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Effect of intrinsic factors in liquid food on the performance of ohmic heating

액체 식품의 내인성 요인이 옴 가열의 성능에 미치는 효과

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석사학위논문

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지도교수 강동현

이 논문을 석사학위 논문으로 제출함

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서울대학교 대학원 농생명공학부

김 상 순

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2015 년 8 월

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ABSTRACT

Ohmic heating is a novel heating technology, which has immense potential for achieving rapid and uniform heating in foods. Even though intrinsic factors in foods such as pH and fat content are important factors influencing the survival of microorganisms, research-investigations about the effect of intrinsic factors on the ohmic heating have been limited. Therefore, effect of pH and fat content on the performance of ohmic heating was investigated in the present study. The influence of pH and fat content was assessed on the heating rate, electrical conductivity, inactivation of foodborne pathogens, and quality aspects of food. Samples with varying pH or fat content were subjected to conventional and ohmic heating after inoculated with *Escherichia coli* O157:H7, *Salmonella enterica* serovar Typhimurium, and *Listeria monocytogenes*. At first, the effect of pH on the efficacy of ohmic heating was identified. For conventional heating, the heating rate was not significantly different ($p > 0.05$) regardless of pH, and pathogens were inactivated more effectively at lower pH. However, different patterns were

observed for ohmic heating. Although heating rate and electrical conductivity were not significantly affected ($p > 0.05$) by lowering pH, heating rate increased with increasing pH due to higher electrical conductivity. Also, the inactivation patterns were different from conventional heating. While *S.* Typhimurium was inactivated most rapidly at pH 2.5, *E. coli* O157:H7 and *L. monocytogenes* were inactivated most rapidly at pH 4.5. Color, pH and ^{o}Brix values of orange juice subjected to ohmic heating was not severely affected while non-thermal effects of ohmic heating were not observed. Additionally, the effect of fat content on the efficacy of ohmic heating was examined. For conventional heating, heating rate of samples and inactivation of pathogens were not significantly different ($p > 0.05$) regardless of fat content. Also, a protective effect of fat on pathogens was not observed for conventional heating. In contrast to conventional heating, ohmic heating was significantly affected by fat content. Heating rate decreased with higher fat content for ohmic heating due to lower electrical conductivity. Also, the protective effect of fat on *E. coli* O157:H7 and *L.*

monocytogenes was observed in samples subjected to ohmic heating. Therefore, pH and fat content should be considered as important factors which have a significant effect on the performance of ohmic heating for inactivation of foodborne pathogens.

Keywords: **Ohmic heating, food-borne pathogens, pH, fat content, electrical conductivity**
Student Number: **2013-23264**

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I. INTRODUCTION

Ohmic heating is a novel heating technology using electric current to generate heat inside of food. It has immense potential for achieving rapid and uniform heating in foods, providing microbiologically safe and high quality foods. Because of these advantages, ohmic heating can be used in diverse food industries. Possible applications for ohmic heating in the food industry include blanching, evaporation, dehydration, fermentation, extraction, sterilization, and pasteurization (Ramaswamy et al., 2014). Ohmic heating can be used for solid-liquid food mixtures because liquid and solid phases can be heated simultaneously (Lee et al., 2013). Regarding pasteurization, thermal and non-thermal effect can be shown for microbial inactivation of ohmic heating (Park and Kang, 2013). However, the non-thermal effect of ohmic heating is still an area of controversy. Some research indicates that ohmic heating may confer mild non-thermal cellular damage due to the presence of the electric field (Knirsch et al., 2010). On the other hand, other research indicates that ohmic heating shows no significant difference compared to conventional heating (Palaniappan et al., 1992). Nevertheless, it is certain that the principal mechanism of microbial inactivation in ohmic heating is the thermal effect.

Electrical conductivity (σ) is the main critical factor determining the rate of heat in ohmic heating technology. There are critical conductivity values below 0.01 Siemens/m (S/m) and above 10 S/m where ohmic heating is not applicable (Piette et al., 2004). Although in some foods electrical conductivity is not temperature-dependent, mostly the electrical conductivity increases with increasing temperature (Palaniappan and Sastry, 1991b). Electrical conductivity is affected by the nature of ions, ionic movement and viscosity of the liquid. Electrical conductivity is also affected by moisture content, starch gelatinization, applied frequency, voltage, type of food, and type and cut of meat (Pongviratchai and Park, 2007; Sarang et al., 2008).

Intrinsic factors in foods such as pH and fat content are important factors influencing the survival of microorganisms. Several studies dealt with the relationship between pH and inactivation of foodborne pathogens. The combination effect of low pH and thermal treatment has been reported on inactivating *Escherichia coli* O157:H7, *Salmonella enteritidis*, and *Listeria monocytogenes* (de W Blackburn et al., 1997; Juneja and Eblen, 1999). And also the combination effect of low pH and non-thermal treatments (ozone and high hydrostatic pressure) for inactivating foodborne pathogens was investigated (Alpas et al., 2000; Patil et al., 2010).

Orange juice enjoys worldwide popularity because of its pleasant taste, fresh flavor, and nutritional value including high vitamin C content. Even though orange juice has been considered a microbiologically safe food due to its acidity, some outbreaks associated with this beverage have been reported (Sospedra, Rubert, Soriano, Mañes, 2012). These outbreaks have resulted in many illnesses and some deaths. For example, a multistate outbreak of *Salmonella* Typhimurium and Saintpaul infections associated with unpasteurized orange juice was reported in the United States in 2005 (Danyluk, Goodrich-Schneider, Schneider, Harris, Worobo, 2012; Jain et al., 2009). These incidents indicate that processing orange juice adequately to ensure microbiological safety is essential. Lee et al. (2015) investigated the inactivation of *E. coli* O157:H7, *S.* Typhimurium, and *L. monoctygoenes* in orange juice samples with varying pH, adjusted with sodium hydroxide and citric acid. They revealed that pH is important factors influencing the inactivation of pathogens at fixed temperature.

Fat is one of the three major nutrients and performs many roles in the human body. Fat content in food varies considerably even within a type of food. Milk fat is the primary component of cream and milk is divided into three types according to fat content: whole milk, low-fat milk, and skimmed milk (Adebamowo et al., 2008). Fat content can be adjusted by adding cream

(Molkentin, 2013) The effect of fat content in various foods on inactivation of pathogens has been of interest. Some research-investigations have reported that fat content had no significant effect on the inactivation of pathogens. Byelashov et al. (2010) identified no differences in the *E. coli* O157:H7 inactivation rate between ground beef knuckle meat (approximately 5 % fat) and ground beef shoulder meat (approximately 15 % fat) subjected to conventional heating in a circulating water bath (75 ^{o}C). Kotrola et al. (1997) and Stoltenberg et el. (2006) also reported that fat content had no significant effect on the inactivation of *E. coli* O157:H7 when processed by thermal treatment. Inactivation of *E. coli* O157:H7, salmonellae, *Campylobacter jejuni*, *Listeria monocytogenes* and *Staphylococcus* spp. in raw ground beef was not influenced by the fat content when treated with gamma irradiation (Clavero et al., 1994; Monk et al., 1994). Inactivation of *L. monocytogenes* Scott A was not significantly different among whole, 2 %, and skimmed milk when treated with pulsed electric field (Reina et al., 1998).

In contrast to these previous research-investigations, the reduction of pathogens was affected by fat content in other studies. Heat resistance of *S.* Typhimurium DT 104 in beef increased with higher fat levels (Juneja and Eblen, 2000). The inactivation rate of *Listeria innocua* decreased with increasing fat content when treated by thermo-sonication (Bermúdez-Aguirre

and Barbosa-Cánovas, 2008). Juneja and Eblen (2000) and Bermúdez-Aguirre and Barbosa-Cánovas (2008) used a shaking water bath or oil bath for conventional heating treatment. However, no previous study has reported the effect of fat content on ohmic heating compared to conventional heating.

E. coli O157:H7, *S.* Typhimurium, and *L. monocytogenes* have been reported as common pathogenic microorganisms implicated in food borne out-break (Murinda et al., 2004). Even though the intrinsic factors such as pH and fat content are important for surviving of pathogens, research about the effect of intrinsic factors on the performance of ohmic heating is limited. Therefore, the objective of present study is to investigate the effect of pH and fat content on the efficacy of ohmic heating compared to conventional heating.

II. MATERIALS AND METHODS

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 19111, ATCC 19115, and ATCC 15313) were obtained from the bacterial culture collection of the School of Food Science, Seoul National University (Seoul, Korea). Cultures were produced as follows: a single colony cultivated from frozen stocks on tryptic soy agar (TSA; Difco, Becton, Dickinson, Sparks, MD) was inoculated into 5 ml of tryptic soy broth (TSB; Difco, Becton, Dickinson, Sparks, MD), incubated at 37 °C for 24 h, collected by centrifugation at 4,000 ×g for 20 min at 4 °C, and washed three times with 0.2 % peptone water (PW; Bacto, Becton, Dickinson, Sparks, MD). The final pellets were resuspended in 0.2 % PW, corresponding to approximately 10^8 to 10^9 CFU/ml. Afterwards, suspended pellets of the three pathogens were combined to comprise a mixed culture cocktail containing approximately equal numbers of cells of each strain of *E. coli* O157:H7 (10^7 CFU/ml), *S.* Typhimurium (10^7 CFU/ml), and *L. monocytogenes* (10^6 CFU/ml).

