

ORIGINAL ARTICLE

Effect of continuous ohmic heating to inactivate *Escherichia coli* O157:H7, *Salmonella* Typhimurium and *Listeria monocytogenes* in orange juice and tomato juice

S.-Y. Lee, H.-G. Sagong, S. Ryu and D.-H. Kang

Department of Food and Animal Biotechnology, Department of Agricultural Biotechnology, Center for Agricultural Biomaterials, and Research Institute for Agriculture and Life Sciences, Seoul National University, Seoul, South Korea

Keywords

continuous ohmic heating, foodborne pathogen, orange juice, tomato juice, vitamin C.

CorrespondenceDong-Hyun Kang, Department of Food and Animal Biotechnology, Seoul National University, Seoul 151-921, South Korea.
E-mail: kang7820@snu.ac.kr

2011/1863: received 31 October 2011, revised 11 January 2012 and accepted 26 January 2012

doi:10.1111/j.1365-2672.2012.05247.x

Abstract**Aims:** The purpose of this study was to investigate the efficacy of continuous ohmic heating for reducing *Escherichia coli* O157:H7, *Salmonella* Typhimurium and *Listeria monocytogenes* in orange juice and tomato juice.**Methods and Results:** Orange juice and tomato juice were treated with electric field strengths in the range of 25–40 V cm⁻¹ for different treatment times. The temperature of the samples increased with increasing treatment time and electric field strength. The rate of temperature change for tomato juice was higher than for orange juice at all voltage gradients applied. Higher electric field strength or longer treatment time resulted in a greater reduction of pathogens. *Escherichia coli* O157:H7 was reduced by more than 5 log after 60-, 90- and 180-s treatments in orange juice with 40, 35 and 30 V cm⁻¹ electric field strength, respectively. In tomato juice, treatment with 25 V cm⁻¹ for 30 s was sufficient to achieve a 5-log reduction in *E. coli* O157:H7. Similar results were observed in *Salm.* Typhimurium and *L. monocytogenes*. The concentration of vitamin C in continuous ohmic heated juice was significantly higher than in conventionally heated juice ($P < 0.05$).**Conclusions:** Continuous ohmic heating can be effective in killing foodborne pathogens on orange juice and tomato juice with lower degradation of quality than conventional heating.**Significance and Impact of the Study:** These results suggest that continuous ohmic heating might be effectively used to pasteurize fruit and vegetable juices in a short operating time and that the effect of inactivation depends on applied electric field strengths, treatment time and electric conductivity.**Introduction**

Fruit and vegetable juices were not recognized until recently as vehicles of foodborne illness because of their acidity (Mazzotta 2001). But recently, several outbreaks of *Escherichia coli* O157:H7, *Salmonella* Typhimurium and *Listeria monocytogenes* have occurred in fruit and vegetable juices. In the United States, 21 juice-associated outbreaks of human illness were reported to the Center for Disease Control and Prevention (CDC) between 1995 and

2005 (Vojdani *et al.* 2008). An outbreak of diarrhoea and haemolytic uraemic syndrome from *E. coli* O157:H7 linked to apple cider in the United States occurred in October 1996, when at least 70 people became sick and one child died (Cody *et al.* 1999). In 2005, a multistate outbreak of *Salm.* Typhimurium infection from orange juice occurred (Jain *et al.* 2009), which caused diarrhoea, abdominal pain, mild fever and chills (Baird-Parker 1990). No outbreaks involving *L. monocytogenes* have been reported in fruit juices; however, the National

Advisory Committee on Microbiological Criteria for Foods recommended that in absence of known specific pathogen-product associations, *E. coli* O157:H7 or *L. monocytogenes*, which causes epidemic listeriosis, should be used as target organisms as appropriate (FDA 1998).

These incidents of outbreaks led the United States Food and Drug Administration (FDA) to issue hazard analysis and critical control point (HACCP) regulations for safe and sanitary processing and importing of juice (USFDA 2001). To assure the juice safety, juice processors are required to implement a system to achieve a 5-log pathogen reduction in an appropriate target organism in the juice process. A common method for achieving a 5-log reduction in pathogens in fruit juices is conventional thermal processing (Oyarzabal *et al.* 2003). But thermal treatment causes the degradation of fresh juice flavour as well as the reduction in nutrients (Braddock 1999). Because of these perceived adverse effects, alternative technologies offering improved nutritional and flavour quality are being explored.

Ohmic heating is a highly attractive technology for continuous food processing, and it has been used in blanching, evaporation, dehydration, fermentation, extraction and pasteurization (Halden *et al.* 1990; Sastry and Barach 2000). The development of a continuous flow ohmic heating with electrode systems has opened new possibilities for industries interested in continuous and instantaneous sterilization of liquid-particle mixtures (Sastry 1992). The basic principle of ohmic heating is the passage of electrical current through a food product that serves as an electrical resistance (Reznick 1996); thus, heat is generated immediately inside the food (Icier and Ilicali 2005). This technology provides a rapid and uniform heating, and the absence of a cold spot in the sample reduces the fouling problems and thermal abuse to the product in comparison with conventional heating (Leizeron and Shimoni 2005a,b). Therefore, a high-quality food product with minimal structural, nutritional or organoleptic changes as well as one that is microbiologically safe can be successfully manufactured in a short processing time (Rahman 1999).

