A THESIS FOR THE DEGREE OF MASTER OF SCIENCE

Inactivation of foodborne pathogens by UV-LED treatment

UV-LED 를 이용한 병원균 저감화 연구

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ABSTRACT

Ultraviolet-C light is a widely used sterilization technology as type of lamp. But UV-lamps have several limitations including low activity at refrigeration temperatures, a long warm-up time, and risk of mercury exposure. As an alternative, UV-LEDs have been developed which can render desired wavelengths while UV-type lamps only emit light at 254 nm. In this study, I validated the inactivation efficacy of UV-LED with different wavelengths and compared results with those of conventional UV-lamps. Selective media inoculated with *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* were irradiated with UV-LEDs of 266, 270, 275, and 279 nm in the UV-C spectrum at 0.1, 0.2, 0.5, and 0.7 mJ/cm^2, respectively. The radiation intensity of UV-LEDs was about 4 μW/cm^2, and UV-lamps were covered with polypropylene films to adjust the light intensity similar to those of UV-LEDs. Additionally, I applied UV-LED to sliced cheese at doses of 1, 2, and 3 mJ/cm^2. My results showed that inactivation rates following UV-

LED treatment were much higher than those of UV-lamps at a same intensity. On microbiological media, UV-LED treatment at 266 and 270 nm showed a slightly higher inactivation effect than for other high wavelength modules, but there were no critical differences by wavelength. For sliced cheeses, 4 to 5 log reductions occurred after treatment at a level of 3 mJ/cm^2 for all three pathogens with negligible generation of injured cells.

To determine primary factors affecting reduction trends shown in several bacterial groups, I investigated the efficacy of UV-LED to Gram negative (GN) or Gram positive bacteria (GP). Four major foodborne pathogens (*E. coli* O157:H7, *Salmonella* spp. *L. monocytogenes*, and *S. aureus*) 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 GN and GP. By using fluorescence dyes ($DiBAC_4(3)$, PI, and Hoechst 33258), the effects of UVC-LED treatment on cell membranes or DNA were investigated. Loss of membrane potential measured by $DiBAC_4(3)$ increased as peak wavelength

increased for every bacteria studied. Similar results were observed in membrane integrity measured by PI. However, there were contrasting results which showed that greater DNA damage occurred at a lower peak wavelength as measured by Hoechst 33258. 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 bacterium studied, DNA damage was the primary factor for inactivating microorganisms through UVC-LED treatment.

Keywords: **ultraviolet irradiation, UVC, wavelength, foodborne pathogens, sliced cheese, Light Emitting Diodes (LED), Gram negative bacteria, Gram positive bacteria**

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I. INTRODUCTION

Ultraviolet light covers a wavelength spectrum from 100 to 380 nm and is subdivided into 3 regions by wavelength: UV-A (320-400 nm), UV-B (280-320 nm), and UV-C (200-280 nm) (Guerrero-Beltrán and Barbosa-Cánovas, 2004). Among them, UV-C has the strongest germicidal effect and is widely used in the form of mercury lamps to inactivate microorganisms. However, UV mercury lamps have several critical limitations. First, UV-lamps are fragile and thus present a risk of mercury leakage through breakage when subjected to any shock. Also the warm-up time is long and moreover cannot exhibit maximum efficacy at low temperatures according to a preceding study (Shin et al., 2016). Due to these critical weaknesses of mercury lamps, UV-LED (light emitting diode) technology has been developed recently as an alternative. LED construction commonly consists of a junction between ‘n-type’ and ‘p-type’ semiconducting materials. Current is caused by mobile electrons in the ‘n-type’ layer and carriers are positively charged holes in the ‘p-type’ layer. To emit light, the electrons and holes reconnect at the junction (Dume, 2006). UV-LEDs are of very small size compared to conventional lamps, so they can be easily incorporated into diverse designs of device (Hamamoto et al., 2007). Also, UV-LEDs emit high intensity light as soon as

they are turned on; in other words, there is no warm-up time. Furthermore, Shin et al (2016) demonstrated that UV-LEDs contain no mercury and yield a consistent irradiation output regardless of temperature which makes them effective even under refrigeration. While UV mercury lamps emit only one wavelength (254 nm), UV-LEDs can be configured to emit certain target wavelengths. The most effective germicidal wavelength occurs at a peak of 260-265 nm at which DNA absorbs UV the most (Kalisvaart. 2004; Sharma, 2000), and LEDs can be designed to produce these specific wavelengths.

Listeria monocytogenes is the most important and critical pathogen of concern to the cheese industry. Every year, 1600 people are hospitalized and 260 people die from listeriosis in the United States (http://www.cdc.gov/listeria/outbreaks/index.html). *Listeria* outbreaks are commonly traced to soft cheese made from unpasteurized milk. Soft cheeses contain 45-50% moisture which are generally smooth and easy to ladle or spread. Soft cheeses made from unpasteurized milk are a very high-risk food, and are 50 to 160 times more likely to be contaminated with *Listeria* than those made from pasteurized milk. *Escherichia coli* O157:H7 and *Salmonella* spp. are also important pathogens of concern to the dairy industry. In 2010, 38 persons were infected with *E. coli* O157:H7 in five states of the United States after consuming cheese. Due to this outbreak, 15 people were hospitalized and one person had hemolytic uremic syndrome

(http://www.cdc.gov/ecoli/2010/cheese0157/index.html). Additionally, several cases of salmonellosis have been reported from Canada and the United States that were traced to consumption of cheese (http://www.cdc.gov/mmwr/preview/mmwrhtml/00000370.htm, http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5716a4.htm).

Not only is it risky to use unpasteurized milk as an ingredient to make cheese, cheeses can also be contaminated with pathogens during cheese-making operations. Even if raw milk is pasteurized, it may become contaminated with pathogens when processed in an unsanitary environment (http://www.cdc.gov/foodsafety/specific-foods/listeria-and-food.html). For these reasons, I chose sliced cheese as a target food in this study and their flat and even surface were suitable for applying UV light.

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 irradiated 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 and 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). Also, some research studies reported that UV treatment induced cell membrane damage which was assessed by the fluorescent dye propidium iodide (PI) (Ha and Kang, 2014; Ha and Kang, 2013; Schenk et al., 2011). Membrane damage induced by UV can be a potential mechanism of bactericidal inactivation.

Fluorescent dyes such as PI, Bis-(1,3-Dibutylbarbituric acid) Trimethine oxonol ($DiBAC_4(3)$), 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. By using $DiBAC_4(3)$, membrane potential change, which is a prior symptom of membrane damage, can be assessed since depolarized cells cannot exclude cation/anion molecules such as $DiBAC_4(3)$ so that accumulation of charged particles proceeds (Diaz et al., 2010). Membrane integrity can be assessed by using PI which can enter the interior of cells and bind to nucleic acids only when the cell membrane is damaged. Hoechst, which is able to permeate cell membranes, binds and stains minor grooves of the Adenine-Thymine rich region in double-stranded DNA (ds-DNA) (Pjura et al., 1987) so that the dye can be used as a way to evaluate DNA damage because it prefers ds-DNA.

Since the fluorescent dyes can determine certain mechanisms of changes in cell physiology, they have been used in various research investigations (Ha and Kang, 2014; Ha and Kang, 2013; da Silveira et al., 2002; Siva et al., 2011; Sanchez et al., 2010).