2.2. Ohmic heating system

The ohmic heating system (Fig. 1) consisted of a function generator (catalog number 33210A; Agilent Technologies, Palo Alto, CA, USA), a precision power amplifier (catalog number 4510; NF Corp., Yokohama, Japan), a two-channel digital-storage oscilloscope (catalog number TDS2001C; Tektronix, Inc., Beaverton, CO, USA), a data logger (catalog number 34970A; Agilent Technologies), and an ohmic heating chamber. The function generator produced various waveforms at frequencies from 1 mHz to 10 MHz and a maximum output level of 5 V. The signals generated through the power amplifier were amplified up to a maximum output of 141 V alternating current (AC). The signals expanded by the power amplifier were delivered to each of two titanium electrodes. The two-channel digital storage oscilloscope was used to measure signals, including waveform, frequency, voltage, and current. Temperature was controlled by Labview software (National Instruments, Austin, TX, USA). K-type thermocouples were inserted at the center of the ohmic heating chamber, and temperatures were recorded at 0.6-s intervals by a data logger and function generator operated according to whether the target temperature was reached or not. The

distance between the two electrodes was 2 cm, and the cross-sectional area was 60 cm^2.

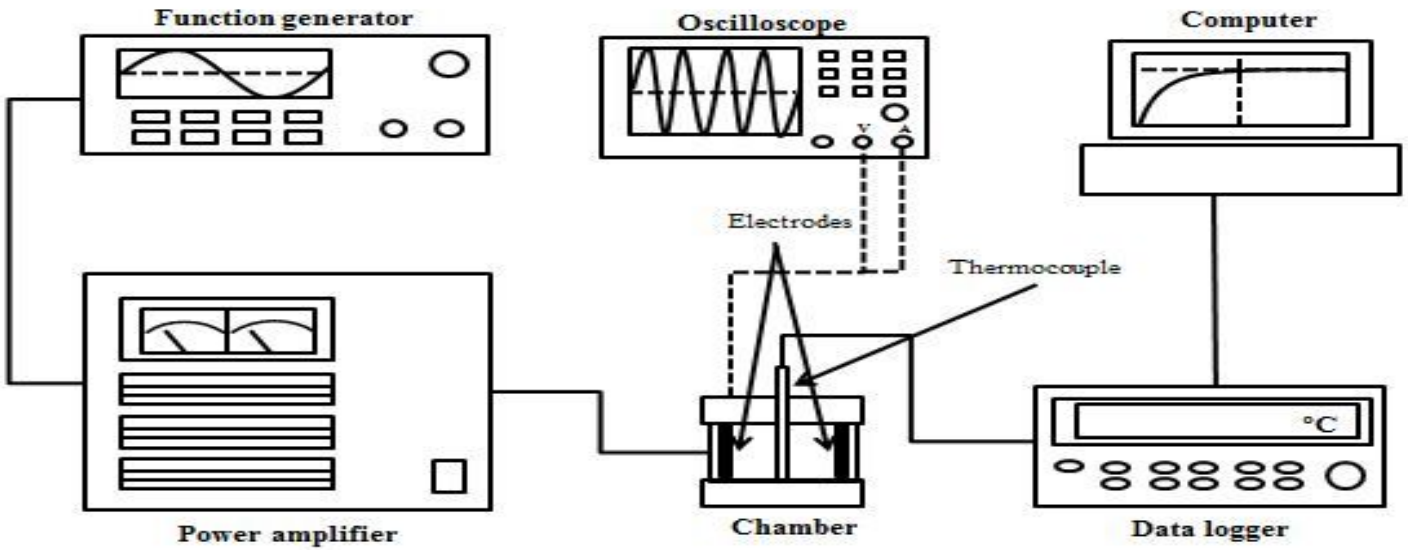


Fig. 1. Schematic diagram of the ohmic heating system at Seoul National University (Seoul, Korea).

2.3. Electrical conductivity measurement

Electrical conductivity of samples was determined from current and voltage data (Palaniappan and Sasatry, 1991a) and calculated as follows (equation 1) :

$$\sigma = \frac{LI}{AV} \quad (1)$$

where σ is the electrical conductivity (S/m), L is the distance between electrodes (m), I is the current (A), A is the cross-sectional area of the electrodes (m^2), and V is the voltage (V). Voltage and current were measured using the two channel digital storage oscilloscope.

2.4. Experiments of pH influencing the performance of ohmic heating

2.4.1. Sample preparation and inoculation

Pasteurized orange juice concentrate (pH 3.6; 66 °Brix), free of any preservatives, was purchased from a local grocery store and adjusted to 13.0 °Brix using distilled water. The pH of orange juice was adjusted to 2.5, 3.0 and 3.5 with 10 % citric acid (w/v) and to 4.0 and 4.5 with 1N sodium

hydroxide. A mixed-culture cocktail (0.2 ml) was inoculated into 25 ml of each prepared orange juice sample (before treatment) or after reaching target temperature for each experiment.

2.4.2. Conventional heating treatment

For conventional heating, a constant-temperature water bath (BW-10G; Jeio Tech, Seoul, Korea) was used. Temperature of the water bath was fixed at 75 ^{o}C. A conventional heating chamber was made of stainless steel (2 x 15 x 6 cm) of 0.2 cm thickness. A fiber optic temperature sensor (FOT-L; FISO Technologies Inc., Quebec, Canada) connected to a signal conditioner (TMI-4; FISO Technologies Inc., Quebec, Canada) was used to measure the temperature in the middle of the sample. Twenty-five ml of sample was placed into the chamber and inoculated with 0.2 ml of mixed culture cocktail. Prepared samples were treated for 0, 20, 40, 60, 80, and 100 s.

2.4.3. Ohmic heating treatment

For ohmic heating experiments, an ohmic heating system with a 20 kHz frequency and sine waveform was used. The electric field strength was fixed

at 25.6 V/cm. The ohmic heating chamber, which had the same shape and size as the conventional heating chamber, was made of polyvinyl chloride (2 x 15 x 6 cm) of 0.5 cm thickness. Twenty-five ml of sample was placed into the ohmic heating chamber and inoculated with 0.2 ml of mixed-culture cocktail before treatment. Appropriate treatment times were established for each pH level relative to inactivation of each pathogen.

2.4.4. Non-thermal effect

Temperature was controlled by Labview software (National Instruments, Austin, TX, USA) to identify the non-thermal effect of ohmic heating. The electric field was fixed at 9.6 V/cm after reaching the target temperature (50 ^{o}C) to maintain temperature stability, and the function generator was operated according to whether the target temperature was reached or not. For conventional heating treatment, the temperature of the water bath was fixed at 51.5 ^{o}C until the sample reached the targeted temperature. After each sample reached the targeted temperature (50 ^{o}C) for each heating method, 0.2 ml of mixed-culture cocktail was inoculated into the sample and treated for 2 min. Treatment temperature and time was chosen based on the

preliminary experiments to moderately inactivate pathogens (2~3 log reduction for non-adjusted samples).

2.4.5. Color, pH and ^{o}Brix measurement

For quality measurements, ohmic heating treatment was performed for the minimum treatment time required to inactivate all three pathogens to below the detection limit. The minimum treatment time was 60, 70, 75, 65, and 45 s for pH 2.5, 3.0, 3.5, 4.0, and 4.5, respectively. Untreated orange juice at each pH level was used as the control. After being treated for the minimum treatment time, samples were cooled rapidly in crushed ice. The color of treated and untreated samples was measured using a Minolta colorimeter (CR400; Minolta Co., Osaka, Japan). Color values for L^{*}, a^{*}, and b^{*} (lightness, redness, and yellowness, respectively) were recorded to evaluate color changes between treated and untreated samples. pH and ^{o}Brix of treated and untreated samples were measured with a Seven Multi 8603 pH meter (Mettler Toledo, Greifensee, Switzerland) and a digital refractometer (ATAGO PR-101, ATAGO CO., Tokyo, Japan), respectively.

2.5. Experiments of fat content influencing the performance of ohmic heating

2.5.1. Sample preparation and inoculation

Sterile buffered peptone water (BPW; Difco, Sparks, MD) was used in this experiment**.** Sterile cream containing 37 % fat and emulsifier were purchased from a local grocery store (Seoul, Korea). BPW was mixed with pasteurized cream to achieve fat contents of 0 % (without cream) 3 %, 7 %, and 10 % (w/v). Samples were mixed using a magnetic stirrer and stir bar. A mixed-culture cocktail (0.2 mL) was inoculated into 25 mL of prepared sample tempered to room temperature before treatment in experiments involving temperature increase. On the other hand, mixed-culture cocktail was inoculated into the sample after it reached target temperature for experiments performed at a fixed temperature.