Although considerable research studies of ohmic heating have been carried out for decades, there is a little information concerning the inactivation of *E. coli* O157:H7, *Salm. Typhimurium* and *L. monocytogenes* in fruit and vegetable juices. The objective of this study was to evaluate the efficacy of continuous ohmic heating for inactivating *E. coli* O157:H7, *Salm. Typhimurium* and *L. monocytogenes* in orange and tomato juices with various treatment times and electric field strengths. Additionally, we investigated the quality of orange juice and morphological changes in the cell after continuous ohmic and conventional heating treatments.

Materials and methods

Bacterial strains

Three strains each of *E. coli* O157:H7 (ATCC 35150, ATCC 43889 and ATCC 43890), *Salm. Typhimurium* (ATCC 19585, ATCC 43971 and DT 104) and *L. monocytogenes* (ATCC 15315, ATCC 19114 and ATCC 19115) were obtained from the Food Science and Human Nutrition culture collection at Seoul National University (Seoul, Korea) for this study and used to inoculate into orange juice. Stock cultures were stored in 0.7 ml of tryptic soy broth (TSB; Difco, Becton Dickinson, Sparks, MD, USA) with 0.3 ml of 50% glycerol at -80°C prior to use. Working cultures were streaked onto tryptic soy agar (TSA; Difco), incubated at 37°C for 24 h and stored at 4°C .

Preparation of cell suspension

All strains of *E. coli* O157:H7, *Salm. Typhimurium* and *L. monocytogenes* were cultured individually in 5 ml TSB at 37°C for 24 h, collected by centrifugation at 4000 g for 20 min at 4°C and washed three times with buffered peptone water (BPW; Difco). The final pellets were resuspended in BPW, corresponding to $c. 10^7$ – 10^8 CFU ml $^{-1}$. To inoculate into orange juice, suspended pellets of the three strains of all species were combined to construct a culture cocktail. These cell suspensions were used in subsequent experiments.

Sample preparation and inoculation

Pasteurized single-strength orange juice (pH 3.94, 11.8°Brix) and tomato juice (pH 4.14, 11.2°Brix) were purchased at a local supermarket (Seoul, Korea). The sodium content of orange juice and tomato juice was 2.86 mg per 100 ml and 26.67 mg per 100 ml, respectively. Ten millilitres of the mixed culture cocktail (*E. coli* O157:H7, *Salm. Typhimurium* and *L. monocytogenes*) was inoculated into three litres of juice at room temperature ($22 \pm 2^{\circ}\text{C}$) and mixed using a magnetic stir bar for 3 min. The final cell concentration was 10^6 – 10^7 CFU ml $^{-1}$. Inoculated juice samples were then immediately treated with continuous ohmic heating.

Experimental apparatus

The experimental device (Fig. 1) consisted of a continuous ohmic heating chamber, a power supply, a data logger (34790A; Agilent Technologies, Palo Alto, CA, USA), a product tank and a volumetric pump (JWS600; Jeniewell, Seoul, Korea). The continuous ohmic heating

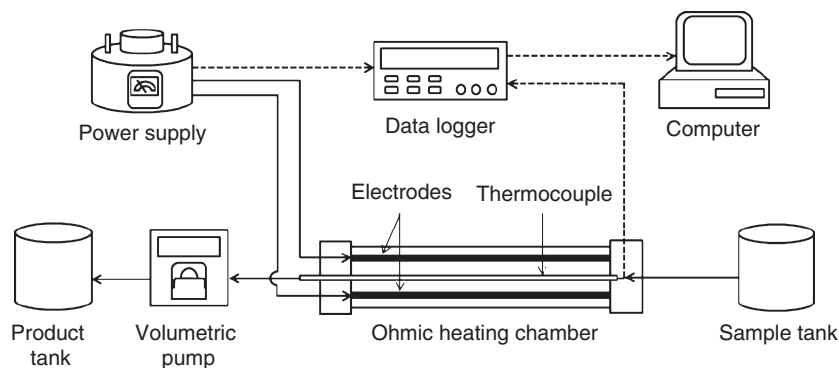


Figure 1 Schematic diagram of the continuous ohmic heating system at Seoul National University (Seoul, Korea).

chamber (33cm × 5cm diameter) was composed of polyvinyl chloride (PVC) pipe of 0.51 cm thickness, PVC caps and two titanium plate electrodes. To reduce the thermal loss and evaporation, both ends of the heating chamber were covered with PVC caps. The distance between the two electrodes was 2 cm, and the cross-sectional area was 120 cm². The product flows along the axis between the electrodes. Temperatures were monitored using a K-type thermocouple, placed at the exit and the geometric centre of the chamber. The temperature of each sample was nearly uniform in the chamber, because the maximum difference among the measured temperatures at different locations was approximately within 1°C. The supplied power in the chamber was alternating current at 60 Hz, and voltage was controlled by a variable transformer with a range of 100–200 V. Temperature, voltage, current and time were recorded at 5-s intervals by a data logger linked to a computer.