Recently, interest in UV-LED technology has been increasing but the inactivating ability of UV-LED with different wavelengths has never been evaluated before. So in this study, I examined the efficacy of UV-LED to inactivate three major foodborne pathogens, *Escherichia coli* O157:H7, *Salmonella* Typhimurium, and *Listeria monocytogenes*, on solid media and compared its germicidal ability relative to UV-C wavelength. Also, the application of UV-LED to sliced cheese was implemented to assess its suitability as an antimicrobial control intervention. Furthermore, I 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 microorganisms: Gram negative bacteria (GN) and Gram positive bacteria (GP).

II. MATERIALS AND METHODS

2.1. Experimental apparatus

Four UV-LED modules (LG Innotek Co., Republic of Korea), each with a same peak wavelength, were connected onto electronic printed circuit boards (PCB) and each set of PCBs had a different peak wavelength (266, 270, 275, or 279 nm). The specifications of UV-LED modules used in this experiment are indicated in Table 1. DC voltage from a power supply (TPM series, Toyotech, Korea) was applied to all the PCBs in accordance with pre-set available current that provided 23 mA for 266 nm PCB, and 20 mA for 270 nm, 275 nm, and 279 nm PCBs. Based on my previous research study, I elected to use the 4-corners arrangement of modules in this experiment with 6 cm distance between modules and 4 cm distance between LEDs and sample (90 mm-diameter petri dish, sliced cheese) for equally distributed irradiance and optimal LED configuration. The PCBs and inoculated media were placed in a treatment chamber (TH-TG-300, JEIO TECH, Korea). A UV-C lamp (G10T5/4P; 357mm; Sankyo, Japan) which has a nominal output power of 16 W was used in order to compare the two UV emitting sources

for efficacy of pathogen inactivation. The peak wavelength of the UV-lamp was 254.31 nm.

Table 1. Specifications of UV-LED modules used in the experiments.

		Wavelength (nm)			
		266	270	275	279
Current (mA)		23	20	20	20
Voltage (V)	1	6.70	6.49	6.47	6.33
	2	6.92	6.50	6.48	6.37
	3	7.12	6.52	6.47	6.35
	4	6.72	6.50	6.47	6.37

2.2. Irradiance measurements

Intensity of the UV-LED modules was measured with a spectrometer (AvaSpec-ULS2048-USB2-UA-50, Avantes, Netherlands) calibrated for a range of 200 to 400 nm to include the entire UV spectrum. For sample treatment, the distance between collimated LEDs and an optical probe was 4 cm and irradiance value of the spectrum at the peak wavelength was measured. Petri factor, which indicates evenness of UV irradiance reaching the petri dish, was calculated by scanning the surface of the petri dish every 5 mm with the probe (Bolton and Linden, 2003). For calculation of corrected intensity, the maximum intensity value was multiplied by the obtained petri factor.

For the purpose of reducing the natural intensity of UV-lamps in order to render comparable irradiance from UV-LEDs, which ranges from about 4 to 5 $\mu W/cm^2$, the UV-lamp was covered with 52 sheets of polypropylene (PP) film (thickness: 0.05 mm), and the distance between probe and lamp was set at 20 cm. Petri factor and corrected intensity were calculated by same method as for UV-LEDs.

2.3. Comparison of inactivation efficacy between UV-LED and UV-lamp

2.3.1. Bacterial strains

Three strains each of *Escherichia coli* O157:H7 (ATCC 35150, ATCC 43889, and ATCC 43890), *Salmonella* Typhimurium (ATCC 19585, ATCC 43971, and DT 104), and *Listeria monocytogenes* (ATCC 19111, ATCC 19115, and ATCC 15313) were obtained from the Food Science and Human Nutrition culture collection at Seoul National University (Seoul, Korea). Stock cultures were kept frozen at –80°C in 0.7 ml of Tryptic Soy Broth (TSB; MB Cell) 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.

2.3.2. Culture preparation

Each strain of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* was cultured in 5 ml TSB at 37°C for 24 h and harvested by centrifugation at 4000 × g for 20 min at 4°C. Cell pellets were obtained by washing with sterile 0.2% peptone water (PW; Bacto, Sparks, MD) three times and the final pellets were resuspended in 9 ml PW, corresponding to approximately

10^8 to 10^9 CFU/ml. Resuspended pellets of each strain of all pathogen species were combined to constitute a 3-pathogen mixed culture cocktail.

2.3.3. Inoculation

The cocktail suspension was 10-fold serially diluted three times with 0.2% sterile PW so that the initial concentration of the inoculum was approximately 10^5 to 10^6 CFU/ml. Also, the culture suspension was subjected to an additional 10-fold serial dilution in 0.2% PW, and 0.1 ml of diluent was inoculated and spread onto selective media or non-selective agar. Sorbitol MacConkey Agar (SMAC; Difco), Xylose Lysine Desoxycholate Agar (XLD; Difco), and Oxford Agar Base with antimicrobial supplement (OAB; MB Cell, Seoul, Korea) were used as selective media to enumerate *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes*, respectively. After inoculation, the media were dried for approximately 30 min at room temperature prior to UV treatment.

2.3.4. UV treatments

Media inoculated by *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogens* were treated in the chamber at room temperature with 4

different peak wavelength UV-LED PCBs or PP-covered UV-lamp at dosages of 0.1, 0.2, 0.5, and 0.7 mJ/cm^2. Treatment times for the doses were calculated by dividing UV doses by intensities with an appropriate conversion factor. After treatments, in order to minimize photoreactivation, all UV-treated petri dishes were covered with aluminum foil before incubating.

2.4. Inactivation of foodborne pathogens on media and sliced cheese

2.4.1. 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 Food Science and Human Nutrition culture collection at Seoul National University (Seoul, Korea). Stock cultures were kept frozen at –80°C in 0.7 ml of TSB and 0.3 ml of 50% glycerol. Working cultures were streaked onto TSA, incubated at 37°C for 24 h and stored at 4°C.

2.4.2. Culture preparation

Each strain of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* was cultured in 5 ml TSB at 37°C for 24 h and harvested by centrifugation at 4000 × g for 20 min at 4°C. Cell pellets were obtained by washing with sterile 0.2% PW three times and the final pellets were resuspended in 9 ml PW, corresponding to approximately 10^8 to 10^9 CFU/ml. Resuspended pellets of each strain of all pathogen species were combined to constitute a 3-pathogen mixed culture cocktail.

2.4.3. Sample preparation and inoculation

Commercially processed sliced camembert cheese was purchased at a local grocery store (Seoul, South Korea). The sliced cheese was 85 by 85 by 2 mm. Samples were stored under refrigeration (4°C) and used within 2 days. For the media surface experiments, the cocktail suspension was 10-fold serially diluted three times with 0.2% PW so that the initial concentration of the inoculum was approximately 10^5 to 10^6 CFU/ml. Also, the culture suspension was subjected to an additional 10-fold serial dilution in 0.2% PW, and 0.1 ml of diluent was inoculated and spread onto selective media or non-selective agar. SMAC, XLD, and OAB were used as selective media to

enumerate *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes*, respectively. Phenol Red agar base (Difco) with 1% sorbitol (MB Cell) (SPRAB) was used to enumerate injured cells of *E. coli* O157:H7 and the overlay (OV) method was used to enumerate injured cells of *S.* Typhimurium. and *L. monocytogenes*. After inoculation, the media were dried for approximately 30 min at room temperature prior to UV treatment.

