International Journal of Food Microbiology 243 (2017) 96–102

Contents lists available at ScienceDirect

International Journal of Food Microbiology

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

Application of a 222-nm krypton-chlorine excilamp to control foodborne pathogens on sliced cheese surfaces and characterization of the bactericidal mechanisms

Jae-Won Ha [a,1], Jae-Ik Lee [b,1], Dong-Hyun Kang [b,*]

[a] Department of Food and Biotechnology, College of Engineering, Food & Bio-industry Research Center, Hankyong National University, Anseong-si 17579, South Korea
[b] Department of Food and Animal Biotechnology, Department of Agricultural Biotechnology, Center for Food and Bioconvergence, Institute of GreenBio Science & Technology, Seoul National University, Seoul 08826, South Korea

ARTICLE INFO

Article history:
Received 31 July 2016
Received in revised form 7 November 2016
Accepted 12 December 2016
Available online 13 December 2016

Keywords:
UV-C irradiation
222-nm
254-nm
Sliced cheese
Foodborne pathogen

ABSTRACT

This study was conducted to investigate the basic spectral properties of a 222-nm krypton-chlorine (KrCl) excilamp and its inactivation efficacy against major foodborne pathogens on solid media, as well as on sliced cheese compared to a conventional 254-nm low-pressure mercury (LP Hg) lamp. Selective media and sliced cheese inoculated with *Escherichia coli* O157:H7, *Salmonella enterica* serovar Typhimurium, and *Listeria monocytogenes* were irradiated with a KrCl excilamp and a LP Hg lamp at the same dose. The KrCl excilamp showed full radiant intensity from the outset at a wide range of working temperatures, especially at low temperatures of around 0 to 10 °C. Irradiation with 222 nm UV-C showed significantly ($P < 0.05$) higher inactivation capacity against all three pathogens than 254-nm radiation on both media and sliced cheese surfaces without generating many sublethally injured cells which potentially could recover. The underlying inactivation mechanisms of 222-nm KrCl excilamp treatment were evaluated by fluorescent staining methods and damage to cellular membranes and intracellular enzyme inactivation were the primary factors contributing to the enhanced bactericidal effect. The results of this study suggest that a 222-nm UV-C surface disinfecting system can be applied as an alternative to conventional LP Hg lamp treatment by the dairy industry.

1. Introduction

Processed cheese products, such as sliced cheese, are common packaged ready-to-eat (RTE) foods and can be main carriers of foodborne pathogens if they become contaminated after pasteurization, particularly during product transferring, cutting, slicing, and packaging (Silva et al., 2003; Zhu et al., 2005). Since they are consumed without any additional cooking by consumers, cross-contaminated cheese products can cause serious foodborne illness. According to the US Centers for Disease Control and Prevention (CDC), over 90 food poisoning outbreak cases related to processed cheese consumption were reported in the United States from 1998 to 2011 (Proulx et al., 2015). The most critically important recurring pathogen in these outbreaks has been *Listeria monocytogenes*, a ubiquitous, psychrotolerant bacterium (Donnelly, 2001). In 2010, 41 people across 5 states of the United States became infected with *Escherichia coli* O157:H7 and the majority of them reported the consumption of Gouda cheese (McCollum et al., 2012). Sporadic cases of salmonellosis have been traced to cheese products in Canada and the United States (CDC, 1998, 2008).

The primary approach to preventing post-pasteurization microbial contamination of cheese products is compliance with good manufacturing practices and proper sanitation. Nonetheless, given the high number of outbreaks involving pasteurized milk cheeses still occurring (Gould et al., 2014), an additional antimicrobial intervention could be extremely beneficial. As one of several non-thermal methods for reducing a broad range of microorganisms, ultraviolet-C (UV-C) radiation has been widely used for the surface sterilization of many foods, including fruits, vegetables, and processed foods, as well as equipment. UV-C radiation is an U.S. Food and Drug Administration (FDA) approved technology that can be used to inactivate pathogenic bacteria in liquid foods and water, and on food contact surfaces (USFDA, 2000). In the majority of studies or industrial applications, UV-C disinfection is typically achieved by using low-pressure mercury (LP Hg) lamps with monochromatic output at 254-nm. However, these lamps have several drawbacks, such as a risk of mercury leakage through breakage, a short lifetime, a long warm-up time, and variability of the radiation intensity according to temperature (Bowker et al., 2011; Shin et al., 2016).

Dielectric barrier discharge (DBD)-driven excilamps (excimer or exciplex lamps) have been introduced as a relatively new form of UV-

* Corresponding author at: Department of Food and Animal Biotechnology, Department of Agricultural Biotechnology, Seoul National University, Seoul, South Korea.
E-mail address: Kang7820@snu.ac.kr (D.-H. Kang).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.ijfoodmicro.2016.12.005
0168-1605/

C emitters, which are based on transitions of rare gas excited dimers, halogen excited dimers or rare gas halide excited complexes. They emit high power narrow-band radiation at defined wavelengths ranging from 172- to 345-nm depending on the type of rare gas and halogen used (Matafonova et al., 2008; Matafonova and Batoev, 2012). In the last decade excilamps have continued to receive attention as attractive alternatives to LP Hg lamps due to the absence of elemental mercury, wavelength-selective applications, long lifetime, geometric freedom of bulbs, high radiant intensity and other advantages (Matafonova and Batoev, 2012; Orlowska et al., 2015).

Recently, two novel monochromatic UV-C light excilamps with wavelengths of 222-nm (KrCl) and 282-nm (XeBr) have been studied mainly for bacterial disinfection (Matafonova et al., 2008; Matafonova and Batoev, 2012; Orlowska et al., 2015; Wang et al., 2010; Yin et al., 2015). A KrCl excilamp (222-nm) was shown to be effective in the rapid inactivation of Gram-positive and -negative bacteria in liquid suspensions (Matafonova et al., 2008). Wang et al. (2010) found that reduction of *Bacillus subtilis* spores suspended in aqueous solution increases in the order 172-nm (Xe_2 excilamp) < 254-nm (LP Hg lamp) < 222-nm (KrCl excilamp). Yin et al. (2015) also reported that inactivation of *E. coli* O157:H7 following exposure to UV-C light at 222-nm (KrCl) was higher than inactivation caused by irradiation at 254-nm (LP Hg) or at 282-nm (XeBr) in apple juice at similar levels of UV fluence (~75 mJ/cm^2). These previous research studies show that disinfection using a 222-nm KrCl excilamp was more efficient than that of conventional LP Hg lamps in aqueous media. However, to our knowledge, the antimicrobial effect of 222-nm KrCl excilamps on solid food surfaces and comparison with LP Hg lamps at an identical dose base has never been evaluated before. The physicochemical state of the treatment medium can affect the bactericidal efficacy of most food preservation technologies (Restaino et al., 1980).

