



# Comparison of pH effects on ohmic heating and conventional heating for inactivation of *Escherichia coli* O157:H7, *Salmonella enterica* Serovar Typhimurium and *Listeria monocytogenes* in orange juice

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## ABSTRACT

The objective of the current study is to identify the influence of acidity on ohmic heating compared to conventional heating for inactivation of food-borne pathogens in orange juice. 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 temperature and electrical conductivity were not greatly affected by lowering pH, temperature increased more rapidly with increasing pH due to higher electrical conductivity. Also, the inactivation patterns were significantly different ( $P < 0.05$ ) from conventional heating. While *Salmonella* Typhimurium was inactivated most rapidly at pH 2.5, *Escherichia coli* O157:H7 and *Listeria monocytogenes* were inactivated most rapidly at pH 4.5. When pathogens were exposed to each heating method at a fixed temperature, additional effects of ohmic heating were not observed. Also, the overall quality of orange juice subjected to ohmic heating was not greatly affected at any pH level. Therefore, increasing as well as lowering pH can also be considered effective ways to optimize pasteurization of orange juice when using ohmic heating. The different characteristics of ohmic heating compared to conventional heating indicate the necessity of a new approach.

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## 1. Introduction

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.

Although heat treatment has been widely used for pasteurizing orange juice, conventional thermal treatment can cause irreversible loss of fresh juice flavor and nutrients (Vikram, Ramesh, & Prapulla, 2005). Therefore, several alternative technologies have been proposed (Ait-Ouazzou, Espina, García-Gonzalo, & Pagán, 2013). Non-thermal treatments such as pulsed electric field (PEF) and high pressure have been investigated for pasteurizing fruit juice (Parish, 1998; Timmermans et al., 2014). The combination effect of non-thermal treatment (PEF or ozone) with mild heat was also reported for inactivation of microorganisms in fruit juice (Hodgins, Mittal, & Griffiths, 2002; Sung, Song, Kim, Ryu, & Kang, 2014). Some alternative thermal treatments such as radio frequency, electric fields, microwave, and ohmic heating have been studied for pasteurizing fruit juice.

Ohmic heating is an alternative thermal technology for

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inactivating food-borne pathogens in orange juice due to its lack of overheating and high retention of sensorial attributes. The advantage of ohmic heating over conventional heating comes from the different mode of heat transfer. In conventional heating, heat transfer occurs via conduction and convection and thermal conductivity is a major factor controlling the rate of conventional heating. Therefore, a cold spot exists inside the food sample at or near the geometric center. On the other hand, ohmic heating occurs in foods due to the presence of ionic species, where an electric current can be made to pass through and generate heat inside of a food. This difference is responsible for the major advantage of ohmic heating: uniform and rapid heating of food products (Leizerson & Shimoni, 2005).

Electrical conductivity of food is a major factor controlling the rate of ohmic heating and it can be affected by many factors such as electric field strength, particle size of solids, fat content of food, and so on. Generally, the electrical conductivity of foods increases with temperature (Castro, Teixeira, Salengke, Sastry, & Vicente, 2003; Sarang, Sastry, & Knipe, 2008). Another research study revealed that soaking vegetable and meat samples in salt solutions increased electrical conductivity while soaking in water resulted in reduced electrical conductivity (Palaniappan & Sastry, 1991a). In some cases, increasing electrical conductivity due to enhanced diffusion of cell fluids induced by an electric field was also reported (Halden, De Alwis, & Fryer, 1990). Even though electrical conductivity may also be affected by the nature of ions, research investigating the electrical conductivity of juice with adjusted pH is limited.

Several research studies investigating the relationship between pH and inactivation of foodborne pathogens are reported. The combination effect of low pH and thermal treatment for inactivating *Escherichia coli* O157:H7, *Salmonella enteritidis*, and *Listeria monocytogenes* has been reported (de W Blackburn, Curtis, Humpheson, Billon, & McClure, 1997; Juneja & 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, Kalchayanand, Bozoglu, & Ray, 2000; Patil, Valdramidis, Cullen, Frias, & Bourke, 2010). However, research about the effect of pH on ohmic heating is limited. The objective of this study was to determine the effect of pH on ohmic heating compared to conventional heating in orange juice. More specifically, the effect of pH on ohmic heating including electrical conductivity, heating rate, inactivation of pathogens, and quality aspects was investigated and compared with conventional heating.

