borne pathogens achieved more than a 5-log reduction. On the other hand, *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* were

reduced by more than 5-log at 60V/cm on 48, 36, 24, and 18 °Brix apple juice within 20s. During 30V/cm treatment for 60s, temperatures of 24.93, 63.62, 74.07, 68.97, and 61.45°C were attained in 72, 48, 36, 24, and 18 °Brix juice, respectively. All apple juice samples subjected to 60V/cm exceeded the target temperatures for inactivating microorganisms (Fig. 2). The time duration required for 5-log reduction at 30V/cm in 36 °Brix apple juice was three times longer than for 60V/cm in all apple juice concentrations with the exception of 72 °Brix. With respect to SPC values, treatment time, and bactericidal efficiency, ohmic heating application of 60V/cm on 48 °Brix apple juice was more effective than that of 30V/cm on 36°Brix apple juice.

These overall results suggest that SPC value can be ignored when the time duration for inactivation of food-borne pathogens is relatively short and production yield is sufficiently high. The optimization of several factors is very important in order to apply ohmic heating by the fruit juice industry. The inactivation of food-borne pathogens by ohmic heating is superior to that of conventional heating, as was demonstrated by previous studies.

Furthermore, the optimization of applied voltage gradient and juice concentration for ohmic heating does give a distinct advantage in terms of both economic and bactericidal aspects but also ensures minimal quality loss by the fruit juice industry.

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Ⅵ. 국문초록

본 연구에서는 펩톤식염완충액 (Buffered peptone water; BPW pH7.2)과 사과주스 (pH3.5, 11.8 °Brix) 내에서 옴가열의 전기장이 *Escherichia coli* O157:H7, *Salmonella* Typhimurium, *Listeria monocytogenes* 의 저감화에 미치는 영향에 대하여 살펴보았다. 펩톤식염완충액과 사과주스를 일반 가열과 옴가열 처리를 55, 58, 60 도에서 0, 10, 20, 25, 30 초 동안 각각 처리하였다. 펩톤식염완충액에서는 30V/cm, 사과주스에서는 60V/cm 각각의 옴가열 전기장 세기가 사용되었다. 펩톤식염완충액에서는 58, 60 도 처리에서 각각의 시간동안 옴가열과 일반 가열 사이에 유의적으로 저감화 효과 차이를 보였으며 사과주스에서는 55, 58, 60 도에서 유의적으로 차이를 나타냈다. 이 결과는 옴가열의 전기장은 준치사 온도에서 추가적인 미생물 저감화 효과가 있음을 보여준다. 옴가열의 전기장이 세포막의 투과성 증대를 초래하여 세포의

파괴시키는 정도를 알아보기 위하여 펩톤식염완충액에 병원성 미생물을 접종 후에 60 도에 0, 10, 20, 25, 30 초 동안 옴가열 처리 후 투과 전자 현미경 (TEM) 분석과 프로피디움 요오드화물 (PI) 침투 시험을 진행하였다. 옴가열과 일반 가열에 대해서 프로피디움 요오드화물 침투량이 유의적으로 차이가 있었으며, 병원성 미생물의 저감화 정도가 증가할수록 프로피디움 요오드화물 값 또한 증가하였다. 이 결과는 옴가열이 병원성 미생물을 더 낮은 온도에서 더 짧은 시간에서 효과적으로 저감화 시킬 수 있다는 것을 입증하며, 특히 사과주스와 같은 산성 조건의 과일 주스에서 더욱 효과적이라는 것을 알 수 있다. 따라서, 살균 시스템에 옴 가열을 도입으로 인해 제품 손상을 최소화 할 수 있다. 이에 더해 72, 48, 36, 24, 18 브릭스의 사과 주스내에서 병원성 미생물 저감화를 위한 옴가열 조건의 최적화 연구를 수행하였다. 각각의 사과 주스 농도에 대해서 30, 40, 50, 60V/cm 의 옴가열에 따른 온도를 측정하였다. 30, 40, 50,

60V/cm 의 옴가열을 적용하였을 경우 모든 실험에 대해서 36 브릭스의 사과주스의 온도가 가장 빠르게 증가하였다. 30, 40, 50, 60V/cm 의 전기장에서 72, 48, 36, 24, 18 브릭스의 사과 주스 각각에 대해서 시스템 효율 계수(SPC)를 측정하였다. 30V/cm 의 전기장을 24 브릭스의 사과주스에 가했을 때 시스템 효율 모든 전기장 세기에 대해서 가장 뛰어났다. 또한 30V/cm 전기장에서 24 브릭스와 36 브릭스의 시스템 효율 계수는 유의적으로 차이가 나타나지 않았다. 따라서 사과주스의 생산수율적 측면을 고려한다면 36 브릭스의 사과 주스 살균이 더욱 효율적이다. 한편, 60V/cm 전기장 세기에서는 48 브릭스의 사과 주스에서 시스템 효율 계수가 가장 높게 나타났다. 30V/cm 에서의 36 브릭스 사과주스와 60V/cm 에서의 48 브릭스 사과 주스 사이의 시스템 효율 계수가 유의적으로 차이는 있지만 8%의 효율 차이로 수치적으로 미미하다. 게다가, 옴가열로 30V/cm 에서 36 브릭스 사과주스를 60 초 처리했을 때만이 모든 병원성 미생물대하여

5 로그의 저감화 효과를 보였지만 48 브릭스의 경우 60V/cm 에서 20 초 처리시에 5 로그의 저감화 효과를 보였다. 이 결과로부터 48 브릭스 사과주스를 60V/cm 의 옴 가열로 살균하는 것이 경제적인 측면과 살균적인 측면에서 가장 월등하다는 것을 알 수 있다.

주요어: 일렉트로포레이션, 최적화, 옴가열, 식중독균, 전기장, 살균
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