The objectives of this study were to examine the fundamental characteristics of a modern DBD-driven KrCl excilamp, such as warm-up time and stability of UV irradiance according to ambient air temperature, and to compare the efficacy of a KrCl excilamp and a conventional LP Hg lamp for reducing populations of foodborne pathogens, including *E. coli* O157:H7, *Salmonella enterica* serovar Typhimurium, and *L. monocytogenes*, on solid media and sliced cheese at the same UV fluences. Also, the mechanisms of inactivation were explored.

2. Materials and methods

2.1. Bacterial strains

Three strains each of *E. coli* O157:H7 (ATCC 35150, ATCC 43889, and ATCC 43890), *S.* Typhimurium (ATCC 19585, ATCC 43971, and DT 104), and *L. monocytogenes* (ATCC 19111, ATCC 19115, and ATCC 15313), were obtained from the bacterial culture collection of Seoul National University (Seoul, South Korea) and used in this investigation. Stock cultures were stored frozen at −80 °C in 0.7 ml of tryptic soy broth (TSB; MB Cell, CA, USA) and 0.3 ml of 50% glycerol. Working cultures were streaked onto tryptic soy agar (TSA; MB Cell), incubated at 37 °C for 24 h, and stored at 4 °C.

2.2. Preparation of pathogen inocula

All strains of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* were cultured individually in 5 ml of TSB at 37 °C for 24 h and harvested by centrifugation (4000 × *g* for 20 min at 4 °C). The obtained pelleted cells were washed three times with 0.2% sterile peptone water (PW). Final pelleted cells were resuspended in 9 ml of PW, corresponding to ca. 10^7 to 10^8 CFU/ml. Subsequently, suspended pellets of each strain of the three pathogenic species (nine strains total) were combined to construct mixed culture cocktails. These cocktails were used in this inactivation study at a final concentration of approximately 10^8 CFU/ml. To analyze the mechanism of inactivation, each final pellet of *E. coli* O157:H7, *S.* Typhimurium, or *L. monocytogenes* was resuspended in 5 ml of phosphate-buffered saline (PBS; 0.1 M) and poured into a crystal grade polystyrene petri dish (15 mm [height] by 60 mm [inside diameter]), respectively.

2.3. Sample preparation and inoculation

Commercially processed sliced camembert cheese (85 by 85 by 2 mm) was purchased at a local grocery store (Seoul, South Korea). Samples were stored under refrigeration (4 °C) and used within 2 days. 100 μl of cocktail suspension was applied to one piece of sliced cheese (ca. 25 g). The inoculum was spread by means of a sterile glass spreader for 1 min for even distribution of pathogens, and the samples were dried inside a biosafety hood for 3 min without the fan running to avoid excessive surface aridity. The final cell concentration was approximately 10^5 to 10^6 CFU/25 g. For surface inoculation of microbiological media, the cocktail suspension was subjected to an additional 10-fold serial dilution in 0.2% sterile PW, and 0.1 ml of diluent was inoculated and spread onto selective media or nonselective agar for injured-cell enumeration. Each type of medium was duplicate spread-plated with three sequential 10-fold dilutions. Sorbitol MacConkey agar (SMAC; Oxoid, NY, USA), xylose lysine desoxycholate agar (XLD; Oxoid), and Oxford agar base with antimicrobial supplement (OAB; MB Cell) were used as selective media to enumerate *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes*, respectively.

2.4. Experimental apparatus and treatment

A dielectric barrier discharge (DBD)-driven excilamp (29 by 9 by 8 cm; UNILAM, Ulsan, South Korea) filled with a KrCl gas mixture with a nominal output power of 20 W (light intensity of 0.29 mW/cm^2 at the sample location) was used in this study for 222-nm UV irradiation. The excilamp was of cylindrical geometry covered by a metal case having an UV exit window with an area of 60 cm^2 (10 × 6 cm) (Fig. 1-b). A modulated electrical field was applied to a quartz glass body filled with KrCl gas. The quartz glass served as a dielectric barrier and prevented the forming plasma from short-circuiting the electrodes (inner-outer) (Fig. 1-a). A 254-nm germicidal lamp (G10T5/4P, 357 mm; Sankyo, Japan) with a nominal output power of 16 W (light intensity of 0.87 mW/cm^2 at the sample location) was used as a conventional LP

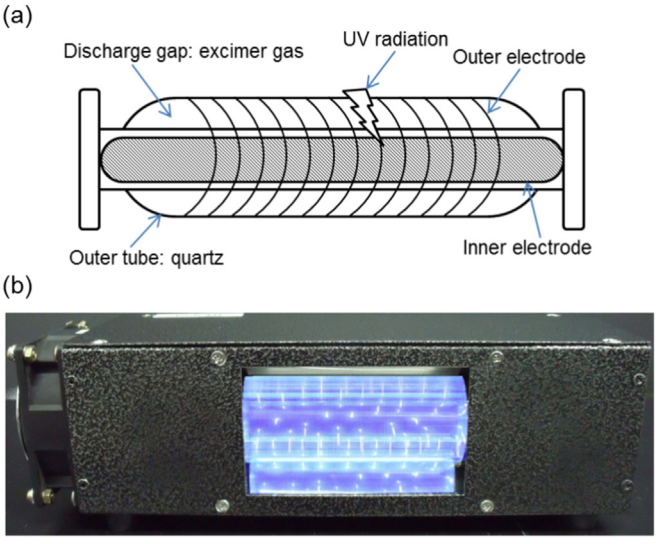

Fig. 1. Schematic diagram (a) and photograph (b) of the experimental 222-nm KrCl excilamp used in this study.