Continuous ohmic heating treatment

For the continuous ohmic heating treatment, 3 l of inoculated juice was placed in a 3-l product tank. The sample was pumped to the ohmic heating chamber by a volumetric pump, and the flow rate was maintained at 120 ml min⁻¹. When the sample passed through the exit of the chamber, power was then turned on and ohmic heating applied. Four different electric field strengths (25, 30, 35 and 40 V cm⁻¹) were applied to the sample with various treatment times. Following heat treatment, samples were taken at 30- to 60-s intervals for up to 300 s and immediately immersed in ice water until the samples were cooled to 4°C. To enumerate surviving pathogens, tenfold serial dilutions were performed in BPW and samples or diluents were spread-plated onto selective medium. *Escherichia coli* O157:H7, *Salm.* Typhimurium and *L. monocytogenes* were enumerated on Sorbitol MacConkey Agar (SMAC; Difco), Xylose Lysine Desoxycholate

Agar (XLD; Difco) and Oxford Agar Base (OAB; Difco) with antimicrobial supplement (Bacto™ Oxford Antimicrobial Supplement; Difco), respectively. When low levels of surviving cells were anticipated, 250 µl of sample was spread-plated onto each of four plates. All plates were incubated at 37°C for 24–48 h before counting.

Conventional heating treatment

To achieve identical thermal histories of ohmic heating and conventional heating, the conventional heating conditions were selected to be the same heating profile as in continuous ohmic heating treatment with 30 V cm⁻¹ of electric field strength. For conventional heating treatment, pasteurized single-strength orange juice (250 ml) purchased at a local supermarket was transferred into a clean 500-ml conical flask (Duran, Schott, Mainz, Germany) and was processed at 150°C in an oil bath (OSB-2000; EYELA, New York, NY, USA) for 180 s. To obtain a uniform temperature, the samples were stirred with glass rod by hand while heating treatment. A data logger was used to measure the juice temperature during the experiments.

Transmission electron microscopy

To obtain the transmission electron microscopy (TEM) electron micrographs, *E. coli* O157:H7 cells in orange juice were rinsed three times with 0.1 mol l⁻¹ phosphate-buffered saline (PBS) and collected by centrifugation at 4000 g for 10 min. The pellet was fixed in modified Karnovsky's (2% paraformaldehyde and 2% glutaraldehyde in 0.05 mol l⁻¹ sodium cacodylate buffer) at 4°C for 2–4 h. After the primary fixation, cells were centrifuged and washed three times with 0.05 mol l⁻¹ sodium cacodylate buffer. The cells were then fixed in 1% osmium tetroxide in 0.05 mol l⁻¹ sodium cacodylate buffer (pH 7.2) at 4°C for 2 h and washed two times briefly with distilled water.

The cells were then dehydrated using a graded ethanol series of 30, 50, 70, 80, 90 and three times at 100% for 10 min each. After dehydration, the cells were placed in 100% propylene oxide at 4°C for 15 min and infiltrated using solutions of propylene oxide and Spurr's resin. A 1 : 1 solution of propylene oxide and Spurr's resin was placed on the cells for 2 h and then placed in Spurr's resin overnight. Infiltrated samples were polymerized at 70°C for 24 h. The samples were sectioned (slices 70 nm in thickness) using an ultramicrotome (MT-X; RMC, Tucson, AZ, USA) and then stained with 2% uranyl acetate for 7 min and Reynold's lead citrate for 7 min. The sections were examined by TEM (LIBRA 120; Carl Zeiss, Heidenheim, Germany) and digitally photographed.

Colour measurement

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

Vitamin C measurement

Ascorbic acid concentration in juice was determined using high-performance liquid chromatography (HPLC) (Ultimate 3000, Dionex, Sunnyvale, CA, USA) equipped with an autosampler and an UV detector set at 265 nm. A reversed-phase C18 column (5- μ m particle size, 4.6 mm diameter, 250 mm length; Dionex) was used to separate the ascorbic acid using 50 mmol l⁻¹ potassium phosphate buffer and acetonitrile (95 : 5, v/v) as a mobile phase. The mobile phase was filtered using a 0.45- μ m membrane filter (Micron separations Inc., Westboro, MA, USA) and degassed via vacuum before being used on the column. A flow rate of 0.8 ml min⁻¹ was employed, and retention time was 3.7 min. A standard calibration curve was obtained by using L-ascorbic acid (Sigma Chemical Co., St Louis, MO, USA) in concentrations ranging from 5 to 80 mg per 100 ml. The samples were centrifuged at 12 500 g for 10 min in a Beckman Microfuge E (Beckman Instruments Inc., Palo Alto, CA, USA) to remove the pulp and coarse cloud particles. Ten microlitres of supernatant was injected into the column using the HPLC autosampler.

Statistical analysis

All experiments were duplicate-plated and replicated three times. All data were analysed by analysis of variance using the ANOVA procedure of 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 significant level of $P = 0.05$.

