International Journal of Food Microbiology 171 (2014) 147–153

Contents lists available at ScienceDirect

International Journal of Food Microbiology

journal homepage: www.elsevier.com/locate/ijfoodmicro

Combination effect of ozone and heat treatments for the inactivation of *Escherichia coli* O157:H7, *Salmonella* Typhimurium, and *Listeria monocytogenes* in apple juice ☆

Hye-Jung Sung [a,b,1], Won-Jae Song [a,b,1], Kwang-Pyo Kim [b], Sangryeol Ryu [a,b], Dong-Hyun Kang [a,b,*]

[a] Department of Food and Animal Biotechnology, School of Agricultural Biotechnology, Center for Food and Bioconvergence, and Research Institute for Agricultural and Life Science, Seoul National University, San 56-1, Sillim-dong, Kwanak-gu, Seoul 151-742, Republic of Korea
[b] Department of Food Science and Technology, College of Agriculture and Life Sciences, Chonbuk National University, Jeonju, Republic of Korea

ARTICLE INFO

Article history:
Received 26 April 2013
Received in revised form 25 October 2013
Accepted 3 November 2013
Available online 10 November 2013

Keywords:
Ozone treatment
Apple juice
Temperature
Foodborne pathogens
Food safety

ABSTRACT

We investigated the combination effect of ozone and heat treatments in apple juice for the inactivation of *Escherichia coli* O157:H7, *Salmonella* Typhimurium, and *Listeria monocytogenes*. Apple juices inoculated with the three pathogens were treated with gaseous ozone and heat simultaneously for up to 1 min. Gaseous ozone treatment was progressed at a flow rate of 3.0 l/min with a concentration of 2.0–3.0 g/m^3 and heat treatment was performed at temperatures of 25, 45, 50, and 55 °C. Populations of surviving pathogens decreased in all samples as treatment temperature increased from 25 to 55 °C. Heat treatment alone (25, 45, 50 and 55 °C) resulted in 0.20, 0.37, 2.16 and 2.54 log CFU/ml reductions of *E. coli* O157:H7, respectively, in apple juice. Combination treatment of ozone and heat for 1 min reduced this pathogen by 1.50 and 1.60 log CFU/ml, respectively, at 25 and 45 °C, and below the detection limit (1 log CFU/ml) at 50 and 55 °C. We found a synergistic effect in the inactivation of pathogens in apple juice treated with ozone and heated at 50 °C. The reduction trend of *S.* Typhimurium and *L. monocytogenes* in apple juice was similar to that of *E. coli* O157:H7. There were no significant changes of Hunter color values when apple juices were treated with heat only and the combination of ozone and heat. Residual ozone was measured following ozone treatment. In all ozone treated samples, the concentration of residual ozone was reduced to under acceptable levels (<0.4 mg/l). In conclusion, the combination treatment of ozone and heat was significantly effective in the inactivation of foodborne pathogens while maintaining acceptable apple juice quality.

1. Introduction

Fruit juice is a popular beverage because it is an important source of bioactive compounds including vitamins, phenolic compounds, anthocyanins, and carotenoids and also has good sensory qualities (Cullen et al., 2010). In the past, fruit juices were believed to be free from foodborne pathogens due to their relatively low pH (Liao et al., 2007). However, there have been several outbreaks of foodborne illnesses caused by consumption of fruit juices containing acid-resistant pathogens such as *Escherichia coli* O157:H7 and *Salmonella* spp. (Choi et al., 2012; Williams et al., 2005). From 1995 to 2005, 21 juice-associated outbreaks were reported to the CDC (Centers for Disease Control and Prevention) in the United States. These outbreaks indicate that fruit juices including apple juice can harbor foodborne pathogens (Vojdani et al., 2008). Although *Listeria monocytogenes* has not been directly related to outbreaks of foodborne illnesses associated with juice, it was identified as a bacterial pathogen pertinent to juice safety along with *E. coli* O157:H7 and *Salmonella enterica* in the final ruling published in the Federal Register [FR] (Gabriel and Nakano, 2009).

As a result, these outbreaks led the United States Food and Drug Administration (US FDA) to issue hazard analysis and critical control points (HACCP) regulations for safe and sanitary processing of juice. A main performance standard in HACCP regulations to improve sanitary processing of juice is a minimum 5-log reduction of the pathogens in the juice being processed (US FDA, 2001). In general, conventional thermal processing technology is used as a method for achieving a 5-log reduction of pathogens in fruit juices. But, thermal treatment damages the nutritional and physicochemical properties of foods. In the case of fresh juice, important factors such as flavor or nutrients can be affected by thermal pasteurization (Braddock, 1999). Recently, many consumers have come to prefer fresh extracted juices due to their fresher taste with fewer flavor or vitamin losses (Bignon, 1997). This consumer

* Corresponding author at: Department of Agricultural Biotechnology, Seoul National University, Seoul, 151-921, Republic of Korea. Tel.: +82 2 880 4927; fax: +82 2 883 4928.
E-mail address: kang7820@snu.ac.kr (D.-H. Kang).

[1] These authors contributed equally to this work.

0168-1605/$ – see front matter © 2013 The Authors. Published by Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.ijfoodmicro.2013.11.001

trend, along with the disadvantages of thermal treatment, leads food researchers and processors to explore novel and alternative technologies which can improve the quality as well as achieve 5-log reductions of pathogens in juices (Lee et al., 2012).

In 2001, ozone in the gaseous and aqueous phases was approved by the US FDA as an antimicrobial agent for the treatment, storage and processing of foods (Khadre et al., 2001). This approval resulted in the active utilization and study of ozone for pathogen inactivation by the food industry (Vojdani et al., 2008). Many food researchers have applied ozone to various fruit juices during processing, for example, apple cider, orange juice, strawberry juice, and apple juice (Choi et al., 2012; Patil et al., 2009; Tiwari et al., 2009b; Williams et al., 2004).

