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# Effect of pH for inactivation of *Escherichia coli* O157:H7, *Salmonella* Typhimurium and *Listeria monocytogenes* in orange juice by ohmic heating

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ABSTRACT

We investigated the combination effect of ohmic heating and pH for inactivation of *Escherichia coli* O157:H7, *Salmonella* Typhimurium and *Listeria monocytogenes* in orange juice. Juice of varying pH (2.5, 3.5, and 4.5) was inoculated with the pathogens and subjected to ohmic heating at different temperatures (50, 55, and 60 °C) for different times (from 0 to 60 s). The reduction of pathogens was higher as treatment temperature and time increased and juice pH decreased. At pH 2.5, populations of *E. coli* O157:H7 were reduced by > 1.81 log, to below the detection limit within 60, 30, and 20 s at 50, 55, and 60 °C, respectively. *S.* Typhimurium was reduced by 5.15 log within 60 s at 50 °C and to below the detection limit within 10 s at 55 and 60 °C. *L. monocytogenes* was little affected at 50 °C but was reduced by 2.15 and 3.37 log after 60 s treatment at 55 and 60 °C. There was a similar pattern of inactivation of the pathogens at pH 3.5 and 4.5. There were no significant quality changes after treatment. In conclusion, our results indicate that ohmic heating can effectively inactivate the pathogens and antimicrobial effect significantly increases when combined with higher acidity.



## 1. Introduction

Fruit juices are beverages enjoyed worldwide (Teo, Ravishankar, & Sizer, 2001). Among them, orange juice is very popular with all age groups because it is very tasty and contains compounds which contribute to health such as vitamin C (Cullen et al., 2010). In the past, orange juice was not recognized as a vehicle of foodborne illness due to its high brix and acidity (Enache & Chen, 2007; Larkin, 2000). The acidity of fruit juice was traditionally thought to be an important barrier against growth of food-borne pathogens (Vojdani, Beuchat, & Tauxe, 2008). However, many research studies about food-borne illness outbreaks involving consumption of fruit juice have been published which illustrate the potential for acidic juices to carry human pathogens (Castillo, Villarruel-López, Navarro-Hidalgo, Martínez-González, & Torres-Vitela, 2006; Cook et al., 1998; Enache & Chen, 2007; Oyarzabal, Nogueira, & Gombas, 2003).

*Escherichia coli* O157:H7, *Salmonella* Typhimurium and *Listeria monocytogenes* are important pathogens that can cause serious foodborne illnesses (Linton, McClemens, & Patterson, 1999; Sagong, Park, Choi, Ryu, & Kang, 2011). *E. coli* O157:H7 can survive well in acidic foods and beverages because it tolerates some organic acids and has acid-adaptability (Koodie & Dhople, 2001; Mazzotta, 2001). Similarly, *Salmonella* has an acid defense network operating in diverse ecosystems (Audia, Webb, & Foster, 2001). Moreover, there have been several outbreaks involving unpasteurized orange juice adulterated with *Salmonella* (CDC, 1999; Cook et al., 1998; Krause, Terzagian, & Hammond, 2001). Illnesses traced to *L. monocytogenes* in fruit juice have not been reported. However, *L. monocytogenes* can survive at low temperature, has acid-resistance (Enache & Chen, 2007; Farber, Sanders, Dunfield, & Prescott, 1989; Ita & Hutkins, 1991) and has been isolated from unpasteurized juices (Sado, Jinneman, Husby, Sorg, & Omiecinski, 1998). Thus, these pathogens are of great importance to the fruit juice industry.

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Juice concentrate and raw juice are thermally processed to destroy pathogens (Nogueira, Oyarzábal, & Gombas, 2003), but heating may or can adversely affect quality. Therefore, alternative technologies to traditional thermal processing, which do not involve direct heat, have been studied (Knorr et al., 2011). Nowadays, there are many novel thermal technologies such as ohmic heating, radio frequency heating, infrared heating, superheated steam and microwave heating. Ohmic heating has attracted attention as a promising technology; it is an intervention that can increase temperature of materials through the passage of electric currents. Thus, ohmic heating treatment can provide a product with rapid and uniform heating without damaging quality because it generates heat within the food internally (Knirsch, dos Santos, de Oliveira Soares Vicente, & Penna, 2010). Additionally, ohmic heating offers better energy efficiency, lower capital cost, shorter treatment time, and is an environmentally-friendly process (Knirsch et al., 2010).