Results

Temperature curves of orange juice and tomato juice

Temperatures of orange juice and tomato juice during continuous ohmic heating treatment with voltage gradients of 25, 30, 35 and 40 V cm⁻¹ are shown in Fig. 2. The maximum treatment time of orange juice, at which the 5-log reduction started, was determined as 60, 90 and 180 s for 30, 35 and 40 V cm⁻¹ electric field strengths, respectively (Fig. 2a). At the same electric field strength, temperature increased with increasing treatment time. Heating times decreased as a result of higher heating rates resulting from higher voltage gradients applied. At 25 V cm⁻¹ electric field strength, the temperature of orange juice did not exceed 60°C until 300 s had elapsed. At 40 V cm⁻¹, orange juice was heated from room temperature to 75.5°C in 60 s. For the same voltage gradients, the maximum treatment time of tomato juice was determined as 30, 55, 65 and 100 s (Fig. 2b). The rate of temperature change for tomato juice was higher than for orange juice at all voltage gradients applied. Unlike orange juice, the temperature of tomato juice reached 76°C at both 25 and 40 V cm⁻¹ electric field strengths after 100 and 30 s, respectively.

A comparison of the thermal histories of orange juice subjected to continuous ohmic heating with 30 V cm⁻¹ of electric field strength and conventional heating is shown in Fig. 3. Both conventionally and continuous ohmic heated samples had a similar temperature profiles. Continuous ohmic heating has more rapid and uniform temperature profile than conventional heating. Conventional heating had a little longer lag period and time to reach targeted temperature.

Inactivation of micro-organisms in orange juice and tomato juice after continuous ohmic heating treatment

The survival of *E. coli* O157:H7, *Salm.* Typhimurium and *L. monocytogenes* in orange juice during continuous ohmic heating is shown in Fig. 4. As electric field strength increased from 25 to 40 V cm⁻¹, surviving populations of the three pathogens decreased more effectively. The levels

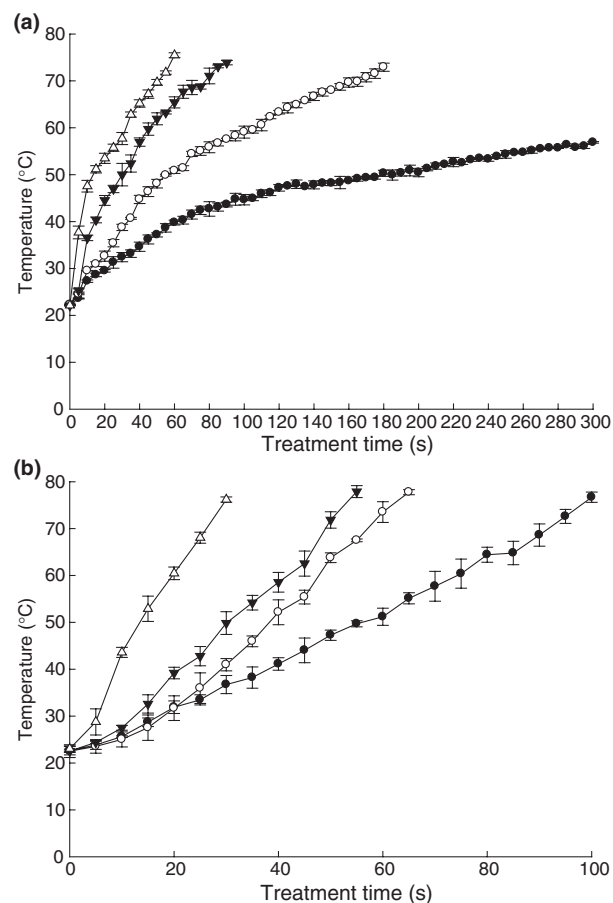


Figure 2 Temperature curves of orange juice (a) and tomato juice (b) during continuous ohmic heating at different electric field strengths. ●, 25 V cm⁻¹, ○, 30 V cm⁻¹, ▼, 35 V cm⁻¹, △, 40 V cm⁻¹.

of surviving cells of the three pathogens were reduced by >5 log within 60 s when treated with 40 V cm⁻¹ electric field strength. At 35 V cm⁻¹, levels of *E. coli* O157:H7 were reduced by 1.14 and 6.1 log CFU ml⁻¹ after 60 and 90 s, respectively. Cell numbers of *Salm.* Typhimurium experienced a significant reduction of 1.32 log CFU ml⁻¹ after 60-s treatment and >6.52 log reduction to below the detection limit (<1 CFU ml⁻¹) after 90-s treatment. For *L. monocytogenes*, the reduction was 1.23 log CFU ml⁻¹ after 60 s and 5.1 log CFU ml⁻¹ after 150 s. At 30 V cm⁻¹, the numbers of *E. coli* O157:H7, *Salm.* Typhimurium and *L. monocytogenes* were reduced to below the detection limit after 180-s treatment. However, at 25 V cm⁻¹, there were no appreciable differences in microbial levels between controls.

Figure 5 shows surviving populations of *E. coli* O157:H7, *Salm.* Typhimurium and *L. monocytogenes* from tomato juice treated with the same electric field strengths. Ohmic heating with 40 V cm⁻¹ electric field strength reduced the three pathogens to below the detection limit

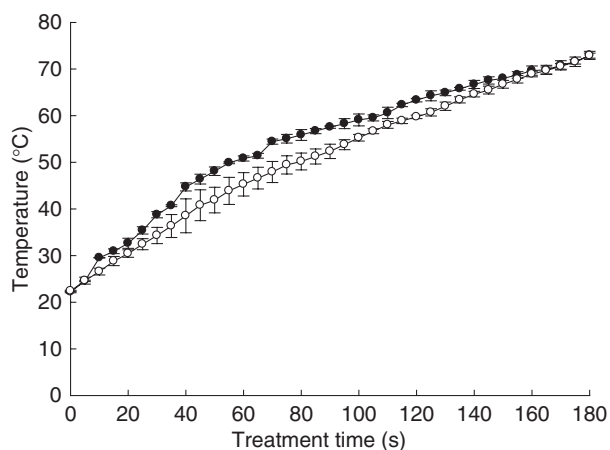


Figure 3 Temperature curves of orange juice processed by continuous ohmic heating at 30 V cm⁻¹ (●) and conventional heating (○).