## 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 obtained from 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<sup>8</sup>–10<sup>9</sup> 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<sup>7</sup> CFU/ml), *S. Typhimurium* (10<sup>7</sup> CFU/ml), and *L. monocytogenes* (10<sup>6</sup> CFU/ml).

### 2.2. 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 1 N 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 (50 °C) for each experiment.

### 2.3. Conventional heating treatment

For conventional heating, a constant-temperature water bath (BW-10G; Jeio Tech, Seoul, South Korea) was used. Temperature of the water bath was fixed at 75 °C. A conventional heating chamber was made of stainless steel (2 × 15 × 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. The temperatures of samples were 20, 28.5, 39.5, 47.5, 52.5, and 56.5 °C for treatment of 0, 20, 40, 60, 80, and 100 s, respectively.

### 2.4. Ohmic heating system

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 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, Tex). 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<sup>2</sup>.

### 2.5. Electrical conductivity measurement

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

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

where  $\sigma$  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<sup>2</sup>), and  $V$  is the voltage (V). Voltage and Current was measured using the two channel digital storage oscilloscope.

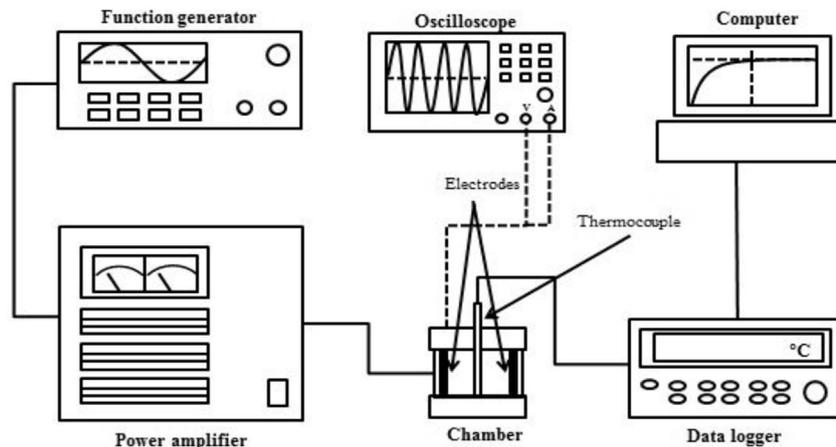


Fig. 1. Schematic diagram of the ohmic heating system.

## 2.6. Ohmic heating treatment

For ohmic heating experiments, an ohmic heating system with a 20 kHz frequency and sine waveform were used. The electric field strength was fixed at 25.6 V/cm. The ohmic heating chamber, which was same shape and size as the conventional heating chamber, was made of polyvinyl chloride ( $2 \times 15 \times 6$  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.

## 2.7. Ohmic and conventional heating treatment at fixed temperature

Temperature was controlled by Labview software (National Instruments, Austin, Tex.) to identify the additional effect of ohmic heating. The electric field was fixed at 9.6 V/cm after reaching the target temperature ( $50\text{ }^{\circ}\text{C}$ ) to maintain temperature stability and the function generator operated according to whether the target temperature was reached or not. For conventional heating treatment, the temperature of the water bath was fixed at  $51.5\text{ }^{\circ}\text{C}$  until the sample reached the targeted temperature. After each sample reached the targeted temperature ( $50\text{ }^{\circ}\text{C}$ ) 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 preliminary experiments to moderately inactivate pathogens (2–3 log reduction for non-adjusted samples).

## 2.8. 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 tenfold 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 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. All agar media were incubated at  $37\text{ }^{\circ}\text{C}$  for 24–48 h before colonies were counted. Random colonies were selected and subjected to biochemical and serological tests to confirm the identity of the pathogens. *E. coli* O157:H7 latex agglutination assay (RIM; Remel, Lenexa, KS), *Salmonella* latex agglutination assay (Oxoid, Ogdensburg, NY), and API *Listeria* test (bioMérieux, Hazelwood, MO) was conducted for each pathogen.