2.5.2. Conventional heating treatment

For conventional heating, a constant-temperature water bath (BW-10G; Jeio Tech, Seoul, Korea) was used in the present study. The conventional heating chamber was made of stainless steel (2 x 15 x 6 cm) of 0.2 cm thickness. Twenty-five mL of sample was placed into the conventional

heating chamber and inoculated with 0.2 mL of mixed culture cocktail. Temperature of the water bath was fixed at 75 ^{o}C. A fiber optic temperature sensor (FOT-L; FISO Technologies Inc.) connected to a signal conditioner (TMI-4; FISO Technologies Inc.) was used to measure the temperature in the middle of the sample. Prepared sample was treated for 0, 30, 60, 90, 120, 150, and 180 s.

2.5.3. Ohmic heating treatment

For ohmic heating experiments, an ohmic heating system with a 20 kHz frequency and sine waveform were used. The ohmic heating chamber was made of polyvinyl chloride (2 x 15 x 6 cm) of 0.5 cm thickness. Twenty-five mL of sample was placed into the ohmic heating chamber and inoculated with 0.2 mL of mixed-culture cocktail before treatment. Each sample was treated for 0, 10, 20, 30, 35, 40, 45 and 50 s with 19.2 V/cm ohmc heating.

2.5.4. Protective effect

Temperature was controlled by Labview software (National Instruments, Austin, TX, USA) to identify the protective effect of fat content.

Temperature of sample was fixed using a water bath in conventional heating. After sample reaching targeted temperature (60 ^{o}C) for each heating method, 0.2 mL of mixed-culture cocktail was inoculated into the sample and treated for 2 min. Treatment temperature (60 ^{o}C) and time (2 min) were chosen from the preliminary experiment to inactivate pathogens moderately.

2.6. Microbial enumeration

For microbial enumeration, each treated 25 mL sample was immediately transferred into a sterile stomacher bag (Labplas, Inc., Sainte-Julie, Quebec, Canada) containing 225 mL of 0.2 % PW and homogenized for 2 min in a stomacher (Easy Mix; AES Chemunex, Rennes, France). After homogenization, 1 mL aliquots withdrawn from stomacher bags were serially diluted tenfold in 0.2 % PW and 0.1 mL of appropriate diluents were spread-plated onto each selective medium. Sorbitol MacConkey (SMAC) agar (Difco), xylose lysine deoxycholate (XLD) agar (Difco), and Oxford agar base (OAB; Difco) with selective supplement (Bacto Oxford antimicrobial 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, 1 mL

aliquots withdrawn from stomacher bags were divided between four plates of each medium and spread plated. After all plates were incubated at 37 °C for 24 to 48 h, colonies were counted.

2.7. Statistical analysis

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

III. RESULTS

3.1. Effect of pH on the performance of ohmic heating for inactivation of foodborne pathogens

3.1.1. Effect of pH on the temperature increase and electrical conductivity

The temperature histories of samples exposed to ohmic and conventional heating are shown in Fig. 2. The heating rate of orange juice subjected to conventional heating was not affected by adjusting pH (Fig. 2A). On the other hand, the heating rate and electrical conductivity of orange juice subjected to ohmic heating was affected by differing pH (Fig. 2B and Fig. 3). Lowering the pH to 2.5, 3.0, and 3.5 with citric acid produced no obvious effect on the heating rate and electrical conductivity of ohmic heated orange juice samples. However, temperature and electrical conductivity increased more rapidly in orange juice of pH 4.0 and 4.5. Thirty-six s was needed for pH 4.5 samples to reach ca. 70 ^{o}C, but 51 s and 63 s were needed for samples of pH 4.0 and 3.5, respectively.

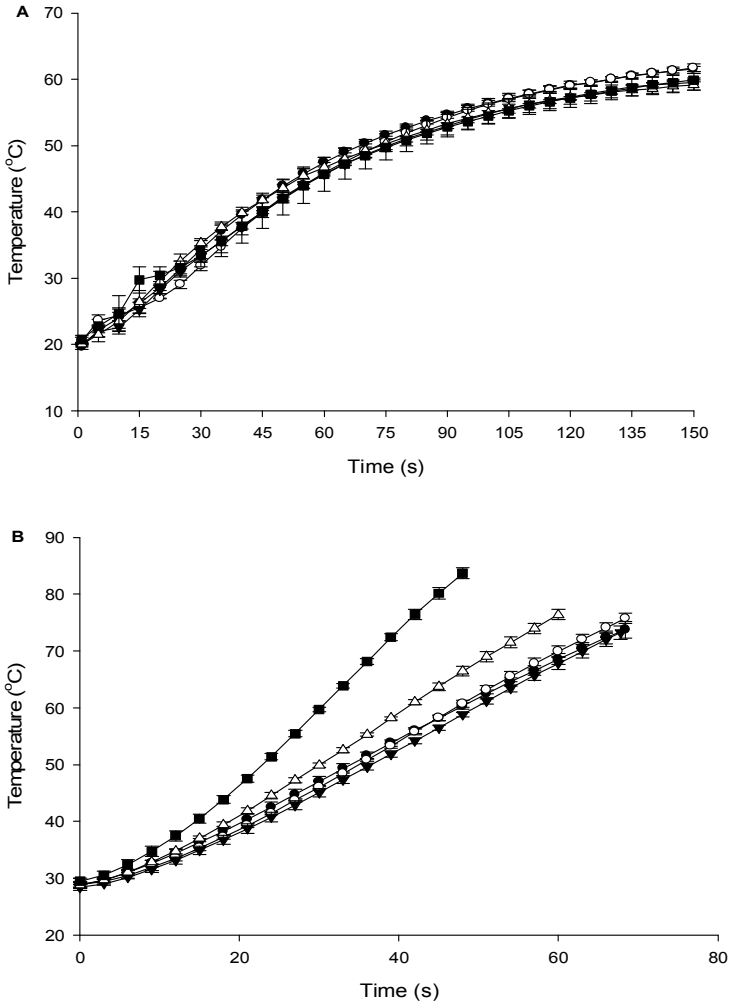


Fig. 2. Temperature histories of samples subjected to conventional (A) and ohmic heating (B) at pH 2.5 (●), 3.0 (○), 3.5 (▼), 4.0 (△) and 4.5 (■). The results are means from three experiments, and error bars indicate standard errors.

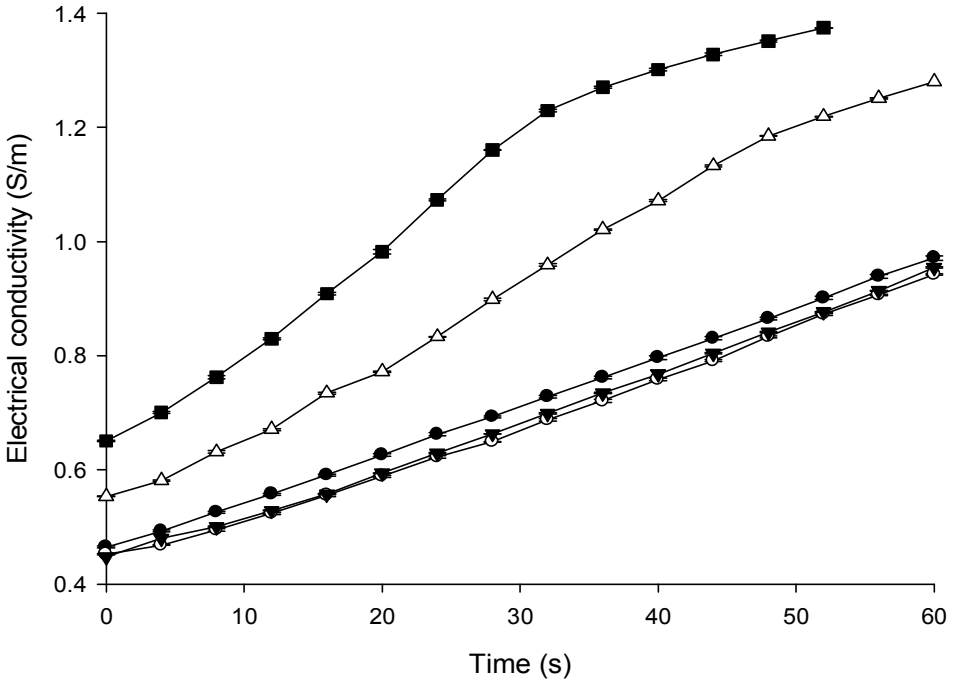
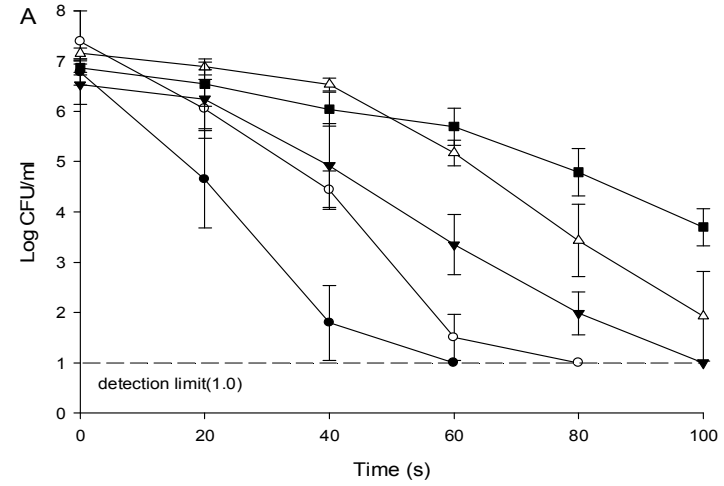


Fig. 3. Electrical conductivity histories of samples subjected to ohmic heating at pH 2.5 (●), 3.0 (○), 3.5 (▼), 4.0 (△) and 4.5 (■). Results are means from three experiments, and error bars indicate standard errors.