(<1 CFU ml⁻¹) after 30-s treatment. At 35 V cm⁻¹, *E. coli* O157:H7, *Salm.* Typhimurium and *L. monocytogenes* were reduced to below undetectable levels after 55-s treatment and 30 V cm⁻¹ reduced these pathogens to below the detection limit after 65 s. Unlike orange juice, levels of *Salm.* Typhimurium in tomato juice were greatly reduced to undetectable levels after 100-s treatment at 25 V cm⁻¹. Populations of *E. coli* O157:H7 and *L. monocytogenes* decreased by 6.19 and 5.41 log CFU ml⁻¹ after 100 s, respectively.

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

TEM examination of inactivated bacterial cell

TEM was used to examine the ultrastructural changes of continuous ohmic heating on *E. coli* O157:H7 cells in comparison with conventional heating. The aggregation of cytoplasm was observed in both continuous ohmic heating and conventional heating treated cells as compared to the untreated cells (Fig. 6). However, *E. coli* O157:H7 cells treated with continuous ohmic heating had undergone significant changes compared with conventionally heated cells. An enlarged periplasmic space and uneven cell wall were observed only in continuous ohmic heating treated cells.

Effects of continuous ohmic heating on colour in orange juice

Colour measurements of orange juice after continuous ohmic and conventional heating treatments are shown in

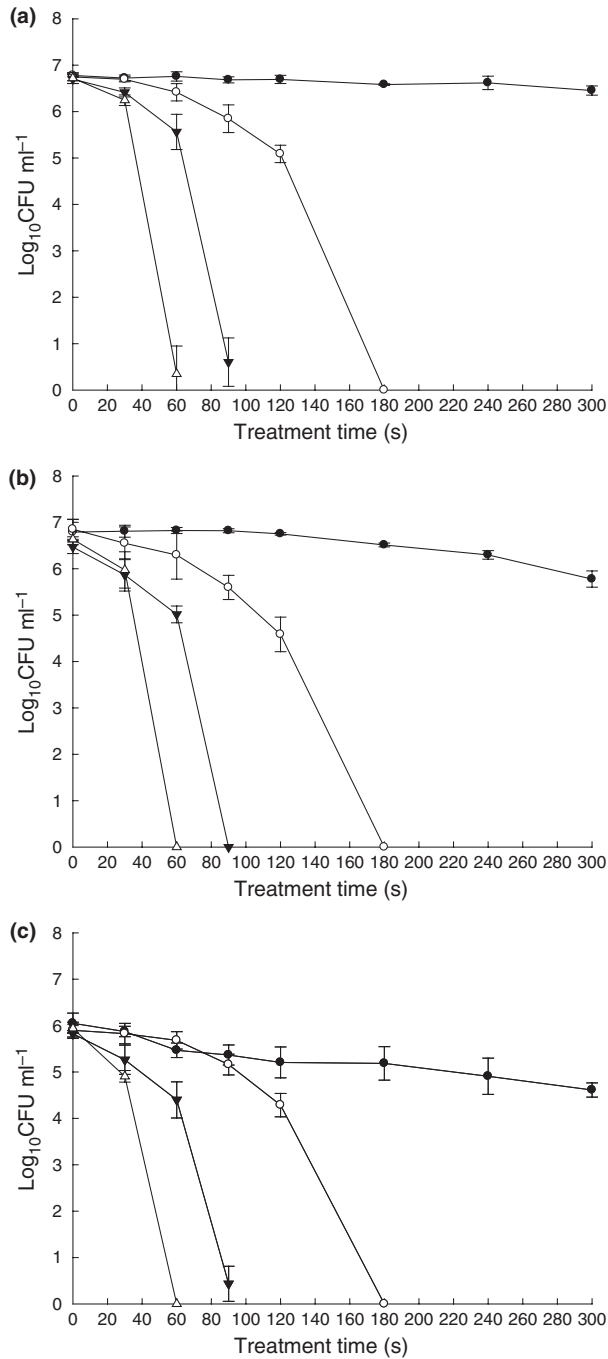


Figure 4 Survival curves for *Escherichia coli* O157:H7 (a), *Salmonella* Typhimurium (b) and *Listeria monocytogenes* (c) in orange juice subjected to ohmic heating at 25 V cm⁻¹ (●), 30 V cm⁻¹ (○), 35 V cm⁻¹ (▼) and 40 V cm⁻¹ (△). The results are means from three experiments, and error bars indicate standard errors.