## 2.9. Color, pH and °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 °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., Japan), respectively.

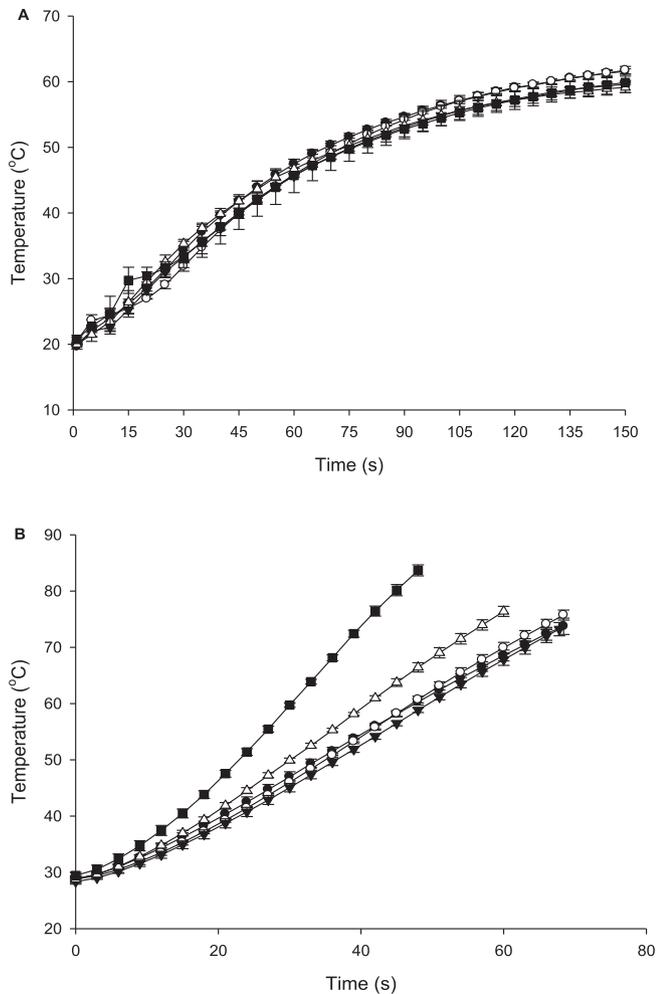
## 2.10. Statistical analysis

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

## 3. Results

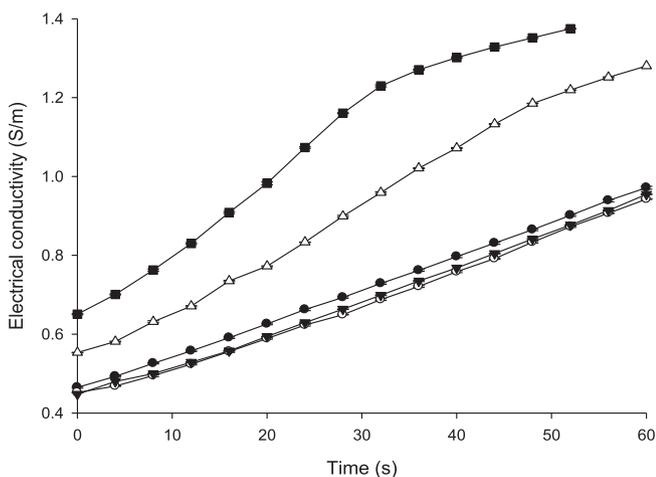
### 3.1. Effect of pH on temperature increase and electrical conductivity

The temperature histories of orange juice 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



**Fig. 2.** Temperature histories of orange juice 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.

heating was affected by differing pH (Figs. 2B and 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



**Fig. 3.** Electrical conductivity histories of orange juice 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.

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 °C, but 51 s and 63 s were needed for samples of pH 4.0 and 3.5, respectively.

