Synergistic effect of ohmic heating and UV-C irradiation for inactivation of *Escherichia coli* O157:H7, *Salmonella* Typhimurium and *Listeria monocytogenes* in buffered peptone water and tomato juice

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ARTICLE INFO

ABSTRACT

Hurdle technology, in which preservation techniques are combined, has been of interest to scientists aiming to ensure microbiological food safety. We investigated the synergistic effect of UV-C irradiation and ohmic heating on the inactivation of foodborne pathogens. UV-C irradiation and ohmic heating were applied to buffered peptone water and tomato juice inoculated with pathogens simultaneously or sequentially. A synergistic bactericidal effect of the simultaneous treatment was observed against *Escherichia coli* O157:H7, *Salmonella* Typhimurium and *Listeria monocytogenes*. For example, the reductions (log CFU/ml) in *E. coli* O157:H7 by UV-C irradiation, ohmic heating and simultaneous treatment in tomato juice were 0.48, 1.84 and 3.83, respectively. Additive effect was observed for cell membrane damage and lipid oxidation values with the simultaneous treatment Therefore, the proposed synergistic bactericidal mechanism by the simultaneous treatment consists of an acceleration of lipid oxidation, which results in an additive effect on cell membrane pore formation. Sequential treatment of UV-C irradiation after ohmic heating showed the least antibacterial effect in buffered peptone water. On the other hand, the reductions levels of all three pathogens in tomato juice were not significantly different between the two treatments regardless of the sequence. The color and lycopene content of tomato juice were not significantly deteriorated by either ohmic heating or the simultaneous treatment. Therefore, the combination treatment of UV-C irradiation and ohmic heating including simultaneous and sequential treatments, and regardless of treatment sequence, can be used as an effective hurdle technology ensuring microbiological safety in juice products.

1. Introduction

Foodborne outbreaks have been reported worldwide, of which foodborne pathogens are known to be the most significant hazards (J.-Y. Lee, Kim, & Kang, 2015). To control biological infectious agents, thermal processing methods such as microwave, steam, ohmic and radio frequency heating and nonthermal processing methods such as pulsed electric field, plasma and high pressure are utilized in the food industry (Jeong & Kang, 2014; Yong et al., 2015). However, the organoleptic properties of food can be adversely affected by these treatments resulting in consumer negative perceptions (Khan, Tango, Miskeen, Lee, & Oh, 2017). For these reasons, hurdle technology, in which preservation techniques are combined, has been of interest to

scientists aiming to maintain the nutritional value and sensory properties of foods. A synergistic bactericidal effect exhibited by the hurdle technology contributes reducing treatment time and intensity. Sung, Song, Kim, Ryu, and Kang (2014) indicated that a synergistic bactericidal effect was observed for *Escherichia coli* O157:H7, *Salmonella enterica* serovar Typhimurium and *Listeria monocytogenes* in apple juice subjected to a combination treatment of ozone and mild heat. Ha and Kang (2014) reported that the combination treatment of near-infrared (NIR) heating and UV irradiation showed a synergistic effect on the inactivation of *C. sakazakii* in infant formula. Many other hurdle technologies have been reported and various mechanisms of synergistic effects have been suggested depending on the type of system, treatment conditions, etc. Generally, different targets of each treatment were

reported as a primary factor of the synergistic bactericidal effect by the hurdle technology. From this perspective, a combination treatment of thermal and nonthermal processing that has different bactericidal targets would be effective to inactivate foodborne pathogens (Ha & Kang, 2015).

Ohmic heating is a novel technology enabling uniform and rapid heating. In particular, many liquid and liquid-solid products can be processed by ohmic heating in a short time (Su-Yeon Lee, Ryu, & Kang, 2013). Many investigations reported that juice products are pasteurized effectively by ohmic heating (S. Y. Lee, Sagong, Ryu, & Kang, 2012; Leizerson & Shimoni, 2005; Sagong, Park, Choi, Ryu, & Kang, 2011). The performance of ohmic heating is influenced by extrinsic factors such as electric field and frequency and intrinsic factors such as pH and sugar concentration (S.-S. Kim & Kang, 2015; Park, Ha, & Kang, 2017).