Table 1. The results are expressed in *L*, *a*, and *b* values. No significant differences ($P > 0.05$) in *L* value change were found among the orange juice samples subject to any heating treatment. The *a* value of continuous ohmic

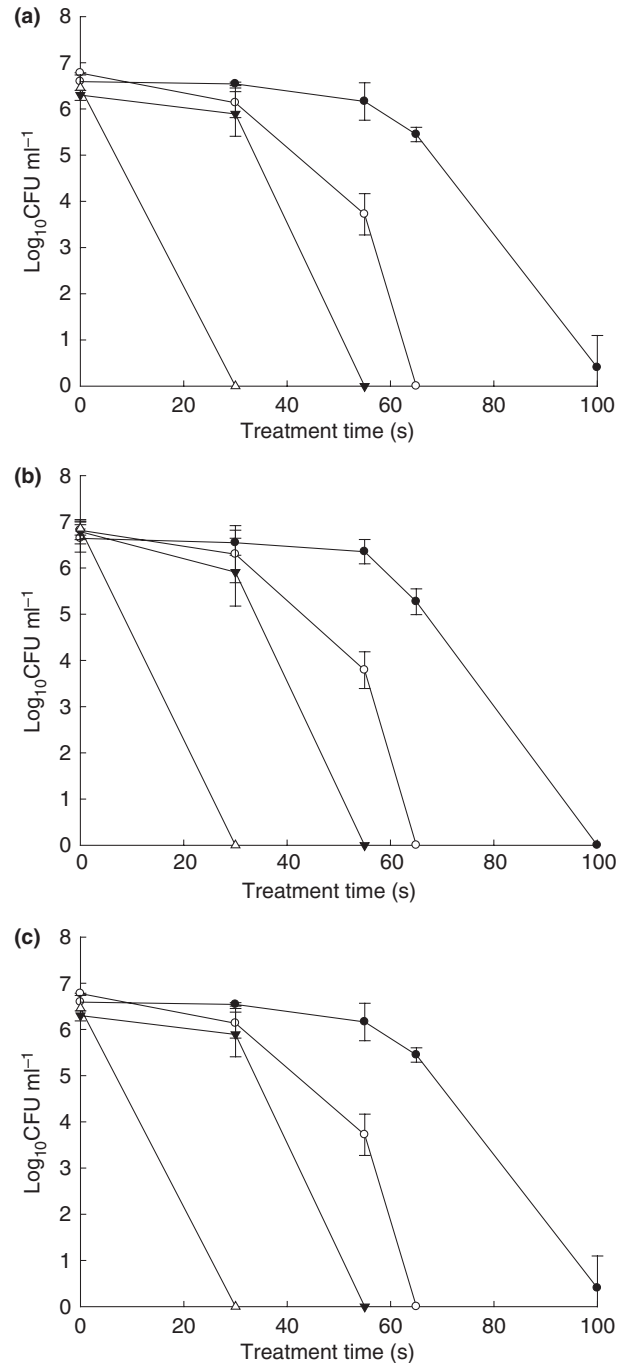


Figure 5 Survival curves for *Escherichia coli* O157:H7 (a), *Salmonella* Typhimurium (b) and *Listeria monocytogenes* (c) in tomato juice subjected to ohmic heating at 25 V cm⁻¹ (●), 30 V cm⁻¹ (○), 35 V cm⁻¹ (▼) and 40 V cm⁻¹ (△). The results are means from three experiments, and error bars indicate standard errors.

heated orange juice was not significantly different ($P > 0.05$) from that of nontreated samples. However, conventional heating was significantly different ($P < 0.05$) and also showed a greater reduction in *b* value than in

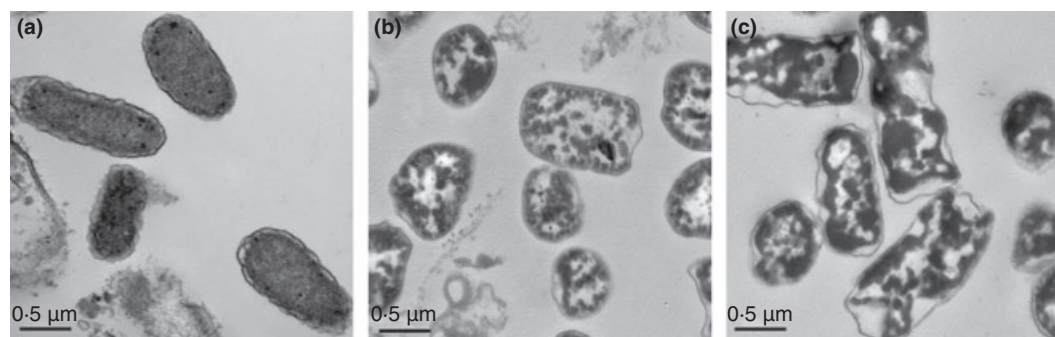


Figure 6 TEM electron micrographs of *Escherichia coli* O157:H7 in orange juice, untreated (a), conventional heated for 180 s (b) and continuous ohmic heated at 30 V cm⁻¹ for 180 s (c).

Table 1 Colour values*, ascorbic acid content of ohmic and conventional heating treated orange juice

Heating method	Colour (<i>L</i>)	Colour (<i>a</i>)	Colour (<i>b</i>)	Ascorbic acid (mg per 100 ml)
Control	57.0 ± 0.5 ^a	-6.6 ± 0.0 ^a	32.3 ± 0.2 ^a	56.9 ± 0.4 ^a
Ohmic	56.1 ± 0.3 ^a	-8.2 ± 0.4 ^{ab}	29.1 ± 0.2 ^b	42.0 ± 0.8 ^b
Conventional	57.5 ± 0.4 ^a	-9.9 ± 0.1 ^b	26.3 ± 0.1 ^c	38.6 ± 0.8 ^c

Mean values ± standard deviation.