3.1.2. Effect of pH on the inactivation of pathogens subjected with conventional heating

The survival of pathogens subjected to conventional heating at various pH levels is shown in Fig. 4. All three pathogens were inactivated more rapidly at lower pH. *S.* Typhimurium was the most sensitive to acidic conditions followed by *E. coli* O157:H7, then finally by *L. monocytogenes*. Also, the time needed for populations of all three pathogens to decrease to below the detection limit was shortened relative to decreasing pH level.


A
Log CFU/ml
detection limit(1.0)
Time (s)
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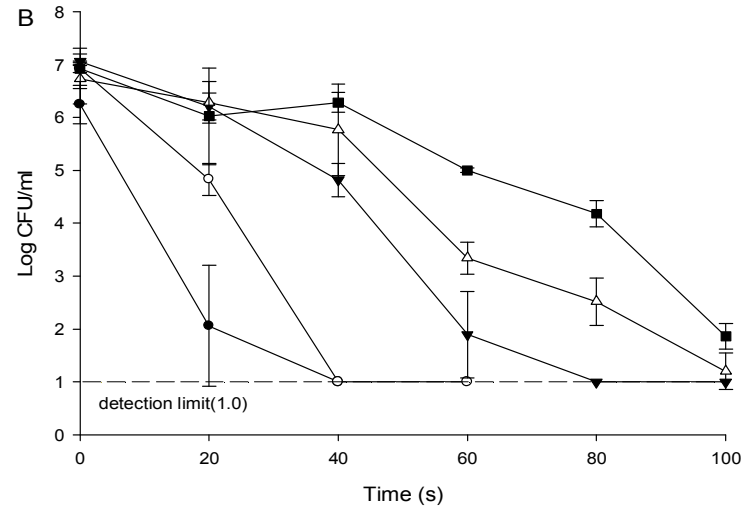
20
40
60
80
100
1
2
3
4
5
6
7
8


B
Log CFU/ml
detection limit(1.0)
Time (s)
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40
60
80
100
1
2
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4
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6
7
8

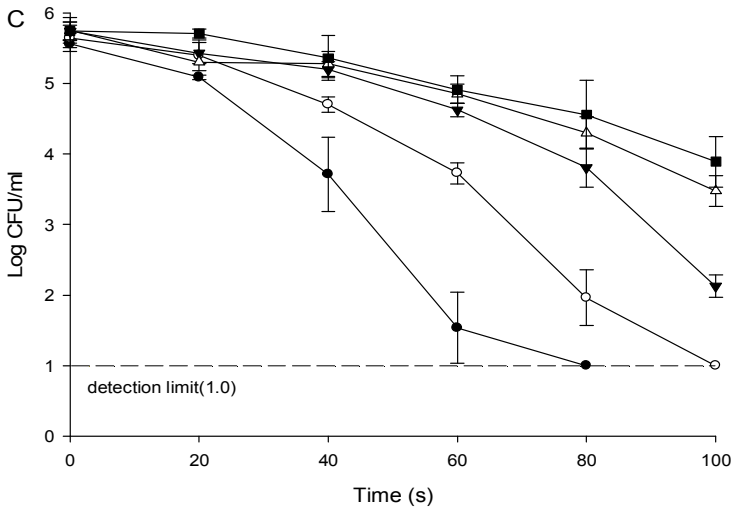
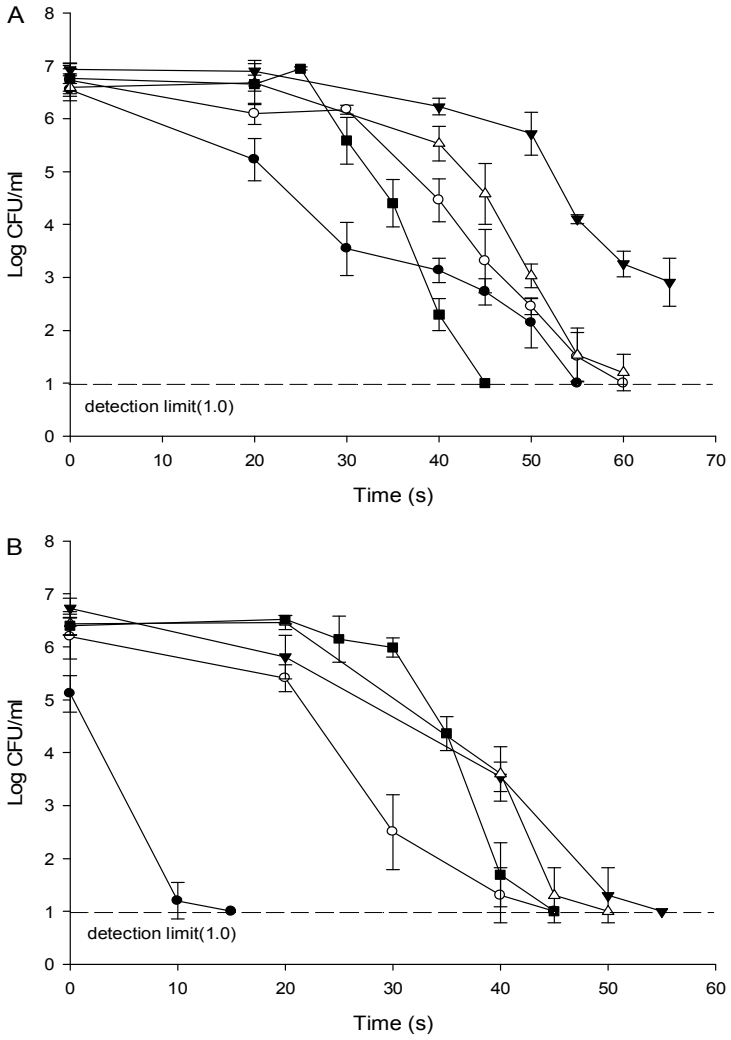


Fig. 4. Survival curves of *E. coli* O157:H7 (A), *S.* Typhimurium (B), and *L. monocytogenes* (C) corresponding to microbial inactivation in samples subjected to conventional heating at pH 2.5 (●), 3.0 (○), 3.5 (▼), 4.0 (△) and 4.5 (■). The results are means from three experiments, and error bars indicate standard errors.

3.1.3. Effect of pH on the inactivation of pathogens subjected with ohmic heating

The survival of pathogens subjected to ohmic heating at various pH levels is shown in Fig. 5. *E. coli* O157:H7 was inactivated most rapidly at pH 4.5 followed by 2.5, 3.0, 4.0 and 3.5. *S.* Typhimurium was inactivated most rapidly at pH 2.5 followed by 3.0, 4.5, 4.0 and 3.5. Finally, *L. monocytogenes* was inactivated most rapidly at pH 4.5 followed by 4.0, 2.5, 3.0 and 3.5. The shortest time required to reduce populations of *E. coli* O157:H7 and *L. monocytogenes* to below the detection limit was 45 s at pH 4.5. On the other hand, the shortest time necessary to decrease populations of *S.* Typhimurium to below the detection limit was 15 s at pH 2.5.


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

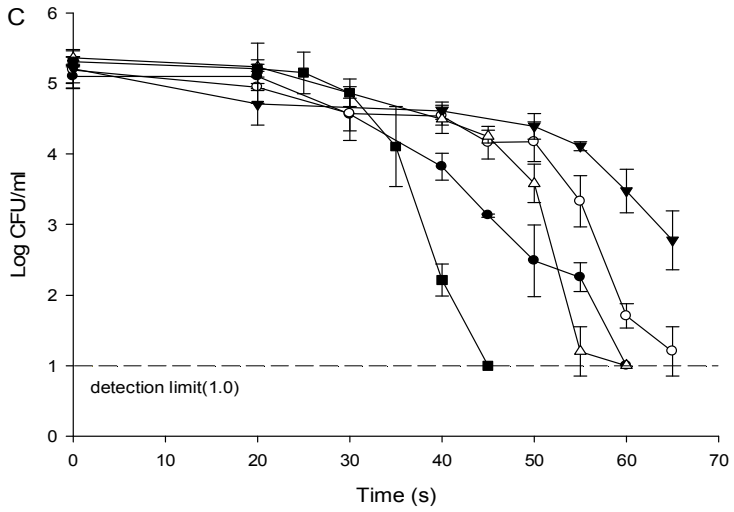


Fig. 5. Survival curves of *E. coli* O157:H7 (A), *S.* Typhimurium (B), and *L. monocytogenes* (C) corresponding to microbial inactivation in samples subjected to ohmic heating at pH 2.5 (●), 3.0 (○), 3.5 (▼), 4.0 (△) and 4.5 (■). The results are means from three experiments, and error bars indicate standard errors.