Nonthermal processing can be used effectively to inactivate foodborne pathogens while maintaining the nutritional and sensory characteristics of foods (D.-K. Kim, Kim, & Kang, 2017). Among the many types of nonthermal treatments, UV-C irradiation still widely used in food industry because the equipment is relatively inexpensive and easy to use (Bintsis, Litopoulou-Tzanetaki, & Robinson, 2000). Many investigations have reported that foodborne pathogens can be effectively inactivated by UV-C irradiation (Chun, Kim, Chung, Won, & Song, 2009; Sommers, Sites, & Musgrove, 2010). In particular, UV-C irradiation can be used to pasteurize juice products without causing quality deterioration (Caminiti et al., 2012; Keyser, Müller, Cilliers, Nel, & Gouws, 2008). The mechanism of inactivation UV-C irradiation is the cross-linking between adjacent pyrimidine nucleo bases, which interrupts vital metabolic functions such as DNA replication (Ha, Back, Kim, & Kang, 2016; D.; Wu, You, Jin, & Li, 2011). However, short penetration depth is a major limitation of UV-C irradiation and can be a critical obstacle to processing opaque liquid products. Combination treatments of UV-C irradiation with other mild preservative techniques has shown promise to overcome this limitation.

Thus, ohmic heating and UV-C irradiation are effective pathogen-inactivating technologies which have different bactericidal targets. The combination of ohmic heating and UV-C irradiation is promising technology minimizing deterioration of food quality by reducing treatment intensity and time. However, to the best of our knowledge, inactivation of pathogens by the combination treatment has not been reported. Therefore, we investigated the effect of combination of ohmic heating and UV-C irradiation on the inactivation of *Escherichia coli* O157:H7, *Salmonella* Typhimurium and *Listeria monocytogenes* in tomato juice.

2. Materials and methods

2.1. Bacterial cultures and cell suspension

Three strains each of *E. coli* O157:H7 (ATCC 35150 (American Type Culture Collection, Rockville, MD), 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 bacteria culture collection of Seoul National University (Seoul, South Korea). Stock and working cultures were prepared according to a previously described method (S.-S. Kim & Kang, 2017). Single colonies cultivated from frozen stocks on tryptic soy agar (TSA; Difco, Becton, Dickinson, Sparks, MD) were inoculated into 5 ml of tryptic soy broth (TSB; Difco, Becton, Dickinson, Sparks, MD), incubated at 37 °C for 24 h, and collected by centrifugation at $4000 \times g$ for 20 min at 4 °C. The pellets were resuspended in 0.2% peptone water (PW; Bacto, Becton, Dickinson, Sparks, MD). Afterwards, suspended pellets of the three inoculums were combined to constitute a mixed-culture cocktail containing *E. coli* O157:H7 (10^9 CFU/ml), *S.* Typhimurium (10^9 CFU/ml) and *L. monocytogenes* (10^8 CFU/ml).

2.2. Sample preparation and inoculation

Sterile BPW (Difco, Sparks, MD, pH 7.2) and pasteurized tomato juice (pH 3.6; 11.8°Brix, packaged with polyethylene terephthalate) were used in this experiment. Each sample was stored in a refrigerator (4 °C) and removed at least 12 h prior to inoculation to equilibrate to room temperature (22 ± 1 °C). Fifty ml of each sample was put into the acrylic plastic ohmic heating chamber. A mixed-culture cocktail (0.2 ml) was inoculated into each prepared sample before treatment. The final bacterial populations were 10^6–10^7 CFU/ml for *E. coli* O157:H7 and *S.* Typhimurium and 10^5–10^6 CFU/ml for *L. monocytogenes*.

2.3. Bactericidal treatments

Each inoculated sample was subjected to individual or combination treatments of UV-C irradiation and ohmic heating. UV-C irradiation was carried out with a 254-nm germicidal low-pressure mercury lamp (LP lamp, G8T5, Sankyo Denki Co. Hiratsuka, Japan). Radiation intensities were measured using an UV-fiber optic spectrometer (AvaSpec-ULS2048; Avantes, Eerbeek, Netherlands). The light doses, which were calculated by multiplying the radiation intensity by the irradiation time, were 45.6 and 191.5 mJ/cm² for BPW and tomato juice, respectively. Ohmic heating treatments were carried out in a previously described apparatus (S.-S. Kim & Kang, 2017). The ohmic heating system 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 electric field strength was fixed at 13.4 V_{rms}/cm with a pulse waveform (0.05 duty ratio, 500 Hz). The treatment times were 50 s and 210 s for BPW and tomato juice, respectively. The target temperatures were 60 °C and 63 °C for BPW and tomato juice, respectively, because resistance of pathogen is higher in food sample than in buffer. The combination treatments were carried out by applying ohmic heating and UV-C irradiation simultaneously or sequentially. UV-C irradiation did not significantly influence the temperature history of pulsed ohmic heating (data not shown). To compare the sequential and simultaneous treatments, an experiment in which all samples were cooled for the same time after ohmic heating was conducted because the samples were cooled when UV-C irradiation was applied after ohmic heating. Individual treatments of ohmic heating and UV-C irradiation was represented with OH and UV, respectively. Simultaneous treatment of OH and UV was represented with OH + UV while sequential treatments of OH after UV and UV after OH were represented with UV-OH and OH-UV, respectively.

