A THESIS FOR THE DEGREE OF MASTER OF SCIENCE

Application of KrCl excilamp for surface disinfection of foodborne pathogen

크립톤 클로라인 엑실램프를 이용한 식품관련 표면 살균

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석사학위논문

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지도교수 강동현

이 논문을 석사학위 논문으로 제출함

2017 년 8 월

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이 재 익

이재익의 석사 학위논문을 인준함

2017 년 8 월

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ABSTRACT

This study was conducted to investigate the basic spectral properties of 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 conventional 254-nm low-pressure Hg (LP Hg) lamp. The KrCl excilamp showed full radiant intensity from the outset at a wide range of working temperatures, especially at low temperature around 0 to 10 °C. 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. Irradiation with 222-nm UV-C showed significantly ($P < 0.05$) higher inactivation capacity against all three pathogens than 254-nm radiation on both of media and sliced cheese surfaces without generating many sublethally injured cells which potentially could recover. The underlying inactivation mechanisms of the 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. And this study was also conducted investigate the resistance of *Escherichia coli* O157:H7 to 222-nm KrCl excilamp and 254-nm LP Hg lamp treatment according to growth temperature. As growth temperature decreased, lag time of *E. coli* O157:H7

significantly increased while the growth rate significantly decreased. Regardless of growth temperature the KrCl excilamp showed higher disinfection capacity compared to the LP Hg lamp at stationary growth phase. KrCl excilamp treatment showed significantly higher reduction as growth temperature decreased. Conversely, reduction levels according to growth temperature were not significantly different when the pathogen was subjected to LP Hg lamp treatment. Inactivation mechanisms were evaluated by the thiobarbituric acid reactive substances (TBARS) assay and SYBR green assay, and confirmed that lipid oxidation capacity following KrCl excilamp treatment increased as growth temperature decreased, which was significantly higher than that of LP Hg lamp treated samples regardless of growth temperature. DNA damage level was significantly higher for LP Hg lamp treated samples compared to those subjected to the KrCl excilamp, but no significant difference pursuant to growth temperature was observed. Consequently, resistance of *E. coli* O157:H7 to the KrCl excilamp decreased as growth temperature decreased because the ratio of unsaturated fatty acid composition increased at low growth temperature resulting in higher lipid oxidation levels. 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 in the surface disinfection.

Keywords: **KrCl Excilamp, food-borne pathogens, surface disinfection, growth temperature**

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CONTENTS

LIST OF TABLE

LIST OF FIGURES

I. INTRODUCTION

Processed cheese products, such as sliced cheese are a common packaged ready-to-eat (RTE) food and can be main carriers of foodborne pathogens if they become contaminated after pasteurization, particularly during the product transferring, cutting, slicing, and packaging (1, 2). Since they are consumed without any additional cooking at the consumers, cross-contaminated chees 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 (3). The most critically important recurring pathogen in these outbreaks has been *Listeria monocytogenes*, a ubiquitous, psychrotolerant bacterium (4). In 2010, 41 people across 5 states of the United States became infected by *Escherichia coli* O157:H7 and majority of them reported the consumption of Gouda cheese (5). Sporadic cases of salmonellosis have been traced to cheese products in Canada and the United States (6, 7).

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 (8), an additional antimicrobial intervention could be extremely beneficial. And one of the main issue of surface disinfection is utensils and equipment surfaces Because cross-contamination during processing commonly occurs from utensils and surfaces (9), even slight contamination of the pathogens onto surfaces or work areas may cause serious outbreaks (10). For example, stainless steel, which is still widely used for constructing processing equipment, can be contaminated with the pathogen. Several research investigations reported that *E. coli* O157:H7 can survive on stainless steel for an extended time (28 – 60 days) at low temperatures (11, 12). These results indicate that the pathogen can survive on stainless steel at numerous temperatures and result in cross contamination from stainless steel to food or medical equipment. Through the present time, sodium hypochlorite (NaOCl) has been used widely to prevent outbreaks and sanitize stainless steel in numerous facilities. However, several disadvantages of NaOCl have been reported including, corrosion of a variety of substances (13).

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, processed foods, and equipment surface. 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 food contact surfaces (14). In majority of the studies or industrial fields, 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 (15). In particular, mercury leakage from LP Hg lamps can cause severe human health hazards as well as environmental damage. Moreover, a new international binding treaty instrument called the Minamata Convention was approved, which restricts the usage of mercury (16). For the reasons given above, the necessity of developing a new source of ultraviolet-C irradiation has been emphasized recently.

Dielectric barrier discharge (DBD)-driven excilamps (excimer or exciplex lamp) have been introduced as a relatively new form of UV-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 (17, 18). 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 (18, 19).

Recently, two novel monochromatic UV-C light excilamps with wavelength of 222-nm (KrCl) and 282-nm (XeBr) have been studied mainly for bacterial disinfection (17-21). A KrCl excilamp (222-nm) was shown to be effective in the rapid inactivation of Gram-positive and –negative bacteria in liquid suspensions (17). Wang et al. (20) found the 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. (21) 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) and at 282-nm (XeBr) in apple juice at similar levels of UV fluence (~75 mJ/cm^2). These previous research show that disinfection using 222-nm KrCl excilamp was more efficient than that of conventional LP Hg lamp in aqueous media. However, to our knowledge, the antimicrobial effect of 222-nm KrCl excilamps on solid food surfaces and equipment surfaces and comparison with LP Hg lamp at 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 (22).

The objectives of this study were to examine the fundamental characteristics of modern DBD-driven KrCl excilamp, such as warm-up time and stability of UV irradiance according to the ambient air temperature, and to compare the efficacy of KrCl excilamp and 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. And in case of equipment surface disinfection, to identify the resistance of *E. coli* O157:H7 grown at various temperatures to LP Hg lamp and KrCl excilamp treatment. Lipid oxidation, and DNA damage to *E. coli* O157:H7 were compared for each growth temperatures to identify the inactivation mechanisms. Moreover, lipid composition of *E. coli* O157:H7 were analyzed in the present study.