The reason why ozone is widely used in the food industry is that it has many advantages over other treatments. Ozone is a triatomic allotrope of oxygen and decomposes automatically and rapidly to a nontoxic product, oxygen, leaving no residues in foods (Burleson et al., 1975; Graham, 1997). It has a high oxidation potential of 2.07 V in alkaline solution compared to that of chlorine (1.36 V), so it can be used as an effective antimicrobial agent (Fisher et al., 2000; Kim et al., 1999). Also, it can destroy all forms of microorganisms at relatively low concentrations. Ozone achieves inactivation of bacteria by having an effect on various cellular components like proteins, peptidoglycans in cell envelopes, enzymes and nucleic acids in the cytoplasm. Oxidation of unsaturated lipids in the cell envelope causes leakage of inner contents and finally results in lysis (Das et al., 2006; Khadre et al., 2001).

In general, food products are treated with gaseous and aqueous forms of ozone. The form of ozone treatment is determined by the types of food products being processed (Cullen et al., 2010). The bactericidal effect of gaseous ozone on apple juice has been reported by several studies. Choi et al. (2012) investigated the effect of the solid content of apple juice on gaseous ozone against *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes*. Patil et al. (2010b) studied the antimicrobial efficacy of gaseous ozone against *E. coli* in apple juice of various pH levels. However, there have been very few research studies investigating the bactericidal effect when apple juice is treated with both heat and ozone gas simultaneously and their effect on quality changes of apple juice. Therefore, in this study, we investigated the combination or synergistic effect of ozone and heat treatments on apple juice to inactivate *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes*. Also, changes in color and residual ozone of apple juice after treatment were investigated.

2. Materials and methods

2.1. Bacterial strains and preparation of inoculums

Strains of *E. coli* O157:H7 (ATCC 35150, ATCC 43889, ATCC 43890), *S.* Typhimurium (ATCC 19585, ATCC 43971, DT 104), and *L. monocytogenes* (ATCC 19114, ATCC 19115, ATCC 15313) were obtained from the bacteria culture collection of Seoul National University (Seoul, Korea). Stock cultures were prepared by combining 0.7 ml of Tryptic Soy Broth (TSB; Difco, Becton Dickinson, Sparks, MD, USA) and 0.3 ml of 50% glycerol and storing at −80 °C. Working cultures were streaked onto Tryptic Soy Agar (TSA; Difco), incubated at 37 °C for 24 h, and stored at 4 °C.

Each strain of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* was cultured in 5 ml TSB at 37 °C for 24 h, harvested by centrifugation at 4000 *g* for 20 min at 4 °C and washed three times with sterile 0.2% peptone (Bacto, Sparks, MD). The final pellets were resuspended in 0.2% sterile peptone water, corresponding to approximately 10^8–10^9 CFU/ml. Mixed culture cocktails were prepared by blending together equal volumes of each test strain.

2.2. Sample preparation and inoculation

Pasteurized apple juice was purchased at a local supermarket (Seoul, Korea) and stored at 4 °C. One hundred milliliters of apple juice was dispensed into a 500 ml bottle and 0.1 ml of antifoam B emulsion (Sigma Aldrich, Ireland Ltd.) was added to the apple juice to prevent excess foaming. Apple juice was inoculated with 0.1 ml of the mixed culture cocktail (*E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes*). The final cell concentration was 10^5–10^6 CFU/ml.

2.3. Ozone and heat treatments

As shown in Fig. 1, ozone gas was produced by an ozone generator (Ozonetech Co., Ltd, Korea) at generation rates of 2.0–3.0 g/m^3 from ambient air at a flow rate of 3.0 l/min. The concentration of ozone was controlled by an ozone monitor (Okitrotec Co., Japan). Ozone was pumped directly into the juice through a delivery tube and sparged through a perforated tube into a 500 ml bottle. As soon as the preset temperature (25, 45, 50 and 55 °C) was stabilized by using a water bath, heat treatments were conducted. Juice samples not subjected to ozone treatment, but heat treated, were designated as the heat treatment alone group. Each apple juice sample treated at 25 °C without or

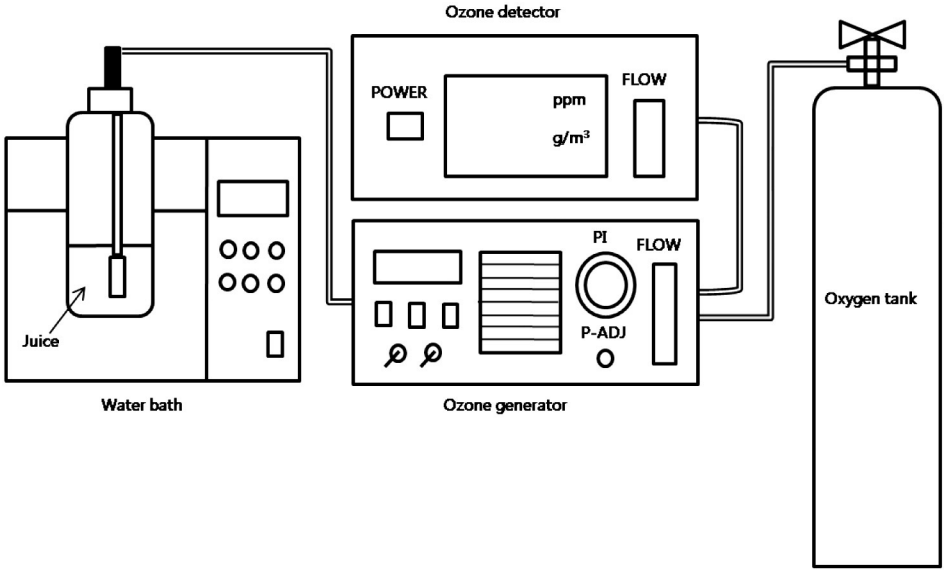

Fig. 1. Schematic diagram of the ozone treatment apparatus at Seoul National University (Seoul, Korea).

with ozone was regarded as control group for confirming the effect of heat and ozone at 25 °C. All samples were treated for 20 s, 40 s or 1 min. During ozone and heat treatments, juice samples were mixed at 150 rpm for the entire treatment time in a shaking water bath to ensure even distribution of inoculum and dispersal of ozone. All experiments were conducted in a fume hood. Excess gaseous ozone was passed into an ozone decomposer.