2.4. Bacterial enumeration

For microbial enumeration, each 50 ml treated sample was transferred into a sterile stomacher bag (Labplas, Inc., Sainte-Julie, Quebec, Canada) containing 100 ml of sterile 0.2% PW (4 °C) and homogenized for 2 min using a stomacher (Easy Mix; AES Chemunex, Rennes, France). After homogenization, 1 ml samples were serially diluted 10-fold with 9 ml of sterile 0.2% peptone water and 0.1 ml of stomached or diluted samples were spread-plated onto each medium. Sorbitol MacConkey with Cefixime Tellurite selective supplement (CT-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 the enumeration of *E. coli* O157:H7, *S.* Typhimurium and *L. monocytogenes*, respectively. All plates were incubated at 37 °C for 24–48 h before counting colonies characteristic of the pathogens. The overlay method (OV), developed by Hartman, Hartman, and Lanz (1975) and verified by Sun-Young Lee and Kang (2001), was used to recover sublethally

injured cells of *S.* Typhimurium and *L. monocytogenes*. After the cells were resuscitated on tryptic soy agar (TSA; Difco) at 37 °C for 2 h, plates were overlaid with 7–8 ml of XLD and OAB for *S.* Typhimurium (XLD-OV) and *L. monocytogenes* (OAB-OV), respectively. The plates were further incubated for 22–46 h at 37 °C before colonies were counted. Phenol red agar base with 1% sorbitol (SPRAB; Difco) was used to recover injured cells of *E. coli* O157:H7 (Sun-Young Lee & Baek, 2008). After incubation at 37 °C for 24 h, typical white colonies characteristic of *E. coli* O157:H7 were enumerated. Randomly selected isolates from SPRAB plates were subjected to serological confirmation as *E. coli* O157:H7 (RIM, *E. coli* O157:H7 latex agglutination test; Remel, Lenexa, KS), because SPRAB is not typically used as a selective agar for enumerating *E. coli* O157:H7.

2.5. Pathogen inactivation curve fitting

GInaFit was used to identify the time required to achieve a 5-log reduction in pathogens (Geeraerd, Valdramidis, & Van Impe, 2005). The survival curves were analyzed by the shoulder log-linear model.

The parameters of the shoulder log-linear model are:

$$\log_{10}(N) = \log_{10}(N_0) - \frac{k_{max} \cdot (t)}{\ln(10)} + \log_{10}\left(\frac{e^{k_{max} \cdot S_l}}{1 + (e^{k_{max} \cdot S_l} - 1) \cdot e^{-k_{max} \cdot t}}\right) \quad (1)$$

where S_l is the shoulder length and k_{max} is the inactivation rate (min^{-1}). The time required to achieve a 5-log reduction $(t_5 d)$ was calculated using Eq. (2)

$$t_x d = S_l + (x) \cdot \frac{\ln(10)}{k_{max}} \quad (2)$$

2.6. Bactericidal mechanism

2.6.1. Propidium iodide uptake test

The fluorescent dye propidium iodide (PI; Sigma-Aldrich, P4170) was used to determine cell membrane damage. The PI uptake test was conducted according to the method described by (S.-S. Kim & Kang, 2017) with slight modifications. Treatment conditions of BPW was not different from the conditions described in bactericidal treatments. Untreated and treated BPW were centrifuged at $10,000 \times g$ for 10 min at 4 °C. The supernatants were discarded, and the cell pellets were resuspended in 3.5 ml of phosphate-buffered saline (PBS; Corning, pH 7.4) to an optical density at 680 nm of approximately 0.35, 0.25 and 0.2 (SpectraMax M2e; Molecular Devices, Sunnyvale, CA) for *E. coli* O157:H7, *S.* Typhimurium and *L. monocytogenes*, respectively. Fluorescent dye PI was added to a final concentration of 2.9 μM and incubated for 10 min at room temperature. After incubation, the samples were resuspended in 1 ml of PBS and centrifuged two times under the same conditions. The final cell pellets were resuspended in 1 ml of PBS and, fluorescence was measured with a spectrofluorophotometer (Spectramax M2e; Molecular Devices, Sunnyvale, CA) at an excitation wavelength of 493 nm and an emission wavelength of 630 nm. The fluorescence data obtained for untreated cells were subtracted from those obtained for all treated cells and were normalized to OD_{680}.