Hg lamp. Since LP Hg lamps radiate in all directions, it was placed within aluminum reflectors to focus onto the process line and the UV output window size was modified to equal that of the KrCl excilamp (10 × 6 cm). The two lamp systems were arranged vertically and directly above the agar medium and sliced cheese samples. The vertical distance between the emitters and the sample was 13 cm (5.1 in.).

Inoculated microbiological media and sliced cheese were treated at room temperature (21 ± 1 °C) with the 222-nm KrCl excilamp and 254-nm LP Hg lamp at equal dosages of 0.87, 1.74 and 2.61 mJ/cm^2. UV doses were calculated by multiplying irradiance values by the irradiation times (1 to 9 s). For the inactivation mechanism study, 5 ml of cell suspensions placed in petri dishes were treated with the two lamp systems at a dose of 3.48 or 8.7 mJ/cm^2 under identical conditions. The volume of the cell suspension (5 ml) and the treatment doses (3.48 and 8.7 mJ/cm^2) were selected after preliminary experiments were performed.

2.5. Irradiance measurement

Radiation intensities of the KrCl excilamp and LP Hg lamp were measured with a UV fiber optic spectrometer (AvaSpec-ULS2048; Avantes, Eerbeek, Netherlands) calibrated to a range of 200- to 400-nm within the UV-C spectrum. The irradiance levels between the two types of lamps were compared by increasing the time and temperature for assessing warm-up time and temperature-dependent irradiation intensity.

2.6. Bacterial enumeration

After UV treatment in the agar medium surface experiment, treated media plates were immediately incubated at 37 °C for 24 h. For food samples, treated sliced cheese was transferred into sterile stomacher bags (Labplas, Inc., Canada), along with 225 ml of sterile 0.2% PW and homogenized for 2 min using a stomacher (EasyMix; AES Chemunex, France). Aliquots (1 ml) of sample were 10-fold serially diluted in 9 ml blanks of PW, and 0.1 ml of diluent was spread plated onto each selective medium (described previously). All agar media were incubated at 37 °C for 24 to 48 h, and typical colonies were counted. To confirm the identity of the pathogens, random colonies were selected from the enumeration plates and subjected to biochemical and serological tests. These tests consisted of the *E. coli* O157:H7 latex agglutination assay (RIM; Remel, KS, USA), the *Salmonella* latex agglutination assay (Oxoid), and the API *Listeria* test (bioMérieux, MO, USA).

2.7. Enumeration of injured cells

The overlay (OV) method was used to enumerate injured cells of *S.* Typhimurium and *L. monocytogenes* (Lee and Kang, 2001). TSA was used as a nonselective medium to repair injured cells. One hundred microliters of the appropriate dilutions were spread plated onto TSA medium, and the plates were incubated at 37 °C for 2 h to allow injured cells to resuscitate (Kang and Siragusa, 1999). The plates were then overlaid with 7 to 8 ml of selective medium (XLD or OAB). After solidification, the plates were further incubated for an additional 22 to 46 h at 37 °C. Following incubation, typical black colonies were counted. In the case of *E. coli* O157:H7, it is not appropriate to overlay with SMAC medium. Instead, phenol red agar base with 1% sorbitol (SPRAB; Difco, Becton, Dickinson, MD, USA) was used (Rhee et al., 2003). After incubation at 37 °C for 24 h, typical white colonies characteristic of *E. coli* O157:H7 were enumerated. Isolates randomly selected from SPRAB plates were subjected to serological confirmation as *E. coli* O157:H7 (*E. coli* O157:H7 latex agglutination assay; Remel), because SPRAB is not typically used as a selective agar for enumerating *E. coli* O157:H7.

2.8. Investigation of the bactericidal mechanism

The fluorescent dyes propidium iodine (PI; Sigma-Aldrich, MO, USA) and carboxyfluorescein diacetate (cFDA; Sigma-Aldrich) were used to quantitatively assess membrane or intracellular enzyme damage to pathogen cells induced by each treatment. For PI uptake value, *E. coli* O157:H7, *S.* Typhimurium, or *Listeria monocytogenes* cells adjusted to an optical density at 680 nm (OD_{680}) of approximately 0.4 in PBS were treated with each UV spectrum (3.48 mJ/cm^2) and then centrifuged (10,000 × *g* for 10 min). The cell pellets were resuspended in PBS and then mixed with PI solution to a final concentration of 2.9 μM. After incubation at 37 °C for 10 min, samples were centrifuged at 10,000 × *g* for 10 min and washed twice in PBS to remove excess dye. The final cell pellets were resuspended in PBS, and fluorescence was measured with a spectrofluorophotometer (Spectramax M2e; Molecular Devices, CA, USA) at an excitation wavelength of 493 nm and an emission wavelength of 630 nm. In the case of the cFDA conversion value, each pathogen cell suspension with an OD_{680} of approximately 0.2 was treated (8.7 mJ/cm^2) and incubated with 50 μM cFDA at 37 °C for 15 min to allow intracellular enzymatic conversion of cFDA into carboxyfluorescein (cF). The cells were then washed to remove excess cFDA as described above. cF emits fluorescence at 517 nm following excitation with light at 492 nm. PI and cFDA fluorescence values for each sample were normalized with the OD_{680} of the cell suspensions, and data obtained for untreated cells were subtracted from those for treated cells.

2.9. Statistical analysis

All experiments were repeated three times with duplicate samples. Triplicate data were analyzed by analysis of variance (ANOVA) and Duncan's multiple-range test of a statistical analysis system (SAS Institute, Cary, NC, USA). A P value of <0.05 was used to indicate significant differences.