3.1.4. Non-thermal effect of ohmic heating on the inactivation of pathogens

The reduction of pathogens subjected to each heating at fixed temperature and time (50 ^{o}C, 2 min) is shown in Table 1. The inactivation aspects of ohmic heating at the same temperature were similar to those of conventional heating. The reduction of all three pathogens increased with decreasing pH. The reductions of all three pathogens subjected to ohmic heating at each pH level were not different from those of conventional heating, and non-thermal effects of ohmic heating were not observed.

Table 1. Reduction of foodborne pathogens subjected to conventional and ohmic heating with various pH levels[a]

Organisms	Treatment	pH				
		2.5	3.0	3.5	4.0	4.5
E. coli O157:H7	Ohmic[b]	5.51 ± 0.08 Aa	3.62 ± 0.24 Ab	2.29 ± 0.72 Ac	0.02 ± 0.03 Ad	0.19 ± 0.34 Ad
	Conventional	5.78 ± 0.18 Aa	3.20 ± 0.19 Ab	2.29 ± 0.46 Ac	0.14 ± 0.12 Ad	0.20 ± 0.18 Ad
S. Typhimurium	Ohmic	5.70 ± 0.26 Aa	5.47 ± 0.26 Aa	3.91 ± 0.91 Ab	1.65 ± 0.56 Ac	0.27 ± 0.28 Ad
	Conventional	5.93 ± 0.07 Aa	5.55 ± 0.46 Aa	3.15 ± 0.33 Ab	1.59 ± 0.38 Ac	0.51 ± 0.21 Ad
L. monocytogenes	Ohmic	1.90 ± 0.17 Aa	0.73 ± 0.06 Ab	0.59 ± 0.62 Abc	0.43 ± 0.17 Abc	0.11 ± 0.10 Ac
	Conventional	1.77 ± 0.28 Aa	0.87 ± 0.20 Ab	0.49 ± 0.20 Abc	0.43 ± 0.31 Abc	0.16 ± 0.28 Ac

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

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

3.1.5. Color, pH and ^{o}Brix measurement

Color, pH, and ^{o}Brix values of ohmic treated and untreated orange juice samples are shown in Table 2. In part, some values of treated orange juice were significantly different from those of untreated orange juice. L^{*} value decreased slightly at pH 2.5. L^{*} and a^{*} values were affected at pH 3.0, and pH values decreased slightly when pH 3.5 juice was treated. Finally, pH and ^{o}Brix values were affected by ohmic heating at pH 4.5. However, the differences were very small and can be ignored.

Table 2
Color, pH and °Brix values of ohmic treated and untreated juice at various pH levels[a]

pH	Treatment	pH	°Brix	Color		
				L*	a*	b*
2.5	Untreated	2.50 ± 0.00 A	12.97 ± 0.06 A	36.97 ± 0.10 A	-3.04 ± 0.05 A	6.11 ± 0.37 A
	Treated (60s)	2.49 ± 0.00 A	13.07 ± 0.06 A	36.26 ± 0.39 B	-2.85 ± 0.13 A	5.78 ± 0.20 A
3.0	Untreated	2.99 ± 0.01 A	13.03 ± 0.06 A	38.65 ± 0.16 A	-3.85 ± 0.05 A	7.89 ± 0.15 A
	Treated (70s)	2.99 ± 0.01 A	13.05 ± 0.07 A	37.16 ± 0.74 B	-3.24 ± 0.33 B	6.60 ± 0.87 A
3.5	Untreated	3.47 ± 0.02 A	13.07 ± 0.06 A	38.52 ± 0.42 A	-3.99 ± 0.20 A	8.52 ± 0.39 A
	Treated (75s)	3.43 ± 0.01 B	13.03 ± 0.06 A	37.59 ± 1.68 A	-3.43 ± 0.62 A	7.17 ± 1.55 A
4.0	Untreated	4.00 ± 0.00 A	12.97 ± 0.06 A	38.43 ± 0.38 A	-4.00 ± 0.15 A	8.44 ± 0.34 A
	Treated (65s)	3.98 ± 0.03 A	13.00 ± 0.10 A	37.85 ± 1.96 A	-3.54 ± 0.85 A	7.27 ± 2.42 A
4.5	Untreated	4.50 ± 0.01 A	13.00 ± 0.00 A	37.68 ± 0.18 A	-3.71 ± 0.08 A	7.61 ± 0.20 A
	Treated (45s)	4.46 ± 0.01 B	13.23 ± 0.06 B	36.77 ± 2.52 A	-3.03 ± 0.82 A	6.00 ± 1.98 A

Mean values ± standard deviation
[a]Values in the same column that are followed by the same uppercase letter are not significantly different at each pH ($p > 0.05$).
[b] Color values are L* (lightness), a* (redness) and b* (yellowness).

3.2. Effect of fat content on the performance of ohmic heating for inactivation of foodborne pathogens

3.2.1. Effect of fat content on the temperature increase and electrical conductivity

The temperature histories of samples subjected to ohmic heating are shown in Figure 6B. Temperature increased with increasing treatment time at each fat content sample. The heating rate of samples with 3 % fat content did not differ from those of 0 % fat content. However, the heating rate of samples with 7 % fat content decreased significantly ($p < 0.05$) followed by 10% fat content. Temperature curves including error bars for samples of 3, 7 and 10 % fat content did not overlap with each other. The electrical conductivity of samples with fat content ranging from 0 to 10 % subjected to ohmic heating is shown in Figure 3. Electrical conductivity increased with increasing time. For each time interval, electrical conductivity was smaller as fat content increased. In contrast to ohmic heating, there was no significant effect ($p > 0.05$) of fat content (0~10 %) on the temperature increase of conventionally heated samples. Temperature curves, including error bars, for those of varying fat content (0~ 10 %) are shown in Figure 6A; these curves

overlap each other. In contrast to ohmic heating, the rate of conventional heating decreased as temperature approached the target temperature. The different pattern and heating rate of conventional compared to ohmic heating resulted from the existence of external heat source in the case of conventional heating. Significant differences ($p = 0.05$) between temperature curves in both ohmic and conventional heating were verified using statistical analysis.

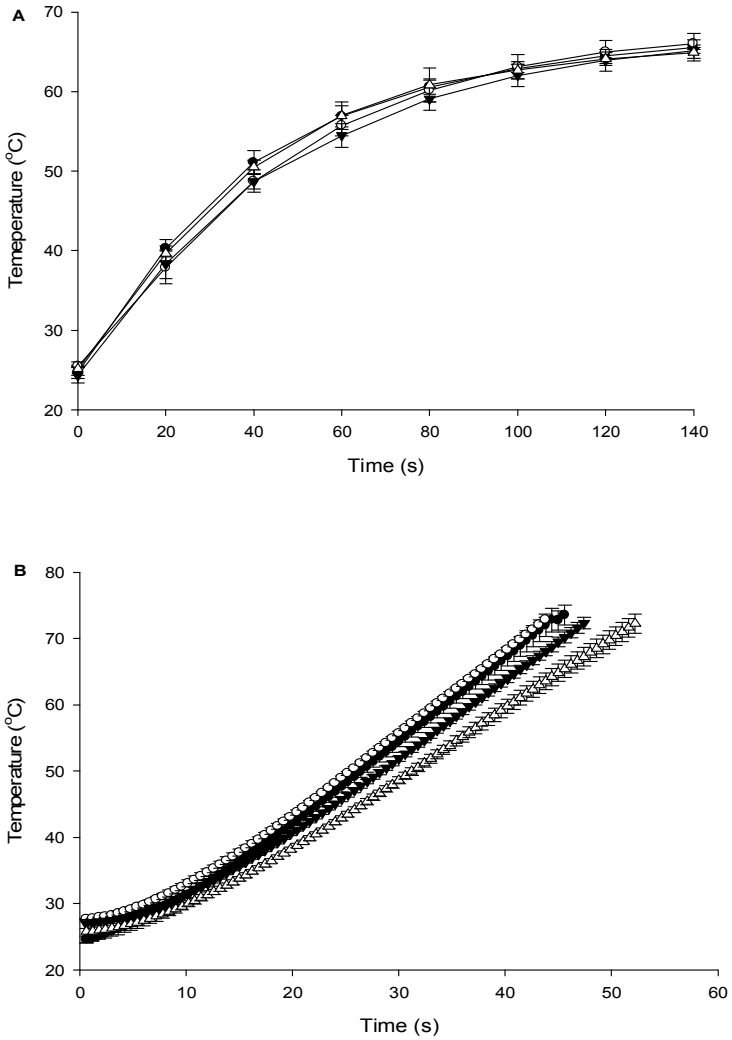


Fig. 6 Temperature histories of samples subjected to conventional (A) and ohmic heating (B) with 0 % (●), 3 % (○), 7 % (▼), and 10 % (△) fat content. The results are means from three experiments, and error bars indicate standard errors.

