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

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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-

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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

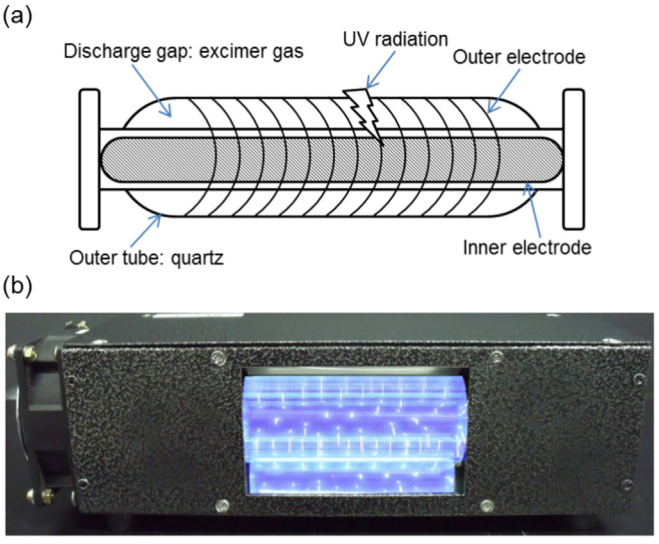

Fig. 1. Schematic diagram (a) and photograph (b) of the experimental 222-nm KrCl excilamp used in this study.