2.4. Bacteriological analysis

For enumeration of pathogens, sample aliquots (1 ml) were transferred into test tubes containing 9 ml of D/E neutralizing broth (Difco, Becton Dickinson, Sparks, MD, USA) after each treatment and homogenized using a vortex mixer (VM-10, Daihan Scientific Co., Ltd, Korea). After homogenization, samples were 10-fold serially diluted with 9 ml of 0.2% sterile buffered peptone water and 0.1 ml of the samples was spread plated onto selective media (*E. coli* O157:H7: Sorbitol MacConkey Agar (SMAC), Difco; *S.* Typhimurium: Xylose Lysine Desoxycholate Agar (XLD), Difco; and *L. monocytogenes*: Oxford Agar Base with antimicrobial supplement MB Cell (MOX), MB Cell). All plates were incubated at 37 °C for 24 h before counting.

2.5. Color measurement

Color was measured by using a Minolta colorimeter (model CR400; Minolta Co., Osaka, Japan). The values for L^*, a^* and b^* were recorded to evaluate the color changes of apple juice after each treatment with heat or combination of ozone and heat. Untreated apple juice was used as the control. A 2 ml of sample was poured into the bottom half of the measurement equipment. The measuring head of the colorimeter was placed on top of the measurement equipment. Before measurement, the treated juice was cooled to about 15 °C by dipping the bottle in crushed ice. 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 performed in triplicate.

2.6. Residual ozone measurement

To measure ozone concentration, distilled water was substituted for apple juice, as will be explained in the Discussion section. The ozone concentration of distilled water treated with ozone in the same way as apple juice was measured by the indigo method (Bader and Hoigni, 1981). An indigo stock reagent was prepared by the following method (Gordon and Bubnis, 2002). Indigo stock solution was prepared by dissolving 770 mg of potassium indigotrisulfonate (Sigma Aldrich Co., LLC) into a 1 l flask containing 500 ml of distilled water and 1 ml of phosphoric acid (85%) and diluting to volume (1 l) with distilled water. Indigo reagent II was prepared in a 1 l flask by adding 100 ml of indigo stock solution, 10 g of sodium dihydrogen phosphate (NaH_2PO_4) and 7 ml of phosphoric acid (85%). This was stirred and diluted to volume (1 l) with distilled water. The solution was stored in the dark. After each treatment, the treated sample was cooled to about 15 °C and then 90 ml of the sample was transferred to a flask containing 10 ml of indigo reagent II. The absorbance was measured by a spectrophotometer at 600 nm. Residual ozone in treated samples was obtained by the equation,

$$\text{mg}\frac{O_3}{L} = \frac{100 \times A}{f \times b \times V}$$

where A is the difference of the absorbance between the blank solution (control) and treated sample, f is an indigo sensitivity coefficient (0.42), b is the path length of the cell (1 cm), and V is the volume of the sample (ml). The blank solution was untreated distilled water.

2.7. Statistical analysis

All experiments were duplicate-plated and replicated three times. All data were analyzed with ANOVA using Statistical Analysis System (SAS Institute, Cary, NC, USA) and Duncan's multiple range test to determine if there were significant differences ($P < 0.05$) in the mean values of microorganism populations.

3. Results

Initial populations of *E. coli* O157:H7, *S.* Typhimurium and *L. monocytogenes* in inoculated apple juice were approximately 10^5–10^6 CFU/ml and the limit of detection was 1.0 log CFU/ml. The combination of ozone and heat treatments exhibited a great effect in reducing *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* in apple juice. The levels of surviving cells of the three pathogens in apple juice after the combination treatment are shown in Figs. 2–4.

Fig. 2 shows the bactericidal effect of the combination treatment of ozone and heat against *E. coli* O157:H7 in apple juice. The populations of surviving pathogens were decreased in all samples as ozone is combined with higher temperature among 4 temperatures (25, 45, 50, 55 °C). Counts of *E. coli* O157:H7 in apple juice treated only with heat (25, 45, 50 and 55 °C) for 1 min were reduced by 0.20, 0.37, 2.16 and 2.54 log CFU/ml, respectively. In the case of the combination treatment of ozone and heat for 1 min, *E. coli* O157:H7 in apple juice was reduced by 1.50 and 1.60 log CFU/ml at 25 and 45 °C, respectively, and was below the detection limit after treatment at 50 and 55 °C.

The reduction of *S.* Typhimurium in apple juice is shown in Fig. 3. The trend of reduction is similar to that of *E. coli* O157:H7. The population of surviving pathogens (25, 45, 50 and 55 °C) was decreased to 0.09, 0.62, 2.50 and 5.21 log CFU/ml after 1 min of heat treatment alone. In apple juice treated with both ozone and heat, *S.* Typhimurium was reduced by 1.84 and 2.20 log CFU/ml at 25 and 45 °C, respectively and below the detection limit at 50 and 55 °C. Fig. 4 shows the reduction of *L. monocytogenes*. It did not differ from the reduction trends of *E.* coli O157:H7 and *S.* Typhimurium. When apple juice was treated with the combination of ozone and heat for 1 min, the population of *L. monocytogenes* was reduced by 0.79 and 0.93 log CFU/ml at 25 and 45 °C, respectively, and was below the detection limit (1 log CFU/ml) at 50 and 55 °C.