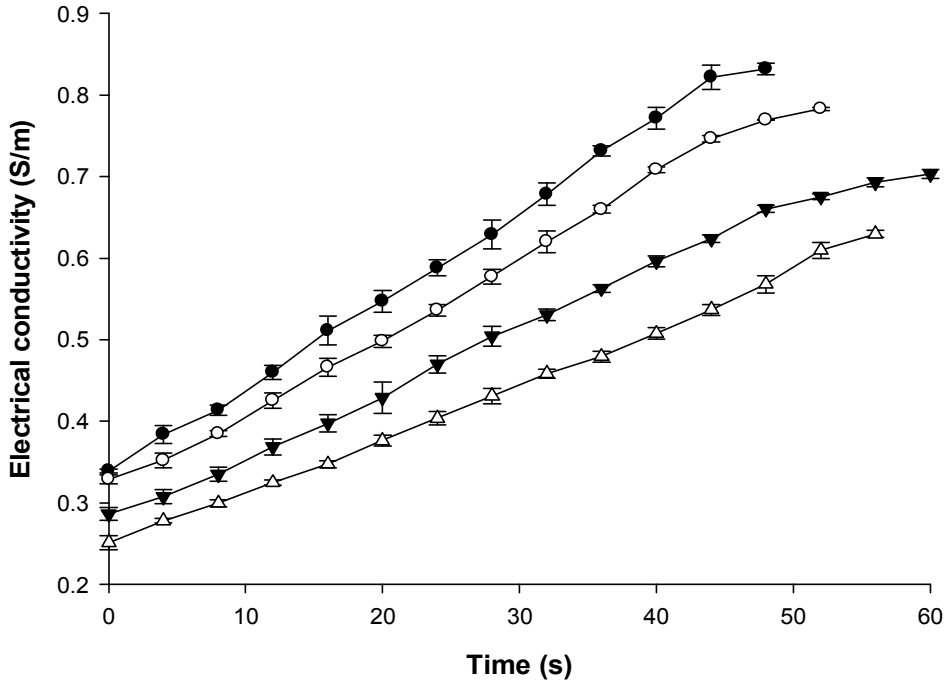
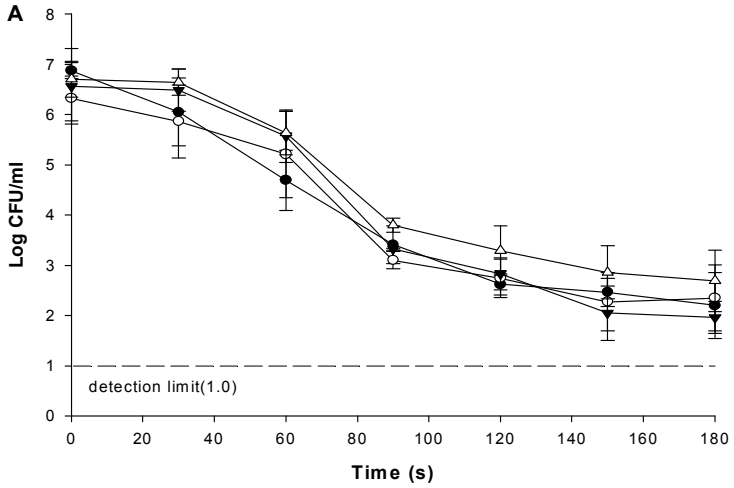
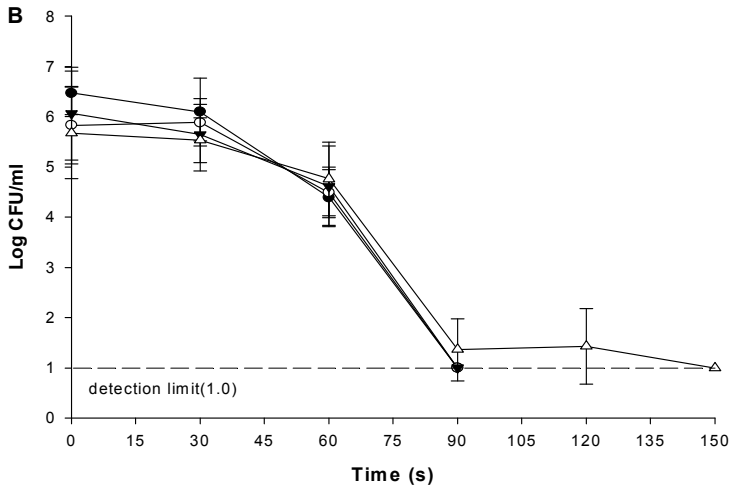


Fig. 7 Electrical conductivity histories of samples subjected to ohmic heating with 0 % (●), 3 % (○), 7 % (▼), and 10 % (△) fat content. The results are means from three experiments, and error bars indicate standard errors.

3.2.2. Effect of fat content on the inactivation of foodborne pathogens subjected with conventional heating

The survival of pathogens in the samples corresponding to temperature histories (Figure 6A) is shown in Figure 8. The reduction of *E. coli* O157: H7, *S.*Typhimurium and *L. monocytogenes* was not different among the samples with 0, 3, and 7 % fat content. Although reduction of three pathogens decreased in the samples with 10 % fat content, survival curves including error bars were overlapped with survival curves in the samples with 0, 3, and 7 % fat content. The survivals of *E. coli* O157:H7, *S.* Typhimurium, and *L.monocytogenes* in the samples with varying fat content (0 ~ 10 %) were not significantly different ($p > 0.05$).


A
Log CFU/ml
detection limit(1.0)
Time (s)
0
1
2
3
4
5
6
7
8
0
20
40
60
80
100
120
140
160
180


B
Log CFU/ml
detection limit(1.0)
Time (s)
0
1
2
3
4
5
6
7
8
0
15
30
45
60
75
90
105
120
135
150

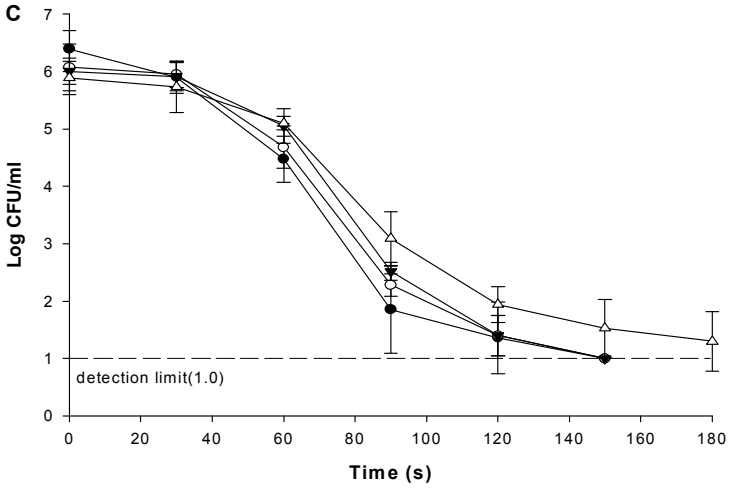
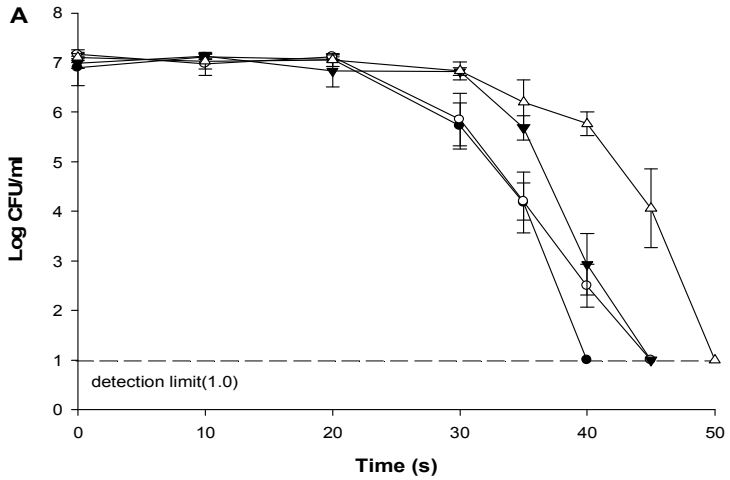
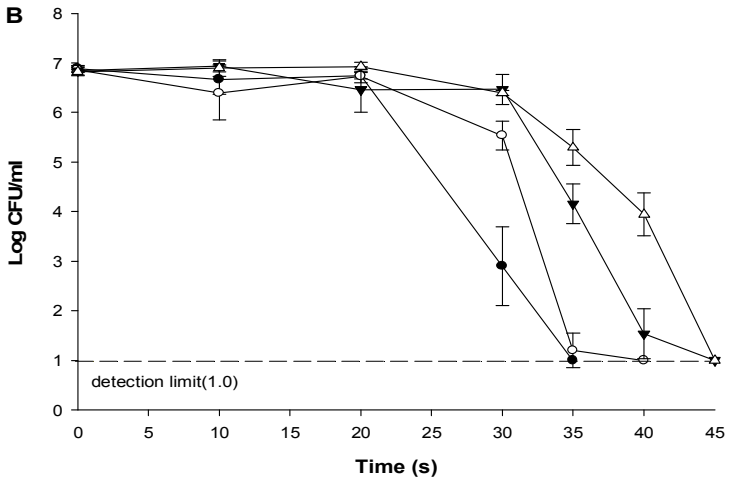


Fig. 8 Survival curves of *E. coli* O157:H7 (A), *S.* Typhimurium (B), and *L. monocytogenes* (C) corresponding to temperature increase (Figure 6A) in samples subjected to conventional heating with 0 % (●), 3 % (○), 7 % (▼), and 10 % (△) fat content. The results are means from three experiments, and error bars indicate standard errors.