II. MATERIALS AND METHODS

2.1 KrCl excilamp and LP Hg lamp system

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 serves 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 Hg lamp. Since LP Hg lamp radiate in all directions, it was placed within aluminum reflectors to focus onto the process line and the UV output window size was modified equal to the KrCl excilamp (10 × 6 cm). Two lamp systems were arranged verticality and

directly above the medium, sliced cheese and stainless steel samples. The vertical distance between the emitters and the sample was 13 cm (5.1 in.).

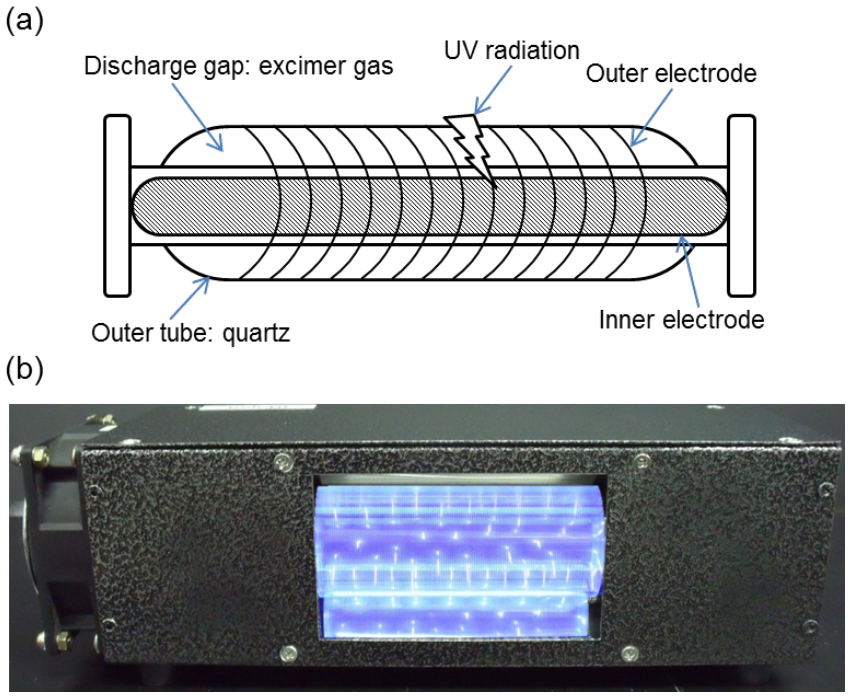


FIG. 1. Schematic diagram (a) and an experimental device (b) of the 222-nm KrCl excilamp used in this study.

2.2. Irradiance measurement

Radiation intensities of the KrCl excilamp and LP Hg lamp were measured using an UV fiber optic spectrometer (AvaSpec-ULS2048; Avantes, Eerbeek, Netherlands). Lamp dosages were calculated by multiplying irradiance values by the irradiation times.

2.3. Application of 222-nm krypton-chlorine excilamp to control foodborne pathogens on sliced cheese surfaces and mechanism of the bactericidal action

2.3.1. Bacterial cultures and cell suspension

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. All strains of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* was cultured individually in 5 ml of TSB at 37 °C for 24 h and harvested by centrifugation (4,000 × 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, and *L. monocytogenes* was resuspended in 5 ml of phosphate-buffered saline (PBS; 0.1 M) and poured into a crystal grade polystyrene petri dish (15mm [height] by 60mm [inside diameter]).

2.3.2. 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. 0.1 ml 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. Every 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.

2.3.3. KrCl excilamp and LP Hg lamp treatment

Inoculated microbiological media and sliced cheese were treated at room temperature(21±1°C) with 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 9s).

For the inactivation mechanism study, 5 ml of cell suspensions placed in petri dishes were treated with two lamp systems at 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.

2.3.4. 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, *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, tsamples 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 case of cFDA conversion value, each pathogen cell suspension with an OD_{680} of approximately 0.2 were 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.

2.4. Susceptibility of Escherichia coli O157:H7 grown at low temperatures to the krypton-chlorine excilamp

2.4.1. Growth condition and Growth curves

Three strains each of *E. coli* O157:H7 (ATCC 35150, ATCC 43889, ATCC 43890) were obtained from the bacteria culture collection of Seoul National University (Seoul, South Korea). A single colony cultivated from frozen stocks on TSA was inoculated into 5 ml of TSB, incubated for

overnight in a shaking incubator at 37°C and 250 rpm. Optical density at 600 nm of the cell suspension was determined and adjusted to 0.1 (ca. 10^6 CFU/ml) with 50 ml TSB. Approximately equal populations of cell suspension were combined with TSB, and incubated in a shaking incubator at different temperature (15, 25, and 37^{o}C) and 250 rpm.

Growth of *E. coli* O157:H7 was determined by withdrawing 200 µl of samples at appropriate time intervals. Absorbance of samples was determined with a spectrofluorophotometer at 600 nm (data not shown). Populations of viable cells withdrawn at the adjusted time intervals (based on absorbance data) were enumerated by plating on SMAC agar. Plate counts were used to determine early stationary phase, which was 52, 12, and 7h for 15, 25, and 37^{o}C, respectively.

2.4.2. Cell suspension and Inoculation

Cell suspensions, incubated at different temperatures, were collected by centrifugation at 4,000 × g for 20 min at 4°C. The pellets were resuspended in 0.2% PW. The cell concentration of suspended pellets was 10^{10} to 10^{11} CFU/ml. One-tenth ml of each resuspended cell culture was inoculated by distributing as 10 droplets onto a pieces of stainless steel(2 x 5 cm, No 4)

with a micropipettor. The inoculated stainless steel pieces were dried inside a biosafety hood for 1 h. The final cell concentration was approximately 10^7 to 10^8 CFU/cm^2.

2.4.3. KrCl excilamp and LP Hg lamp treatment

An KrCl excilamp and a 254-nm germicidal lamp using LP Hg lamp was used in this study. Lamp dosages were calculated by multiplying irradiance values by the irradiation times. Inoculated stainless steel was treated at room temperature with the KrCl excilamp or LP Hg lamp at equal dosages of 26.1, 69.6, 113.1, and 156.6 mJ/cm^2. For microbial enumeration, each treated stainless steel piece immediately transferred into a sterile plastic tube (Corning Science Mexico, Reynosa, Mexico) containing 30 ml of sterile 0.2% PW and 3 g glass beads (425–600 μm; Sigma–Aldrich, St. Louis, MO, USA) and homogenized for 2 min using a vortex mixer (WISE Mix VM-10, DAIHAN Scientific Co., Seoul, Korea) at a maximum speed (37).