Lee, Sagong, Ryu, and Kang (2012) studied the effect of continuous ohmic heating to inactivate food-borne pathogens in orange and tomato juice. Icier and Ilicali (2005) examined the effects of soluble solids concentration (°Brix) on electrical conductivity of orange juice during ohmic heating. Baysal and Icier (2010) investigated the effects of voltage gradient and temperature on inactivation of *Alicyclobacillus acidoterrestris* spores in orange juice using ohmic heating. Park and Kang (2013) investigated the effect of electropermeabilization by ohmic heating for inactivation of food-borne pathogens in apple juice. In addition, a variety of research focused on ohmic heating has been conducted but there have been no studies targeting the effect of pH on ohmic heating for inactivation of food-borne pathogens. Thus, the objective of this study was to investigate the effect of different levels of pH on inactivation of representative food-borne pathogens by ohmic heating of orange juice. We also examined the effect of pH on orange juice quality at different temperatures following ohmic heating treatment.

## 2. 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 provided by the bacterial culture collection of the School of Food Science, Seoul National University (Seoul, South 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 4000 × g for 20 min at 4 °C, and washed three times with 0.2% peptone water (PW; Bacto, Becton, Dickinson, Sparks, MD). The final pellets were resuspended in 0.2% PW, corresponding to approximately $10^8$–$10^9$ CFU/ml. Afterward, suspended pellets of the three pathogens were combined to comprise a mixed culture cocktail containing approximately equal numbers of cells of each strain of *E. coli* O157:H7 ($10^7$ CFU/ml), *S.* Typhimurium ($10^7$ CFU/ml), and *L. monocytogenes* ($10^6$ CFU/ml).

### 2.2. Sample preparation and inoculation

Pasteurized orange juice concentrates (pH 3.6; 66 °Brix), free of any preservatives, was purchased from a local grocery store. The orange juice concentrates was adjusted to 13.0 °Brix using distilled water. The pH of orange juice concentrates was adjusted to 2.5 and 3.5 with 10% citric acid (w/w) and to 4.5 with 1 N NaOH. A mixed-culture cocktail (0.2 ml) was inoculated into 25 ml of adjusted orange juice at target temperatures for each treatment.

### 2.3. Experimental apparatus

The ohmic heating system (Fig. 1) consisted of a function generator (catalog number 33210A; Agilent Technologies, Palo Alto, CA), a precision power amplifier (catalog number 4510; NF Corp., Yokohama, Japan), a two-channel digital-storage oscilloscope (catalog number TDS2001C; Tektronix, Inc., Beaverton, CO), 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 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 Instrument, Austin, Tex.). 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 ohmic heating chamber was composed of two titanium plate electrodes in contact with the sample and K-type thermocouples inserted at the center of a rectangular (2 by 15 by 6 cm) Pyrex glass container of 0.5 cm thickness. The distance between the two electrodes was 2 cm, and the cross-sectional area was 60 $cm^2$.

### 2.4. Ohmic heat treatment

For all experiments, 25 ml of sample was placed into the ohmic heating chamber. Electric field strength was fixed at 16 V/cm to maintain a constant temperature, and a 20 kHz frequency and sine waveform were used. Treatments were conducted at 50, 55, and 60 °C for 0, 10, 20, 30, 40, 50 and 60 s.

### 2.5. 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 with a stomacher (Easy Mix; AES Chemunex, Rennes, France). After homogenization, 1 ml aliquots withdrawn from stomacher bags were serially diluted 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 antimicrobial supplement (Bacto Oxford antimicrobial supplement; Difco) were used as selective media for enumeration

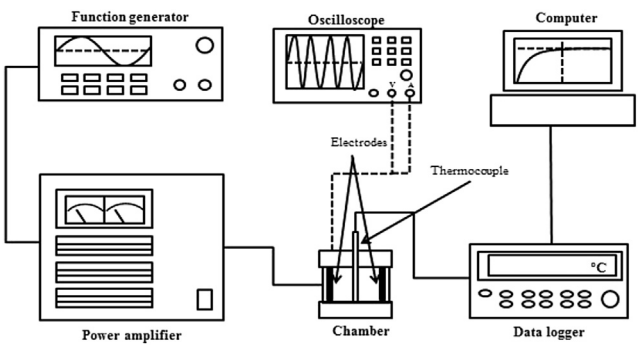


**Fig. 1.** Schematic diagram of the ohmic heating system.

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 24–48 h, colonies were counted. All experiments were duplicate-plated and replicated three times.