3. Results

3.1. Comparison of properties between 222-nm KrCl excilamp and 254-nm LP lamp

Fig. 2 shows typical spectral irradiance of the 222-nm KrCl excilamp. The full width at half maximum, defined as the wavelength gap between the output half-peak-intensity values, was ca. 2.1 nm for the

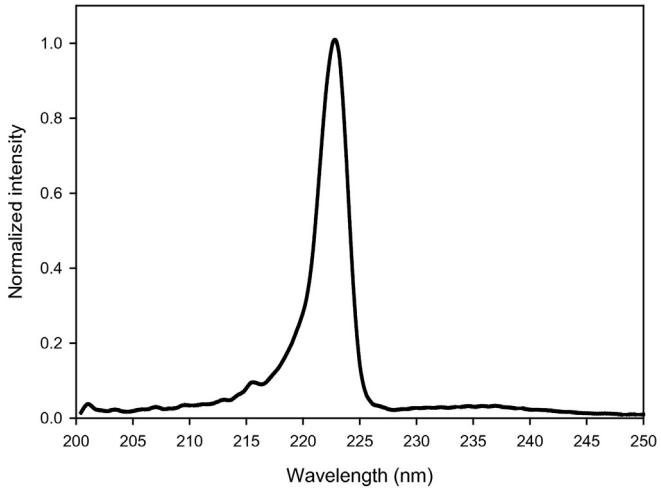

Fig. 2. Emission spectrum of the 222-nm KrCl excilamp (intensity unit: μW/cm^2).

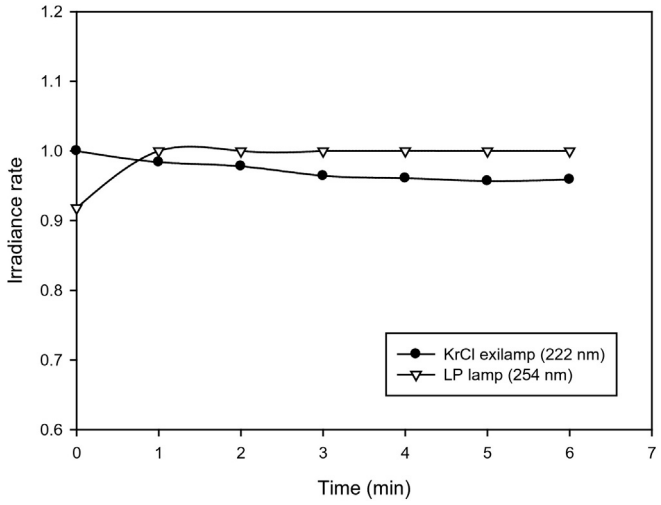

Fig. 3. Comparison of warm-up time between the KrCl excilamp and LP-UV lamp.

222-nm excilamp. The 254-nm LP lamp needed 1 min to reach maximum irradiance; however, the 222-nm excilamp needed no warm-up time and radiant power was maintained within a range of 96 to 100% (Fig. 3). Fig. 4 shows variation of radiation output relative to air temperature. Radiation intensity of the LP Hg lamp was highest at around 30 to 35 °C, and the intensity greatly decreased at refrigeration temperatures. Conversely, the output of the 222-nm excilamp was not affected by ambient temperature (from 0 to 45 °C).

3.2. Bactericidal effect of UV irradiation on media and sliced cheese surfaces

The viable-count reduction levels of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* cells spread onto selective media following 222-nm excilamp and 254-nm LP lamp treatment are presented in Table 1. As irradiation dose of the 222-nm KrCl excilamp increased from 0 to 2.61 mJ/cm^2, all pathogens experienced over 4.5 log reductions. The 254-nm LP lamp also induced higher levels of inactivation at higher doses. However, for the LP lamp at a dose of 2.61 mJ/cm^2, inactivation levels were 3.34-, 2.45-, and 2.02-log for *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes*, respectively, which were significantly less ($P < 0.05$) than for the KrCl excilamp at the same dose. At the other doses (0.87, and 1.74 mJ/cm^2) significant differences between

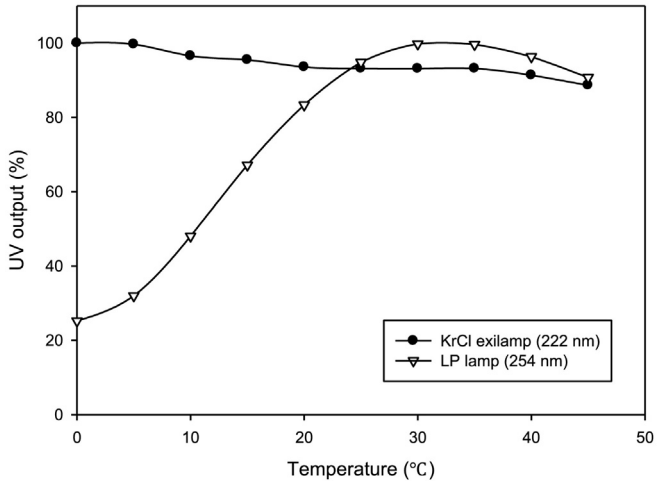

Fig. 4. Comparison of the temperature effect on intensity between the KrCl excilamp and LP-UV lamp.

reductions of the three pathogens treated with 222-nm and 254-nm irradiation occurred.

Log reductions of foodborne pathogens on sliced cheese samples after treatment with 222-nm and 254-nm UV lamps are presented in Table 2. The relationship between reduction levels and treatment doses was similar to that of selective media experiments. Approximately 2-log reductions were accomplished with the 222-nm excilamp at 2.61 mJ/cm^2 for the three pathogens. With regard to the 254-nm LP lamp, pathogen reductions ranged from 0.99 to 1.33 log CFU/g at the same dose of 2.61 mJ/cm^2.

3.3. Resuscitation of UV-injured cells

Tables 1 and 2 show levels of sublethally injured cells of the three pathogens on media or sliced cheese following 222-nm and 254-nm UV treatment, respectively. Determining the difference between inactivation of samples subjected to injured cell recovery methods and those plated directly on selective media revealed the presence of 0.98, 0.91, and 0.47 log units of injured *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* cells, respectively, after 254-nm LP lamp treatment at 2.61 mJ/cm^2. For 222-nm excilamp irradiation, injured cell levels of 0.16, 0.50, and 0.17 log CFU/g were observed after treatment at the same dose of 2.61 mJ/cm^2 (Table 1).

Following treatment of inoculated sliced cheese with 254-nm radiation at2.61 mJ/cm^2, injured cell levels of 0.47, 0.69, and 0.54 log CFU/g were detected for *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes*, respectively. For 222-nm excilamp treatment, however, there were no significant ($P > 0.05$) differences between the reduction levels enumerated on selective agar versus those on agar used for recovery over the entire range of treatment doses (Table 2).