The Hunter color values of apple juice after heat treatment only or the combination treatment of ozone and heat are shown in Table 1. *L*-, *a*-, and *b*-values of all treated samples were not significantly different ($P > 0.05$) from those of the control. All treatments had no effect on the color value of apple juice.

Residual ozone was measured by the indigo method after ozone treatment on distilled water for 1 min at each temperature (25, 45, 50 and 55 °C). As shown in Table 2, in all ozone treated samples, the concentrations of residual ozone were in the range from 0.08 to 0.31 mg/l when treated at 25, 45, 50 and 55 °C. Also, the concentration of ozone decreased as the treatment temperature increased.

4. Discussion

The traditional belief that apple juice is safe from pathogens due to its relatively low pH (3.1–4.4) has changed due to several outbreaks and research investigations proving the survivability of pathogens in juice. Recently, many novel treatments have been evaluated for the inactivation of pathogens in apple juice, including gamma irradiation (Buchanan et al., 1998), ultraviolet light (Gachovska et al., 2008; Keyser et al., 2008), pulsed electric fields (Evrendilek et al., 1999; Gachovska et al., 2008), hydrostatic high pressure (Bayındırlı et al., 2006), dense phase carbon dioxide (Liao et al., 2007), and natural antimicrobials and essential oils (Yuste and Fung, 2004). Also, ozone treatment is one of the most actively researched and applied technologies for reducing harmful bacteria in apple juice.

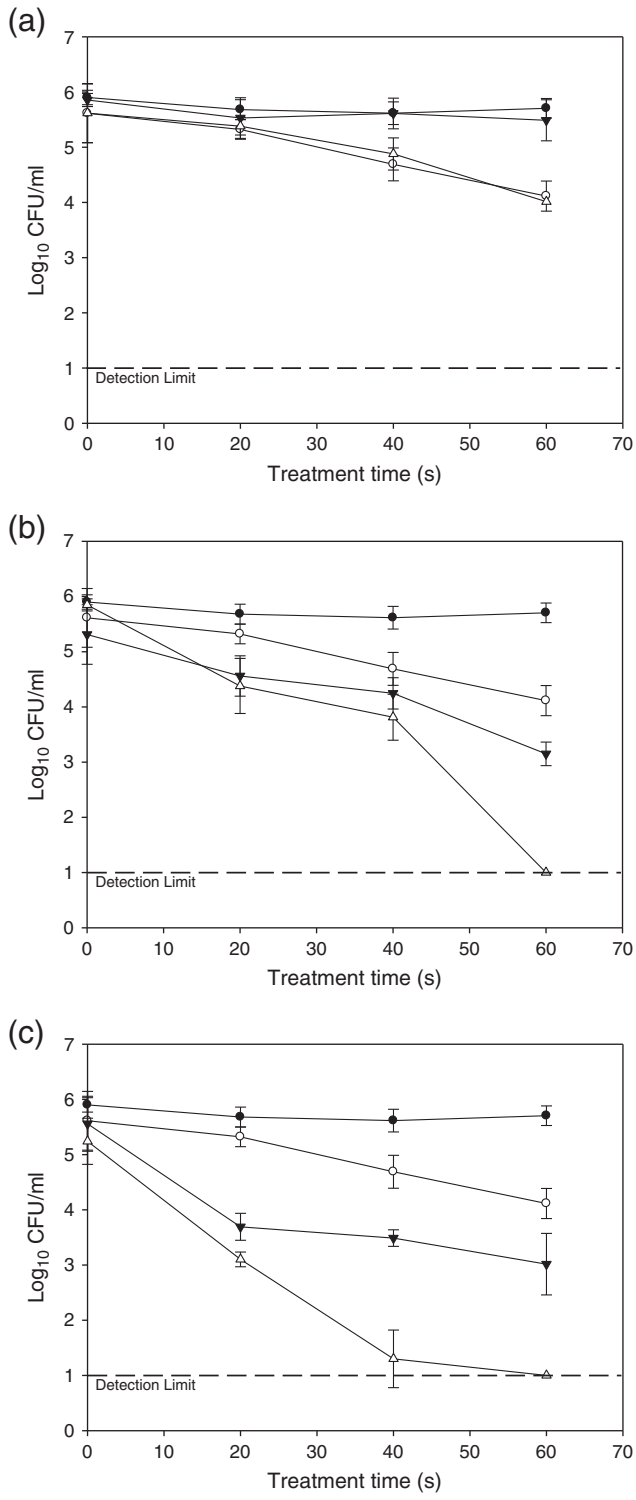

Fig. 2. Inactivation of *E. coli* O157:H7 in apple juice treated with heat and ozone. (a) ● 25 °C; ○ 25 °C + ozone; ▼ 45 °C; Δ 45 °C + ozone, (b) ● 25 °C; ○ 25 °C + ozone; ▼ 50 °C; Δ 50 °C + ozone, (c) ● 25 °C; ○ 25 °C + ozone; ▼ 55 °C; Δ 55 °C + ozone.