2.4.4. Bactericidal mechanism

The quantity of malondialdehyde (MDA) was analyzed using the OxiSelect TBARS Assay Kit (Cell BioLabs, Inc., San Diego, CA), following the manufacturer's directions. One milliliter of treated sample was centrifuged at 10,000 x g for 10 min and the supernatant was discarded and the pellet resuspended in PBS containing butylated hydroxytoluene (BHT). Each sample was mixed with 100 µl SDS lysis solution and incubated at room temperature for 5 min. Following incubation, each sample was reacted with 250 µl of TBA reagent at 95^{o}C for 1 h and cooled to room temperature in an ice bath for 5 min. Fluorescence of the sample was measured with a spectrofluorophotometer (Spectramax M2e; Molecular Devices, Sunnyvale, CA) at excitation and emission wavelengths of 540 and 590 nm, respectively.

DNA damage values were observed according to the method described by L. Han et al. (23). Following each treatment, samples were incubated with 100 µg/ml lysozyme at 37^{o}C for 4 h to break the cell envelope. Then, the solutions were incubated with SYBR green I (1:10,000 dilution; Sigma-Aldrich Ltd., Dublin, Ireland) at working concentration (1:1) for 15 min at 37^{o}C. Fluorescence of the aliquot of each sample was measured with a spectrofluorophotometer (Spectramax M2e; Molecular Devices, Sunnyvale, CA) at excitation and emission wavelengths of 485 and 525 nm, respectively.

2.4.5. Membrane composition

Membrane fatty acid profiles of *E. coli* O157:H7 grown at different temperatures were analyzed. *E. coli* O157:H7 was incubated to early stationary phase and collected by centrifugation at 4,000 × g for 20 min at 4°C. Each pellet was subjected to fatty acid extraction as described in MIDI Technical note no. 101 (24). Mixtures of hexane and methyl tert-butyl ether were used to extract the fatty acid methyl esters (FAMEs). FAMEs in the upper phase were analyzed on an Agilent gas chromatograph (model 7890A, Agilent Technologies, Santa Clara, CA, USA) equipped with a split-capillary injector and a flame ionization detector (25). Separations were obtained using a DB-23 column (60 mm x 0.25 mm I. d., 0.25 um, Agilent Technologies). The injector temperature was set at 250^{o}C, the column oven at 50^{o}C for 1 min, followed by increasing at a rate of 15 ^{o}C/min to 130^{o}C, 8 ^{o}C/min to 170^{o}C, and 2 ^{o}C/min to 215^{o}C, and holding for 10 min. Hydrogen, air, and helium were used as the carrier gas, and the flow rate was set to 35 mL/min, 350 mL/min, and 35 mL/min, respectively. The detector temperature was held at 280^{o}C. Supelco 37 component FAME mix (Supelco, Inc., PA, USA) was used for analyzing fatty acid profiles.

2.5. Microbial enumeration

After UV treatment in the medium surface experiment, treated media were immediately incubated at 37°C for 24 h. For food samples, treated sliced cheeses were 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). For stainless steel pieces, each treated stainless steel piece immediately transferred into a sterile plastic tube (Corning Science Mexico, Reynosa, Mexico) containing 30 ml of sterile 0.2% peptone water and 3 g glass beads (425–600 μm; Sigma–Aldrich, St. Louis, MO, USA) and homogenized for 2 min using a vortex mixer (WISE Mix VM-10, DAIHAN Scientific Co., Seoul, Korea) at a maximum speed (26). After homogenization, 1 ml samples were 10-fold serially diluted with 9 ml of sterile 0.2% peptone water and 0.1 ml of stomached or diluted samples were spread plated onto Sorbitol MacConkey (SMAC) agar (Difco). 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).

The overlay (OV) method was used to enumerate injured cells of *S.* Typhimurium and *L. monocytogenes* (27). TSA was used as a nonselective medium to repair injured cells. One hundred microliters of the appropriate dilutions was spread plated onto TSA medium, and the plates were incubated at 37°C for 2 h to allow injured cells to resuscitate (28). 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 (29). 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.

2.6. Statistical analysis

All experiments were duplicate-plated and replicated three times. All data were analyzed by the analysis of variance (ANOVA) procedure of the Statistical Analysis System (SAS Institute, Cary, NC, USA) and mean values were separated using Duncan's multiple-range test. Significant differences in the processing treatments were determined at a significance level of $p = 0.05$.

III. RESULTS

3.1. Application of 222-nm krypton-chlorine excilamp to control foodborne pathogens on sliced cheese surfaces and mechanism of the bactericidal action

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

Fig. 2 shows typical spectral irradiance of 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 222-nm excilmap. 254-nm LP Hg lamp need 1 min to reach maximum irradiance; however, 222-nm excilmap needed no warm-up time and the radiant power was maintained within range of 96 to 100 % (Fig. 3). Fig. 4 shows variation of radiation output according to the air temperature. Radiation intensity of LP Hg lamp was highest at around 30 to 35 °C, and the intensity greatly decreased at refrigeration temperatures. Conversely, the output of 222-nm excilamp was not affected by the ambient temperature (from 0 to 45 °C).

