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ORIGINAL ARTICLE

Combined ohmic heating and krypton-chlorine excilamp treatment for the inactivation of *Listeria monocytogenes*, *Salmonella* Typhimurium, and *Escherichia coli* O157:H7 in apple juice

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Abstract

The combined effect of the 222-nm krypton-chlorine (KrCl) excilamp and ohmic heating for the inactivation of *Listeria monocytogenes*, *Salmonella enterica* serovar Typhimurium, and *Escherichia coli* O157:H7 in apple juice was investigated in this study. When ohmic heating and a KrCl excilamp were applied to apple juice simultaneously, the reduction level of *E. coli* O157:H7 following 70 s (target temperature of 65.9°C) of combination treatment reaching 4.6 log CFU/ml was significantly higher than that of each treatment alone (2.7 log CFU/ml). The same trend, indicating a synergistic bactericidal effect, was observed for *L. monocytogenes* and *S.* Typhimurium. Therefore, the combination treatment of the KrCl excilamp and ohmic heating can be used effectively to control bacterial pathogens in apple juice with a reduced processing time.

Practical applications

Demands for energy-efficient and environmentally friendly bactericidal apparatuses have been increasing. Although the mercury UV lamp has been widely used to inactivate foodborne pathogens in water or juice products individually or combined with heat treatment, the use of this conventional lamp will be limited continuously in accordance with the Minamata Convention treaty, which restricts the use of mercury. Thus, it is of interest to identify the bactericidal effect of an alternative UV-C lamp and its combination with heat treatment. The synergistic bactericidal effect of the KrCl excilamp and ohmic heating, which are alternative nonthermal and thermal technologies, respectively, was identified in the present study. The results indicated in this study could be utilized by juice processors to achieve a 5-log reduction in foodborne pathogens.

1 | INTRODUCTION

Foodborne pathogens such as *Listeria monocytogenes*, *Salmonella enterica* serovar Typhimurium, *Escherichia coli* O157:H7 and are still significant microbiological hazards causing foodborne outbreaks (S. H. Kim & Rhee, 2018). UV-C irradiation has long been used to control these foodborne pathogens in juice (Falguera, Pagán, Garza, Garvín, & Ibarz, 2011). Nucleic acids are a major target of UV-C

irradiation and pyrimidine dimers can form following UV-C irradiation, which inhibit cell replication and transcription. Even though low pressure mercury lamps (LP Hg lamp) emitting 254-nm UV radiation have long been used to inactivate foodborne pathogens (Franz, Specht, Cho, Graef, & Stahl, 2009), new methods for generating UV radiation have been under development as the Minamata Convention was approved which restricts the usage of mercury (Mackey, Contreras, & Liang, 2014). Excimer lamps and light emitting diodes are representative methods for generating UV radiation without mercury (D.-K. Kim & Kang, 2018). In particular, application of 222-nm krypton-chlorine (KrCl) excilamps for inactivation of foodborne pathogens has been of interest recently because their bactericidal efficacy is known to superior to that of LP Hg lamps (Ha, Lee, & Kang, 2017). Recent studies about the application of KrCl excilamp for inactivation of pathogens in water (Ha & Kang, 2018), sliced cheese (Ha et al., 2017), and apples or bell peppers (Kang & Kang, 2019a) were reported, and bactericidal mechanism of KrCl excilamp is known to damage not only the DNA but also the cellular membrane and intracellular enzyme (Ha et al., 2017). Even though using KrCl excilamps is an effective method to control foodborne pathogens, it still requires a long treatment time and has limitations in penetration depth. Considering increasing demands for energy efficient and environmentally friendly bactericidal apparatus, it is promising to combine this new irradiation method with ohmic heating, a thermal technology which has been used for apple juice processing (N. Kim, Ryang, Lee, Kim, & Rhee, 2017a).