### 3.2. Effect of pH on inactivation of *E. coli* O157:H7, *S. Typhimurium*, and *L. monocytogenes* corresponding to temperature increase (Fig. 2A) of conventionally heated orange juice

The survival of *E. coli* O157:H7, *S. Typhimurium*, and *L. monocytogenes* in orange juice at various pH levels subjected to conventional heating 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.

### 3.3. Effect of pH on inactivation of *E. coli* O157:H7, *S. Typhimurium* and *L. monocytogenes* corresponding to temperature increase (Fig. 2B) of ohmic heated orange juice

The survival of *E. coli* O157:H7, *S. Typhimurium* and *L. monocytogenes* in samples at various pH levels subjected to ohmic heating 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.

### 3.4. Inactivation of *E. coli* O157:H7, *S. Typhimurium* and *L. monocytogenes* in orange juice at fixed temperature

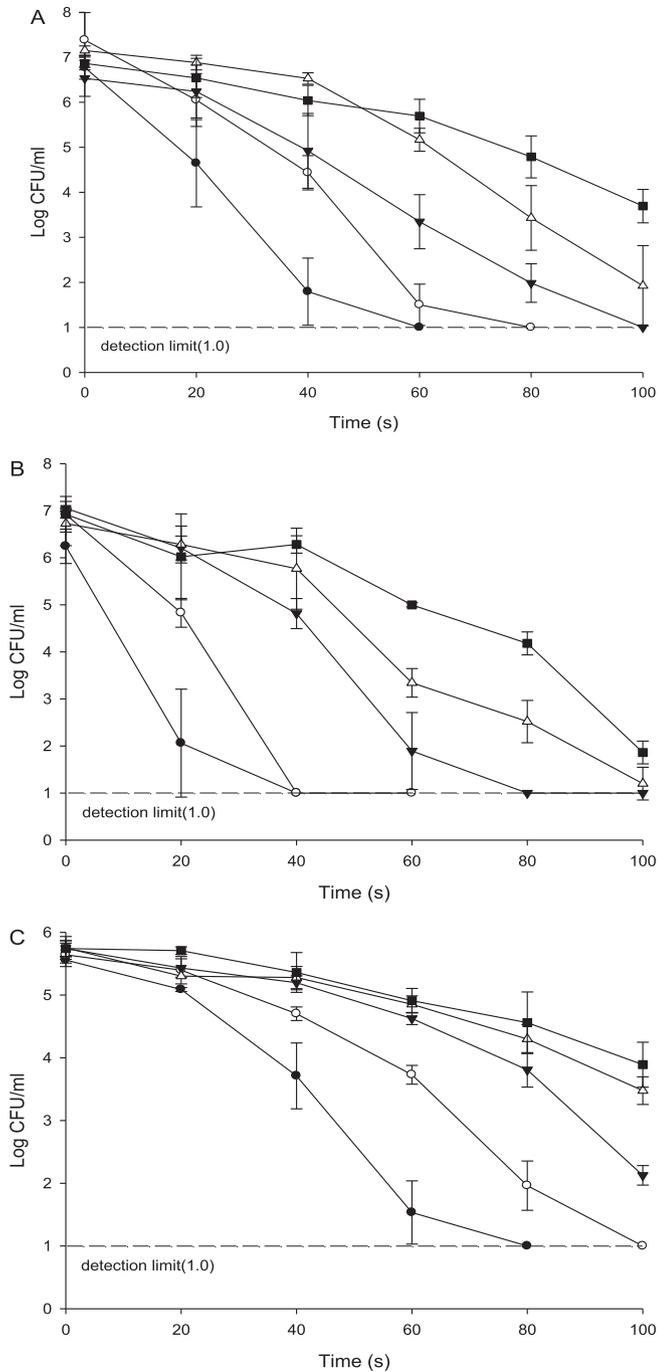
The reduction of *E. coli* O157:H7, *S. Typhimurium*, and *L. monocytogenes* in orange juice at various pH levels subjected to ohmic and conventional heating at a fixed temperature and time (50 °C, 2 min) is shown in Table 1. The inactivation aspects of ohmic heating at the same temperature were similar 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 additional effects of ohmic heating were not observed.

