



Bactericidal effect of 266 to 279 nm wavelength UVC-LEDs for inactivation of Gram positive and Gram negative foodborne pathogenic bacteria and yeasts



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ABSTRACT

Recently, UVC-LED technology has been validated as an alternative to irradiation with conventional mercury UV lamps. In this study, we sought to determine primary factors affecting reduction trends shown in several microorganisms. Four major foodborne pathogens (*Escherichia coli* O157:H7, *Salmonella* spp. *Listeria monocytogenes*, *Staphylococcus aureus*) and spoilage yeasts (*Saccharomyces pastorianus*, *Pichia membranaefaciens*), important to the brewing industry, were inoculated onto selective and non-selective media in order to investigate reduction tendencies at 4 different peak wavelengths (266 to 279 nm). As irradiation dose increased, inactivation levels for every microorganism were enhanced, but there were different UV-sensitivities in Gram positive bacteria (GP), Gram negative bacteria (GN), and yeasts (Y). Loss of membrane integrity measured by propidium iodide (PI) increased as peak wavelength increased for every microorganism studied. Similar results were observed in membrane potential measured by DiBAC₄(3). However, there were contrasting results which showed that greater DNA damage occurred at a lower peak wavelength as measured by Hoechst 33,258. The level of DNA damage was strongly related to trends of microbial inactivation. This study showed that even though membrane damage was present in every microorganism studied, DNA damage was the primary factor for inactivating microorganisms through UVC-LED treatment.

1. Introduction

Minimally processed fresh produce has become popular due to consumers' increasing interest in wellness, a trend not unnoticed by the food industry (Sivapalasingam, Friedman, Cohen, & Tauxe, 2004). In this cultural context, non-thermal pasteurization technology, which can fulfill the goal of minimizing overall quality damage such as texture change and flavor deterioration, could be an alternative method in order to overcome some flaws inherent in conventional thermal treatment. As one of the non-thermal inactivation interventions, ultraviolet light irradiation is one of the methods that has potential to control foodborne pathogens (Bintsis, Litopoulou-Tzanetaki, & Robinson, 2000). UV light falls within the electromagnetic wave spectrum ranging from 100 to 380 nm, and is classified as UV-A, UV-B, and UV-C regions according to wavelength range (Guerrero-Beltran & Barbosa-Canovas, 2004). Of these, UV-C has the maximum bactericidal effect and mercury lamps have been used to generate light

in the UV-C region. Because UV lamps have some disadvantages such as leakage of mercury, necessity of warm-up time for maximum power and temperature dependency, light emitting diode (LED) type UV-C generating technology has been developed (Shin, Kim, Kim, & Kang, 2016). Temperature-independent irradiance output and prompt maximum power were observed in using UVC-LEDs while UVC lamps were affected by temperature and operating time. Also, small size UVC-LED modules compared to mercury lamps could be expeditious in applying into various device shapes. Above all, the peak wavelength of UV-LEDs can be manipulated to emit the target wavelength, so that maximum UV-light absorbance by DNA (ranging from 260 to 265 nm) (Kalisvaart, 2004) can be precisely adjusted with this new technology, whereas UV lamps can only emit a peak wavelength of 254 nm.

Formation of photoproducts by UV irradiation is generally known to be the key bactericidal effect of this treatment. Especially, when DNA absorbs UVC light, nucleic acid damage induced by pyrimidine dimer formation occurs, which leads to bacterial cell death provided irra-

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diated DNA molecules generate a sufficient level of dimers (Bintsis et al., 2000). Also, DNA destruction can occur by means of reactive oxygen species (ROS) such as hydroxyl radicals and hydrogen peroxide generated by UV irradiation (Cadet & Wagner, 2013). The ROS stress inside bacterial cells causes damage to not only cellular components, but also nucleic acids so that changes in the pattern of gene expression arises (Scharffetter-Kochanek et al., 1997). Some research studies reported that UV treatment induced cell membrane damage which was assessed by the fluorescent dye propidium iodide (PI) (Ha & Kang, 2013, 2014; Schenk, Raffellini, Guerrero, Blanco, & Alzamora, 2011). Membrane damage induced by UV can be a potential mechanism of bactericidal inactivation.

Fluorescent dyes such as propidium iodide (PI), Bis-(1,3-Dibutylbarbituric acid) Trimethine oxonol (DiBAC₄(3)), and Hoechst staining are powerful techniques to investigate physiological properties during cellular changes. The fluorescent labelling dyes can be used in combination with flow cytometry, and the signals are analyzed and sorted in accordance with changes in membrane potential, membrane integrity, enzymatic activity and so forth.

In the current study, we evaluated not only the inactivation efficacy of UVC-LEDs but also the inactivating mechanism by using the fluorescent dyes in terms of peak wavelengths and types of micro-organisms: Gram positive bacteria (GP), Gram negative bacteria (GN), and yeasts (Y).