3.4. Determination of injury sites in 222-nm excilamp treated cells

As a quantitative analysis of membrane disruption and intracellular esterase denaturation, 222-nm and 254-nm UV treated cells were stained with the fluorescent dyes. The PI and cFDA fluorescence values of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* after each treatment are shown in Table 3. For both PI uptake and cFDA conversion values, overall result patterns between the three pathogens were similar. Based on PI uptake values, there was no significant ($P > 0.05$) damage to pathogen cellular membranes following 254-nm LP lamp treatment. The degree of PI uptake for 222-nm UV treated cells was much higher than that of 254-nm irradiated cells. Furthermore, cells subjected to 222-nm excilamp treatment showed significantly ($P < 0.05$) higher cFDA conversion values than did cells subjected to the 254-nm LP lamp treatment, indicating that 222-nm UV irradiation resulted in increasing perturbation of enzymatic activity in pathogenic bacterial cells (Table 3).

4. Discussion

The slicing step has been known for many years to serve as a vehicle for major foodborne pathogens in various deli foods (Chen et al., 2014). According to a recent FDA report, deli slicers have been identified as the primary source of *L. monocytogenes* cross-contamination in RTE foods (USFDA, 2013). Since sliced cheese contamination occurs primarily via contact with surfaces, an additional superficial decontamination step may become imperative in order to control pathogenic bacteria on sliced cheese products. In this respect, the mercury-free 222-nm excilamp treatment could become an attractive solution to mitigate surface contamination of cheese in manufacturing, distribution, and retail environments as an alternative to conventional LP UV-C lamps.

As shown in Fig. 3, just after initiation, the KrCl excilamp achieved maximum irradiance, whereas the LP Hg lamp needed about 1 min for its initial heating to provide stable luminous flux. However, this would not be a problem in a continuous operation. In addition, it is well-

Table 1
Log reductions of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* on culture media treated with a 254-nm LP lamp and 222-nm KrCl excilamp at equal doses.

Treatment type	Dose (mJ/cm^2)	Log reduction [$\log_{10}$ (N_0/N)][a] by organism and selection medium											
		E. coli O157:H7				*S.* Typhimurium				*L. monocytogenes*			
		SMAC		SPRAB		XLD		OV-XLD		OAB		OV-OAB	
LP lamp (254-nm)	0	0.00 ± 0.00	Aa	0.00 ± 0.00	Aa	0.00 ± 0.00	Aa	0.00 ± 0.00	Aa	0.00 ± 0.00	Aa	0.00 ± 0.00	Aa
	0.87	1.26 ± 0.21	Ba	0.84 ± 0.45	ABa	0.64 ± 0.18	Ba	0.12 ± 0.34	Aa	0.41 ± 0.15	Aa	0.04 ± 0.41	Aa
	1.74	2.18 ± 0.68	Ca	1.55 ± 1.09	BCa	1.63 ± 0.32	Ca	0.77 ± 0.37	Bb	1.21 ± 0.09	Ba	0.64 ± 0.27	Ab
	2.61	3.34 ± 0.26	Da	2.36 ± 0.51	Cb	2.45 ± 0.47	Da	1.54 ± 0.13	Cb	2.02 ± 0.64	Ca	1.55 ± 0.66	Ba
KrCl excilamp (222-nm)	0	0.00 ± 0.00	Aa	0.00 ± 0.00	Aa	0.00 ± 0.00	Aa	0.00 ± 0.00	Aa	0.00 ± 0.00	Aa	0.00 ± 0.00	Aa
	0.87	2.46 ± 0.49	Ba	2.09 ± 0.50	Ba	1.99 ± 0.10	Ba	1.66 ± 0.23	Ba	2.01 ± 0.49	Ba	1.14 ± 0.20	Bb
	1.74	4.00 ± 0.08	Ca	3.56 ± 0.56	Ca	3.55 ± 0.28	Ca	2.44 ± 0.17	Cb	3.04 ± 0.36	Ca	2.84 ± 0.37	Ca
	2.61	4.66 ± 0.23	Da	4.82 ± 0.15	Da	4.86 ± 0.34	Da	4.36 ± 0.23	Da	4.58 ± 0.02	Da	4.41 ± 0.15	Da

[a] The values are means ± standard deviations from three replications. Values in the same column followed by the same uppercase letter are not significantly different ($P > 0.05$). Means with the same lowercase letter in the same row are not significantly different ($P > 0.05$). SMAC, sorbitol MacConkey agar; SPRAB, phenol red agar base with 1% sorbitol; XLD, xylose lysine desoxycholate agar; OV-XLD, overlay XLD agar on TSA; OAB; Oxford agar base with antimicrobial supplement; OV-OAB, overlay OAB agar on TSA.

known that the radiant power of LP Hg lamps is very sensitive to ambient thermal changes (Crawford et al., 2005). Fig. 4 shows the temperature stability of a KrCl excilamp compared to that of a LP Hg lamp. Especially at low temperatures, around 0 to 10 °C, radiation output of the LP Hg lamp declined 64.7% point on average, whereas full radiant power was observed with KrCl excilamp irradiation. This property of KrCl excilamps facilitates applications at refrigeration temperatures for inactivation of numerous psychrotrophic microorganisms, particularly *L. monocytogenes*, which are capable of growing at low temperatures.

Sublethally injured cells resulting from UV-C irradiation should be considered since they can recover and regain their pathogenicity under suitable conditions (Wu, 2008). In the present study, the extent to which sublethally injured pathogens survived after 222- or 254-nm UV treatment was assessed by using the OV method or SPRAB agar. On both agar media and sliced cheese, more sublethally injured cells were observed following LP Hg lamp treatment than with the KrCl excilamp as treatment dose increased. Especially, *S.* Typhimurium produced significant numbers of injured cells when the agar medium or cheese surfaces were treated with the 254-nm LP Hg lamp (Tables 1 and 2). Similar increases in *S.* Typhimurium injured cells after UV-C irradiation were also reported by other researchers (Choi et al., 2015; Kim et al., 2016). However, there were no significant ($P > 0.05$) differences in levels of *S.* Typhimurium cells enumerated on XLD and OV-XLD following 222-nm excilamp treatment, even at maximum irradiance (2.61 mJ/cm^2) (Tables 1 and 2). This proves that 222-nm KrCl excilamp treatment can effectively inactivate all major foodborne pathogens on solid food surfaces without generating appreciable injury to bacterial cells.