Hurdle technology, combining several treatments at mild intensity, has been of great interest to the food industry (N. H. Kim, Cho, & Rhee, 2017b). Synergistic bactericidal effects of thermal and nonthermal treatments have been reported including ozone-mild heat (Song, Sung, & Kang, 2015) and UV-mild heat (Cheon, Shin, Park, Chung, & Kang, 2015). It is well-known that different bactericidal targets in each treatment can result in the synergistic effect (Ha & Kang, 2015). In light of this, the combination treatment of KrCl excilamps and ohmic heating is promising because the bactericidal target of ohmic heating is the cell membrane whereas KrCl excilamps affect various cellular materials simultaneously (Kang, Kim, & Kang, 2018). However, the combination treatment of 222-nm KrCl excilamps and ohmic heating for inactivation of foodborne pathogens has not been reported to the best of our knowledge. Because acid-adapted foodborne pathogens including *L. monocytogenes*, *S.* Typhimurium, and *E. coli* O157:H7 showed increased resistance to KrCl excilamp due to the change in cell membrane fatty acid (Kang & Kang, 2019b), it is of interest combining KrCl excilamp with ohmic heating to pasteurize apple juice. Accordingly, the combination bactericidal effect of ohmic heating and KrCl excilamp treatment in apple juice was investigated in the present study using *L. monocytogenes*, *S.* Typhimurium, and *E. coli* O157:H7 as representative foodborne pathogens.

2 | MATERIALS AND METHODS

2.1 | Bacterial cultures and cell suspension

Three strains each of *L. monocytogenes* (ATCC 15313, ATCC 19111, and ATCC 19115), *S.* Typhimurium (ATCC 43971, ATCC19585, and DT 104), and *E. coli* O157:H7 (ATCC 43889 [American Type Culture Collection, Rockville, MD], ATCC 43890, and ATCC 35150) 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, Park, & Kang, 2018). 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 hr, and collected by centrifugation at 4,000*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 pathogens were combined to constitute a mixed-culture cocktail containing approximately equal numbers of cells of each strain of *L. monocytogenes* (10^8 CFU/ml), *S.* Typhimurium (10^9 CFU/ml), and *E. coli* O157:H7 (10^9 CFU/ml).

2.2 | Sample preparation and inoculation

Pasteurized apple juice (pH 2.95, 12.6 °Brix), free from chemical preservative, was purchased from a local grocery store (Seoul, South Korea) and used in this experiment. Each sample was stored in a refrigerator (4°C). Fifty-milliliter samples were prepared and inoculated with 0.2 ml of mixed-culture cocktail and subjected to each treatment within 5 min after inoculation. The final bacterial populations were 10^5–10^6 CFU/ml for *L. monocytogenes* and 10^6–10^7 CFU/ml for *S.* Typhimurium and *E. coli* O157:H7.

2.3 | Bactericidal treatments

The inoculated 50 ml samples were subjected to KrCl excilamp and ohmic heating treatment. Ohmic heating treatments were carried out in a previously described apparatus (S.-S. Kim, Choi, & Kang, 2017c). The ohmic heating system consisted of a precision power amplifier (catalog number 4510; NF Corp., Yokohama, Japan), a function generator (catalog number 33210A; Agilent Technologies, Palo Alto, CA), a data logger (catalog number 34970A; Agilent Technologies), a two-channel digital-storage oscilloscope (catalog number TDS2001C; Tektronix, Inc., Beaverton, CO), and an ohmic heating chamber. The electric field strength was fixed at 33 V_{rms}/cm with a pulse waveform (0.3 duty ratio, 500 Hz). The treatment times for ohmic heating were 0, 40, 60, 70, 80, and 85 s. The combination experiment was carried out by applying ohmic heating and KrCl excilamp treatments individually or simultaneously (Figure 1) for 70 s. KrCl excilamp treatments were carried out with a previously described apparatus (Ha et al., 2017). A dielectric barrier discharge driven excilamp filled with a KrCl gas mixture was used with a 20 W nominal output power. The KrCl