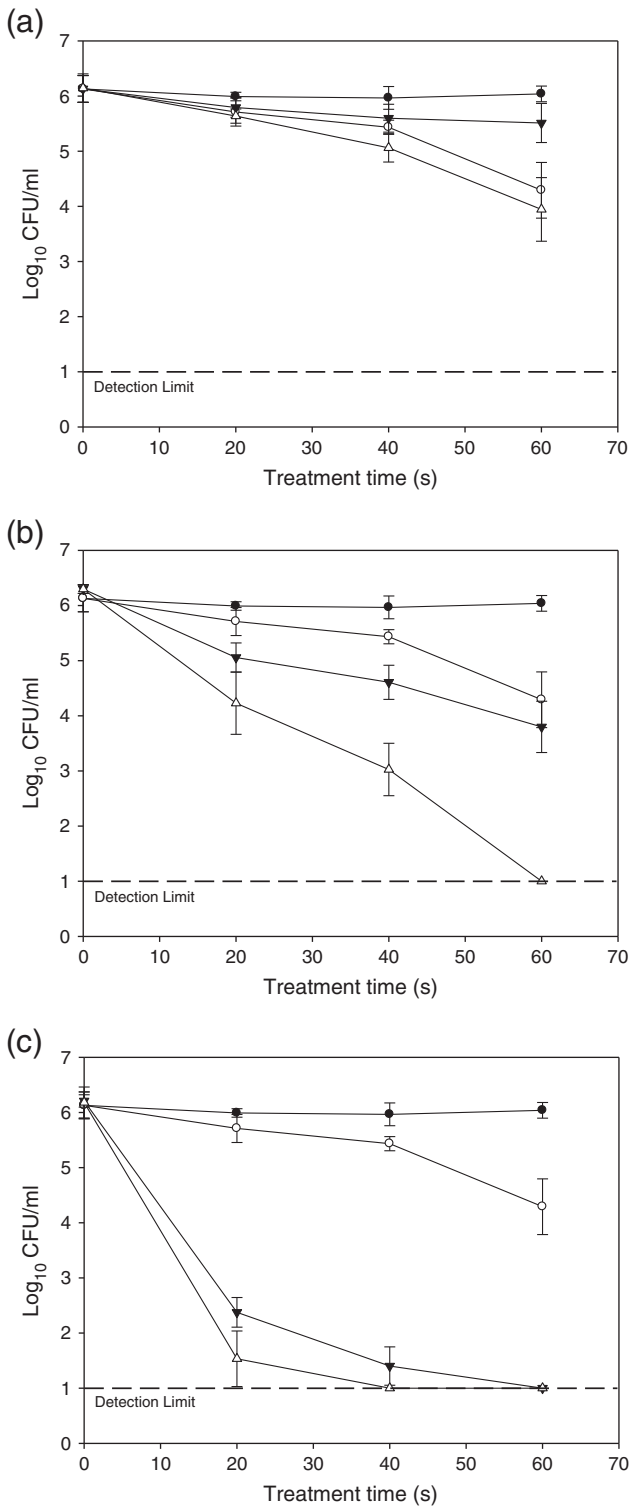

Fig. 3. Inactivation of *S.* Typhimurium in apple juice treated with heat and ozone. (a) ● 25 °C; ○ 25 °C + ozone; ▼45 °C; Δ 45 °C + ozone, (b) ● 25 °C; ○ 25 °C + ozone; ▼50 °C; Δ 50 °C + ozone, (c) ● 25 °C; ○ 25 °C + ozone; ▼55 °C; Δ 55 °C + ozone.

Williams et al. (2005) reported lower efficacy of single ozone treatment for the inactivation of *E. coli* O157:H7 and *Salmonella* in unpasteurized apple cider and orange juice compared to a combination treatment of ozone and antimicrobial agents such as dimethyl dicarbonate and hydrogen peroxide, which achieved a 5-log reduction. When apple juice (18 °Brix) was treated with 0.90 g/h ozone gas at room temperature, about 0.5 and 4.5 log CFU/ml reductions of *E. coli* O157:H7 were observed after 30 s and 60 s treatment, respectively (Choi et al., 2012). Unpasteurized apple cider and orange juice containing *E. coli* O157:H7 and *S.* Typhimurium were treated with gaseous ozone at 4, 20, and 50 °C (Williams et al., 2004). The antimicrobial efficacy of ozone treatment dependent on temperature for their study can be expressed relatively as follows: 50 °C > 4 °C > 20 °C. While less than 5.0 log CFU/ml reduction resulted after treatment at 20 °C for 3–4 h, >5.0 log CFU/ml populations of *E. coli* were inactivated at 50 °C