For cheese inoculation, one-tenth ml of same cocktail suspension with media experiments was applied to one piece of sliced cheese (ca. 25 g). The inoculum was spread by using a sterile glass spreader every 5 min for even distribution of pathogens, and the samples were dried inside of biosafety hood for 15 min without the fan running to avoid excessive surface aridity. The final cell concentration was approximately 10^6 to 10^7 CFU/25g.

2.4.4. UV treatments

Media inoculated by *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogens* were treated in the chamber at room temperature with 4 different peak wavelength UV-LED PCBs at dosages of 0.1, 0.2, 0.5, and 0.7 mJ/cm^2. Treatment times for the doses were calculated by dividing UV doses by intensities with an appropriate conversion factor. After treatments, in order to minimize photoreactivation, all UV-treated petri dishes were

covered with aluminum foil before incubating. Also, pieces of inoculated sliced cheese were treated with the same UV-LED PCBs with dosages of 1, 2, and 3 mJ/cm^2 in the same environment and treatment chamber.

2.4.5. Color measurement of sliced cheese

A Minolta colorimeter (model CR400; Minolta Co., Japan) was used to quantify the color changes of treated samples to determine the effect of UV-LED treatment on the color of sliced cheese. CIE LAB measurement was implemented and *L** (lightness), *a** (green-red), and *b** (blue-yellow) of chromaticity were used for the test. Three randomly selected locations on sliced cheese surfaces were analyzed and averaged to compare changes in color during the UV-LED treatments.

2.5. Comparison of the effects of UV-LED to Gram negative and Gram positive bacteria

2.5.1. Bacterial strains

Three strains each of *E. coli* O157:H7 (ATCC 35150, ATCC 43889, and ATCC 43890), *L. monocytogenes* (ATCC 19111, ATCC 19115, and ATCC

15313), *Staphylococcus aureus* (ATCC 10390, ATCC 12598, and ATCC 27644), and *Salmonella* Enteritidis PT 30 (ATCC BAA1045) were obtained from the Food Science and Human Nutrition 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). Stock cultures were kept frozen at –80°C in 0.7 ml of TSB and 0.3 ml of 50% glycerol. Working cultures were streaked onto TSA, incubated at 37°C for 24 h and stored at 4°C.

2.5.2. Culture preparation

Each strain of *E. coli* O157:H7, Salmonella spp., *L. monocytogenes*, and *S. aureus* was cultured in 5 ml TSB at 37°C for 24 h and harvested by centrifugation at 4000 × g for 20 min at 4°C. Cell pellets were obtained by washing with sterile 0.2% PW three times and the final pellets were resuspended in 9 ml PW, corresponding to approximately 10^8 to 10^9 CFU/ml. Resuspended pellets of each strain of all pathogen species were combined to constitute a 3-pathogen mixed culture cocktail.

2.5.3. Inoculation

The cocktail suspension was 10-fold serially diluted three times with 0.2% sterile PW so that the initial concentration of the inoculum was approximately 10^5 to 10^6 CFU/ml. Also, the culture suspension was subjected to an additional 10-fold serial dilution in 0.2% PW, and 0.1 ml of diluent was inoculated and spread onto selective media or non-selective agar. SMAC, XLD, OAB, and Baird Parker Agar (BPA; Difco) were used as selective media to enumerate *E. coli* O157:H7, *Salmonella* spp., *L. monocytogenes*, and *S. aureus*, respectively. SPRAB was used to enumerate injured cells of *E. coli* O157:H7 and the overlay (OV) method was used to enumerate injured cells of *Salmonella* spp. and *L. monocytogenes*. TSA was used as non-selective medium for *S. aureus*. After inoculation, the media were dried for approximately 30 min at room temperature prior to UV treatment.

2.5.4. UV treatments

Media inoculated by *E. coli* O157:H7, *Salmonella* spp., *L. monocytogenes*, and *S. aureus* were treated in the chamber at room temperature with 4 different peak wavelength UV-LED PCBs at dosages of

0.1, 0.2, 0.3, 0.4, 0.5, and 0.6 mJ/cm^2. Treatment times for the doses were calculated by dividing UV doses by intensities with an appropriate conversion factor. After treatments, in order to minimize photoreactivation, all UV-treated petri dishes were covered with aluminum foil before incubating.

2.6. Bacterial enumeration

Following UV treatment in the media 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). One ml aliquots of sample were 10-fold serially diluted in 9 ml blanks of 0.2% PW, and 0.1 ml of diluent was spread-plated onto each selective medium (described previously). All agar media from food sample treatments were incubated at 37°C for 24-48 h and typical colonies were counted.

2.7. Enumeration of injured cells

To enumerate injured cells, the overlay (OV) method was used for *Salmonella* spp. and *L. monocytogenes* (Lee and Kang, 2001). For the first step of the overlay method, non-selective TSA medium was used, which enables injured cells to resuscitate. Plated and UV-treated TSA was incubated at 37°C for 2 h to permit injured cells to recover. The plates were then overlaid with 7 ml of the selective medium XLD for *Salmonella* spp. or OAB for *L. monocytogenes*, respectively. Solidified plates were further incubated for an additional 22 h at 37°C. After incubation, typical black colonies of both pathogens were enumerated. Enumeration of injured *E. coli* O157:H7 on SPRAB was implemented to count typical white colonies after incubation at 37°C for 24 h with simultaneous serological confirmation of randomly selected characteristic white colonies representing 10% of the total (RIM, *E. coli* O157:H7 latex agglutination test; Remel, KS, USA). Injured cells of *S. aureus* were calculated after colony counts were taken on TSA after incubation at 37°C for 24 h and compared with counts made on BPA plated simultaneously.

2.8. Identification of inactivation mechanism of UV-LED with different wavelengths

2.8.1. UV treatments

Each bacterial suspension as *E. coli* O157:H7, *Salmonella* spp., *L. monocytogenes*, and *S. aureus* was appropriately diluted with Phosphate-Buffered Saline (PBS, Corning Inc.; Corning, NY, USA) to adjust optical density (OD) to approximately 0.4, and 5 ml of suspensions were pipetted into empty petri dishes. Irradiation of 1 mJ/cm^2 by means of UV-LEDs was applied to the bacterial suspension; this dose corresponds to media treatment of 0.6 mJ/cm^2 which induces a similar inactivation level (ca. 5 log reduction). After irradiation, 5 ml aliquots of suspensions were delivered to sterile conical tubes, and placed in the dark until assessments using fluorescence dye.

2.8.2. Cellular membrane damage measurement

To investigate cellular membrane damage after UVC-LED irradiation, $DiBAC_4(3)$ accumulation and PI uptake values were measured. Membrane potential is the difference of electrical state between inner and outer sides of

a cell membrane which can be an indicator of cellular damage (Rottenberg, 1979). To evaluate membrane potential of cells, bis-(1,3-Dibutylbarbituric Acid) Trimethine Oxonol ($DiBAC_4(3)$; Molecular Probes, Invitrogen, Thermo Fisher Scientific; Waltham, MA, USA; BOX) was used as a fluorescent dye. GN cells were incubated in 2.5 μg $DiBAC_4(3)$/ml in PBS with 4 mM ethylenediaminetetraacetic acid (EDTA) and Suspensions of GP were incubated in 0.5 μg $DiBAC_4(3)$/ml. GN suspensions were incubated for 15 min at 37°C, and GP were incubated for 2 min at room temperature in a dark room. Propidium Iodide (PI; Sigma-Aldrich Corp; St. Louis, MO, USA) is a fluorescent dye used to quantitatively assess membrane destruction of bacteria cells induced by UV treatment. Treated bacteria were diluted in PBS to adjust optical density at 680 nm (OD680) to approximately 0.4 and then mixed with PI solution to a final concentration of 2.9 μM, followed by 10 min incubation in the dark at room temperature.