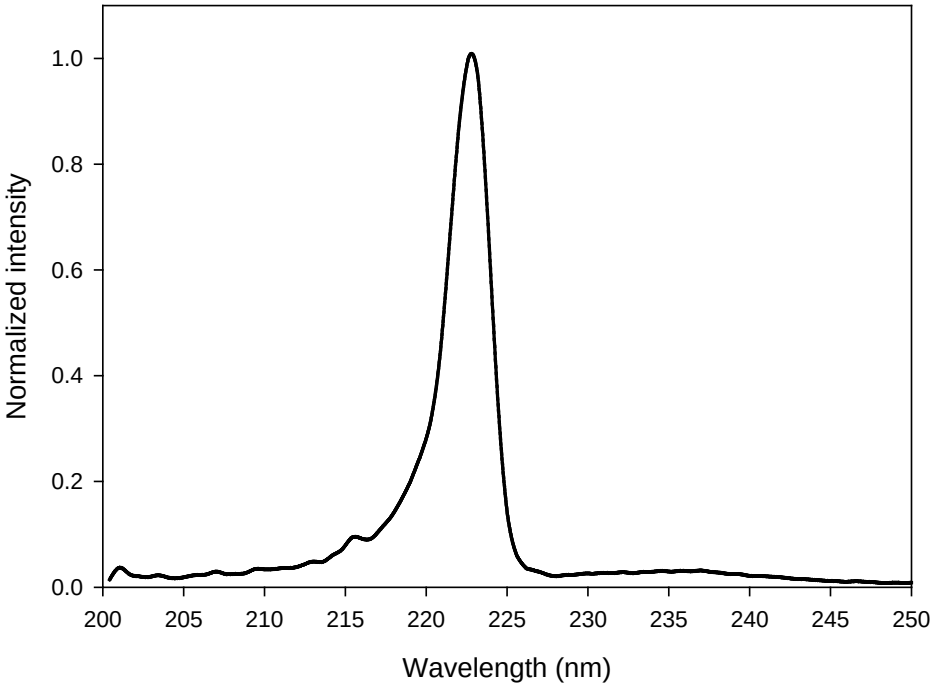


Fig. 2. Emission spectrum of the 222-nm KrCl excilamp.

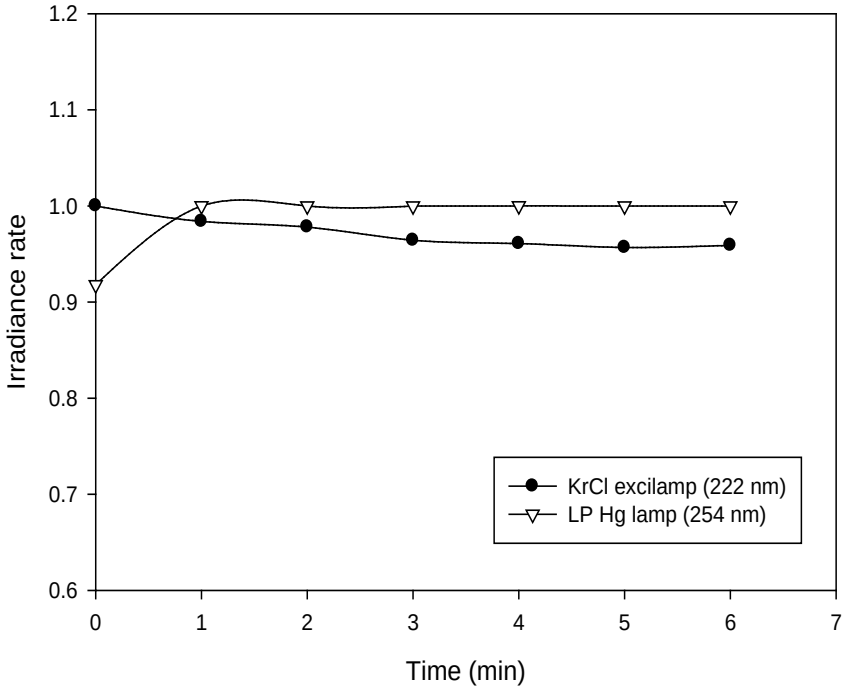


Fig. 3. Comparison between KrCl excilamp and LP Hg lamp for warm-up time.

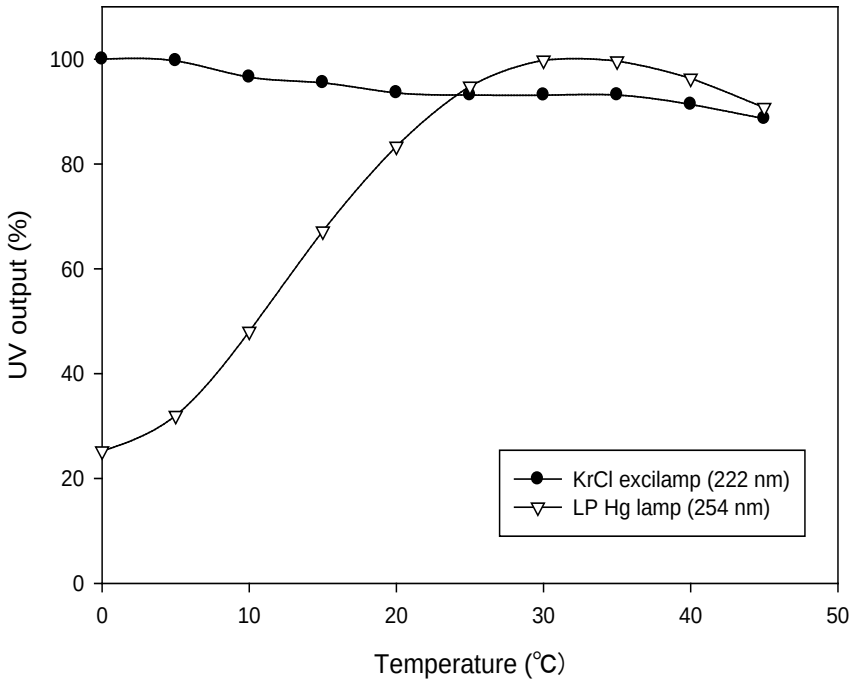


Fig. 4. Comparison between KrCl excilamp and LP Hg lamp for variation of intensity according to temperature.

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

Log reductions of foodborne pathogens on sliced cheese samples after treating 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 KrCl excilamp at 2.61 mJ/cm^2 for three

pathogens. With regard to the 254-nm LP Hg lamp, the pathogen reductions ranged from 0.99 to 1.33 log CFU/g at same dose of 2.61 mJ/cm^2.

3.1.3. Resuscitation of UV-injured cells

Table 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 Hg 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 at 2.61 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 the selective agar versus those on the agar used for recovery over the entire range of treatment dose (Table 2).

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

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

Treatment type	Dose (mJ/cm2)	Log reduction [log10 (N0/N)]a by organism and selection medium											
		E. coli O157:H7				*S.* Typhimurium				*L. monocytogenes*			
		SMAC		SPRAB		XLD		OV-XLD		OAB		OV-OAB	
LP Hg 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 exilamp (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 capital 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.