PI value = (fluorescence value of treated cells/OD_{680}) – (fluorescence value of untreated cells/OD_{680}) (3)

2.6.2. Diphenyl-1-pyrenyl-phosphine (DPPP) test

Fluorescent dyeDPPP was used to evaluate membrane lipid peroxidation (Rahman, Ninomiya, Ogino, & Shimizu, 2010). DPPP was solubilized in di-methyl sulfoxide (DMSO) to prepare a 50 mM stock solution and was stored in the dark at −20 °C prior to use. Untreated and treated BPW were centrifuged at $10,000 \times g$ for 10 min at 4 °C. The supernatants were discarded, and the cell pellets were resuspended in

3.5 ml phosphate-buffered saline (PBS; Corning, pH 7.4). DPPP was added to the untreated or treated samples to a final concentration of 50 μM and incubated for 60 min in the dark at room temperature. Fluorescence was measured with the spectrofluorophotometer (SpectraMax M2e; CA) at an excitation wavelength of 351 nm and an emission wavelength of 380 nm. The fluorescence data obtained for untreated sample were subtracted from those obtained for all treated cells and were normalized to OD_{680}.

DPPP value = (fluorescence value of treated cells/OD_{680}) – (fluorescence value of untreated cells/OD_{680}) (4)

2.6.3. SYBR green I test

SYBR green I was used to evaluate DNA damage (Han et al., 2016; Kang, Kim, & Kang, 2018). Following UV-C irradiation, *E. coli* O157:H7 and *S.* Typhimurium samples were incubated with 100 μg/ml lysozyme at 37 °C for 4 h while *L. monocytogenes* sample was incubated with not only 100 μg/ml lysozyme but also 10 μg/ml lysostaphin at 37 °C for 4 h. After incubation, SYBR green I (1:10,000 dilution; Molecular Probes, Eugene, OR, USA) at a working concentration (1:1) was applied for 15 min at 37 °C. Fluorescence of the aliquot of each sample was measured with the spectrofluorophotometer (SpectraMax M2e; CA) at an excitation wavelength of 485 nm and an emission wavelength of 525 nm.

2.7. Color and lycopene measurement

Tomato juice sample was subjected to ohmic heating or simultaneous treatment for 190, 210, 230 and 250 s to identify the 5 log-reduction conditions for *E. coli* O157:H7, *S.* Typhimurium and *L. monocytogenes* and enumerated by resuscitation media to assess food quality. After calculating 5 log-reduction conditions $(t_5 d$ values) of each pathogen, the color and lycopene content of untreated (control) and treated tomato juice samples were measured. All treated samples were cooled immediately in a crushed ice-water mixture. The color values were measured with a Minolta colorimeter (model CR400; Minolta Co., Osaka, Japan). The values for L^*, a^* and b^* were measured to evaluate color changes. L^* is a measure of lightness, a^* is an indicator of redness and b^* is a measure of yellowness (Chen, Zhu, Zhang, Niu, & Du, 2010). The lycopene content in tomato juice was measured according to a previously described method (S.-S. Kim, Choi, & Kang, 2017). The concentration of lycopene in tomato juice was determined using absorbance values and sample weights with equation (3). Absorbance was measured with a spectrofluorophotometer (SpectraMax M2e; Molecular Devices, Sunnyvale, CA) at 503 nm.

Lycopene (mg/kg tissue) = A_{503} * 31.2/g sample (5)

2.8. Statistical analysis

All inactivation experiments and quality measurements were replicated three times. The data were analyzed by the analysis of variance procedure of the Statistical Analysis System (version 9.3, SAS Institute, Cary, NC) and mean values were separated using Duncan's multiple-range test. Significant differences were determined at a significance level of $p < 0.05$.

The fitness of the shoulder log-linear model analyzed by GInaFit was evaluated by the root mean squared error (RMSE) and the regression coefficient (R^2).

$$RMSE = \sqrt{\sum_{i=1}^{n_t} \frac{(y_{expi} - y_{pre})^2}{n_t - n_p}}$$

where y_{expi} refers to the experimental observations, y_{pre} refers to the model predictions, n_t refers to the number of data and n_p refers to the number of parameters.

Table 1

Reduction levels (log CFU/ml) of *E. coli* O157:H7, *S.* Typhimurium and L. *monocytogenes* in buffered peptone water (BPW) and tomato juice following UV-C irradiation (UV), ohmic heating (OH) and simultaneous treatment of OH and UV (OH + UV).[a,b]