For positive controls, which were considered to receive complete cell membrane damage, cells were heat-shocked at 55°C for 15 min. Also, untreated groups were assigned as negative controls.

After incubation, each dyed sample was centrifuged at 10,000 × g for 10 min and washed twice with PBS to remove excess dye. The cell pellet was resuspended in PBS, and fluorescence was measured with a spectrofluorophotometer (Spectramax M2e; Molecular Devices, Sunnyvale,

CA, USA) at an excitation wavelength (Ex) of 488 nm and an emission wavelength (Em) of 525 nm for $DiBAC_4(3)$ accumulation and an Ex of 495 nm and an Em of 615 nm for PI uptake. Fluorescence values from untreated cells were subtracted from those of treated cells, and the data were normalized against the OD680 of the cell suspensions (Siva et al., 2011; Park and Kang, 2013; Lloyd et al., 2002). Each value was expressed as a ratio to that of the positive control.

2.8.3. DNA damage measurement

It is well-known that the major inactivation mechanism of ultraviolet rays is DNA damage. I measured membrane damage of bacteria after UV irradiation, and in order to compare the damage ratio between membranes and cellular DNA, Hoechst 33258 (Molecular Probes, Invitrogen, Thermo Fisher Scientific; Waltham, MA, USA) was used to examine DNA damage after exposure to UVC-LED. Two μg/ml of Hoechst 33258 dye was mixed with UVC-LED treated bacteria cells and incubated in the dark at room temperature for 10 min. For the positive control, which was considered to be a state of complete DNA damage, cells were irradiated at 250 mJ/cm^2 with a conventional UV-lamp (G10T5/4P; 16W; 357mm; Sankyo, Japan). Untreated groups were assigned as negative groups.

After incubation, each dyed sample was centrifuged at 10,000 × g for 10 min and washed twice with PBS to remove excess dye. The cell pellet was resuspended in PBS, and fluorescence was measured with a spectrofluorophotometer at an Ex of 352 nm and an Em of 461 nm. Fluorescence values from negative controls (untreated cells) were subtracted from those of treated cells, and the data were normalized against the OD680 of the cell suspensions. Measured values were expressed as a ratio to that of the positive control and the calculated ratio was subtracted from 100% to quantify DNA damage.

2.9. Statistical analysis

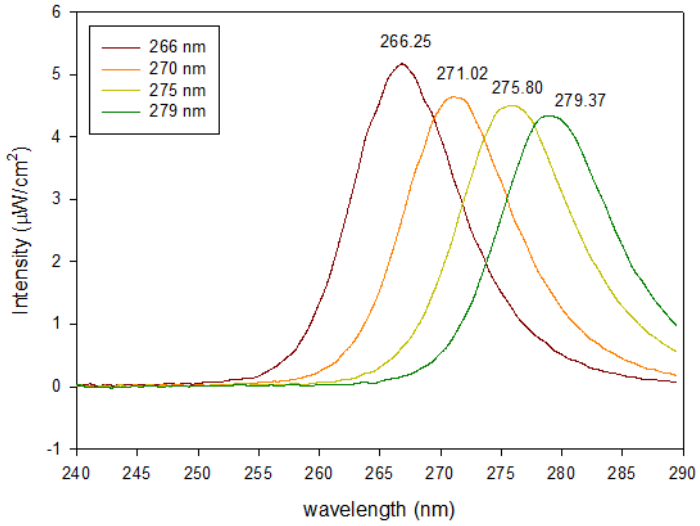
All experiments were duplicate-plated and replicated three times. All data were analyzed with ANOVA using the Statistical Analysis System (SAS Institute, Cary, NC, USA) and Duncan's multiple range test to determine if there were significant differences ($P < 0.05$) in mean values of log reduction of microorganism populations, color changes or fluorescence value ratios.

III. RESULTS

3.1. Emission spectrum of UV-lamp and UV-LED

Spectral intensity of the 254 nm UV-lamp covered with PP films was measured with a spectrometer, and presented in Fig. 1 (b). The actual peak wavelength was 254.31 nm, and as the number of PP films increased, the irradiance of the UV-lamp decreased. With 52 PP films, the intensity of the 254 nm lamp was found to be 3.97 ± 0.02 $\mu W/cm^2$, which was 0.47% the intensity of the uncovered lamp. Also, the irradiance of UV-LED PCBs is shown in Fig. 1 (a). The actual peak wavelengths of LED PCBs were 266.25 nm, 271.02 nm, 275.80 nm, and 279.37 nm, respectively, and the intensity values ranged from 4 to 5 $\mu W/cm^2$.

(a)



(b)

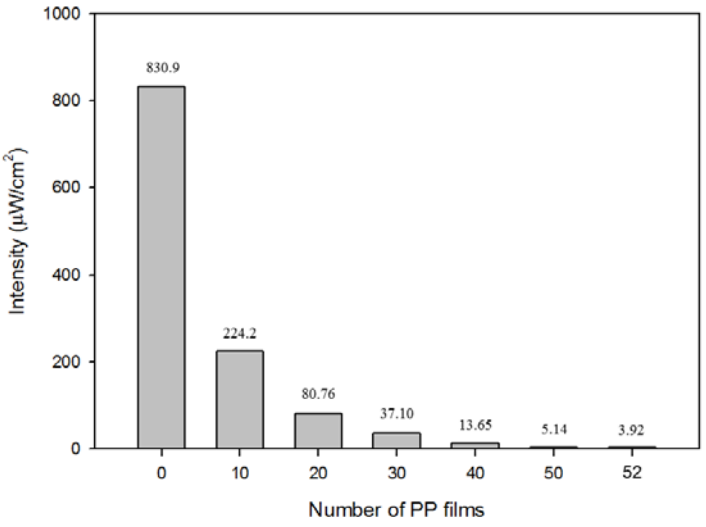


Fig. 1. Emission spectra of 4 different peak wavelengths of UV-LED PCBs (a), and absolute intensity of UV-lamp covered with varying number of PP films (b).

3.2. Comparison of microbial reductions between the UV-lamp and UV-LED

Fig. 2 shows the viable-count reduction levels of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* spread on selective media after treating with the 254 nm UV-lamp or 266 nm UV-LED. Both treatments presented the same pattern of foodborne pathogen reductions; that is, higher doses induced higher levels of inactivation. The 266 nm UV-LED treatment at a dose of 0.7 mJ/cm^2 achieved around 6 log reductions of *E. coli* O157:H7 and *S.* Typhimurium, respectively, and a 5.3 log reduction of *L. monocytogenes*. In other words, 0.7 mJ/cm^2 treatment to 266 nm UV-LED demonstrated that nearly all inoculated pathogens were inactivated at this dose. On the other hand, the reduction levels with UV-lamp treatment were 3.06, 1.42, and 0.34 log for *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes*, respectively, which were significantly less ($P < 0.05$) than the UV-LED inactivation levels at the same dose. The other doses (0.1, 0.2, and 0.5 mJ/cm^2) also showed significant differences between reductions of the 3 foodborne pathogens treated with the UV-lamp and UV-LED. For each dosage, the inactivation level of *L. monocytogenes* was the least compared to *E. coli* O157:H7 and *S.* Typhimurium. Resuscitation of injured cells from either UV-lamp or UV-LED treatment was observed in terms of numerical

level (data not shown) but statistically, there were no significant differences ($P > 0.05$).