### 3.5. Color, pH and °Brix measurement

Color, pH, and °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 °Brix values were affected by ohmic heating at pH 4.5. However, overall quality indicators were not severely degraded by ohmic heating at any pH level.

## 4. Discussion

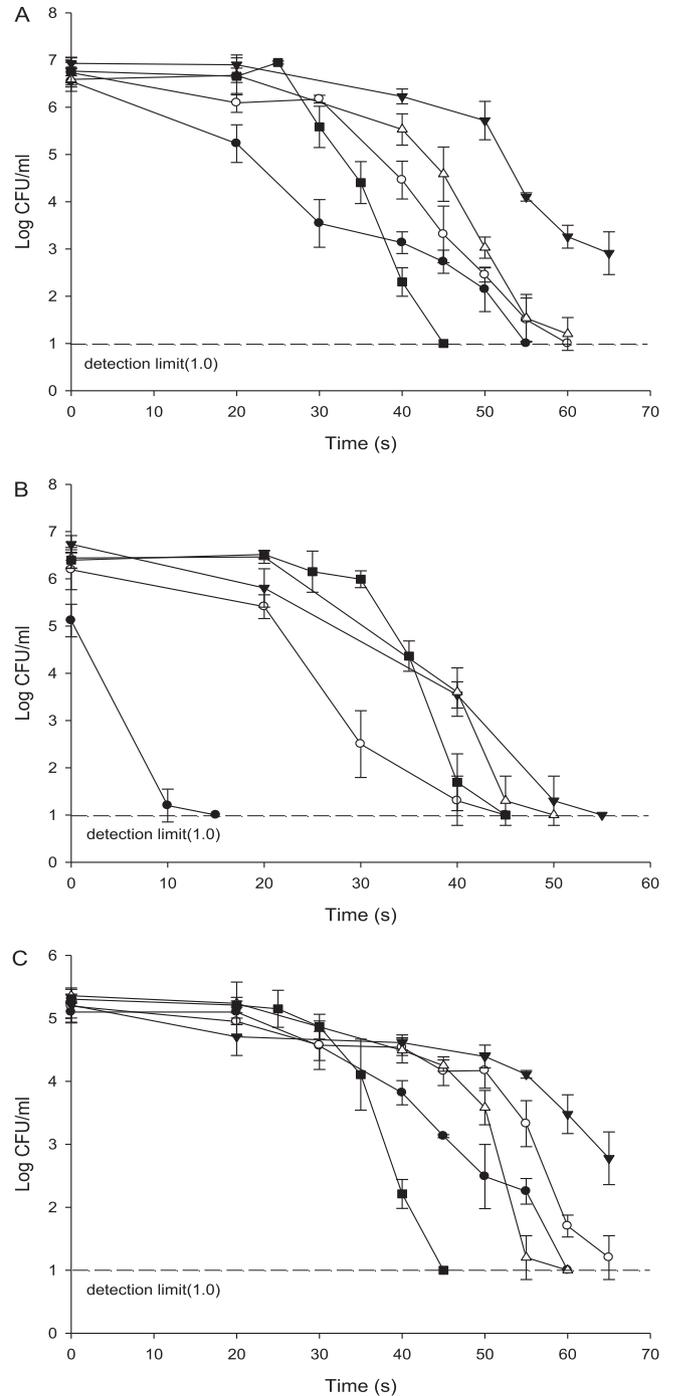
Various aspects of ohmic heated orange juice have been studied including the effect of orange juice concentration and solids content on electrical conductivity of orange juice (Icier & Ilicali, 2005;



**Fig. 4.** Survival curves of *E. coli* O157:H7 (A), *S. Typhimurium* (B), and *L. monocytogenes* (C) corresponding to microbial inactivation in orange juice 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.

Palaniappan & Sastry, 1991b). The effect of electric field strength on inactivation of *E. coli* O157:H7, *S. Typhimurium*, *L. monocytogenes*, and *Alicyclobacillus acidoterrestris* spores was also investigated (Baysal & Icier, 2010; Sagong, Park, Choi, Ryu, & Kang, 2011). However, none of these research studies has determined the pH effect of orange juice. Understanding the effects of pH is crucial to pasteurize orange juice effectively with ohmic heating.