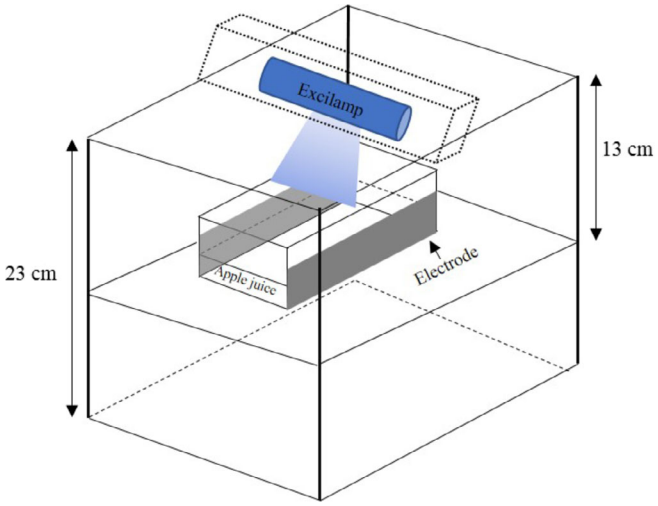

FIGURE 1 Schematic diagram of 222-nm krypton-chlorine excilamp and ohmic heating combination treatment chamber

excilamp (cylindrical geometry) was covered by a metal case having an UV exit window (10 cm x 6 cm).

2.4 | Bacterial enumeration

Fifty milliliter untreated or treated samples were transferred into a sterile stomacher bag (Labplas, Inc., Sainte-Julie, Quebec, Canada) containing 100 ml of 0.2% sterile PW. The stomacher bag was homogenized using a stomacher (Easy Mix; AES Chemunex, Rennes, France) for 2 min and 1 ml samples were 10-fold serially diluted with 9 ml of sterile PW. One hundred µl of diluted or stomached samples were spread-plated onto each selective medium. Oxford agar base (OAB; Difco) with supplement (Bacto Oxford antimicrobial supplement; Difco), xylose lysine deoxycholate (XLD) agar (Difco), and Sorbitol MacConkey (SMAC) agar (Difco) were used as selective media for the enumeration of *L. monocytogenes*, *S.* Typhimurium, and *E. coli* O157:H7, respectively. Before counting colonies characteristic of the pathogens, all plates were incubated at 37°C for 24 to 48 hr.

2.5 | Electrical conductivity measurement

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

$$\sigma = \frac{LI}{AV} \tag{1}$$

Where σ is the electrical conductivity (S/m), *A* is the cross-sectional area of the electrodes (m^2), *L* is the distance between electrodes (m), *V* is the voltage (V), and *I* is the current (A). Two channel digital storage oscilloscope was used to measure voltage and current.

2.6 | GInaFit analysis

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 Weibull model and the log-linear + shoulder model.

The parameters of the Weibull model (δ and p) are calculated from the following (Equation 2):

$$\log_{10}(N) = \log_{10}(N_0) - \left(\frac{t}{\delta}\right)^p \tag{2}$$

where *N* (CFU/ml) is the population of the microorganisms, N_o is the initial population, *t* (min) is the treatment time, δ (min) is the time for the first decimal reduction, and p is the parameter related to the scale and shape of the survival curve. The Weibull distribution corresponds to a concave downward survival curve if $p > 1$ and upward if $p < 1$. The time required to achieve a 3 log (t_3d) and a 5 log reduction (t_5d) was calculated using Equation 3.

$$t_x d = \delta \times (x)^{\frac{1}{p}} \tag{3}$$

The parameters of the log-linear + shoulder model are follows (Equation 4):

$$\log_{10}(N) = \log_{10}(N_0) - \frac{k_{max} \cdot (t)}{\ln(10)} + \log_{10}\left(\frac{e^{k_{max} \cdot S_l}}{1 + (e^{k_{max} \cdot S_l} - 1) \cdot e^{-k_{max} \cdot t}}\right) \tag{4}$$

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_5d) was calculated using Equation (5)

$$t_x d = S_l + (x) \cdot \frac{\ln(10)}{k_{max}} \tag{5}$$

2.7 | Statistical analysis

All inactivation experiments were replicated three times. Analysis of variance procedure of the Statistical Analysis System (version 9.3, SAS Institute, Cary, NC) was used to analyze the data. Duncan's multiple-range test was used to separate the mean values. Significant differences were determined at a significance level of $p = .05$.