In the agar medium surface experiments, *L. monocytogenes*, a Gram-positive bacterium, showed higher resistance to 254-nm UV-C radiation than did Gram-negative bacteria, such as *E. coli* O157:H7 and *S.* Typhimurium (Table 1). This greater resistance may be attributed to the thick peptidoglycan wall that surrounds the cytoplasmic membrane in Gram-positive bacteria, whereas Gram-negative bacteria possess only an external membrane (Virto et al., 2005). Additionally, *L. monocytogenes* generates fewer photoproducts (cyclobutane pyrimidine dimers and pyrimidine 6–4 pyrimidone photoproducts) than *E. coli* during a UV-C lamp irradiation. These photoproducts can lead to structural distortion in DNA and disrupt its replication (Beauchamp and Lacroix, 2012). Interestingly, after treatment with 222-nm UV radiation, the inactivation level of *L. monocytogenes* was comparatively similar to that of *E. coli* O157:H7 or *S.* Typhimurium (Table 1).

One of the purposes of the current study was to examine the mechanism of the enhanced lethal effect of 222-nm excilamp treatment. The reason could be that different UV light wavelengths cause different damage to bacteria and it can be speculated that cell damage caused by 222-nm UV radiation is even more serious than that of 254-nm UV lamps; however, further evidence is needed to validate that hypothesis. It is well known that LP Hg lamps emit near the DNA peak absorption coefficient of 265 nm. This absorption causes damage to DNA by altering nucleotide base paring. However, bacteria generally possess molecular mechanisms to compensate for UV-induced damages to DNA (Zimmer and Slawson, 2002). Light-dependent photo-reactivation which uses a single enzyme called photolyase is particularly effective at reversing the formation of pyrimidine dimers (Thoma, 1999). In order to reduce this photo-reactivation mechanism, the use of a lower wavelength of ca. 220 nm could be a solution. All proteins have an additional higher absorption from 180 to 230 nm due to the peptide bonds in the amino acid chain. The KrCl excilamp shows a relatively sharp emission

Table 2
Log reductions of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* on sliced cheese treated with a 254-nm LP lamp and 222-nm KrCl excilamp at equal doses.

Treatment type	Dose (mJ/cm^2)	Log reduction [$\log_{10}$ (N_0/N)][a] by organism and selection medium											
		E. coli O157:H7				*S.* Typhimurium				*L. monocytogenes*			
		SMAC		SPRAB		XLD		OV-XLD		OAB		OV-OAB	
LP lamp (254-nm)	0	0.00 ± 0.00	Aa	0.00 ± 0.00	Aa	0.00 ± 0.00	Aa	0.00 ± 0.00	Aa	0.00 ± 0.00	Aa	0.00 ± 0.00	Aa
	0.87	0.74 ± 0.47	Ba	0.34 ± 0.31	ABa	0.43 ± 0.28	Ba	0.01 ± 0.28	Aa	0.55 ± 0.06	Ba	0.07 ± 0.09	Ab
	1.74	1.02 ± 0.33	Ba	0.45 ± 0.37	ABa	0.79 ± 0.29	BCa	0.28 ± 0.09	Ab	0.73 ± 0.15	Ca	0.32 ± 0.30	ABa
	2.61	1.33 ± 0.20	Ba	0.86 ± 0.42	Ba	0.99 ± 0.07	Ca	0.30 ± 0.08	Ab	0.99 ± 0.10	Da	0.45 ± 0.17	Bb
KrCl excilamp (222-nm)	0	0.00 ± 0.00	Aa	0.00 ± 0.00	Aa	0.00 ± 0.00	Aa	0.00 ± 0.00	Aa	0.00 ± 0.00	Aa	0.00 ± 0.00	Aa
	0.87	1.22 ± 0.47	Ba	1.11 ± 0.36	Ba	1.14 ± 0.16	Ba	1.05 ± 0.06	Ba	1.08 ± 0.44	Ba	0.67 ± 0.15	Ba
	1.74	1.37 ± 0.48	BCa	1.15 ± 0.18	Ba	1.50 ± 0.10	Ca	1.27 ± 0.14	BCa	1.57 ± 0.50	Ba	1.13 ± 0.25	Ba
	2.61	2.02 ± 0.41	Ca	1.93 ± 0.32	Ca	1.99 ± 0.11	Da	1.80 ± 0.64	Ca	1.78 ± 0.35	Ba	1.66 ± 0.46	Ca

[a] The values are means ± standard deviations from three replications. Values in the same column followed by the same uppercase letter are not significantly different ($P > 0.05$). Means with the same lowercase letter in the same row are not significantly different ($P > 0.05$). SMAC, sorbitol MacConkey agar; SPRAB, phenol red agar base with 1% sorbitol; XLD, xylose lysine desoxycholate agar; OV-XLD, overlay XLD agar on TSA; OAB; Oxford agar base with antimicrobial supplement; OV-OAB, overlay OAB agar on TSA.

Table 3
Levels of membrane damage and intracellular enzyme inactivation of 254-nm LP lamp or 222-nm KrCl excilamp treated cells inferred from PI uptake and cFDA conversion tests.

Value[a]	Treatment type	Microorganism					
		E. coli O157:H7		*S.* Typhimurium		*L. monocytogenes*	
PI uptake	Untreated control	0 ± 0	A	0 ± 0	A	0 ± 0	A
	254-nm LP lamp	0.23 ± 0.06	A	0.41 ± 0.15	A	1.44 ± 0.39	A
	222-nm KrCl excilamp	3.59 ± 1.13	B	3.91 ± 0.58	B	7.49 ± 2.06	B
cFDA conversion	Untreated control	0 ± 0	A	0 ± 0	A	0 ± 0	A
	254-nm LP lamp	188 ± 33	A	359 ± 70	B	259 ± 26	B
	222-nm KrCl excilamp	988 ± 245	B	2486 ± 273	C	3847 ± 130	C

[a] Values are means of three replications ± standard deviations. Values followed by the same letters within the column for each value and microorganism are not significantly different ($P > 0.05$). The data were normalized by subtracting fluorescence values obtained from untreated cells and against OD_{680} as follows: PI uptake value = (fluorescence value after treatment − fluorescence value of non-treated)/OD_{680}; cFDA conversion value = |(fluorescence value after treatment − fluorescence value of non-treated)/OD_{680}|.

spectrum with a peak at 222-nm targeting the protein molecules of microorganisms. Clauß and Grotjohann (2008) showed that photodegradation of proteins and inactivation of enzymes is much more effective with the 222-nm excimer lamp compared to the 254-nm mercury lamp. Furthermore, the integrity of the bacterial membrane could be very important for evaluation of the effect of 222-nm UV light irradiation on food borne pathogens, because UV light at a wavelength of 222-nm seems specific for destroying bacterial outer membrane proteins (Abdallah et al., 2012). Yin et al. (2015) indicated that the higher disinfection efficiency at 222-nm versus those of 254-nm and 282-nm UV sources can result from damage to the cell envelope. Thus, various mechanisms of protein damage can presumably be held responsible for inactivation of microorganisms.