### 2.6. Color and pH measurement

For color and pH measurements, ohmic heating treatment was performed at the maximum treatment time (1 min) and untreated orange juice was used as the control. The pH of treated and untreated samples was measured with a Seven Multi 8603 pH meter (Mettler Toledo, Greifensee, Switzerland). The color of

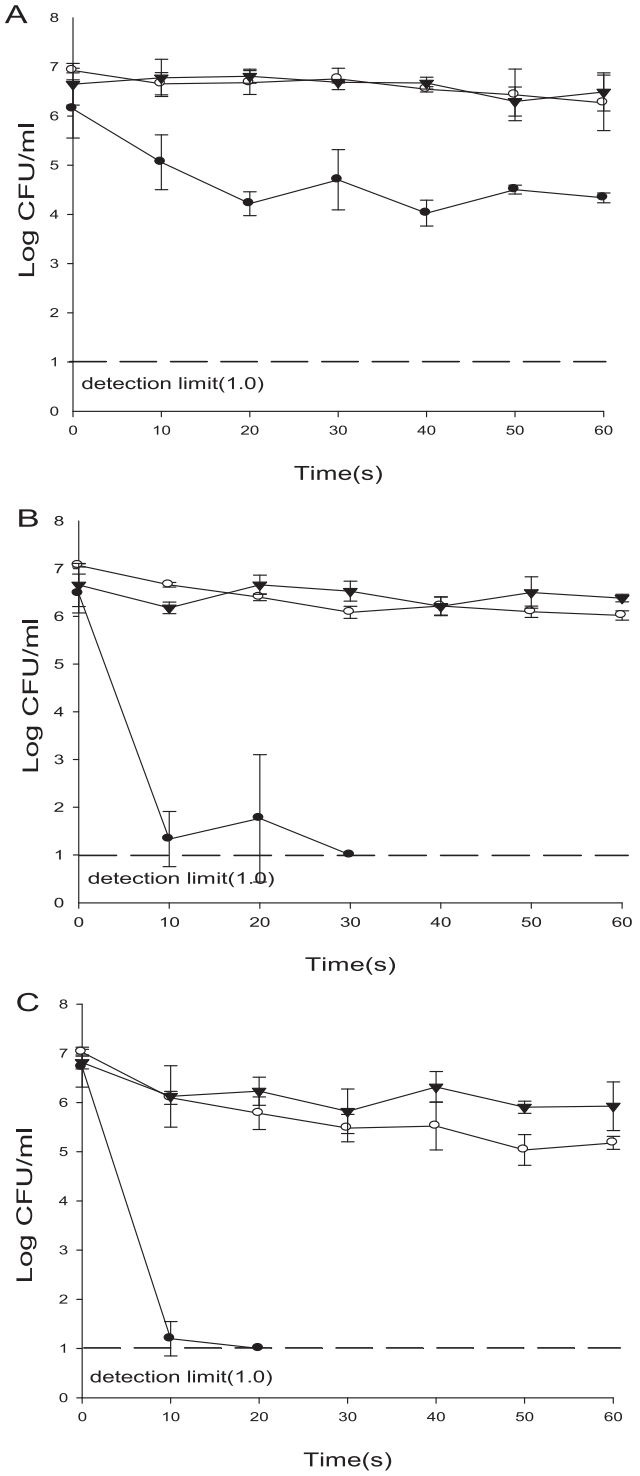


**Fig. 2.** Survival curves of *E. coli* O157:H7 corresponding to microbial inactivation by ohmic heat in orange juice (13 °Brix) at 50 °C (A), 55 °C (B), and 60 °C (C). Symbols: ●, pH 2.5; ○, pH 3.5; ▼, pH 4.5.