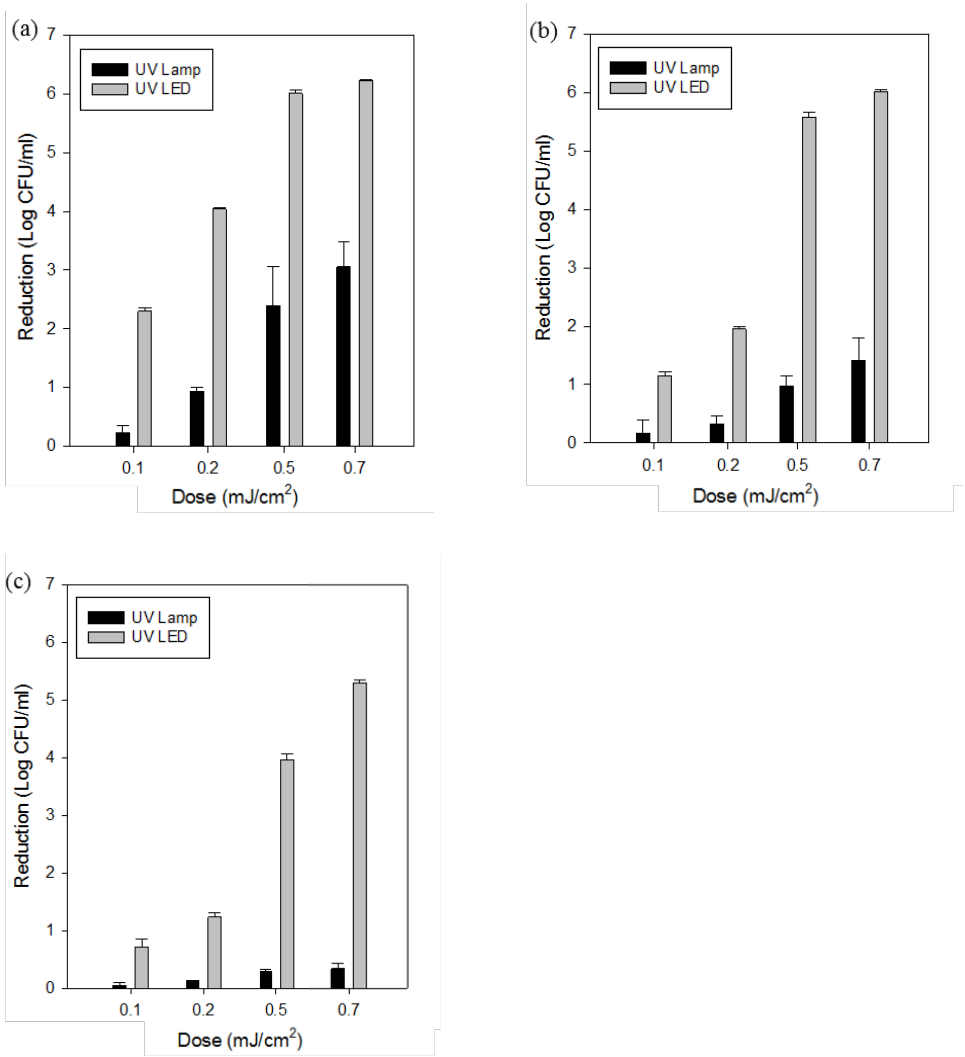


Fig. 2. Reduction of *E. coli* O157:H7 (a), *S.* Typhimurium (b), and *L. monocytogenes* (c) cells on each selective medium treated with UV-lamp and UV-LED PCBs.

3.3. Inactivation effect of UV-LED on media with different wavelengths

The log reductions of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* on media treated with 4 different wavelengths of UV-LED are shown in Table 2. Reduction levels showed an increasing tendency in accordance with treatment dose, achieving an approximately 6 log reduction for *E. coli* O157:H7 and *S.* Typhimurium, and 5 log reduction for *L. monocytogenes* at a dose of 0.7 mJ/cm^2. Comparison of the inactivation of foodborne pathogens with respect to wavelengths demonstrated that UV treatment with relatively short wavelengths (266 nm and 270 nm) had a pronounced bactericidal effect at low dosage levels. In the case of *E. coli* O157:H7, over 4 log reduction was demonstrated at 0.2 mJ/cm^2 with 270 nm PCB treatment, and the PCB treatments of other wavelength achieved 3-4 log reductions at the same dose, which were significantly lower ($P < 0.05$). At 0.5 mJ/cm^2, over 5 log reductions were achieved with the 266 and 270 nm PCBs on *S.* Typhimurium, significantly greater than the reductions obtained with the longer wavelengths. Also, *L. monocytogenes* showed around 4 log reductions only at 266 and 270 nm UV-LED treatments which were 1.0-1.5 log greater compared to 279 nm treatment.

With regard to resuscitation of sublethally injured cells, only in the case of *S.* Typhimurium at 0.5 and 0.7 mJ/cm^2 doses were there any significant

differences (0.6-1 log unit) between inactivation of samples subjected to injured-cell recovery methods and those plated directly onto selective media. Slightly lower reductions of *E. coli* O157:H7 and *S.* Typhimurium were observed for the OV agar method (SPRAB in the case of *E. coli* O157:H7) than for selective agar. However, significant differences between the inactivation levels obtained on each selective agar (SMAC, XLD, and OAB) versus the agar for recovering injured-cells were not observed except for high dose treatments (0.5, and 0.7 mJ/cm^2) on *S.* Typhimurium, as already mentioned.

Table 2. Log reductions of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* on culture media after treatment with UV-LED PCBs at 4 different wavelengths.