3 | RESULTS AND DISCUSSION

3.1 | Temperature, electrical conductivity, and pathogen inactivation levels by ohmic heating

Temperature (°C) and electrical conductivity (S/m) of apple juice increased as ohmic heating treatment time increased (Figure 2). Electrical conductivity increased linearly with treatment time while heating rate accelerated as treatment time increased. Increased

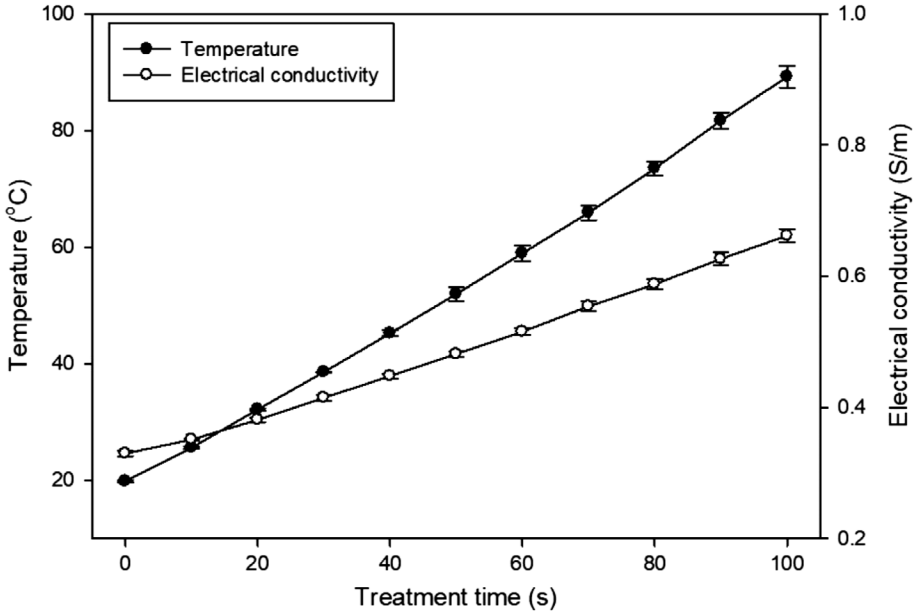

FIGURE 2 Temperature (°C) and electrical conductivity (S/m) histories of apple juice subjected to pulsed ohmic heating (0.3 duty ratio, 500 Hz, and 33 V_{rms}/cm)

TABLE 1 Populations (log CFU/ml) of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* subjected to ohmic heating

Treatment time (s)	*E. coli* O157:H7	*S.* Typhimurium	*L. monocytogenes*
0	6.06 ± 0.56 Aa	5.53 ± 0.29 Aa	5.76 ± 0.07 Aa
40	5.97 ± 0.34 Aa	5.54 ± 0.31 Aa	5.45 ± 0.17 ABa
60	5.59 ± 0.58 Aa	5.57 ± 0.34 Aa	5.36 ± 0.27 ABa
70	5.52 ± 0.46 Aa	4.71 ± 0.75 Aa	5.06 ± 0.19 Ba
80	3.37 ± 0.95 Ba	1.27 ± 0.71 Bab	2.18 ± 0.48 Cb
85	< 0.48 Ca	< 0.48 Ba	< 0.48 Da

Mean values ± standard deviation.
Values in the same column followed by the same uppercase letter are not significantly different ($p > .05$).
Values in the same row followed by the same lowercase letter are not significantly different ($p > .05$).