3.1.4. 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, the overall result patterns of results 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 Hg 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 Hg lamp treatment, indicating that 222-nm UV irradiation resulted in increasing perturbation of enzymatic activity in pathogenic bacteria cells (Table 3).

TABLE 3. Level of membrane damages and intracellular enzyme inactivation of 254-nm LP Hg lamp or 222-nm KrCl excilamp treated cells inferred from the PI uptake and cFDA conversion tests

		Microorganism					
Value [a]	Treatment type	*E. coli* O157:H7		*S.* Typhimurium		*L. monocytogenes*	
PI uptake	Untreated control	0±0	A	0±0	A	0±0	A
	254-nm LP Hg lamp	0.23±0.06	A	0.41±0.15	A	1.44±0.39	A
	222-nm KrCl exilamp	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 Hg lamp	188.21±33.22	A	359.42±70.18	B	259.25±25.61	B
	222-nm KrCl exilamp	988.37±245.44	B	2486.17±273.21	C	3847.43±130.37	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}| .

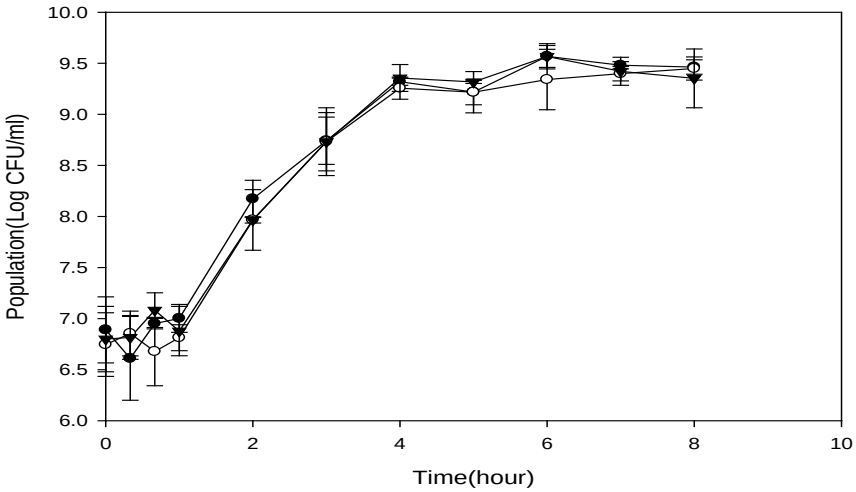
3.2. Susceptibility of Escherichia coli O157:H7 grown at low temperatures to the krypton-chlorine excilamp

3.2.1. Growth curves of E. coli O157:H7 after growing at different temperatures

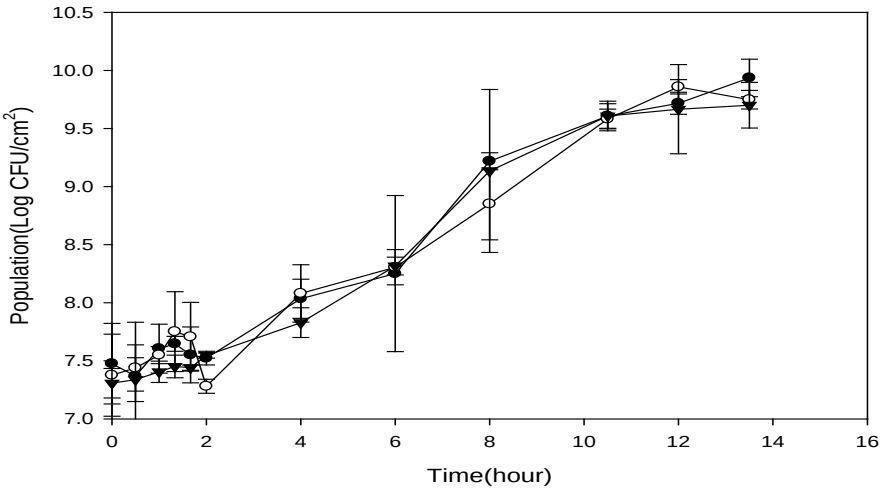
Temperature has a significant effect on the growth of *E. coli* O157:H7 (Fig.5). Lag time and growth rate were significantly affected by temperature while final cell numbers were not significantly different. Lag time increased as temperature decreased. The mean lag times were 0.9, 2.7, and 11.5 h for *E. coli* O157:H7 grown at 37, 25, and 15 ^{o}C, respectively. On the other hand, growth rate decreased as temperature decreased. The incubation times for the pathogens to enter stationary phase were 6, 12, and 42 h for 37, 25, and 15 ^{o}C, respectively. It is widely accepted that stationary phase is the stage most resistant to at various stresses. D. Jenkins et al. (30) found that stationary phase cells are extremely resistant to various stresses and inimical processes because the rpoS gene is expressed at stationary phase, which is associated with many stress resistance gene such as appR (acid phosphatase expression), nur (near UV resistance) and csi(carbon starvation) (31). Therefore, I investigated the resistance of *E. coli* O157:H7

to the LP Hg lamp or KrCl exilamp at stationary phase. Final cell numbers at the stationary phase were about $10^{10\text{-}11}$ CFU/ml regardless of growth temperature.

(A)


Population(Log CFU/ml)
Time(hour)
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
0
2
4
6
8
10

(B)


Population(Log CFU/cm2)
Time(hour)
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
0
2
4
6
8
10
12
14
16

(C)

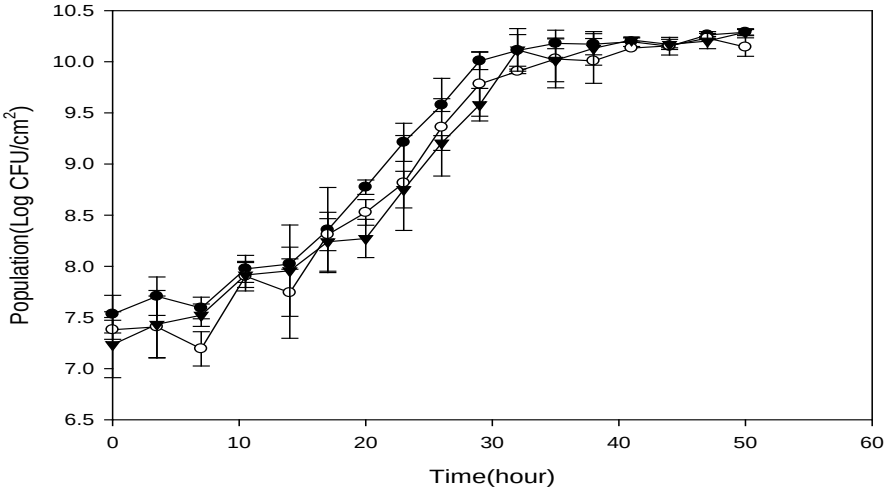


Fig.5. Growth curves of *E. coli* O157:H7 35150 (●), ATCC 43889 (○), and ATCC 43890 (▼) grown at 37ºC (A), 25ºC (B), and 15ºC (C)