In this study, to clarify the mechanism of the enhanced lethal effect of 222-nm UV irradiation, membrane and intracellular protein damage to *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* cells caused by KrCl excilamp and LP Hg lamp treatment was compared quantitatively by fluorescent staining methods. Fluorescent stains that bind to intracellular components are useful in determining the viability or the process-induced changes of microorganisms. In particular, PI emits red fluorescence when binding to nucleic acids (DNA and RNA) and does not pass through intact cell membranes (Breeuwer and Abee, 2000). By contrast, cFDA, a lipophilic non-fluorescent precursor that diffuses freely across cell membranes, is widely used for the assessment of non-specific enzymatic activity in cells. In cytoplasm, cFDA is converted by nonspecific esterases into a polar, membrane-impermeant green fluorescent compound, carboxyfluorescein (Li et al., 2016). As shown in Table 3, the improved inactivation of foodborne pathogens by the 222-nm KrCl excilamp might be related to disruptions of bacterial cell membranes and enzymatic activities. Therefore, although the DNA absorption coefficient decreases as UV-C wavelength decreases from 254-nm to 222-nm, the synergistic effect of outer membrane damage and a lower rate of photo-reactivation probably gives rise to the enhanced disinfection effect of UV light at 222-nm.

In conclusion, the efficacy of 222-nm KrCl excilamp treatment for controlling major foodborne pathogens on microbiological media and sliced cheese surfaces was superior to that of conventional 254-nm LP Hg lamps, and shows that narrow-band UV-C irradiation at 222-nm could be an alternative surface disinfection method having several advantages. However, as shown in Tables 1 and 2, 222-nm KrCl excilamp as well as 254-nm LP Hg lamp exposure was less effective on sliced cheese than on agar plates at the same irradiance levels. In other words, the efficacy of 222-nm UV-C irradiation was greatly affected by surface characteristics of the sample subject. To achieve >3 log reductions of the three pathogens on sliced cheese, a much higher irradiation dose was required compared to microbiological media. In many studies, sterilization methods have shown very different results when applied to actual foods (Kim et al., 2016; Noriega et al., 2011). Noriega et al. (2011) showed that cold plasma treatment led to a maximal inactivation of *L. innocua* on membrane filters, but was less effective on chicken muscle and skin. These results might be attributed to the surface topography of samples. The surface of the sliced cheese may be rougher than that of the agar medium due to slicing process, providing numerous sites for pathogens to attach and potentially escape UV-C radiation. Therefore, further research with various model solid foods having different surface properties are necessary to optimize treatment conditions. In addition, future plans include determining the capital and operating costs of a 222-nm KrCl excilamp system for treating large quantities of food samples with a high throughput.

Acknowledgments

This research was supported by the Agriculture, Food and Rural Affairs Research Center Support Program, Ministry of Agriculture, Food and Rural Affairs, Republic of Korea. This research was also 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).

References

Abdallah, F.B., Lagha, R., Ellafi, A., Namane, A., Rousselle, J.C., Lenormand, P., Kallel, H., 2012. Identification of outer membrane proteins altered in response to UVC-radiation in *Vibrio parahaemolyticus* and *Vibrio alginolyticus*. Indian J. Microbiol. 52, 660–665.

Beauchamp, S., Lacroix, M., 2012. Resistance of the genome of *Escherichia coli* and *Listeria monocytogenes* to irradiation evaluated by the induction of cyclobutane pyrimidine dimers and 6–4 photoproducts using gamma and UV-C radiations. Radiat. Physics Chem. 81, 1193–1197.

Bowker, C., Sain, A., Shatalov, M., Ducoste, J., 2011. Microbial UV fluence-response assessment using a novel UV-LED collimated beam system. Water Res. 45, 2011–2019.

Breeuwer, P., Abee, T., 2000. Assessment of viability of microorganisms employing fluorescence techniques. Int. J. Food Microbiol. 55, 193–200.

Centers for Disease Control and Prevention, 1998. Salmonellosis Associated With Cheese Consumption—Canada. Centers for Disease Control and Prevention, Atlanta, GA. http://www.cdc.gov/mmwr/preview/mmwrhtml/00000370.htm.

Centers for Disease Control and Prevention, 2008. Outbreak of Multidrug-resistant *Salmonella enterica* Serotype Newport Infections Associated With Consumption of Unpasteurized Mexican-style Aged Cheese—Illinois, March 2006–April 2007. Centers for Disease Control and Prevention, Atlanta, GA. http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5716a4.htm.

Chen, D., Zhao, T., Doyle, M.P., 2014. Transfer of foodborne pathogens during mechanical slicing and their inactivation by levulinic acid-based sanitizer on slicers. Food Microbiol. 38, 263–269.

Choi, D.S., Park, S.H., Choi, S.R., Kim, J.S., Chun, H.H., 2015. The combined effects of ultraviolet-C irradiation and modified atmosphere packaging for inactivating *Salmonella enterica* serovar Typhimurium and extending the shelf life of cherry tomatoes during cold storage. Food Packag. Shelf Life 3, 19–30.

Clauß, M., Grotjohann, N., 2008. Effective photoinactivation of alpha-amylase, catalase and urease at 222 nm emitted by an KrCl-excimer lamp. Clean 36, 754–759.