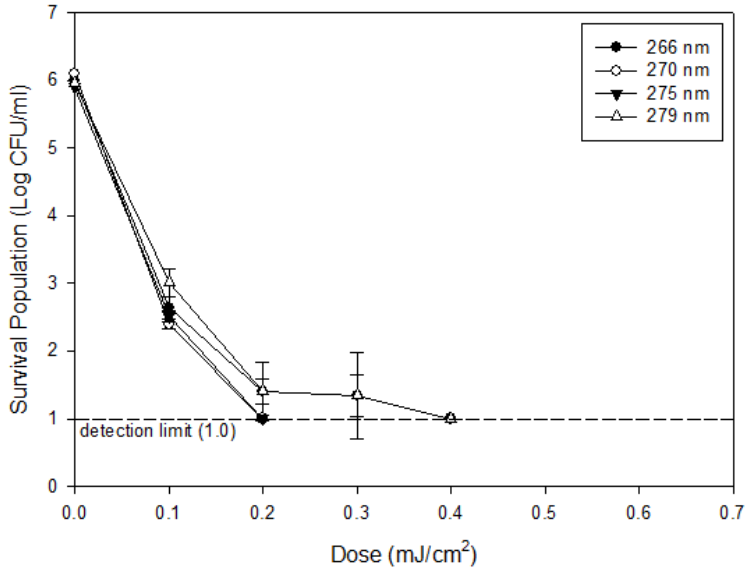
	Log reduction[a] (log_{10} CFU/ml)							
Dose (mJ/cm^2)	0.1		0.2		0.5		0.7	
Wavelength (nm)								
E. coli O157:H7	SMAC	SPRAB	SMAC	SPRAB	SMAC	SPRAB	SMAC	SPRAB
266	2.30 ± 0.06 Ba	2.86 ± 0.51 Aa	4.04 ± 0.03 Ba	4.05 ± 0.41 Aa	6.01 ± 0.05 Aa	5.83 ± 0.09 Ab	6.23 ± 0.01 Aa	5.82 ± 0.51 Aa
270	2.93 ± 0.27 Aa	2.75 ± 0.22 Aa	4.49 ± 0.34 Aa	4.27 ± 0.29 Aa	5.85 ± 0.12 Aa	5.92 ± 0.43 Aa	6.17 ± 0.23 Aa	5.88 ± 0.84 Aa
275	2.10 ± 0.03 BCa	2.72 ± 0.41 Aa	3.79 ± 0.04 Ba	4.17 ± 0.49 Aa	6.02 ± 0.20 Aa	5.83 ± 0.35 Aa	6.27 ± 0.11 Aa	6.31 ± 0.09 Aa
279	1.89 ± 0.24 Cb	2.65 ± 0.30 Aa	3.16 ± 0.22 Cb	3.95 ± 0.38 Aa	5.86 ± 0.27 Aa	5.21 ± 0.62 Aa	6.17 ± 0.23 Aa	6.05 ± 0.32 Aa
S. Typhimurium	XLD	OV-XLD	XLD	OV-XLD	XLD	OV-XLD	XLD	OV-XLD
266	1.15 ± 0.07 ABa	0.80 ± 0.39 Aa	1.95 ± 0.04 ABa	1.57 ± 0.46 Aa	5.58 ± 0.09 Aa	4.35 ± 0.44 Ab	6.01 ± 0.03 Aa	5.07 ± 0.15 ABb
270	1.39 ± 0.27 Aa	0.74 ± 0.30 ABb	2.27 ± 0.31 Aa	1.64 ± 0.41 Aa	5.26 ± 0.47 Aa	4.30 ± 0.33 Ab	6.00 ± 0.10 Aa	5.32 ± 0.22 Ab
275	0.97 ± 0.02 Ba	0.84 ± 0.30 Aa	1.76 ± 0.07 Ba	1.97 ± 0.89 Aa	4.59 ± 0.05 Ba	3.90 ± 0.41 ABb	5.81 ± 0.33 Aa	4.79 ± 0.38 Bb
279	0.86 ± 0.21 Ba	0.91 ± 0.50 Aa	1.93 ± 0.26 ABa	1.60 ± 0.38 Aa	4.61 ± 0.23 Ba	3.46 ± 0.12 Bb	5.62 ± 0.37 Aa	4.79 ± 0.38 Ba
L. monocytogenes	OAB	OV-OAB	OAB	OV-OAB	OAB	OV-OAB	OAB	OV-OAB
266	0.71 ± 0.15 Aa	0.49 ± 0.05 Aa	1.23 ± 0.08 Aa	1.03 ± 0.05 Ab	3.97 ± 0.09 Aa	4.13 ± 0.48 Aa	5.31 ± 0.05 Aa	4.91 ± 0.34 Aa
270	0.42 ± 0.11 Ba	0.46 ± 0.07 ABa	0.88 ± 0.18 Ba	0.98 ± 0.18 ABa	3.57 ± 0.05 Ba	3.87 ± 0.44 Aa	5.46 ± 0.26 Aa	4.74 ± 0.57 Aa
275	0.34 ± 0.18 Ba	0.35 ± 0.08 BCa	0.68 ± 0.10 Ba	0.79 ± 0.09 BCa	2.94 ± 0.29 Ca	3.55 ± 0.32 ABa	4.61 ± 0.34 Ba	5.14 ± 0.19 Aa
279	0.29 ± 0.10 Ba	0.32 ± 0.04 CDa	0.68 ± 0.10 Ba	0.74 ± 0.13 Ca	2.27 ± 0.20 Db	3.08 ± 0.24 Ba	4.20 ± 0.23 Ca	4.54 ± 0.07 Aa

[a] Data represent means ± standard deviations from three replications. Values followed by the same uppercase letters within columns and lowercase within rows per each dose are not significantly different.

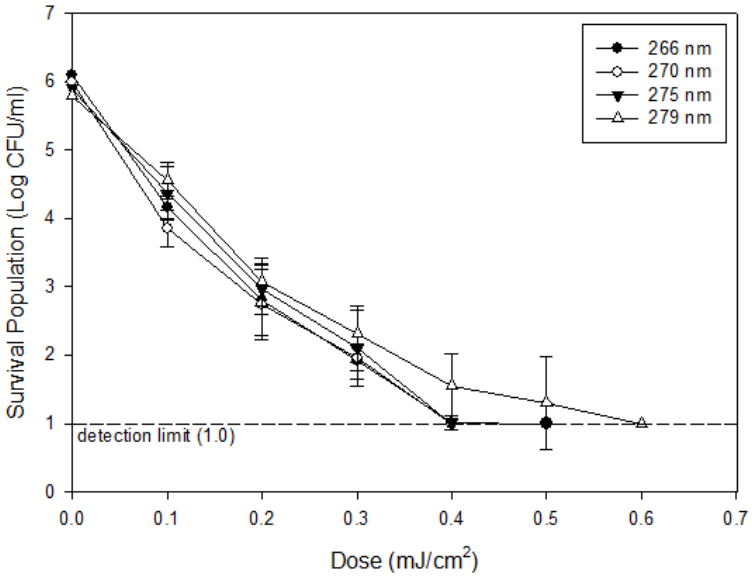
3.4. Comparison of inactivation effect using UV-LED to Gram negative bacteria and Gram positive bacteria on media

Media inoculated with bacteria were treated with the four wavelength UVC-LEDs. Though low wavelength yielded higher bacterial reduction did high wavelength UVCs, there was no significant difference ($P > 0.05$). Fig. 3 indicates log reductions of each bacterium. Increasing irradiation dose led to higher reductions of bacteria. GN, as *E. coli* O157:H7 and *Salmonella* spp., were easily inactivated by UVC-LED (Fig. 3a, 3b). At the low dose of 0.2 mJ/cm^2, *E. coli* O157:H7 was reduced by about 5 log and reduction of *Salmonella* spp. was close to 3 log. At same dose, GP, such as *L. monocytogenes*, achieved less than 1 log reduction and *S. aureus* experienced slightly more than 2 log reduction (Fig. 3c, 3d). *E. coli* O157:H7 was not detected (> 5 log reduction) after 0.4 and 0.6 mJ/cm^2 of irradiance, while reduction of *Salmonella* spp. was over 5 log. GP was reduced by 3-5 log after 0.6 mJ/cm^2. With respect to the injured cell generation, *E. coli* O157:H7 and *Salmonella* spp. showed significant levels of resuscitated injured cells at 0.2 mJ/cm^2 and 0.4 mJ/cm^2 (data not shown). Injured cells were not detected at higher levels of irradiation. As for GP, no injured cells were generated.