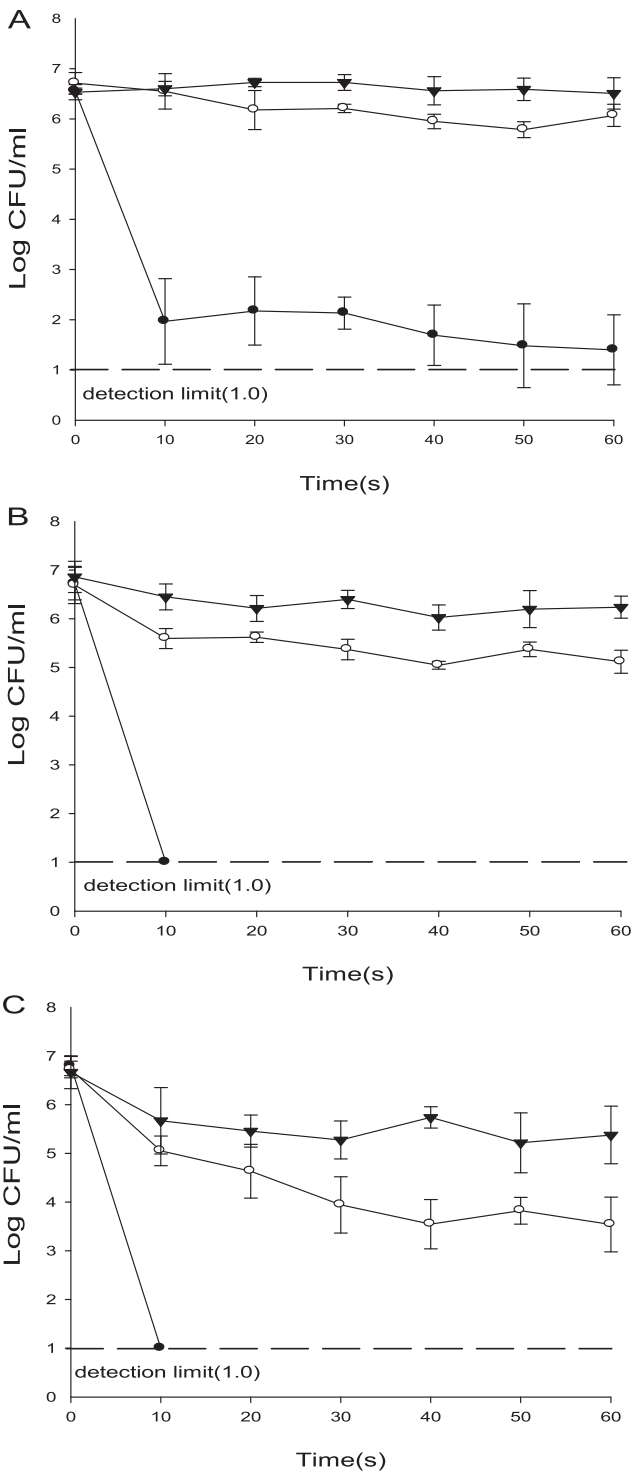


**Fig. 3.** Survival curves of *S.* Typhimurium corresponding to microbial inactivation by ohmic heat in orange juice (13 °Brix) at 50 °C (A), 55 °C (B), and 60 °C (C). Symbols: ●, pH 2.5; ○, pH 3.5; ▼, pH 4.5.

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 of treated and untreated samples.