electrical conductivity has an important role on the accelerated heating rate as indicated by Palaniappan and Sastry (1991). Inactivation levels of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* increased with treatment time as temperature increased (Table 1). Even though the heat resistances differed according to the type of pathogen, all three pathogens were inactivated to the below the detection limit (0.48 CFU/ml) after 85 s treatment in apple juice. Because it is very important to identify the exact processing time to achieve 5 log reduction in juice products (Vojdani, Beuchat, & Tauxe, 2008), inactivation trends were analyzed by Weibull and log-linear + shoulder models, which showed good fit with $R^2 > 0.96$ (Table 2). For all three pathogens, t_5d values predicted by the Weibull model were higher than those of the log-linear + shoulder model. The most challenging scenario in the present study was to inactivate *L. monocytogenes*, which was the most resistant pathogen. According to the Weibull model, it would take 84.34 s to achieve 5 log reduction for *L. monocytogenes*. Because treatment temperature generated after 84.34 s was greater than 75°C, juice quality could suffer due to heat damage. Even though quality deterioration after ohmic heating is significantly less than for conventional heating (Leizerson & Shimoni, 2005b), reduction in nutrients such as vitamin C could still occur due to high temperature (Leizerson & Shimoni, 2005a). Therefore, it is important to reduce the processing time and temperature not only for cost savings but also for preservation of product quality.

3.2 | Synergistic effect of KrCl excilamp and ohmic heating for inactivation of foodborne pathogens

Ohmic heating was combined with a KrCl excilamp to reduce the treatment time and temperature. When ohmic heating and KrCl excilamp treatment was applied simultaneously to apple juice a synergistic bactericidal effect was observed (Figure 3) even though KrCl excilamp treatment did not significantly influence the temperature or electrical conductivity histories of ohmic heating (data not shown). For example, reduction levels of *E. coli* O157:H7 subjected individually to KrCl excilamp or ohmic heating treatment were 1.1 or 1.6 log CFU/ml, respectively. But when KrCl excilamp and ohmic heating treatment were applied simultaneously, the reduction level of *E. coli* O157:H7 was 4.6 log CFU/ml, which was significantly higher than sum of each treatment (1.1 + 1.6 = 2.7 log CFU/ml). The same tendency was observed for *S.* Typhimurium and *L. monocytogenes*. It is well known that the combination of conventional UV-C irradiation (254 nm) and mild conventional heat treatment shows the synergistic bactericidal effect. Cheon et al. (2015) reported that the synergistic bactericidal effect was observed for inactivation of *E. coli* O157:H7 and *S.* Typhimurium in powdered red pepper using combined UV-C irradiation and mild heat treatment. Gayán, Serrano, Raso, Álvarez, and Condón (2012) also reported that simultaneous UV light and heat treatment (UV-H) had a synergistic inactivation effect on *S.* Typhimurium, and identified the external membrane as a major target.

TABLE 2 Parameters of the Weibull (W) and log-linear + shoulder (S) models for inactivation of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* subjected to ohmic heating

		δ *or* S_l (s) ± *SE*	p *or* k_{max} ± *SE*	R^2	t_{5d} (s) ± *SD*
E. coli O157:H7	W	74.26 ± 1.01	12.53 ± 1.25	0.99	84.14 ± 0.66 A
	S	75.83 ± 0.97	1.33 ± 0.18	0.99	83.65 ± 2.77 A
S. Typhimurium	W	66.43 ± 5.23	7.00 ± 2.18	0.96	82.70 ± 2.61 A
	S	66.78 ± 1.50	0.68 ± 0.07	0.99	80.04 ± 0.76 A
L. Monocytogenes	W	70.17 ± 2.54	8.73 ± 1.62	0.99	84.34 ± 0.55 A
	S	69.33 ± 1.00	0.73 ± 0.05	0.99	82.99 ± 2.22 A

For each pathogen, values in the same column followed by the same uppercase letter are not significantly different ($p > .05$).