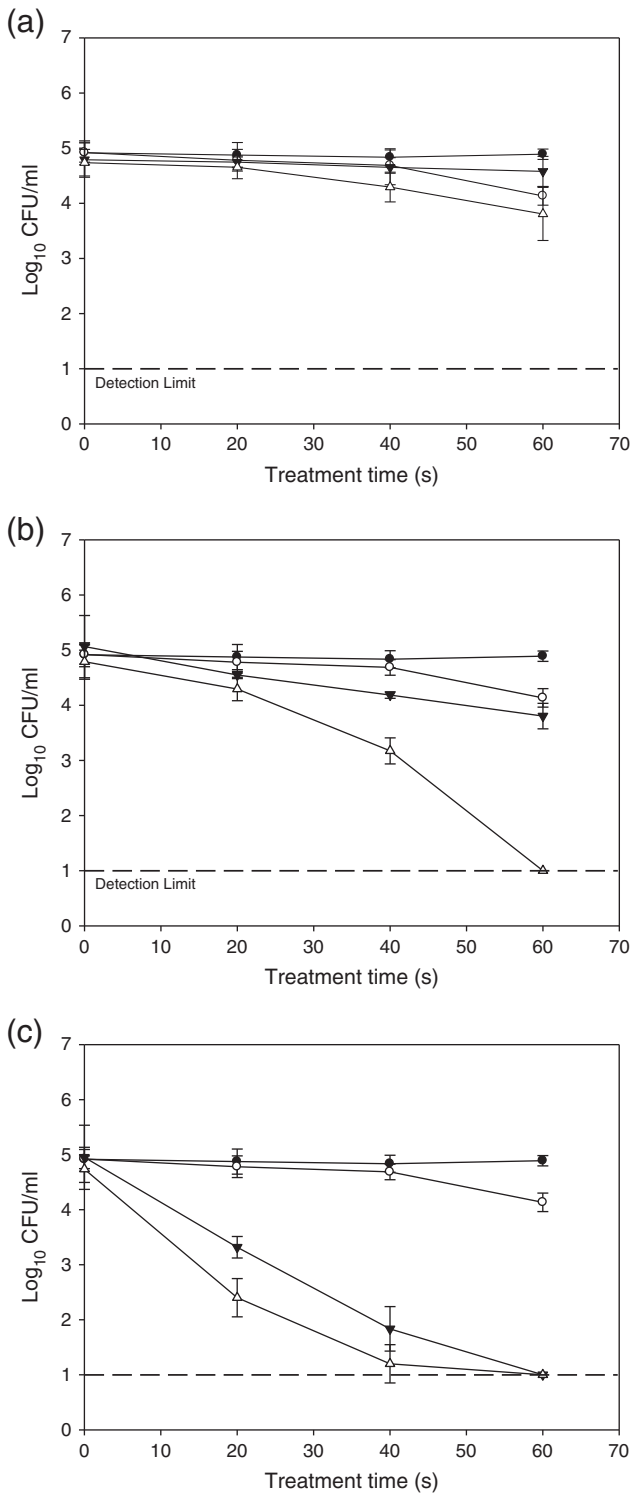

Fig. 4. Inactivation of *L. monocytogenes* in apple juice treated with heat and ozone. (a) ● 25 °C; ○ 25 °C + ozone; ▼45 °C; Δ 45 °C + ozone, (b) ● 25 °C; ○ 25 °C + ozone; ▼50 °C; Δ 50 °C + ozone, (c) ● 25 °C; ○ 25 °C + ozone; ▼55 °C; Δ 55 °C + ozone.

after 45 min of ozone treatment. This supports the findings of the current study in the way that ozone with mild heat treatment showed greater antimicrobial effect than ozone treatment at ambient temperature. Our study confirmed that ozone treatment at 50 °C was highly effective in the reduction of pathogens in apple juice. In the case of *E. coli* O157:H7, the effect of ozone itself resulted in about a 1.50 log CFU/ml

Table 1
Color values of ozone untreated and treated apple juice.

Treatment	Parameter		
	L	*a*	*b*
Control	26.40 ± 2.91^{A}	0.28 ± 0.03^{A}	3.94 ± 0.24^{A}
25 °C	26.61 ± 0.59^{A}	0.25 ± 0.03^{A}	3.95 ± 0.11^{A}
25 °C, ozone	26.93 ± 0.91^{A}	0.24 ± 0.02^{A}	3.78 ± 0.12^{A}
45 °C	27.27 ± 1.77^{A}	0.25 ± 0.03^{A}	3.92 ± 0.06^{A}
45 °C, ozone	27.07 ± 1.26^{A}	0.24 ± 0.03^{A}	3.90 ± 0.04^{A}
50 °C	27.03 ± 1.68^{A}	0.22 ± 0.05^{A}	4.00 ± 0.06^{A}
50 °C, ozone	27.54 ± 1.02^{A}	0.23 ± 0.06^{A}	3.83 ± 0.13^{A}
55 °C	26.95 ± 1.18^{A}	0.28 ± 0.06^{A}	3.83 ± 0.17^{A}
55 °C, ozone	26.93 ± 1.11^{A}	0.26 ± 0.08^{A}	3.88 ± 0.12^{A}

Mean values ± standard deviation. Mean values in the same column followed by the same superscript are not significantly different ($P > 0.05$).

reduction after treatment at 25 °C. Considering that heat treatment alone reduced this pathogen by about 2.16 log at 50 °C, a minimum of 3.66 log reduction could be expected when both ozone and heat treatments are performed at 50 °C. However, approximately 4.85 log reduction was obtained. Similarly, the combination treatment resulted in an enhanced reduction of *S.* Typhimurium and *L. monocytogenes* (about 5.30 and 3.79 log CFU/ml), which is greater than the sum of the reductions (about 4.34 and 2.05 log CFU/ml, respectively) obtained through single treatments of ozone or 50 °C. This indicates that the combination of ozone and heat treatments at 50 °C can produce a synergistic effect in pathogen inactivation of apple juice.

Achen and Yousef (2001) confirmed that there was no significant difference among various temperatures (4, 22, 45 °C) with bubbling ozone treatment in the inactivation of *E. coli* from apple surfaces. They explained that conflict between increasing solubility and decreasing stability and reaction rate reduced the efficacy of ozone at low temperatures. The current study also shows that there was no significant difference between surviving populations after treatments at 25 °C and 45 °C except for *S.* Typhimurium treated for 60 s as shown in (a) of Figs. 2, 3, and 4.

In Figs. 2–4(b), it is evident that microbial reduction following a 50 °C heat treatment alone was much greater than that of ozone treatment at 25 °C, but the combination treatment at 50 °C was more effective than the 50 °C heat treatment alone, and this trend was observed for all pathogens. In Figs. 2–4(c), pathogens were greatly inactivated by the 55 °C heat treatment alone. Therefore, there were no significant differences in pathogen reduction between the 55 °C heat treatment alone and the combination treatment for 60 s except in *E. coli* O157:H7. In other words, both *S.* Typhimurium and *L. monocytogenes* were reduced to below the detection limit (1.0 log CFU/ml) after both heat only and combination treatments for 60 s.

Some researchers reported that ozonation of fruit juices resulted in color change. When apple juice was treated with ozone (1–4.8% w/w) for 10 min, the color of the juice was changed significantly (Torres et al., 2011). Patil et al. (2010a) reported that the color of apple juice samples lightened after ozone treatment (0.048 mg O_3 at a flow rate

Table 2
Residual ozone in distilled water stored at 4 °C for 6 h after ozone and heat treatments.