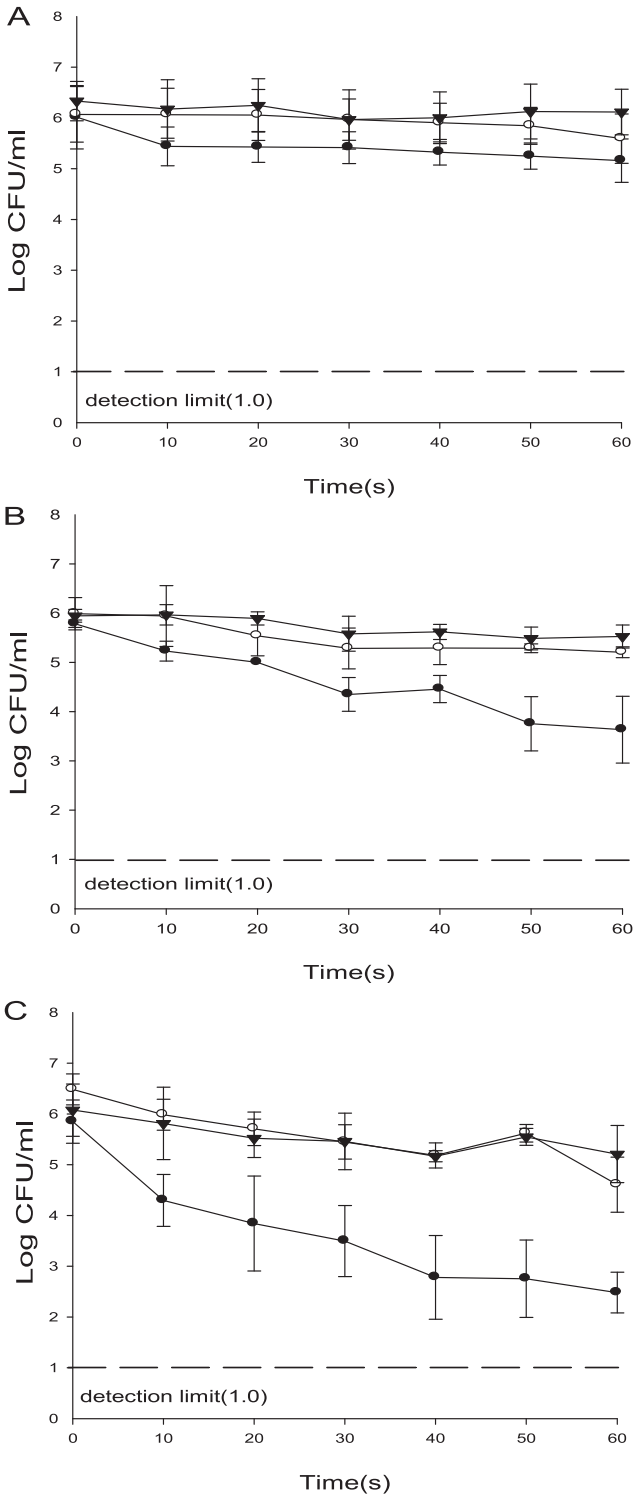


**Fig. 4.** Survival curves of *L. monocytogenes* corresponding to microbial inactivation by ohmic heat in orange juice (13 °Brix) at 50 °C (A), 55 °C (B), and 60 °C (C). Symbols: ●, pH 2.5; ○, pH 3.5; ▼, pH.

### 2.7. Statistical analysis

All experiments were done in triplicate with duplicate samples. Data were analyzed by the Statistical Analysis System (SAS Institute, Cary, NC) and mean values were separated by using Duncan's multiple-range test. Significant differences were determined at a $P$ value of $<0.05$.

## 3. Results

### 3.1. Comparison of the effect of ohmic heating on inactivation of E. coli O157:H7 in orange juice at various pH and temperature levels

The survival of *E. coli* O157:H7 in orange juice following ohmic heating at various pH and temperature levels is shown in Fig. 2. In general, the reduction in numbers of *E. coli* O157:H7 was greater at low pH. Ohmic heating for 60 s at 50 °C accomplished 1.81 log reductions at pH 2.5. But there was little effect (less than 1 log reduction) at pH 3.5 and 4.5. When orange juice at pH 2.5 was subjected to ohmic heating at 55 °C, levels of surviving cells of *E. coli* O157:H7 decreased to below the detection limit (≥5.48 log reductions) within 30 s. Ohmic heating for 60 s at 55 °C accomplished 1.05 and 0.28 log reductions at pH 3.5 and 4.5, respectively. When orange juice at pH 2.5 was treated with ohmic heating at 60 °C, levels of surviving cells of *E. coli* O157:H7 decreased to below the detection limit (≥5.72 log reductions) within 20 s. Ohmic heating for 60 s at 60 °C accomplished 1.84 and 0.89 log reductions at pH 3.5 and 4.5, respectively.

### 3.2. Comparison of the effect of ohmic heating on inactivation of S. Typhimuirum in orange juice at various pH and temperature levels

The survival of *S.* Typhimurium in orange juice following ohmic heating at various pH and temperature levels is shown in Fig. 3. In general, the reduction in numbers of *S.* Typhimurium increased at low pH. Ohmic heating for 60 s at 50 °C accomplished 5.15 log reductions at pH 2.5. But there was little effect (less than 1 log reduction) at pH 3.5 and 4.5. When pH 2.5 orange juice was treated with ohmic heating at 55 °C, levels of surviving cells of *S.* Typhimurium decreased to below the detection limit (≥5.72 log reductions) within 10 s. Ohmic heating for 60 s at 55 °C accomplished 1.58 and 0.62 log reductions at pH 3.5 and 4.5, respectively. When orange juice of pH 2.5 was treated with ohmic heating at 60 °C, levels of surviving cells of *S.* Typhimurium decreased to below the detection limit (≥5.79 log reductions) within 10 s. Ohmic heating for 60 s at 60 °C accomplished 3.18 and 1.29 log reductions at pH 3.5 and 4.5, respectively.