	Treatment	*E. coli* O157:H7	*S.* Typhimurium	*L. monocytogenes*
BPW	UV	0.24 ± 0.51 [Aa]	0.59 ± 0.61 [Aa]	0.38 ± 0.21 [Aa]
	OH	0.80 ± 0.33 [Aa]	0.66 ± 0.39 [Aa]	0.21 ± 0.19 [Aa]
	Sum[c]	1.04 ± 0.19 [Aa]	1.25 ± 0.50 [ABa]	0.60 ± 0.39 [Aa]
	OH + UV	2.54 ± 0.41 [Ba]	2.02 ± 0.74 [Ba]	1.82 ± 0.41 [Ba]
Tomato juice	UV	0.48 ± 0.73 [Aa]	0.70 ± 0.17 [ABa]	0.23 ± 0.11 [Aa]
	OH	1.84 ± 0.13 [Ba]	0.43 ± 0.29 [Ab]	0.63 ± 0.23 [ABb]
	Sum3	2.32 ± 0.62 [Ba]	1.13 ± 0.40 [BCb]	0.86 ± 0.32 [BCb]
	OH + UV	3.83 ± 0.84 [Ca]	2.19 ± 0.22 [Db]	2.70 ± 0.38 [Db]

Mean values ± standard deviation.

[a] Values in the same column followed by the same upper-case letter are not significantly different for each sample ($p > 0.05$).

[b] Values in the same row followed by the same lower-case letter are not significantly different for each sample ($p > 0.05$).

[c] Sum: Sum of UV and OH values.

3. Results

3.1. Inactivation of foodborne pathogens subjected with UV, OH and OH + UV

Simultaneous treatment of UV-C irradiation and ohmic heating (OH + UV) exhibited a synergistic effect on the inactivation of *E. coli* O157:H7, *S.* Typhimurium and L. *monocytogenes* in both BPW and tomato juice (Table 1). In BPW, UV and OH exhibited an insignificant bactericidal effect (< 1 log reduction) for all three pathogens, but the reductions induced by OH + UV were significantly higher ($p < 0.05$) than the sum of the reductions caused by the individual treatments except *S.* Typhimurium. For example, the reductions in *E. coli* O157:H7 by UV, OH and OH + UV in BPW were 0.24, 0.80 and 2.54, respectively. The same trend was observed in tomato juice. The reductions induced by OH + UV were significantly higher ($p < 0.05$) than the sum of the reductions caused by the individual treatments for all three pathogens. For example, reductions in *E. coli* O157:H7 by UV, OH and OH + UV were 0.48, 1.84 and 3.83, respectively.

3.2. PI uptake and DPPP values of foodborne pathogens subjected with UV, OH and OH + UV

Cell membrane damage and lipid peroxidation, as determined by the PI uptake and the DPPP test, respectively, were the highest with OH + UV for all three pathogens followed by OH and UV (Fig. 1). For all three pathogens, the OH + UV had a additive effect on the PI uptake and DPPP values, as the values for OH + UV were similar to the sums corresponding to the individual treatments. For example, PI uptake values (relative fluorescence unit; RFU) for L. *monocytogenens* were 2.16, 36.25 46.39 and 53.93 for the UV, OH sum of individual treatment values and OH + UV, respectively. The PI uptake values for L. *monocytogenes* were significantly higher than those for *E. coli* O157:H7 or *S.* Typhimurium because Gram positive microorganisms such as L. *monocytogenes* and *S. aureus* have a thicker peptidoglycan layer and are lack of an outer membrane (Peabody, Laird, Vlasschaert, Lo, & Brinkman, 2015). The DPPP values (RFU) for L. *monocytogenes* were 8.2, 13.58 21.77 and 29.20 for the UV, OH sum of individual treatment values and OH + UV, respectively.

3.3. Inactivation of foodborne pathogens subjected with OH and UV sequentially or simultaneously

Sequential treatments exhibited no difference from simultaneous treatment in tomato juice, but a significant difference was observed in BPW (Table 2). The reduction levels of all three pathogens by OH-UV was lower than that by the reverse sequential treatment (UV-OH) and by the simultaneous treatment (OH + UV) in BPW. For example, reduction level of *E. coli* O157:H7 in BPW by OH + UV treatment was 3.30-log CFU/ml, which was significantly higher ($p < 0.05$) than reduction byOH-UV treatment resulting in 1.76-log CFU/ml. However, reduction level after the OH + UV or UV-OH treatments were not significantly different from OH-UV treatments for *S.* Typhimurium and L. *monocytogenes* ($p > 0.05$).
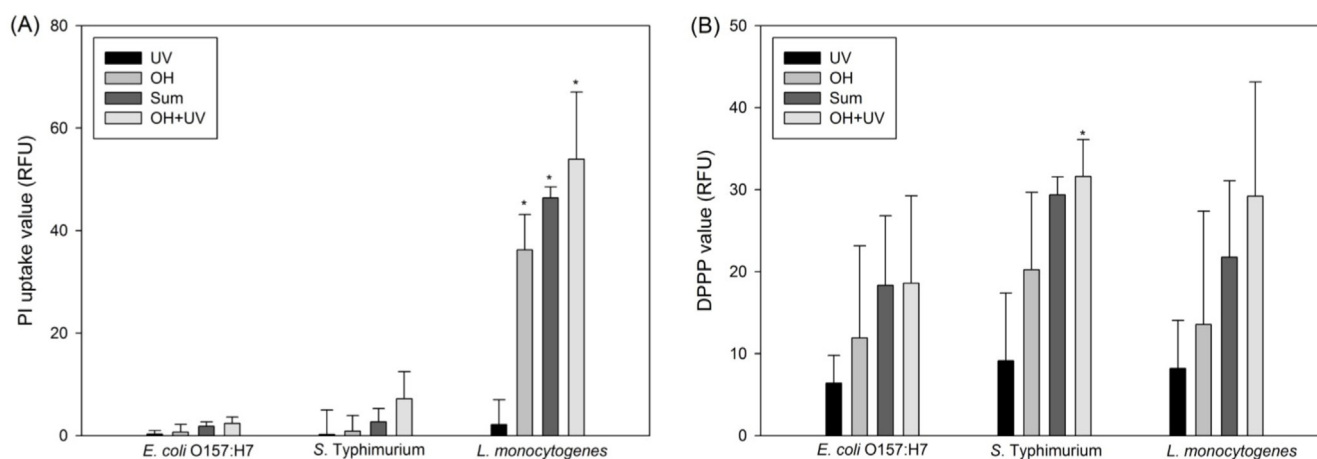