3.2.2. Reduction of E. coli O157:H7

Growth temperature had a significant effect on the inactivation of *E. coli* O157:H7 by KrCl excilamp treatment while significant differences were not observed in LP Hg lamp treated cells (Table 4). Reduction levels (log CFU/cm^2) of *E. coli* O157:H7 subjected to KrCl excilamp exposure increased as growth temperature decreased at same dose treatment (mJ/cm^2). When *E. coli* O157:H7, grown at 37, 25, and 15^{o}C were, subjected to the KrCl excilamp at an dose of 156.6 mJ/cm^2, the reduction levels (log CFU/cm^2) enumerated on SMAC were 2.48, 3.12, and 4.74, respectively. This same tendency was observed when *E. coli* O157:H7 was enumerated on SPRAB, and significant generation of injured cells was not observed (< 1 log) for all treatment conditions. At the identical dose, KrCl exilamp treatment more effectively inactivated *E. coli* O157:H7 than did LP Hg lamp treatment.

Table 4. Reduction (log CFU/cm^2) of *E. coli* O157:H7 subjected to LP Hg lamp and KrCl exilamp treatment after growth at 37ºC, 25ºC, and 15ºC.

	Dose	SMAC			SPRAB		
	(mJ/cm^2)	37ºC	25ºC	15ºC	37ºC	25ºC	15ºC
LP Hg lamp	26.1	0.25 ± 0.08 Aa	0.23 ± 0.21 Aa	0.11 ± 0.20 Aa	0.21 ± 0.03 Aa	0.24 ± 0.12 Aa	0.26 ± 0.20 Aa
	69.6	0.53 ± 0.28 ABa	0.29 ± 0.25 Aa	0.23 ± 0.28 Aa	0.26 ± 0.05 ABa	0.63 ± 0.23 Ba	0.56 ± 0.28 ABa
	131.3	0.65 ± 0.30 ABa	0.78 ± 0.24 Ba	0.76 ± 0.55 Ba	0.35 ± 0.06 BCa	0.97 ± 0.14 Ca	0.88 ± 0.55 ABa
	156.6	0.74 ± 0.21 Ba	1.15 ± 0.22 Ba	0.94 ± 0.47 Ba	0.42 ± 0.07 Ca	1.31 ± 0.06 Db	1.14 ± 0.47 Bb
KrCl excilamp	26.1	0.79 ± 0.15 Aa	1.01 ± 0.27 Aa	1.19 ± 0.50 Aa	0.81 ± 0.21 Aa	1.10 ± 0.44 Aa	1.15 ± 0.18 Aa
	69.6	1.46 ± 0.43 Ba	1.44 ± 0.48 Aa	1.83 ± 0.38 Aa	1.25 ± 0.49 ABa	1.85 ± 0.83 ABa	1.74 ± 0.20 Ba
	131.3	1.72 ± 0.38 Ba	2.47 ± 0.11 Bb	2.79 ± 0.20 Bb	1.69 ± 0.19 BCa	2.32 ± 1.09 ABa	2.73 ± 0.46 Ca
	156.6	2.48 ± 0.26 Ca	3.12 ± 0.21 Cb	4.74 ± 0.33 Cc	2.17 ± 0.12 Ca	2.80 ± 0.64 Ba	4.19 ± 0.32 Db

[a]Values in the same column for each treatment followed by the same uppercase letter are not significantly different ($p > 0.05$)

[b]Values in the same row for each enumeration medium followed by the same lowercase letter are not significantly different ($p > 0.05$)

3.2.3. Lipid oxidation and DNA damage

Growth temperature had a significant effect ($p < 0.05$) on lipid oxidation and DNA damage values of KrCl excilamp-treated *E. coli* O157:H7 (Table 5, Table 6). The lipid oxidation values were 10.74, 14.26, and 28.97 for 37ºC, 25ºC and 15ºC grown cells, respectively. The DNA damage values were 1.06, 1.15 and 4.64 for 37ºC, 25ºC and 15ºC grown cells, respectively. On the other hand, growth temperature had no significant effect ($p > 0.05$) on either lipid oxidation or DNA damage of LP Hg lamp-treated *E. coli* O157:H7. Lipid oxidation values were significantly higher ($p < 0.05$) for KrCl excilamp- treated cells than for LP Hg lamp-treated cells while DNA damage values were significantly higher ($p < 0.05$) for LP Hg lamp-treated cells than for KrCl excilamp-treated cells.

Table 5. Lipid oxidation values of *E. coli* O157:H7 subjected to KrCl excilamp and LP Hg lamp treatment after growth at 37ºC, 25ºC and 15ºC[a,b]

	37ºC	25ºC	15ºC
LP Hg lamp	0.20 ± 0.59 Aa	0.38 ± 0.19 Aa	0.35 ± 0.23 Aa
KrCl excilamp	10.74 ± 2.00 Ba	14.26 ± 0.54 Bb	28.97 ± 2.11 Bc

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

Table 6. DNA damage values of *E. coli* O157:H7 subjected to KrCl excilamp and LP Hg lamp treatment after grown at 37°C, 25°C and 15°C[a,b]

	37°C	25°C	15°C
LP Hg lamp	6.61 ± 1.64 Aa	8.59 ± 1.34 Aa	8.41 ± 2.03 Aa
KrCl excilamp	1.06 ± 0.82 Ba	1.15 ± 0.68 Ba	4.64 ± 0.89 Bb

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

3.2.4. Membrane fatty acid composition

The ratio of unsaturated fatty acids (USFA) to saturated fatty acids (SFA) was higher for the *E. coli* O157:H7 membrane grown at lower rather than higher temperatures (Fig. 6). Relative contents (%) of palmitoleic acid of the cell membrane was 7.2, 24.1, and 39.3 for *E. coli* O157:H7 grown at 37ºC, 25ºC, and 15ºC, respectively. On the other hand, relative content of lauric, myristic, and palmitic acids decreased as growth temperature decreased. The decrements (%) were 16.4, 7.8, and 13.2 for lauric, myristic, and palmitic acid, respectively, when growth temperature decreased from 37ºC to 15ºC. Growth temperature of *E. coli* O157:H7 had no significant effect ($p > 0.05$) on the relative content of the rest of the fatty acids.