2. Materials and methods

2.1. Experimental setup

Electronic printed circuit boards (PCB) connected to UVC-LED modules (LG Innotek Co., Seoul, Korea) corresponding to four separate peak wavelengths (266, 270, 275, and 279 nm) were utilized (Fig. 1). Averaged UVC-LED voltages when appropriate currents (23 mA for 266 nm, 20 mA for 270, 275, 279 nm) were applied ranged from 6.36 V to 6.92 V and nominal power consumptions were 0.16 W (266 nm) or 0.13 W (270, 275, 279 nm). The four LEDs were arranged in the '4 corners' array which indicates 6 cm distance between each LED module and 4 cm distance between PCB and petri dish based on previous investigations conducted in our laboratory (Kim, Kim, & Kang, 2016; Shin et al., 2016). This arrangement and distances were optimized to accommodate a 90 mm diameter petri dish for equally distributed irradiance with high irradiance intensity given the petri factor. Because UVC-LEDs emit parallel and collimated ultraviolet light while radial light is generated by conventional UV lamps, gradient intensity distribution over the surface is developed. Therefore, the petri factor, which indicates averaged surface irradiance, should be taken into consideration in UVC-LEDs treatment. In this research the petri factor of the 4 corners array was over 0.9 (90%) which indicates nearly uniform distribution of light across the petri dish surface (Bolton & Linden, 2003). Constant electric current was provided by a DC power supply (TPM series, Toyotech; Incheon, Korea) which applied 23 mA for a 266 nm PCB, and 20 mA for 270, 275, and 279 nm PCBs.

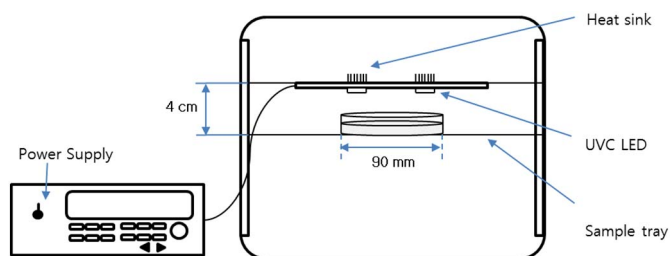


Fig. 1. Schematic diagram of the UVC-LEDs irradiation system in Seoul National University.

2.2. Irradiance measurements

Irradiance intensity of the UV-LED modules was measured with a spectrometer (AvaSpec-ULS2048-USB2-UA-50, Avantes; Apeldoorn, Netherlands) which was able to calibrate within a range of 200 to 400 nm which includes the entire UV wavelength spectrum. For sample treatment, the distance between PCB with LEDs and an optical probe was 4 cm and irradiance value at the peak wavelength was measured. Irradiance for every 5 mm of area corresponding to the dimensions of a petri dish was measured. Each measured intensity was divided by maximum irradiance value and averaged to obtain the petri factor. The final irradiance value was normalized by multiplying maximum intensity by the petri factor.

2.3. Bacterial and yeast strains

Three strains each of *Escherichia coli* O157:H7 (ATCC 35150, ATCC 43889, and ATCC 43890), *Listeria monocytogenes* (ATCC 15313, ATCC 19111, and ATCC 19115), *Staphylococcus aureus* (ATCC 10390, ATCC 12598, and ATCC 27644), and *Salmonella* Enteritidis PT 30 (ATCC BAA1045) were obtained from the Bacterial Culture Collection at Seoul National University (Seoul, Korea). *Salmonella* Senftenberg (KVCC 0590) and *Salmonella* Tennessee (KVCC 0592) were obtained from the Korea Veterinary Culture Collection (Gimcheon, Korea). *Pichia membranaefaciens* (KCCM 12470) and *Saccharomyces pastorianus* (KCCM 11523) were obtained from the Korean Federation of Culture Collections (Seoul, Korea). Stock cultures were grown in Tryptic Soy Broth (TSB; Difco, Becton Dickinson and company; Sparks, MD, USA) at 37 °C for 24 h for bacteria and in Nutrient Yeast Dextrose (NYD) broth (1% nutrient broth + 0.6% yeast extract + 1.3% glucose) at 25 °C for 48 h for yeasts and stored at −80 °C (0.7 ml of TSB culture with 0.3 ml of sterile 50% glycerol solution). To obtain working cultures, bacteria and yeasts were each streaked onto Tryptic Soy Agar (TSA; Difco) and Potato Dextrose Agar (PDA; Difco), respectively, and incubated at 37 °C for 24 h and 25 °C for 48 h, respectively, stored at 4 °C and used within 3 days.

2.4. Culture preparation

Each bacterial strain (*E. coli* O157:H7, *Salmonella* spp., *L. monocytogenes*, and *S. aureus*) was cultured in 5 ml TSB at 37 °C for 24 h. Yeasts (*P. membranaefaciens* and *S. pastorianus*) were incubated in 5 ml NYD broth at 25 °C for 48 h. Each bacterial and yeast strain was cultured to stationary growth phase and harvested by centrifugation at 4000 × g for 20 min at 4 °C and the supernatant discarded. Obtained bacteria or yeast cell pellets were resuspended in sterile 0.2% Bacto peptone (Bacto, Becton, Dickinson and Company; Sparks, MD, USA) and centrifuged. This washing procedure was performed three times. Final pellets were resuspended in 9 ml peptone water (PW), corresponding to approximately 10⁸ to 10⁹ CFU/ml for bacteria and 10⁶ to 10⁷ for yeasts. Resuspended pellets of each strain of all bacterial pathogen species were combined to constitute a 3-pathogen mixed culture cocktail. A mixed culture cocktail comprised of the two yeast strains was similarly prepared.

2.5. Inoculation

In order to set a control solution, mixed bacteria culture cocktails were 10-fold serially diluted three times (10^{−3} dilution) and yeast suspensions were 10-fold (10^{−1}) diluted with 0.2% sterile PW resulting in a final concentration of approximately 5–6 log CFU/ml. For inoculation, the culture suspensions were further serially decimal diluted with 0.2% sterile PW to obtain countable colonies. One-tenth ml aliquots of selected diluents were spread-plated onto selective or nonselective media. Sorbitol MacConkey Agar (SMAC; Difco), Xylose Lysine Desoxycholate Agar (XLD; Difco), Oxford Agar Base with antimicrobial

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