Fig. 1. Cell membrane damage (A) and lipid oxidation (B) values (relative fluorescence unit; RFU) of *E. coli* O157:H7, *S.* Typhimurium and L. *monocytogenes* subjected to UV-C irradiation (UV), ohmic heating (OH) sum of individual treatment values (Sum) and simultaneous treatment of ohmic heating and UV-C irradiation (OH + UV). Asterisk (*) means significant difference from values by UV for each pathogen ($p < 0.05$).

Table 2

Reduction levels (log CFU/ml) of *E. coli* O157:H7, *S.* Typhimurium and L. *monocytogenes* in buffered peptone water (BPW) and tomato juice following sequential treatment of UV-C irradiation after ohmic heating (OH-UV), ohmic heating after UV-C irradiation (UV-OH) and simultaneous treatment of ohmic heating and UV-C irradiation (OH + UV).[a,b]

	Treatment	*E. coli* O157:H7	*S.* Typhimurium	*L. monocytogenes*
BPW	OH-UV	1.76 ± 0.74 [Aa]	0.37 ± 0.44 [Ab]	0.60 ± 0.52 [Aab]
	UV-OH	2.85 ± 0.72 [ABa]	1.05 ± 0.18 [Ab]	1.62 ± 0.95 [Aab]
	OH + UV	3.30 ± 0.12 [Ba]	0.96 ± 0.43 [Ab]	1.39 ± 0.56 [Ab]
Tomato juice	OH-UV	4.78 ± 0.68 [Aa]	2.98 ± 0.28 [Ab]	5.31 ± 0.36 [Aa]
	UV-OH	4.78 ± 0.68 [Aa]	3.06 ± 0.43 [Ab]	5.21 ± 0.52 [Aa]
	OH + UV	5.12 ± 0.17 [Aa]	2.80 ± 0.72 [Ab]	5.51 ± 0.01 [Aa]

Mean values ± standard deviation.

[a] Values in the same column followed by the same upper-case letter are not significantly different for each sample ($p > 0.05$).

[b] Values in the same row followed by the same lower-case letter are not significantly different for each sample ($p > 0.05$).

3.4. Color and lycopene content of tomato juice

Effect of reduced treatment time by OH + UV from individual OH on the tomato juice quality was compared because thermal treatment (OH) has more significant effect than nonthermal treatment (UV). Moreover, sequential treatment had no significant difference ($p > 0.05$) with simultaneous treatment. For the tomato juice quality experiment, the treatment times to ensure 5-log reductions were calculated for all three bacterial pathogens when enumerated on resuscitation media after OH and OH + UV. When analyzed by GInaFit, the shoulder log-linear models fitted well to the inactivation of *E. coli* O157:H7, *S.* Typhimurium and L. *monocytogenes* by OH and OH + UV with a high value of R^2 (≥ 0.93) and a low value of RMSE (≤ 0.84). For OH, the times (min) required to achieve a 5-log reduction (t_5d) were 4.54, 4.06 and 3.96 for *E. coli* O157:H7, *S.* Typhimurium and L. *monocytogenes,* respectively. On the other hand, these values were 4.16, 3.85 and 3.76 for *E. coli* O157:H7, *S.* Typhimurium and L. *monocytogenes,* respectively, after the OH + UV (Table 3). Therefore, at least 4.54 and 4.16 min were required to achieve a 5-log reduction for all three pathogens by OH and by OH + UV, respectively. After tomato juice was subjected to 4.54 min of OH or 4.15 min of OH + UV, its color and lycopene content values were not significantly different from those of untreated sample (Table 4).