### 3.3. Comparison the effect of ohmic heating on inactivation of L. monocytogenes in orange juice at various pH and temperature levels

The survival of *L. monocytogenes* in orange juice following ohmic heating at various pH and temperature levels is shown in Fig. 4. In general, the reduction in numbers of *L. monocytogenes* increased at low pH. There was little effect (less than 1 log reduction) at 50 °C at all tested levels of pH. Ohmic heating for 60 s at 55 °C accomplished 2.15, 0.78 and 0.42 log reductions at pH 2.5, 3.5 and 4.5, respectively. Ohmic heating for 60 s at 60 °C accomplished 3.37, 1.88 and 0.87 log reductions at pH 2.5, 3.5 and 4.5, respectively.

### 3.4. Color and pH measurement

The color and pH values of orange juice, treated or untreated, are measured. The pH values did not differ significantly between

treated and untreated orange juice within all tested levels of pH. The L*, a*, and b* values of treated and untreated juices were not significantly different. Ohmic heating for 1 min at various levels of tested pH and temperature did not affect the color and pH value of orange juice (data not shown).

## 4. Discussion

Ohmic heating technology has been used by the fruit juice, whole fruit and milk processing industries for commercial sterilization as well as various other purposes (Reznick, 1996; Sastry & Barach, 2000). Newly spotlighted ohmic heating as an alternative to conventional heat treatment is effective due to not only the thermal effect of internal heat generation, but also the non-thermal effect of electropermeabilization (usually electroporation), which leads to enormous inactivation of microorganisms, especially food-borne pathogens. Several studies have been performed on inactivation by ohmic heating (Baysal & Icier, 2010; Lee et al., 2012; Park & Kang, 2013; Sagong et al., 2011). Park and Kang (2013) demonstrated the effect of electroporation by ohmic heating for inactivation of foodborne pathogens by comparing conventional heating with ohmic heating. They referred that ohmic heat treatment can effectively inactivate bacterial populations in acidic fruit juices but the direct effect of specific pH range associated with ohmic heating on inactivation of foodborne pathogens in acidic juice has not been clearly investigated. Besides there have been no research studies investigating the effect of pH in the reduction of *E. coli* O157:H7, *S.* Typhimurium and *L. monocytogenes* by ohmic heat treatment. pH, along with temperature, water activity and redox potential, is a critical intrinsic growth factor for microorganisms and one of the major preservative factors for foods. It is widely known that low pH inhibits the growth of microorganisms. There have also been research investigations into the synergistic effect of low pH and heat treatment (Ama, Hamdy, & Toledo, 1994; Seo, Lee, Lim, & Ko, 2012). Through this study, we confirmed the combination effect of low pH and ohmic heating.

We tested three representative food-borne pathogens, although *L. monocytogenes* has not been implicated in any juice-associated outbreaks. However, in the absence of known specific pathogen product associations, the NACMCF recommended that *E. coli* O157:H7 or *L. monocytogenes* be used as the target organism, as appropriate. Because these two organisms are most difficult to control, by inactivating them, other pathogenic organisms will also likely be controlled (US FDA, 1998). We used a voltage frequency of 20 kHz because no electrochemical reactions – which can cause electrode corrosion during ohmic heating occur at that frequency (Lee, Ryu, and Kang, 2013; Park & Kang, 2013). When using ohmic heating, heating rates differed for each pH level because added salts necessary for adjusting pH caused samples to have different electric conductivity. If heating rates of foods differ, the treatment times needed to inactivate pathogens also differ (Lee et al., 2012). Therefore, we used a controlled system to confirm the effect of pH at the same temperature and treatment time. We adjusted pH of orange juice concentrate at 2.5, 3.5 and 4.5 with citric acid and NaOH for confirming effect of pH level in the interval 1.0. Based on results of Park and Kang (2013), we set the temperature at 50, 55, and 60 °C within the range of mild heat.