Treatment	Residual ozone (mg O_3/l)
25 °C, ozone	0.306 ± 0.269^{A}
45 °C, ozone	0.141 ± 0.076^{B}
50 °C, ozone	0.111 ± 0.062^{BC}
55 °C, ozone	0.078 ± 0.046^{C}

Mean values ± standard deviation. Mean values in the same column followed by different superscripts are significantly different ($P < 0.05$).

of 0.12 l/min) for 0 to 10 min. While *L*- and *b*-values showed significant increases, the *a*-value of apple juice samples decreased as treatment time and concentration of ozone increased. In the case of ozone treated freshly squeezed orange juice (Tiwari et al., 2008) and blackberry juice (Tiwari et al., 2009a), an increase in *L*-value and decreases in *a*- and *b*-values resulted. However, in our study, no significant changes of *L*-, *a*-, and *b*-values were found in apple juice treated with ozone and/or heat. The differences in color changes of fruit juices among the various studies may be caused by different systems consisting of various control parameters such as concentration, gas flow of ozone, and treatment time. Especially, treatment time in the current study was shorter than that of the cited studies.

According to the Code of Federal Regulations, the maximum residual ozone level is 0.4 mg/l when water is bottled (FDA, 2012). However, there are no official regulations regarding a measurement method for residual ozone in liquid foods such as apple juice. This may be due to the rapid decomposition property of ozone. Therefore, we analyzed residual ozone by the indigo method which is commonly used to determine the concentration of ozone in water. After ozone treatment, residual ozone concentration in all samples decreased to below 0.4 mg/l when maintained at 4 °C for 6 h. We also confirmed that residual ozone increased as the treatment temperature decreased, correlating with the result of a previous study (Achen and Yousef, 2001). They reported that the concentration of residual ozone was greatest at 4 °C, not at 22 or 45 °C.

It has been reported that ozone treatment is more effective when microorganisms are suspended in pure water or buffers containing less ozone demanding materials than in complex food systems composed of organic compounds (Cho et al., 2003). Ozone concentration in treated apple juice couldn't be determined by the indigo method (data not shown) because of the intrinsic color of juice, but residual ozone in distilled water could be analyzed. Even though we obtained results about residual ozone by using distilled water instead of apple juice, low concentrations of residual ozone may occur in apple juice due to various ozone consuming compounds compared to distilled water. There are organic compounds such as sugars, pectic substances, and antioxidants in apple cider and orange juice. These compounds may react with ozone (Kim et al., 1999; Williams et al., 2004). Antioxidant compounds like polyphenols, phenolic acids, flavonoids and ascorbic acid are ozone consuming materials having an effect on ozone chelation in apple juice (Liao et al., 2007). Therefore, application of the indigo method in this study is considered reasonable for judging the concentration of residual ozone in apple juice by inference through results obtained from distilled water.

In conclusion, the combination of ozone and heat treatments can be used as a novel technology for the inactivation of foodborne pathogens in apple juice. We found a combination effect when apple juice is treated with both ozone and heat simultaneously and a clear synergistic effect at 50 °C. Also, this treatment is effective in that the color of the juice was maintained and the concentration of residual ozone in the juice might decrease to below 0.4 mg/l after ozone treatment. If this intervention is to be used in the food industry, processing for more rapid ozone decomposition is necessary and specific treatment conditions such as temperature, time and ozone concentration should be established considering the inactivation of pathogens and maintenance of sensory quality in apple juice.

Acknowledgments

This research was supported by the Public Welfare & Safety research program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT and Future Planning (NRF-2012M3A2A1051679). This work was supported by National Research Foundation of Korea (NRF) grant funded by the Korea government (MEST) (No. 2010-0026030).