3.2.3. Effect of fat content on the inactivation of foodborne pathogens subjected with ohmic heating

The survival of pathogens in the samples corresponding to temperature histories (Figure 6B) is shown in Figure 9. Regardless of the type of pathogen, ohmic heating in samples of lower, rather than higher, fat content achieved greater reduction for the same treatment time. Also, ohmic heating of lower fat content samples required less time for pathogens to decrease below the detection limit than for those of a higher fat content. For *E. coli* O157:H7, 40 s and 50 s were needed to decrease the population below the detection limit for 0 % and 10 % fat content samples, respectively. For *S.* Typhimurium, 35 s and 45 s were needed to decrease populations below the detection limit for 0 % and 10 % fat content samples, respectively. Finally for *L. monocytogenes*, 40 s and 50 s were needed to decrease populations below the detection limit for 0 % and 10 % fat content samples, respectively.


A
Log CFU/ml
detection limit(1.0)
Time (s)
0
10
20
30
40
50
1
2
3
4
5
6
7
8


B
Log CFU/ml
detection limit(1.0)
Time (s)
0
5
10
15
20
25
30
35
40
45
1
2
3
4
5
6
7
8

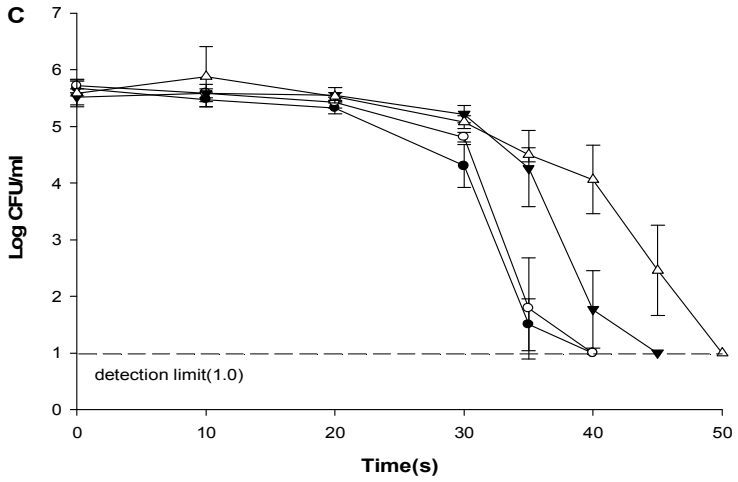


Fig. 9 Survival curves of *E. coli* O157:H7 (A), *S.* Typhimurium (B), and *L. monocytogenes* (C) corresponding to temperature increase (Figure 6B) in samples subjected to ohmic heating with 0 % (●), 3 % (○), 7 % (▼), and 10 % (△) fat content. The results are means from three experiments, and error bars indicate standard errors.

3.2.4. Protective effect of fat content on the inactivation of foodborne pathogens

The reduction of *E.coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* in the samples with 0 % fat content were moderate (2~4 log reduction CFU/ml) in the preliminary experiments (60 ^{o}C, 2min). Figure 6A shows the reduction of pathogens in samples subjected to ohmic heating for a fixed temperature and time (60 ^{o}C, 2 min). The reduction of *S.* Typhimurium was not significantly different regardless of fat content. Also, the reduction of *E. coli* O157: H7 and *L. monocytogenes* was not significantly different among fat content levels of 0 %, 3 % and 7 %. However, the reduction of *E. coli* O157: H7 and *L. monocytogenes* decreased when 10 % fat content samples were treated with ohmic heating. In contrast to ohmic heating, reductions of all three pathogens subjected to conventional heating did not differ significantly regardless of fat content (Figure 10B).

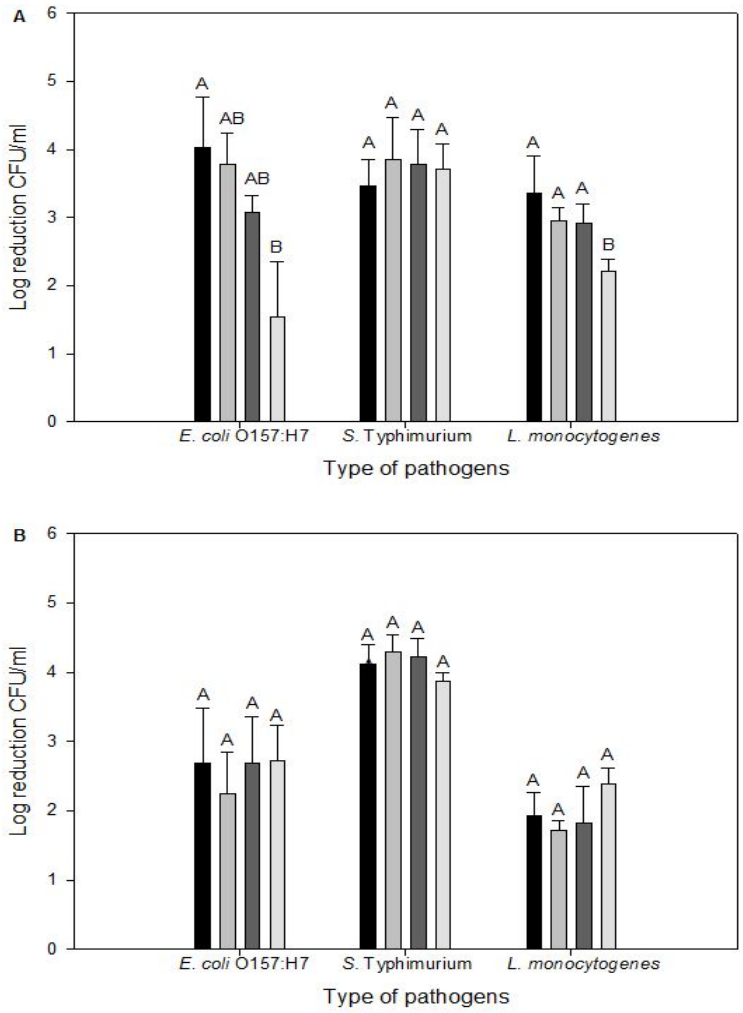


Figure 10. Reduction (log CFU/mL) of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* in samples subjected to ohmic (A) and conventional heating (B) at a fixed temperature and time (60 ^{o}C, 2min) with 0 % (■), 3 % (■), 7 % (■), and 10 % (■) fat content**.** The results are means from three

experiments, and error bars indicate standard errors. Bars for each pathogen with different letters are significantly different for each pathogen ($p < 0.05$).

IV. DISCUSSION

In conventional heating, heat transfer can be achieved via conduction and convection (Zhu et al., 2014). Therefore, adjusted pH had no significant effect ($p > 0.05$) on the conventional heat transfer of samples. In contrast to conventional heating, the heating rate of orange juice subjected to ohmic heating was significantly altered by varying the pH. The heating rate of orange juice adjusted with sodium hydroxide (pH 4.0 and 4.5) clearly increased. On the other hand, the heating rate of orange juice adjusted with citric acid did not significantly increase. The difference in heating rate was due to differences in electrical conductivity. Electrical conductivity of orange juice was affected more by sodium hydroxide rather than by citric acid. Major factors influencing aqueous solution conductivity include the nature and concentration of solutes, the degree to which solutes are dissociated into ions, the amount of electrical charge on each ion, the freedom of ions to move about, and the temperature of solution (Palaniappan and Sastry, 1991b). The electrical conductivity ratio is an indicator of the degree of dissociation, and weak electrolytes such as organic compounds are only partly dissociated in solution (Minear and Keith, 1982). Therefore, dissociation is variable, depending on the type of the acid and base. Sodium hydroxide is a popular

strong base and citric acid is a weak organic acid; both are generally recognized as safe and are used widely as food additives (FDA, 2014). A faster heating rate was observed for juice adjusted with sodium hydroxide, due to its higher electrical conductivity resulting from high dissociation of this molecule. Nevertheless, these results about the temperature increase and electrical conductivity are only applicable to orange juice adjusted with citric acid and sodium hydroxide. For example, results from apple juice adjusted with malic acid and sodium hydroxide were different from those of orange juice (data not shown).