Crawford, M.H., Banas, M.A., Ross, M.P., Ruby, D.S., Nelson, J.S., Boucher, A., Allerman, A.A., 2005. Final LDRD Report: Ultraviolet Water Purification Systems for Rural Environments and Mobile Applications. Sandia Report. US Department of Energy, Washington, DC.

Donnelly, C., 2001. Factors associated with hygienic control and quality of cheeses prepared from raw milk: a review. Bull. Int. Dairy Fed. 369, 16–27.

Gould, L.H., Mungai, E., Barton Behravesh, C., 2014. Outbreaks attributed to cheese: differences between outbreaks caused by unpasteurized and pasteurized dairy products, United States, 1998–2011. Foodborne Pathog. Dis. 11, 545–551.

Kang, D.H., Siragusa, G.R., 1999. Agar underlay method for recovery of sublethally heat-injured bacteria. Appl. Environ. Microbiol. 65, 5334–5337.

Kim, S.J., Kim, D.K., Kang, D.H., 2016. Using UVC light-emitting diodes at wavelengths of 266 to 279 nanometers to inactivate foodborne pathogens and pasteurize sliced cheese. Appl. Environ. Microbiol. 82, 11–17.

Lee, S.Y., Kang, D.H., 2001. Suitability of overlay method for recovery of heat-injured *Listeria monocytogenes* and *Salmonella* Typhimurium. Food Sci. Biotechnol. 10, 323–326.

Li, J., Ahn, J., Liu, D., Chen, S., Ye, X., Ding, T., 2016. Evaluation of ultrasound induced damage to *Escherichia coli* and *Staphylococcus aureus* by flow cytometry and transmission electron microscopy. Appl. Environ. Microbiol. 82, 1828–1837.

Matafonova, G.G., Batoev, V.B., Astakhova, S.A., Gómez, M., Christofi, N., 2008. Efficiency of KrCl excilamp (222 nm) for inactivation of bacteria in suspension. Lett. Appl. Microbiol. 47, 508–513.

Matafonova, G.G., Batoev, V.B., 2012. Recent progress on application of UV excilamps for degradation of organic pollutants and microbial inactivation. Chemosphere 89, 637–647.

McCollum, J.T., Williams, N.J., Beam, S.W., Cosgrove, S., Ettestad, P.J., Ghosh, T.S., 2012. Multistate outbreak of *Escherichia coli* O157:H7 infections associated with in-store sampling of an aged raw-milk Gouda cheese, 2010. J. Food Prot. 75, 1759–1765.

Noriega, E., Shama, G., Laca, A., Díaz, M., Kong, M.G., 2011. Cold atmospheric gas plasma disinfection of chicken meat and chicken skin contaminated with *Listeria innocua*. Food Microbiol. 28, 1293–1300.

Orlowska, M., Koutchma, T., Kostrzynska, M., Tang, J., 2015. Surrogate organisms for pathogenic O157:H7 and non-O157 *Escherichia coli* strains for apple juice treatments by UV-C light at three monochromatic wavelengths. Food Control 47, 647–655.

Proulx, J., Hsu, L.C., Miller, B.M., Sullivan, G., Paradis, K., Moraru, C.I., 2015. Pulsed-light inactivation of pathogenic and spoilage bacteria on cheese surface. J. Dairy Sci. 98, 5890–5898.

Restaino, L., Jeter, W.S., Hill, W.M., 1980. Thermal injury of *Yersinia enterocolitica*. Appl. Environ. Microbiol. 40, 939–949.

Rhee, M.S., Lee, S.Y., Hillers, V.N., McCurdy, S.M., Kang, D.H., 2003. Evaluation of consumer-style cooking methods for reduction of *Escherichia coli* O157:H7 in ground beef. J. Food Prot. 66, 1030–1034.

Shin, J.Y., Kim, S.J., Kim, D.K., Kang, D.H., 2016. Fundamental characteristics of deep-UV light-emitting diodes and their application to control foodborne pathogens. Appl. Environ. Microbiol. 82, 2–10.

Silva, I.M.M., Almeida, R.C.C., Alves, M.A.O., Almeida, P.F., 2003. Occurrence of *Listeria* spp. in critical control points and the environment of minas frescal cheese processing. Intl. J. Food Microbiol. 81, 241–248.

Thoma, F., 1999. Light and dark in chromatin repair: repair of UV-induced DNA lesions by photolyase and nucleotide excision repair. EMBO J. 18, 6585–6598.

US Food and Drug Administration, 2000. Ultraviolet radiation for the processing and treatment of food. Code of Federal Regulations 21 (Part 179.39). FDA, Washington, DC.

US Food and Drug Administration, 2013. Draft Interagency Risk Assessment e *Listeria monocytogenes* in Retail Delicatessens Technical Report. Available at:. http://www.fda.gov/downloads/Food/FoodScienceResearch/RiskSafetyAssessment/UCM351328.pdf.

Virto, R., Manas, P., Alvarez, I., Condon, S., Raso, J., 2005. Membrane damage and microbial inactivation by chlorine in the absence and presence of a chlorine-demanding substrate. Appl. Environ. Microbiol. 71, 5022–5028.

Wang, D., Oppenländer, T., El-Din, M.G., Bolton, J.R., 2010. Comparison of the disinfection effects of vacuum-UV (VUV) and UV light on *Bacillus subtilis* spores in aqueous suspensions at 172, 222 and 254 nm. Photochem. Photobiol. 86, 176–181.

Wu, V.C.H., 2008. A review of microbial injury and recovery methods in food. Food Microbiol. 25, 735–744.

Yin, F., Zhu, Y., Koutchma, T., Gong, J., 2015. Inactivation and potential reactivation of pathogenic *Escherichia coli* O157:H7 in apple juice following ultraviolet light exposure at three monochromatic wavelengths. Food Microbiol. 46, 329–335.

Zhu, M., Du, M., Cordray, J., Ahn, D.U., 2005. Control of *Listeria monocytogenes* contamination in ready-to-eat meat products. Comp. Rev. Food Sci. Food Safety. 4, 34–42.

Zimmer, J.L., Slawson, R.M., 2002. Potential repair of *Escherichia coli* DNA following exposure to UV radiation from both medium-and low-pressure UV sources used in drinking water treatment. Appl. Environ. Microbiol. 68, 3293–3299.