^{a-c}Means with same columns followed by different superscript are significantly different ($P < 0.05$).

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

orange juice treated with continuous ohmic heating. The *b* value of orange juice treated with continuous ohmic heating was 29.06, much higher than the value of 26.34 for orange juice heated conventionally. The *b* value of continuous ohmic heating treated orange juice was much closer to that of the control than was the conventionally heated counterpart.

Influence of continuous ohmic and conventional heating on vitamin C concentration

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

Discussion

All three foodborne pathogens in orange juice and tomato juice were significantly reduced by continuous ohmic

heating treatment. Inactivation of micro-organisms by ohmic heating is mainly because of the thermal effect and heat is generated instantly inside the food. There was an additional killing effect caused by electrical current itself; several studies have been conducted on this mechanism of inactivation (Sun *et al.* 2008). Bacterial death owing to the chemical effects during low-voltage electric current treatment might be due to the presence of chloride-containing compounds in the treatment medium (Pareilleux and Sicard 1970) or due to the formation of hydrogen peroxide (Shimada and Shimahara 1982). Palaniappan *et al.* (1990) reported membrane damage that caused permeability modification and leakage of cellular contents by combination of these chemical factors. Kulshrestha and Sastry (2003) assumed that the electric breakdown or electroporation mechanism of cell membranes by electric current is the predominant nonthermal effect of ohmic heating. In the present study, we examined the morphological change of micro-organisms influenced by continuous ohmic and conventional heating using TEM. Although the shrinkage of intracellular materials was observed in both continuous ohmic and conventionally heated cells, continuous ohmic heated cells show the development of space between cell wall and the membrane and irregular changes in the cell wall. Yoon *et al.* (2002) observed that the electroporation caused by the electric field with ohmic heating increased the

permeability of the yeast cell wall. The enlarged periplasmic space and the severe loss of cytoplasm in continuous ohmic heated cells could be due to the removal of the cell membrane caused by electropermeabilization. Therefore, the TEM electron micrographs verify the destruction of the vegetative cell and suggest that continuous ohmic heating has not only a thermal-lethal effect but also a nonthermal-lethal effect because of the effect of electric current on micro-organisms.

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

Because of the different heating rates between juices, the treatment time needed to achieve a 5-log reduction was also different. At 25 V cm⁻¹ electric field strength, there was no appreciable inactivation of *E. coli* O157:H7 in orange juice after a 300-s treatments, but tomato juice experienced a more than 6-log reduction. Sagong *et al.* (2011) reported that the ohmic heating treatment time required to achieve a minimum 5-log reduction in tomato juice was from 30 to 60 s faster than in orange juice. Similar results were observed for *Salm.* Typhimurium and *L. monocytogenes* in our studies. As the electric field strengths increased, the treatment time needed to achieve a 5-log reduction was reduced for all three foodborne pathogens. Baysal and Icier (2010) suggested that moderate increases in the voltage gradient seemed to enhance the inactivation effect of *Alicyclobacillus acidoterrestris* spores suspended in orange juice during ohmic heating treatment. In the present study, the most effective treatment condition for pasteurization in both orange juice and tomato juice was 40 V cm⁻¹ of electric field strength.

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

Our results indicated that continuous ohmic heating treatment can be effectively used to inactivate *E. coli* O157:H7, *Salm.* Typhimurium and *L. monocytogenes* in orange juice and tomato juice with higher quality than conventional heating. The effects of inactivation are dependent on applied electric field strength, treatment time and electrical conductivity. Continuous ohmic heating treatment could be applied to control foodborne pathogens in fruit juice industry over conventional heating methods. Further investigation is needed to determine the optimum parameters for the shelf life and quality of products.

Acknowledgements

This research was supported by the Agriculture Research Center program of the Ministry for Food, Agriculture, Forestry and Fisheries, Korea. This work was also supported by grant no. R32-2008-000-10183-0 from the World Class University project of the Ministry of Education, Science & Technology and the Korea Science and Engineering Foundation through Seoul National University.