Inactivation corresponding to temperature increase resulting from ohmic heating has a different trend compared with that of conventional heating. Altering pH was obviously effective for pasteurizing orange juice by ohmic heating. Pathogens were inactivated in two different ways (thermal effect and acid effect). While the thermal effect was most pronounced at pH 4.5 due to the higher temperature attained, the acid effect was most pronounced at pH 2.5. Because *S.* Typhimurium has relatively low acid resistance, it was inactivated most effectively at pH 2.5. On the other hand, the inactivation of *E. coli* O157:H7 and *L. monocytogenes* was most prominent at pH 4.5 due to their relatively high acid resistance as well as faster heating as acidity

decreased. Considering the time for all three pathogens to be inactivated, pH 4.5 (45 s) was more effective than pH 2.5 (60 s).

Reductions of pathogens subjected to each heating method at the same temperature and time were compared to identify non-thermal effects of ohmic heating. Inactivation of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* was accelerated by lowering the pH for both heating methods. Although all three pathogens are known to produce acid-resistant bacteria (Lee et al., 1994; Lin et al., 1996; Phan-Thanh et al., 2000), the results in the present study show that *L. monocytogenes* was the most acid resistant followed by *E. coli* O157:H7, then by *S.* Typhimurium. Because the reductions of all three pathogens subjected to ohmic heating were not significantly different from those of conventional heating, non-thermal effects of ohmic heating were not identified in the present study.

Two important factors one has to consider when applying ohmic heating to food processing are (1) quality of treated samples and (2) corrosion of electrodes. Quality aspects were investigated, including pH, ^{o}Brix, and color, which are important quality factors having a profound influence on a food's acceptance (Cortés et al., 2008). Although some quality aspects of treated orange juice were significantly different from untreated, these differences were very small and could be ignored. Also, corrosion of titanium electrodes

operated at high frequency (20 kHz) was not observed in the present study (data not shown). Nevertheless, Samaranayake and Sastry (2005) suggested that titanium electrodes used under more acidic conditions can experience a higher corrosion rate when operated at a low frequency (60 Hz).

Ohmic heating was also significantly affected by fat content (0 to 10 %). Bozkurt and Icier (2010) working with minced beef-fat blends confirmed that samples with lower fat levels have the highest electrical conductivity within tested levels of fat content (2 %, 9 % and 15 %). The effect of fat content (0 to 10 %) on conductivity of samples was investigated in the present study. The results indicate that electrical conductivity was higher in the samples with lower fat content. Because of these differences in electrical conductivity, temperature increased more rapidly in the samples having lower fat content. These results suggest that non-uniform heat can be generated by ohmic heating if food contains non-homogeneous milk fat.

If a fat globule is present within a region of high electrical conductivity, where electric currents can bypass the globule, it may heat more slowly than its surroundings due to its lack of electrical conductivity. Under such conditions, any pathogens potentially present within the fat phase may receive less thermal treatment than the rest of the product (Sastry and Barach, 2000). The possibility of non-uniform heat generation was verified using a

thermal infrared camera (data now shown). Marra (2014) suggested that cold areas can exist at junctions of electrodes. Cold areas (blue section in treated samples) were also observed at junctions of electrodes having lateral sample surfaces in the present study. Although only the surface temperature distribution was measured, lack of heating was observed in the 10 % fat content samples compared to 0 % fat content samples. Therefore, food-borne pathogens in the non-homogeneous high fat-containing food subjected to ohmic heating may exhibit increased survival possibility.

Because the thermal effect is the primary parameter in the inactivation of microorganisms by ohmic heating (Palaniappan and Sastry, 1991b; Leizerson and Shimoni, 2005), it is a reasonable expectation that pathogens are inactivated more rapidly in the samples at the higher temperature. Most of results agree with this hypothesis, but there were some findings which support the exact opposite. Even though the temperature of 10 % fat content samples treated with ohmic heating for 45 s (64.82 ^{o}C) was slightly higher than that of 7 % fat content samples treated for 40 s (63.94 ^{o}C), the populations of *E. coli* O157: H7 and *L. monocytogenes* were larger in the samples of 7 % than 10 % fat content. Populations of *E. coli* O157: H7 in ohmic heated 10 % fat content samples (45 s) and 7 % fat content samples (40 s) were 4.06 and 2.93 log CFU/mL, respectively. Populations of *L.*

monocytogenes in ohmic heated 10 % fat content samples (45 s) and 7 % fat content samples (40 s) were 2.46 and 1.77 log CFU/mL, respectively. However this trend was not observed in case of *S.* Typhimurium. Populations of *S.* Typhimurium of ohmic treated 10 % fat content samples (45 s) and 7 % fat content samples (40 s) were 1.00 and 1.53 log CFU/mL, respectively. These results suggest a protective effect of fat content on *E. coli* O157:H7 and *L. monocytogenes*.

The protective effect was verified by treating samples at the fixed temperature and time. The result showed that inactivation of *S.* Typhimurium was not influenced by fat content (0 to 10 %), whereas survival of *E. coli* O157:H7 and *L. monocytogenes* increased with increasing fat content. Although there were no significant differences among 0, 3, and 7 % fat content, the reduction rate decreased when fat content reached 10 %. The protective effect of 10 % fat content for *E. coli* O157:H7 and *L. monocytogenes* explains why the previous findings (lower reductions of *E.coli* O157:H7 and *L. monocytogenes* in the samples of higher temperature) showed some interesting and unusual aspects.

With regard to conventional heating, pathogens were inactivated more effectively in more acidic and lower fat containing food. In contrast to conventional heating, other factors including heating rate, electrical

conductivity, and electrode corrosion should be considered when seeking to optimize ohmic heating for effective pasteurization. The differing characteristics of ohmic heating compared to conventional heating were identified in the present study. Therefore, intrinsic factors such as pH and fat content should be considered as important factors which have a significant effect on the performance of ohmic heating for inactivation of foodborne pathogens.

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VI. 국문초록

옴 가열은 전류의 흐름을 통해 식품 내부에서 열을 발생시키기 때문에 균일하고 빠른 온도 상승이 가능한 기술이다. 지방질 함량과 pH 는 식품 내 미생물의 성장에 영향을 주는 중요한 내인성 인자임에도 불구하고, 내인성 인자가 옴 가열의 성능에 미치는 영향에 대한 연구는 미미한 실정이다. 이 연구에서는 pH 와 지방질 함량이 옴 가열의 가열속도, 전기전도도, 식중독 균의 저감과 식품의 품질 변화에 미치는 영향을 살펴보았다. 각각의 실험에서 pH 와 지방질 함량을 변화시킨 시료에 *Escherichia coli* O157:H7, *Salmonella enterica* serovar Typhimurium 과 *Listeria monocytogenes* 를 접종시킨 후 기존 가열 처리 또는 옴 가열 처리하여 그 결과를 비교하였다. 먼저, pH 가 옴 가열의 효율에 미치는 영향을 조사하였다. pH 의 변화에 따른 기존 가열 처리의 가열속도는 유의적인 차이를 나타내지 않았으며 ($p > 0.05$), 식중독 균은 낮은 pH 에서 보다 효과적으로 사멸되었다. 옴 가열에서는 이와 다른 양상을 나타냈다. pH 를 낮추는 것은 가열속도와 전기전도도에 유의적인 영향을 주지 못했지만 ($p > 0.05$), pH 를

높이는 것은 전기전도도를 증가시켰으며 이에 따라 가열속도 또한 증가하였다. 식중독 균 저감 양상 역시 기존 가열과는 달랐다. *S.* Typhimurium 은 pH 2.5 에서 가장 빠르게 저감된데 비해 *E. coli* O157:H7 과 *L. monocytogenes* 는 pH 4.5 에서 가장 빠르게 저감되었다. 옴 가열 처리를 통한 비 가열적인 효과는 관찰되지 않았으나, 오렌지 주스의 품질은 옴 가열 처리 전후에 유의적인 차이를 나타내지 않았다. 추가적으로 지방질 함량이 옴 가열의 효율에 미치는 영향을 조사하였다. 지방질 함량 변화에 따른 기존 가열의 가열속도와 식중독 균의 저감은 유의적인 차이를 나타내지 않았다 ($p > 0.05$). 또한, 기존 가열에 대한 지방질의 식중독 균 보호 효과는 관찰되지 않았다. 이와 대조적으로, 지방질 함량 변화에 따라 옴 가열의 성능은 상당한 영향을 받았다. 지방질 함량이 증가하면서 전기전도도가 감소하였으며 이에 따라 가열속도는 감소하였다. 또한, 옴 가열 처리시 지방의 *E. coli* O157:H7 과 *L. monocytogenes* 에 대한 보호 효과가 관찰 되었다. 그러므로 pH 와 지방질 함량과 같은 내인성 인자는 옴 가열을 이용한 식품 내의 식중독 균 저감에 영향을 미치는 중요한 요인으로 고려되어야 할 것이다.

주요어: 옴 가열, 식품매개 병원균, pH, 지방질 함량, 전기전도도

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