In contrast to conventional heating (Fig. 2A), the heating rate of orange juice subjected to ohmic heating was significantly affected by varying the pH (Fig. 2B). The heating rate of orange juice



**Fig. 5.** Survival curves of *E. coli* O157:H7 (A), *S. Typhimurium* (B), and *L. monocytogenes* (C) corresponding to microbial inactivation in orange juice 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.

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 (pH 2.5, 3.0, and 3.5) 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

**Table 1**Reduction of three pathogens in orange juice samples subjected to conventional and ohmic heating at the same temperature and time (50 °C, 2 min) with various pH levels<sup>a</sup>.

Organisms	Treatment	pH				
		2.5	3.0	3.5	4.0	4.5
<i>E. coli</i> O157:H7	Ohmic <sup>b</sup>	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
<i>S. Typhimurium</i>	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
<i>L. monocytogenes</i>	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

Mean values ± standard deviation.

<sup>a</sup> Values in the same column for each pathogen that are followed by the same uppercase letter are not significantly different ( $P > 0.05$ ).<sup>b</sup> Values in the same row that are followed by the same lowercase letter are not significantly different ( $P > 0.05$ ).

freedom of ions to move about, and the temperature of solution. 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 & 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). We observed a faster heating rate for juice adjusted with sodium hydroxide, due to its higher electrical conductivity resulting from high dissociation of this molecule. Nevertheless, our results (Figs. 2B and 3) 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 (Fig. 5) has a different trend compared to that of conventional heating (Fig. 4). Altering pH was obviously effective for pasteurizing orange juice by ohmic heating. Pathogens were inactivated 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 (Table 1). 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, Slonczewski, & Foster, 1994; Lin et al., 1996; Phan-Thanh, Mahouin, & Aligé, 2000), our results 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 ohmic heating were not significantly different from those of conventional heating, an effects of ohmic heating were not identified in this 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, °Brix, and color, which are important quality factors having a profound influence on a food's acceptance (Cortés, Esteve, & Frígola, 2008). Although some quality aspects of treated orange juice were significantly different from untreated, these differences were very small and can be ignored (Table 2). Also, corrosion of titanium electrodes operated at high frequency (20 kHz) was not observed in our study (data not shown). Nevertheless, Samaranyake and Sastry (2005) suggest that titanium electrodes used under more acidic conditions can experience a higher corrosion rate when operated at a low frequency (60 Hz).

With regard to conventional heating, pathogens were inactivated more effectively when using conventional heating in more acidic orange juice. But in contrast to conventional heating, other factors such as heating rate, electrical conductivity, and electrode corrosion should be considered when seeking to optimize ohmic heating for effective juice pasteurization. The heating rate was accelerated in juice of increased pH due to higher electrical conductivity resulting from nearly complete ionization of sodium hydroxide. Also, the time required to completely inactivate all three pathogens was shortest at pH 4.5, while it was shortest at pH 2.5 for conventional heating. Moreover, the quality of orange juice and electrode corrosion were not severely affected regardless of pH.

**Table 2**Color, pH and °Brix values of ohmic treated and untreated juice at various pH levels<sup>a</sup>.

pH	Treatment	pH	°Brix	Color <sup>b</sup>		
				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.

<sup>a</sup> Values in the same column that are followed by the same uppercase letter are not significantly different at each pH ( $P > 0.05$ ).<sup>b</sup> Color values are L\*(lightness), a\*(redness) and b\*(yellowness).

Considering above results, increasing pH can be considered as effective ways to enhance pasteurization of orange juice by ohmic heating, depending on pathogens and ionic species involved. Although these aspects were limited to orange juice adjusted with citric acid and sodium hydroxide, the differing characteristics of ohmic heating compared to conventional heating were verified in this study. Therefore, variables suitable for effective conventional heating may not be optimal when using ohmic heating to pasteurize orange juice.

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