References

Achen, M., Yousef, A.E., 2001. Efficacy of ozone against *Escherichia coli* O157:H7 on apples. Fruit Process. 66, 1380–1384.
Bader, H., Hoigni, J., 1981. Determination of ozone in water by the indigo method. Water Res. 15, 449–456.
Bayındırlı, A., Alpas, H., Bozoğlu, F., Hızal, M., 2006. Efficiency of high pressure treatment on inactivation of pathogenic microorganisms and enzymes in apple, orange, apricot and sour cherry juices. Food Control 17, 52–58.
Bignon, J., 1997. Cold pasteurizers hyperbar for the stabilization of fresh fruit juices. Fruit Process. 6, 46–48.
Braddock, R.J., 1999. Single strength orange juices and concentrate. Handbook of Citrus By-products and Processing Technology. Wiley, New York.
Buchanan, R.L., Edelson, S.G., Snipes, K., Boyd, G., 1998. Inactivation of *Escherichia coli* O157:H7 in apple juice by irradiation. Appl. Environ. Microbiol. 64, 4533–4535.
Burleson, G.R., Murray, T.M., Pollard, M., 1975. Inactivation of viruses and bacteria by ozone with and without sonication. Appl. Microbiol. 29, 340–344.
Cho, M., Chung, H.M., Yoon, J., 2003. Disinfection of water containing natural organic matter by using ozone-initiated radical reactions. Appl. Environ. Microbiol. 69, 2284–2291.
Choi, M.R., Liu, Q., Lee, S.Y., Jin, J.H., Ryu, S.R., Kang, D.H., 2012. Inactivation of *Escherichia coli* O157:H7, *Salmonella* Typhimurium and *Listeria monocytogenes* in apple juice with gaseous ozone. Food Microbiol. 32, 191–195.
Cullen, P.J., Valdramidis, V.P., Tiwari, B.K., Patil, S., Bourke, P., O'Donnell, C.P., 2010. Ozone processing for food preservation: an overview on fruit Juice treatments. Ozone Sci. Eng. 32, 166–179.
Das, E., Gu¨rakan, G.C., Bayındırlı, A., 2006. Effect of controlled atmosphere storage, modified atmosphere packaging and gaseous ozone treatment on the survival of *Salmonella* Enteritidis on cherry tomatoes. Food Microbiol. 23, 430–438.
Evrendilek, G.A., Zhang, Howard Q., Richter, Edward R., 1999. Inactivation of *Escherichia coli* O157:H7 and *Escherichia coli* 8739 in apple juice by pulsed electric fields. J. Food Prot. 62, 793–796.
Fisher, C.W., Lee, D.H., Dodge, B.A., Hamman, K.M., Robbins, J.B., Martin, S.E., 2000. Influence of catalase and superoxide dismutase on ozone inactivation of *Listeria monocytogenes*. Appl. Environ. Microbiol. 66, 1405–1409.
Gabriel, A., Nakano, A.H., 2009. Inactivation of *Salmonella*, *E. coli* and *Listeria monocytogenes* in phosphate-buffered saline and apple juice by ultraviolet and heat treatments. Food Control 20, 443–446.
Gachovska, T.K., Kumar, S., Thippareddi, H., Subbiah, J., Williams, F., 2008. Ultraviolet and pulsed electric field treatments have additive effect on inactivation of *E. coli* in apple juice. J. Food Sci. 73, M412–M417.
Gordon, G., Bubnis, B., 2002. Residual ozone measurement: indigo sensitivity coefficient adjustment. Ozone Sci. Eng. 24, 17–28.
Graham, D.M., 1997. Use of ozone for food processing. Food Technol. 5, 72–75.
Keyser, M., Műller, I.A., Cilliers, F.P., Nel, W., Gouws, P.A., 2008. Ultraviolet radiation as a non-thermal treatment for the inactivation of microorganisms in fruit juice. Innov. Food Sci. Emerg. Technol. 9, 348–354.
Khadre, M.A., Yousef, A.E., Kim, J.G., 2001. Microbiological aspects of ozone applications in food: a review. J. Food Sci. 66, 1242–1252.
Kim, J.G., Yousef, A.E., Dave, S., 1999. Application of ozone for enhancing the microbiological safety and quality of foods: a review. J. Food Prot. 62, 1071–1087.
Lee, S.Y., Sagong, H.G., Ryu, S.R., Kang, D.H., 2012. Effect of continuous ohmic heating to inactivate *Escherichia coli* O157:H7, *Salmonella* Typhimurium and *Listeria monocytogenes* in orange juice and tomato juice. J. Appl. Microbiol. 112, 723–731.
Liao, H., Hu, X., Liao, X., Chen, F., Wu, J., 2007. Inactivation of *Escherichia coli* inoculated into cloudy apple juice exposed to dense phase carbon dioxide. Int. J. Food Microbiol. 118, 126–131.
Patil, S., Bourke, P., Frias, J.M., Tiwari, B.K., Cullen, P.J., 2009. Inactivation of *Escherichia coli* in orange juice using ozone. Innov. Food Sci. Emerg. Technol. 10, 551–557.
Patil, S., Torres, B., Tiwari, B.K., Wijngaard, H.H., Bourke, P., Cullen, P.J., O'Donnell, C.P., Valdramidis, V.P., 2010a. Safety and quality assessment during the ozonation of cloudy apple juice. J. Food Sci. 75, M437–M443.
Patil, S., Valdramidis, V.P., Cullen, P.J., Frias, J., Bourke, P., 2010b. Inactivation of *Escherichia coli* by ozone treatment of apple juice at different pH levels. Food Microbiol. 27, 835–840.
Tiwari, B.K., Muthukumarappan, K., O'Donnell, C.P., Cullen, P.J., 2008. Kinetics of freshly squeezed orange juice quality changes during ozone processing. J. Agric. Food Chem. 56, 6416–6422.
Tiwari, B.K., O'Donnell, C.P., Muthukumarappan, K., Cullen, P.J., 2009a. Anthocyanin and colour degradation in ozone treated blackberry juice. Innov. Food Sci. Emerg. Technol. 10, 70–75.
Tiwari, B.K., O'Donnell, C.P., Patras, A., Brunton, N., Cullen, P.J., 2009b. Effect of ozone processing on anthocyanins and ascorbic acid degradation of strawberry juice. Food Chem. 113, 1119–1126.
Torres, B., Tiwari, B.K., Patras, A., Wijngaard, H.H., Brunton, N., Cullen, P.J., O'Donnell, C.P., 2011. Effect of ozone processing on the colour, rheological properties and phenolic content of apple juice. Food Chem. 124, 721–726.
US Food and Drug Administration (US FDA), 2001. Hazard Analysis and Critical Point (HACCP); procedures for the safe and sanitary processing and importing of juice; final rule. Fed. Regist. 66, 33829–33830.
US Food and Drug Administration (US FDA), 2012. Direct food substances affirmed as generally recognized as safe: Ozone. 21CFR 184.1563. Food and Drug Administration, Washington, DC.

Vojdani, J.D., Beuchat, L.R., Tauxe, R.V., 2008. Juice-associated outbreaks of human illness in the United States, 1995 through 2005. J. Food Prot. 71, 356–364.

Williams, R.C., Sumner, S.S., Golden, D.A., 2004. Survival of *Escherichia coli* O157:H7 and *Salmonella* in apple cider and orange juice as affected by ozone and treatment temperature. J. Food Prot. 67, 2381–2386.

Williams, R.C., Sumner, S.S., Golden, D.A., 2005. Inactivation of *Escherichia coli* O157:H7 and *Salmonella* in apple cider and orange juice treated with combinations of ozone, dimethyl dicarbonate, and hydrogen peroxide. J. Food Sci. 70, M197–M201.

Yuste, J., Fung, D.Y.C., 2004. Inactivation of *Salmonella* Typhimurium and *Escherichia coli* O157:H7 in apple juice by a combination of nisin and cinnamon. J. Food Prot. 67, 371–377.