4. Discussion

In the present study, we investigated the antimicrobial effect of the combined UV and OH treatment to inactivate *E. coli* O157:H7, *S.* Typhimurium and L. *monocytogenes*. First, the OH + UV treatment exhibited a synergistic bactericidal effect. We postulated that the difference in bactericidal targets between UV and OH is the reason for this synergistic bactericidal effect. However, in this study, DNA damage of all three pathogen was not significant after UV (Table 5). Otherwise, the cell membranes of all three pathogens were significantly damaged by OH + UV, followed by OH and UV. The PI uptake values after UV treatment by itself were very small (< 2.2), while the PI uptake values

Table 3

Parameters[a] of the shoulder log-linear models for the inactivation of *E. coli* O157:H7 (E), *S.* Typhimurium (S) and *L. monocytogenes* (L) in tomato juice subjected to ohmic heating and the combination treatment and enumerated in resuscitation media (SPRAB, OV-XLD and OV-OAB).

	Heating method	S_l (min) ± SE	k_{max} ± SE	RMSE	R^2	t_5d (min)
E	Ohmic heating	2.98 ± 0.13	7.39 ± 0.87	0.25	0.99	4.54
	Combination	3.02 ± 0.04	10.14 ± 0.37	0.11	1.00	4.16
S	Ohmic heating	2.73 ± 0.20	8.67 ± 1.38	0.44	0.98	4.06
	Combination	2.72 ± 0.29	10.19 ± 2.32	0.75	0.96	3.85
L	Ohmic heating	2.11 ± 0.73	6.22 ± 2.56	0.83	0.93	3.96
	Combination	2.29 ± 0.52	7.83 ± 2.50	0.81	0.95	3.76

[a] SE: standard error, R^2: regression coefficient.

after the OH + UV were similar with the sum of the uptake values for the individual treatments. These results indicate that UV treatment does not have a significant effect on the cell membrane itself but can accelerate cell membrane damage if pore formation is initiated by ohmic heating treatment. Similar tendency was observed by Ha and Kang (2015), who reported that synergistic bactericidal effect by combination treatment of near-infrared heating (NIR) and ultraviolet irradiation (UV-C) is attributed to the cell membrane damage rather than DNA damage. DNA damage by UV-C was also not significant compared to the untreated sample in this study ($p > 0.05$). After we identified the increased cell membrane damage by simultaneous treatments, it was assumed that more fundamental factor contributes the increased cell membrane damage. Lipid peroxidation was presumed as one of the fundamental factors because lipid peroxidation of the cell membrane by UV is one possible mechanism of damaging foodborne pathogens, as reported previously (D. Wu et al., 2011). Specifically, lipid peroxidation can increase membrane fluidity, reduce its integrity, disrupt cell osmotic balance and lead to cell wall rupture (Alwi & Ali, 2014).

The PI uptake values after treatment with only UV were very small, as indicated in section 3.2, we presumed that lipid peroxidation by UV was not enough to form pores in all three pathogens used in this study. Because the lipid oxidation values after OH were larger than those after UV for all three pathogens, following the same trend as the PI uptake values, we proposed that a threshold lipid oxidation value exists for cell membrane damage. Lipid peroxidation by UV would not exceed the threshold value by itself, but it can exceed the threshold value when combined with OH. Accelerated lipid oxidation would cause severe damage to the cell membrane and simultaneous treatment exhibited an additive effect on the PI uptake values for all three pathogens. It is of interest that lipid oxidation levels of foodborne pathogens by 222 nm KrCl excilamp were higher than those by 254 nm LP Hg lamp when bactericidal mechanism of the 222 nm KrCl excilamp was compared with that of the 254 nm LP Hg lamp (Kang et al., 2018). In this regard, further study is needed to identify bactericidal effect of combination treatment of OH and 222 nm excilamp.