(a)


266 nm
270 nm
275 nm
279 nm
Survival Population (Log CFU/ml)
detection limit (1.0)
Dose (mJ/cm2)
0
1
2
3
4
5
6
7
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7

(b)


266 nm
270 nm
275 nm
279 nm
Survival Population (Log CFU/ml)
detection limit (1.0)
Dose (mJ/cm2)
0
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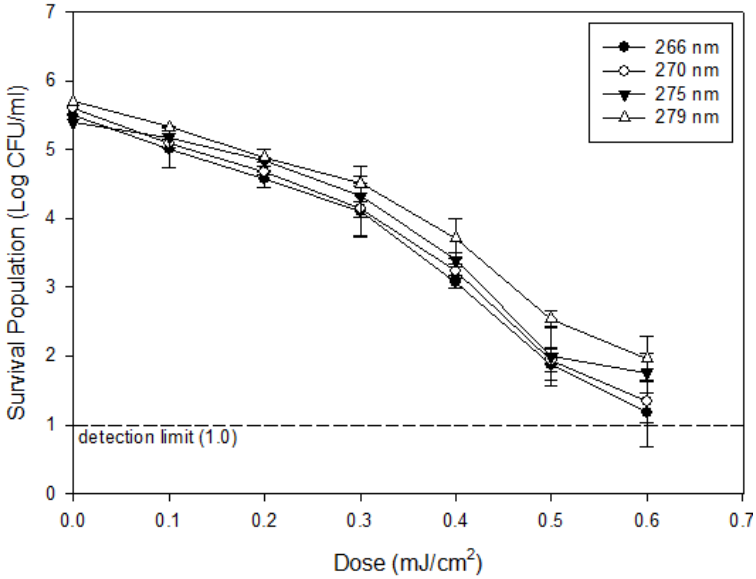
0.4
0.5
0.6
0.7

(c)



(d)

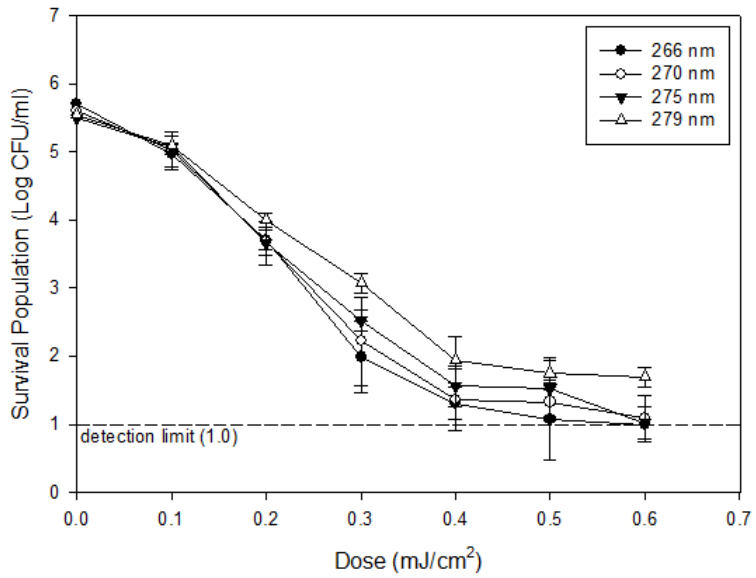


Fig. 3. Survival (Log CFU/ml) of Gram negative bacteria ((a) *E. coli* O157:H7, (b) *Salmonella* spp.) and Gram positive bacteria ((c) *L. monocytogenes*, (d) *S. aureus*) after UV-LED irradiation.

3.5. Bactericidal effect by UV-LED treatment on sliced cheeses

Log reductions of foodborne pathogens on sliced cheese samples following UV-LED treatments are presented in Table 3. A similar relationship between reduction levels and treatment doses was observed as described previously for experiments involving selective media. Approximately 4-5 log reductions were accomplished at 3 mJ/cm^2 radiation intensity for *E. coli* O157:H7 and *S.* Typhimurium, and 3-4 log reductions for *L. monocytogenes*. Furthermore, UV-LED composed of 266 nm modules achieved 4.88, 4.72, and 3.52 log reductions of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes*, respectively, while 279 nm modules achieved 4.04, 3.91, and 3.24 log reductions of each pathogen, respectively. Statistically significant differences ($P < 0.05$) in numbers of surviving cells enumerated on selective media following exposure to relatively short peak wavelengths (266 and 270 nm) versus relatively long peak wavelengths (275 and 279 nm) were observed at 3 mJ/cm^2, the highest treatment dose. The resuscitation of sublethally injured cells following UV-LED treatment was not demonstrated in the overall data.

Table 3. Log reductions of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* on sliced cheese after treatment with UV-LED PCBs at 4 different wavelengths.

	Log reduction[a] (log_{10} CFU/g)					
Dose (mJ/cm^2)	1		2		3	
Wavelength (nm)						
E. coli O157:H7	SMAC	SPRAB	SMAC	SPRAB	SMAC	SPRAB
266	3.50 ± 0.57 Aa	3.21 ± 0.22 Aa	4.09 ± 0.46 Aa	3.43 ± 0.30 Aa	4.88 ± 0.18 Aa	4.49 ± 0.09 Ab
270	2.83 ± 0.43 Aa	3.09 ± 0.72 Aa	3.99 ± 0.10 Aa	3.73 ± 0.10 Ab	4.81 ± 0.10 Aa	4.14 ± 0.72 ABa
275	2.78 ± 0.36 Aa	2.74 ± 0.42 Aa	3.79 ± 0.50 Aa	3.39 ± 0.43 Aa	4.31 ± 0.31 Ba	4.13 ± 0.28 ABa
279	2.80 ± 0.53 Aa	2.86 ± 0.73 Aa	3.46 ± 0.51 Aa	3.38 ± 0.40 Aa	4.04 ± 0.33 Ba	3.64 ± 0.17 Ba
S. Typhimurium	XLD	OV-XLD	XLD	OV-XLD	XLD	OV-XLD
266	3.10 ± 0.24 Aa	3.13 ± 0.25 Aa	3.93 ± 0.68 Aa	3.42 ± 0.46 Aa	4.72 ± 0.02 Aa	4.50 ± 0.37 Aa
270	2.82 ± 0.33 Aa	3.08 ± 0.47 Aa	3.70 ± 0.12 Aa	3.43 ± 0.41 Aa	4.73 ± 0.05 Aa	4.37 ± 0.39 Aa
275	2.83 ± 0.31 Aa	2.91 ± 0.20 Aa	3.24 ± 0.36 Aa	3.35 ± 0.28 Aa	4.24 ± 0.26 Ba	4.04 ± 0.22 Aa
279	2.73 ± 0.38 Aa	2.93 ± 0.37 Aa	3.17 ± 0.39 Aa	2.94 ± 0.61 Aa	3.91 ± 0.05 Ca	3.96 ± 0.28 Aa
L. monocytogenes	OAB	OV-OAB	OAB	OV-OAB	OAB	OV-OAB
266	3.09 ± 0.26 Aa	2.55 ± 0.22 Ab	3.10 ± 0.10 Aa	3.03 ± 0.43 Aa	3.52 ± 0.05 ABa	3.32 ± 0.75 Aa
270	2.89 ± 0.19 Aa	2.66 ± 0.62 Aa	2.97 ± 0.44 Aa	2.73 ± 0.21 Aa	3.94 ± 0.55 Aa	3.06 ± 0.25 ABa
275	2.54 ± 0.41 Aa	2.04 ± 0.11 ABa	2.72 ± 0.34 ABa	2.43 ± 0.30 Aa	3.31 ± 0.22 Ba	2.57 ± 0.18 ABb
279	2.33 ± 0.65 Aa	1.72 ± 0.24 Ba	2.37 ± 0.17 Ba	2.07 ± 0.84 Aa	3.24 ± 0.08 Ba	2.27 ± 0.37 Bb

[a] Data represent means ± standard deviations from three replications. Values followed by the same uppercase letters within columns and lowercase within rows per each dose are not significantly different.