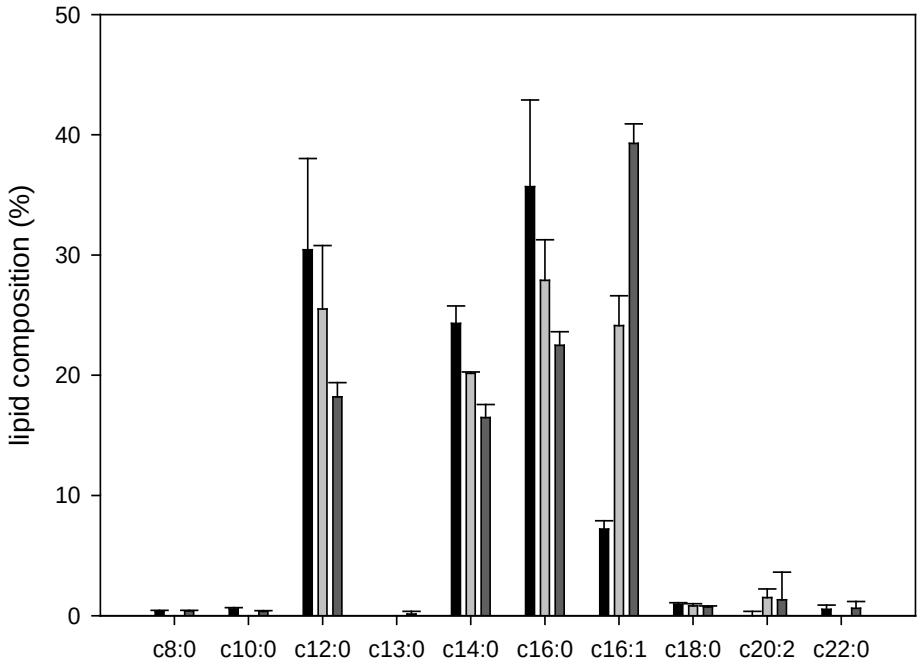


Fig. 6. Membrane fatty acid composition of *E. coli* O157:H7 after grown at 37ºC (■), 25ºC (■), and 15ºC (■).The data represent the mean ± standard deviation (SD, n=3)

IV. DISCUSSION

4.1 Application of 222-nm krypton-chlorine excilamp to control foodborne pathogens on sliced cheese surfaces and mechanism of the bactericidal action

The slicing step has been known for many years to serve as a vehicle for major foodborne pathogens in various deli foods (32). According to a recent FDA report, deli slicers have been identified as the primary source of *L. monocytogenes* cross-contamination in RTE foods (33). Since the 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 the conventional LP Hg lamps.

As shown in Fig. 3, just after the 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-known that the radiant power of LP Hg lamps is very sensitive to ambient thermal changes (34). Figure 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 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 psychrophilic 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 (35). In the present study, the extent to which sublethally injured pathogens survived after 222- or 254-nm UV treatments was assessed by using 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 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 (36, 37). However, there were no significant ($P > 0.05$) differences in levels of *S.* Typhimurium cells enumerate 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 can effectively inactivate all major 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 (38). Additionally, the *L. monocytogenes* generate 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 (39). Interestingly, after treatment of 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 the cell damage caused by the 222-nm UV radiation is even more serious than that of 254-nm UV lights; however, further evidences is needed to validate that hypothesis. It is well known that LP Hg lamp emits 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 (40). Light-dependent photo-reactivation which uses a single enzyme called photolyase is particularly effective to reverse the formation of a pyrimidine dimer (41). 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 spectrum with a peak at 222-nm targeting the protein molecules of microorganism. Clauß and Grotjohann (42) 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 the bacterial outer membrane proteins (43). Yin et al. (21) 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 the microorganisms.

In this study, to clarify the mechanism of the enhanced lethal effect of 222-nm UV irradiation, the membrane and intracellular protein damages of *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 (44). By contrast, cFDA, a lipophilic non-fluorescent precursor that diffuses freely across cell membranes, is widely used for the assessment of nonspecific enzymatic activity in cells. In cytoplasm, cFDA is converted by nonspecific esterases into a polar, membrane-impermeant green fluorescent compound, carboxyfluorescein (45). As shown in Table 3, the

improved inactivation of foodborne pathogens by the 222-nm KrCl excilamp might be related to disruptions of the 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 damages and a lower rate of photo-reactivation probably give rise to the enhanced disinfection effect of UV light at 222-nm.

4.2. Susceptibility of Escherichia coli O157:H7 grown at low temperatures to the krypton-chlorine excilamp

In the present study, we identified that resistance of *E. coli* O157:H7 to KrCl exilamp treatment was significantly different depending on growth temperature. Reduction levels (log CFU/cm^2) of *E. coli* O157:H7 on stainless steel was higher for cells grown at lower temperature (Table 4). I assumed that specific bactericidal targets of *E. coli* O157:H7, such as DNA, proteins, or lipids are affected by growth temperature. At first, I investigated protein photodegradation, which has been revealed as a one of the bactericidal mechanisms of the KrCl excilamp. (42), However, significant differences