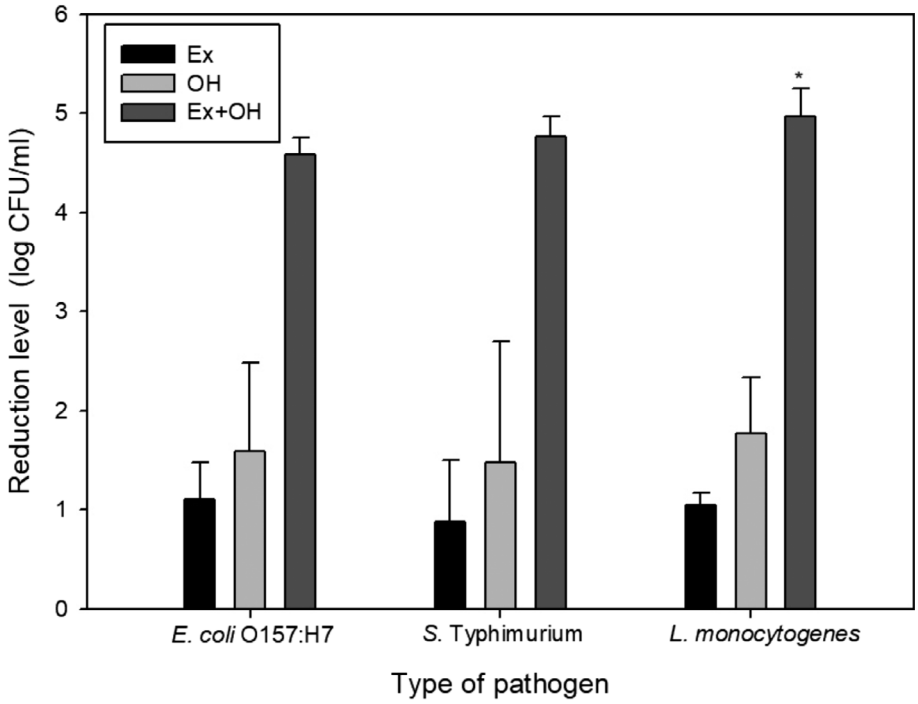

FIGURE 3 Reduction levels (log CFU/ml) of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* subjected to 222-nm krypton-chlorine excilamp (Ex), 33 V_{rms}/cm ohmic heating (OH), or combination treatment (Ex+OH) for 70 s

Even though the synergistic effect incorporating conventional treatments has been reported, it is of importance from the point of view of the food industry to reduce contamination risks as well as treatment time. The KrCl excilamp is a remarkable alternative to conventional Hg UV lamps and it was demonstrated in the present study that the combination treatment of KrCl excilamps with ohmic heating is an effective way to control pathogens in apple juice within a very short time (70 s). Moreover, quality deterioration of juice would be minimized by combining the KrCl excilamp and ohmic heating. KrCl excilamp would not affect the quality of apple juice in the present study because the treatment dosage was significantly lower than Kang and Kang (2019b) study, which indicates no significant difference in quality indicator compared to the untreated controls when same apple juice was used. On the other hand, quality of juice would be damaged when ohmic heating was used alone due to the high temperature. Therefore, we recommend use of ohmic heating with combination with KrCl excilamps for inactivation of foodborne pathogens in apple juice minimizing quality deterioration. Further study is needed to identify the mechanism of the synergistic bactericidal effect with regard to the inactivation mechanism of KrCl excilamps.

4 | CONCLUSION

Foodborne pathogens such as *L. monocytogenes*, *S.* Typhimurium, and *E. coli* O157:H7 were effectively inactivated by ohmic heating in apple juice. Less than 85 s was needed to achieve 5 log reduction of all three pathogens, but high temperature can deteriorate the quality of juice product. Therefore, ohmic heating was combined with a KrCl excilamp to reduce treatment time and temperature. When ohmic heating and a KrCl excilamp were applied simultaneously in apple juice, the synergistic bactericidal effect was observed for all three pathogens. Therefore, combination treatment of ohmic heating and KrCl excilamps can be used effectively to inactivate foodborne pathogens in apple juice with a reduced processing time.

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