References

- Baird-Parker, A.C. (1990) Foodborne salmonellosis. *Lancet* **336**, 1231–1235.
- Baysal, A.H. and Icier, F. (2010) Inactivation kinetics of *Alicyclobacillus acidoterrestris* spores in orange juice by ohmic heating: effects of voltage gradient and temperature on inactivation. *J Food Protect* **73**, 299–304.
- Braddock, R.J. (1999) Single strength orange juices and concentrate. In *Handbook of Citrus By-Products and Processing Technology*. pp. 53–83. New York: Wiley.
- Cody, S.H., Glynn, M.K., Farrar, J.A., Cairns, K.L., Griffin, P.M., Kobayashi, J., Fyfe, M., Hoffman, R. *et al.* (1999) An outbreak of *Escherichia coli* O157:H7 infection from unpasteurized commercial apple juice. *Ann Intern Med* **130**, 202–209.
- Halden, K., Alwis, A.A.P.D. and Fryer, P.J. (1990) Changes in the electrical conductivity of foods during ohmic heating. *Int J Food Sci Technol* **25**, 9–25.
- Icier, F. and Ilicali, C. (2005) Temperature dependent electrical conductivities of fruit purees during ohmic heating. *Food Res Int* **38**, 1135–1142.
- Jain, S., Bidol, S.A., Austin, J.L., Berl, E., Elson, F., Lemaile-Williams, M., Deasy, M., Moll, M.E. *et al.* (2009) Multi-state outbreak of *Salmonella* Typhimurium and saint-paul infections associated with unpasteurized orange juice—United States, 2005. *Clin Infect Dis* **48**, 1065–1071.
- Kulshrestha, S. and Sastry, S.K. (2003) Frequency and voltage effects on enhanced diffusion during moderate electric field (MEF) treatment. *Innov Food Sci Emerg Technol* **4**, 189–194.
- Leizerson, S. and Shimoni, E. (2005a) Effects of ultrahigh-temperature continuous ohmic heating treatment on fresh orange juice. *J Agric Food Chem* **53**, 3519–3524.
- Leizerson, S. and Shimoni, E. (2005b) Stability and sensory shelf life of orange juice pasteurized by continuous ohmic heating. *J Agric Food Chem* **53**, 4012–4018.
- Mazzotta, A.S. (2001) Thermal inactivation of stationary-Phase and acid-adapted *Escherichia coli* O157:H7, *Salmonella*, and *Listeria monocytogenes* in fruit juices. *J Food Protect* **64**, 315–320.
- Oyarzabal, O.A., Nogueira, M.C.L. and Gombas, D.E. (2003) Survival of *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella* in juice concentrates. *J Food Protect* **66**, 1595–1598.
- Palaniappan, S. and Sastry, S.K. (1991) Electrical conductivity of selected juices: influences of temperature, solids content, applied voltage, and particle size. *J Food Process Eng* **14**, 247–260.
- Palaniappan, S., Sastry, S.K. and Richter, E.R. (1990) Effects of electricity on microorganisms: a review. *J Food Process Preserv* **14**, 393–414.
- Pareilleux, A. and Sicard, N. (1970) Lethal effects of electric current on *Escherichia coli*. *J Appl Microbiol* **19**, 421–424.
- Rahman, M.S. (eds) (1999) *Handbook of Food Preservation*. pp. 521–532. New York: Dekker.
- Reznick, D. (1996) Ohmic heating of fluid foods. *Food Technol* **50**, 250–251.
- Ruan, R., Ye, X., Chen, P. and Doona, C.J. (2000) Developments in ohmic heating. In *Improving the Thermal Processing of Foods* ed. Richardson, P. pp. 224–227 Boca Raton, FL: CRC Press.
- Sagong, H.G., Park, S.H., Choi, Y.J., Ryu, S. and Kang, D.H. (2011) Inactivation of *Escherichia coli* O157:H7, *Salmonella* Typhimurium, and *Listeria monocytogenes* in orange and tomato juice using ohmic heating. *J Food Protect* **74**, 899–904.
- Sastry, S.K. (1992) A model for heating of liquid-particle mixtures in a continuous flow ohmic heater. *J Food Process Eng* **15**, 263–278.
- Sastry, S.K. and Barach, J.T. (2000) Ohmic and inductive heating. *J Food Sci* **65**, 42–46.
- Shimada, K. and Shimahara, K. (1982) Responsibility of hydrogen peroxide for the lethality of resting *Escherichia coli* B cells anaerobically exposed to an alternating current in phosphate buffer solution. *Agric Biol Chem* **46**, 1329–1337.
- Sun, H.X., Kawamura, S., Himoto, J.I., Itoh, K., Wada, T. and Kimura, T. (2008) Effects of ohmic heating on microbial counts and denaturation of proteins in milk. *Food Sci Technol Res* **14**, 117–123.
- United States Food and Drug Administration (1998) Hazard analysis and critical control points (HACCP); procedures for the safe and sanitary processing and importing of juice. *Fed Regist* **63**, 20450–20486.
- United States Food and Drug Administration (USFDA) (2001) Hazard analysis and critical point (HACCP); Procedures for the safe and sanitary processing and importing of juice; Final rule. *Fed Regist* **66**, 6137–6202.
- Vikram, V.B., Ramesh, M.N. and Prapulla, S.G. (2005) Thermal degradation kinetics of nutrients in orange juice heated by electromagnetic and conventional methods. *J Food Eng* **69**, 31–40.
- Vojdani, J.D., Beuchat, L.R. and Tauxe, R.V. (2008) Juice-associated outbreaks of human illness in the united states, 1995 through 2005. *J Food Protect* **71**, 356–364.
- Wang, W. and Sastry, S. (1993) Salt diffusion into vegetables tissue as a pretreatment for ohmic heating: electrical conductivity profiles and vacuum infusion studies. *J Food Eng* **20**, 299–309.
- Yoon, S.W., Lee, C.Y.J., Kim, K.M. and Lee, C.H. (2002) Leakage of cellular material from *Saccharomyces cerevisiae* by ohmic heating. *J Microbiol Biotechnol* **12**, 183–188.