Simultaneous application of thermal and nonthermal technologies is ideal for inactivating pathogens without deteriorating food, but in many cases, it is difficult to apply thermal and nonthermal technologies simultaneously due to the limitations of industrial space and equipment size. It is noteworthy that the treatment sequence has a significant effect on the inactivation of pathogens in BPW. Two factors could contribute to this phenomenon. First, heat shock proteins expressed after ohmic heating could play a significant role in the cross-protection against UV irradiation. Estilo and Gabriel (2017) also reported that the UV-C resistance of *Salmonella enterica* increased after exposure to heat stress. Second, the recovery process after UV-C irradiation could be inhibited by ohmic heating. In response to UV-C irradiation used for the inactivation of microorganisms, many pathogens have a mechanism of photoreactivation and dark repair (Escalona, Aguayo, Martínez-Hernández, & Artés, 2010). The enzyme photolyase is needed for

Table 4
Color and lycopene content of tomato juice subjected to ohmic heating or the simultaneous treatment.[a]

	Color			Lycopene content
	L^*	a^*	b^*	
Control	26.86 ± 0.49 [A]	5.28 ± 0.15 [A]	9.35 ± 0.04 [A]	61.54 ± 2.01 [A]
OH	26.87 ± 0.17 [A]	5.37 ± 0.22 [A]	9.37 ± 0.10 [A]	59.53 ± 3.43 [A]
Simultaneous	26.62 ± 0.04 [A]	5.13 ± 0.06 [A]	9.27 ± 0.09 [A]	58.03 ± 1.66 [A]

Mean values ± standard deviation.
[a] Values in the same column followed by the same letter are not significantly different ($p > 0.05$).

Table 5
DNA damage values of *E. coli* O157:H7, *S.* Typhimurium and L. *monocytogenes* following UV-C irradiation.[a]

Treatment	*E. coli* O157:H7	*S.* Typhimurium	*L. monocytogenes*
Control	52.29 ± 0.85 [A]	53.58 ± 4.51 [A]	40.90 ± 2.78 [A]
UV-C	49.66 ± 11.5 [A]	45.88 ± 9.56 [A]	41.21 ± 6.91 [A]

Mean values ± standard deviation.
[a] Values in the same column followed by the same upper-case letter are not significantly different ($p > 0.05$).

photoreactivation and a multienzyme repair process is involved in the dark repair mechanism (Sanz, Davila, Balao, & Alonso, 2007). The enzymes related to the recovery mechanism can be immediately denatured by the high temperature of ohmic heating when UV-C irradiation treatment is performed before or during ohmic heating. However, when UV-C irradiation is carried out after ohmic heating, enzymes related to the recovery mechanism could not be denatured completely. These two factors and other factor may affect simultaneously to inhibit the reduction in pathogens by OH-UV treatment in BPW, and further study is needed. In contrast to BPW, reduction levels of all three pathogens by sequential treatment of UV-C irradiation and ohmic heating in tomato juice are not significantly different ($p > 0.05$) from those of the simultaneous treatment regardless of the treatment sequence. Because the pH in tomato juice (3.6) is significantly lower than that in BPW (7.2), heat stress-related genes would be induced regardless of the treatment sequence because the bacterial heat-shock response is activated not only by heat shock stress but also by many other unfavorable conditions (Ban, Kang, & Yoon, 2015). Moreover, recovery-related enzymes can be denatured because of the low pH. Therefore, reductions in levels of all three pathogens may not be significantly different depending on the treatment sequence in tomato juice.

Juice processors should treat their juices to achieve 5-log reduction in the number of microorganisms (U. S. FDA., 2001). We identified the treatment conditions to achieve 5-log reductions in *E. coli* O157:H7, *S.* Typhimurium and L. *monocytogenes* levels by ohmic heating and simultaneous treatment. Resuscitation medium was used to enumerate pathogens before and after treatment because injured pathogens can recover into healthy cells during storage (V. Wu, 2008). *E. coli* O157:H7 was the most resistant to both ohmic heating and simultaneous treatment and the times (m) required to achieve a 5-log reduction (t_5d) were 4.54 and 4.16 for OH and simultaneous treatment, respectively. The results indicate that we can reduce treatment time of OH by 8.37% by combining it with UV and that the reduced treatment time allowed a 6.77% decrease in treatment temperature. The quality improvement enabled by the reduced temperature was not observed in the present study because the treatment conditions of ohmic heating were not critical to the deterioration in quality of tomato juice, however, other foods can be deteriorated in many cases during pasteurization. In such a situation, combining ohmic heating with UV-C treatment will minimize the quality degradation of juice products. Moreover, the reduced treatment time brings an economic benefit.

In conclusion, a synergistic bactericidal effect by simultaneous treatment of ohmic heating and UV-C irradiation was observed against *E. coli* O157:H7, *S.* Typhimurium and L. *monocytogenes* in tomato juice. The results of PI uptake and DPPP experiments indicates that, UV can accelerate lipid peroxidation when combined with ohmic heating, which results in the synergistic effect on cell membrane damage. The synergistic effect on cell membrane damage in turn induces the synergistic bactericidal effect. Consequently, the time required to achieve a 5-log reduction (t_5d) in pathogens by ohmic heating was significantly decreased by combining it with UV-C irradiation. Therefore, the combination treatment of OH and UV can be used as an alternative hurdle technology to ensure biological safety in juice products.

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