were not observed when quantified protein concentrations of *E. coli* O157:H7 after growing it at various temperatures (data not shown). Therefore, protein degradation is not sufficient to describe different reduction levels of *E. coli* O157:H7 grown at different temperatures. Recently,. M. Gomez et al. (46) found that KrCl excilamp can utilize advanced oxidation processes. Also, lipid oxidation was observed in KrCl excilamp-treated samples, which was significantly higher than that of LP Hg lamp-treated samples. Moreover, lipid oxidation levels of *E. coli* O157:H7 subjected to KrCl exilamp treatment increased as growth temperature decreased. Simultaneously, DNA damage levels were not significantly different relative to growth temperature. From the results above, I postulate that differing lipid oxidation levels is the major reason for different reduction levels of *E. coli* O157:H7 grown at different temperatures when subjected to KrCl excilamp treatment. In contrast to the KrCl excilamp, reduction of *E. coli* O157:H7 grown at different temperatures was not significantly different when subjected to the LP Hg lamp. Because the results can be attributed to low reduction levels (< 1.5 log), confirmed that significant differences were not observed at high reduction levels (about 4 log) relative to the growth temperature (data not shown). The major bactericidal mechanism of LP Hg lamps is known to be DNA damage (40), and identified that DNA damage

values were significantly larger for LP Hg lamp treated samples compared to KrCl excilamp treated samples regardless of growth temperature. Because the amount of DNA did not vary according to growth temperature, damage to DNA of *E. coli* O157:H7 subjected to the LP Hg lamp did not differ significantly according to growth temperatures. This is why reduction levels by the LP Hg lamp were not significantly different ($p > 0.05$) relative to growth temperature.

Membrane fluidity of *E. coli* O157:H7 is also known to be important for adapting to a low temperature environment. I identified that palmitoleic acid content was significantly larger for *E. coli* O157:H7 grown at 15℃ than that grown at 37℃. On the other hand, saturated fatty acid, lauric acid, myristic acid, and palmitic acid content was significantly reduced for *E. coli*O157:H7 grown at 15℃ compared to that grown at 37℃. Because cis-unsaturated bonds make a "kink" into the acyl chain, contributing to molecular packing and molecular motions (47). membrane fluidity increases as the ratio of unsaturated to saturated fatty acid increases. However, lipid oxidation reacts more easily as the degree of *cis*-unsaturated fatty acid increases. Therefore, membrane lipids of *E. coli* O157:H7 grown at low temperatures oxidized more easily than those grown at 37℃, and consequently were more susceptible to KrCl excilamp treatment.

V. Conclusion

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 the 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 Table 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 irradiances. In other words, the efficacy of 222-nm UV-C irradiation was greatly affected by the 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.

At the second experiments, *E. coli* O157:H7 adapt and survive at low growth temperatures. As a result, membrane lipid composition was altered to ensure membrane fluidity. Because unsaturated fatty acid composition of *E. coli* O157:H7 grown at low temperature increased, lipid oxidation level increased when *E. coli* O157:H7 was subjected to KrCl excilamp treatment. Consequently, resistance of *E. coli* O157:H7 to the KrCl excilamp decreased as growth temperature decreased while the resistance was not significantly different for the LP Hg lamp. Because *E. coli* O157:H7 is a major foodborne

pathogen which causes severe risk to public health and to the economy, adequate treatment is necessary to control this pathogen. The KrCl excilamp is a promising non-thermal treatment to replace conventional LP Hg lamps. However, excessive treatment causes quality degradation of treated samples as well as leads to economic loss. Therefore, adequate treatment time for the KrCl excilamp should be determined considering the growth temperature of *E. coli* O157:H7.

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VII. 국문초록

본 연구는 222 nm 의 크립톤 클로라인 엑실램프와 254 nm 의 낮은 압력 수은 램프의 기본적인 특성 차이 및 주요 식중독 균에 대한 저감화 효율을 배지와 치즈 표면에서 비교하였다. 크립톤 클로라인 엑실램프는 온도에 따라서 조사하는 빛의 세기에 영향을 받지 않으며 특히 0 에서 10 도의 낮은 온도에서 조사하는 빛의 세기가 달라지지 않는다. 선택배지와 치즈 표면에 *Escherichia coli* O157:H7, *Salmonella enterica* serovar Typhimurium 과 *Listeria monocytogenes* 를 접종시킨 후 두 램프를 같은 에너지 양만큼 조사하였다. 그 결과, 모든 균에서 222 nm 에서 254 nm 에 비해 유의적으로 더 높은 저감화 효과가 나타났고($P < 0.05$) 추후에 회복할 수 있는 손상된 균들이 많이 발생하지 않았다. 222 nm 의 크립톤 클로라인 엑실램프의 살균기작을 형광염색방법으로 측정한 결과, 세포막 및 효소 손상이 주요한 살균 기작임을 알 수 있었다. 그리고 또한 본 연구에서는 성장온도에 따른 *Escherichia coli* O157:H7 의 두 램프 처리에 대한 저항성을 연구하였다. 성장온도가 내려갈 수록, *E. coli* O157:H7 은 유도기의 시간이

늘어났으며 성장 속도도 감소하였다. 성장온도에 관계없이 크립톤 클로라인 엑실램프는 낮은 압력의 수은 램프에 비해 더 높은 정지상의 *E. coli* O157:H7 의 살균효과를 보였다. 또한, 크립톤 클로라인 엑실램프 처리는 성장온도가 내려갈 수록 더 높은 저감화효율을 보였다. 이에 반해, 낮은 압력의 수은램프는 성장온도에 따른 저감화양상에 변화가 없었다. TBARS 및 SYBR green 을 통한 살균 기작을 본 결과, 크립톤 클로라인 엑실램프 처리에서는 성장온도가 내려갈 수록 지방산화가 더 많이 일어나며 낮은 압력의 수은 램프에 비해 더 높은 지방산화를 보였다. DNA 손상정도는 낮은 압력의 수은램프 처리에서 크립톤 클로라인 엑실램프에 비해 더 높았지만, 성장온도에 따른 차이는 발생하지 않았다. 결과적으로 *E. coli* O157:H7 의 크립톤 클로라인 엑실램프에 대한 저항성은 성장온도가 내려갈수록 불포화지방산의 조성이 많아져 지방산화가 더 많이 일어나기 때문에 감소한다. 본 연구에서의 결론은 222 nm 파장의 자외선은 식품관련 표면살균에 있어서 기존의 낮은 압력의 수은램프를 충분히 대체할 수 있다는 것이다.

주요어: 크립톤 클로라인 엑실램프, 식품매개 병원균, 표면살균,
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