Initial populations of *E. coli* O157:H7, *S.* Typhimurium and *L. monocytogenes* were approximately $10^6$ ~ $10^7$ CFU/ml and the detection limit was 1.0 log CFU/ml. In general, reduction of *E. coli* O157:H7, *S.* Typhimurium and *L. monocytogenes* increased as ohmic heating temperature and treatment time increased in orange juice. These results are similar to those of Lee et al. (2012) and Sagong et al. (2011). In our study, the effect of inactivation was progressively greater as temperature increased at lower pH. At pH 2.5, all three pathogens showed noticeable reduction at various temperatures except for *L. monocytogenes* at 50 °C. However, compared to pH 2.5, reductions at pH 3.5 and 4.5 were much smaller and for the most part did not differ greatly from each other. Splittstoesser, McLellan, and Churey (1995) also reported that pH in the range of 3.6–4.0 in apple juice was not a significant variable in the heat resistance of *E. coli* O157:H7. Ohmic heating at 60 °C, the highest temperature in the present study, demonstrated a clear trend of inactivation of all of three pathogens regardless of pH. In addition, the inactivation trend followed a different pattern depending on the species of pathogen. Among the three pathogens studied, *L. monocytogenes* was the one most resistant to ohmic heating and acidic conditions, followed by *E. coli* O157:H7 and lastly by the most sensitive one, *S.* Typhimurium. Stationary phase cells of *L. monocytogenes* have been shown to be acid-resistant (Davis, Coote, & O'Byrne, 1996) and Gram-positive bacteria have been shown to be more resistant than Gram-negative bacteria in similar environments in previous studies (Lin, Kim, Du, & Wei, 2000). But Sagong et al. (2011) reported results that were a little different from ours: *E. coli* O157:H7 was the most resistant of three pathogens, namely, *E. coli* O157:H7, *S.* Typhimurium and *L. monocytogenes* in tomato and orange juice. This is explained because *E. coli* O157:H7 also has high heat resistance and acid adaptability (Cheville, Arnold, Buchreser, Cheng, & Kaspar, 1996; Mazzotta, 2001). Therefore, microbial growth or survival may depend on the types of microorganisms occurring in acidified foods. In addition, we enumerate microbial counts using selective agar medium. The selective medium containing organic dyes, antibiotics, bile salts and surfactants may inhibit the repair of injured cells by ohmic heat treatment (Wu & Fung, 2001). Further research about induction and resuscitation of injured cells by ohmic heating are also needed.

As a control, we treated orange juice with acid at room temperature for 60 s without ohmic heating to study the effect of applying acid only. Our results did not show reduction of pathogens at any pH level, not even at pH 2.5 (data not shown). Usually, mild heating in the range of 50–60 °C does not induce inactivation of food-borne pathogens. Interestingly, we found effective inactivation of pathogens at 50, 55 and 60 °C when ohmic heating was applied at low pH. Our combination treatment of ohmic heating and pH produced a great antimicrobial effect. This result indicated that ohmic heating at low pH made microorganisms more sensitive to inactivation and yielded a pronounced antimicrobial effect of inactivation within a range of mild heating temperatures. This combination treatment could disturb the homeostasis of the pathogens, simultaneously affecting different targets such as the cell membrane, DNA, enzyme systems, pH and water activity within the microbial cells in several respects (Leistner, 2000). Organic acids such as citric acid, lactic acid and malic acid cause cell inactivation through penetrating plasma membranes and lowering the pH of the cell interior (Booth & Kroll, 1989). In our study, electric current may have produced a thermal lethal effect in concert with the added effect of pH to disrupt cell membranes of the target microorganisms. This suggests an additive or synergistic potential of ohmic heating inactivation with acidic pH. Park and Kang (2013) also mentioned that ohmic heating can more effectively reduce populations of bacteria at reduced temperatures and shorter times in apple juices than buffered peptone water due to its higher acidity. Patil, Valdramidis, Cullen, Frias, and Bourke (2010) reported that low pH enhanced inactivation efficacy of *E. coli* by ozone treatment. Wuytack and Michiels (2001) suggested pressure treatment at low pH made *Bacillus subtilis* spores more sensitive to heat inactivation.

In our study, we detected no significant quality differences, such as color change which is a key factor of orange juice quality between untreated and treated orange juice after ohmic heating

treatment at all pH levels. This result is similar to those of other research studies (Lee et al., 2012, 2013; Leizerson & Shimoni, 2005). In conclusion, we confirmed that ohmic heating technology achieved more effective inactivation of food-borne pathogens in orange juice when combined with higher acidity without damaging quality. Therefore, ohmic heating is a very promising technology for inactivating of food-borne pathogens in juice and could be a desirable intervention for use by the fruit juice industry.

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