3.6. Effect of UV-LED treatment on product color values

The CIE LAB color method was used to determine color changes in sliced cheese samples after 3 mJ/cm^2 of UV-LED treatment, and the results are shown in Table 4. Slight changes in *L**, *a**, *b** values of UV-LED treated sliced cheese were observed, but there were no significant differences ($P > 0.05$) between any of the treatments and the control.

Table 4. Color values[a] of sliced cheese surfaces after UV-LED treatment at a dose of 3 mJ/cm^2.

	Parameter[b]		
Wavelength (nm)	*L**	*a**	*b**
Control[c]	82.42 ± 0.22 A	-4.51 ± 0.01 A	13.37 ± 0.11 A
266	82.73 ± 0.27 A	-4.45 ± 0.02 A	13.37 ± 0.02 A
270	82.79 ± 0.16 A	-4.43 ± 0.07 A	13.10 ± 0.09 A
275	82.75 ± 0.15 A	-4.44 ± 0.05 A	13.27 ± 0.09 A
279	82.82 ± 0.31 A	-4.43 ± 0.06 A	13.60 ± 0.27 A

[a] Color parameters are lightness (*L**), redness (*a**), and yellowness (*b**).

[b] Values are means from three replications ± standard deviation. Values followed by the same letters within the column per parameter are not significantly different ($P > 0.05$).

[c] Settling time which corresponded to a dosage time of 3 mJ/cm^2.

3.7. Cell membrane damage assessment

I measured $DiBAC_4(3)$ accumulation values and PI uptake of each microorganism species after 1 mJ/cm^2 irradiation. $DiBAC_4(3)$ values increased with increasing UV-LED peak wavelengths (Table 5). GP experienced higher $DiBAC_4(3)$ values than GN and PI values displayed the same tendency as $DiBAC_4(3)$ values (Table 6). Because loss of membrane potential occurred prior to physical membrane destruction, $DiBAC_4(3)$ values were higher than PI uptake values but the same trends were observed as already mentioned (Diaz et al., 2010).

Table 5. Ratio of DiBAC4(3) (BOX) accumulation values of treated bacteria to that of the positive control after 1 mJ/cm^2 UVC-LED treatment.

Wavelength (nm)	$DiBAC_4(3)$ percentage[a] (%)			
	Gram negative		Gram positive	
	E. coli O157:H7	*Salmonella* spp.	*L. monocytogenes*	*S. aureus*
266	0.80 ± 0.21 A	3.76 ± 3.03 A	6.42 ± 1.87 A	7.65 ± 0.72 A
270	2.77 ± 0.24 AB	8.20 ± 2.70 AB	5.69 ± 2.36 A	10.72 ± 5.97 A
275	5.04 ± 1.17 BC	13.04 ± 0.51 BC	9.01 ± 1.69 A	19.93 ± 6.39 B
279	6.73 ± 2.12 C	15.35 ± 1.93 C	16.67 ± 3.87 B	35.11 ± 1.21 C

[a] Data represent means ± standard deviations from three replications. Values followed by the same uppercase letters within columns are not significantly different ($P > 0.05$). Normalized data were obtained by subtracting OD680 fluorescence values of untreated cells from those of treated cells and dividing by the positive control value and expressing this value as a percentage ($DiBAC_4(3)$ percentage = [fluorescence value after treatment – fluorescence value of untreated cells] / [OD680·fluorescence value of positive control]).

Table 6. Ratio of propidium iodide (PI) uptake values of bacteria to that of the positive control after 1 mJ/cm^2 UVC-LED treatment.

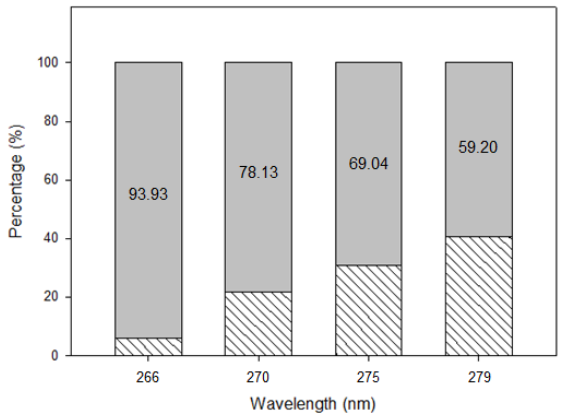
Wavelength (nm)	PI uptake percentage[a](%)			
	Gram negative		Gram positive	
	E. coli O157:H7	*Salmonella* spp.	*L. monocytogenes*	*S. aureus*
266	1.34 ± 1.07 A	1.45 ± 1.32 A	2.69 ± 1.10 A	3.39 ± 2.49 A
270	2.02 ± 0.74 AB	2.03 ± 2.20 A	3.60 ± 1.15 AB	4.66 ± 2.41 A
275	2.36 ± 1.16 AB	4.57 ± 3.49 AB	9.00 ± 5.62 B	14.66 ± 3.20 B
279	5.52 ± 3.30 B	7.91 ± 1.04 B	16.98 ± 1.01 C	18.29 ± 4.64 B

[a] Data represent means ± standard deviations from three replications. Values followed by the same uppercase letters within columns are not significantly different ($P > 0.05$). Normalized data were obtained by subtracting OD680 fluorescence values of untreated cells from those of treated cells and dividing by the positive control value and expressing this value as a percentage (PI percentage = [fluorescence value after treatment – fluorescence value of untreated cells] / [OD680·fluorescence value of positive control]).

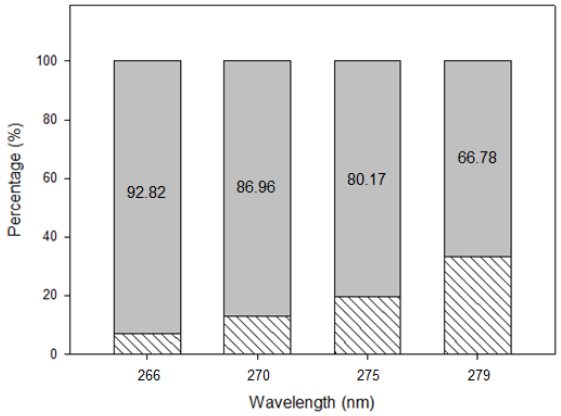
3.8. DNA damage assessment

Hoechst 33258 bonds with double stranded DNA at the AT-rich region (Fornander et al., 2013). Therefore, a low Hoechst 33258 value indicates that many DNA strands were unwound, causing great DNA damage. As shown in Fig. 4, DNA damage was highest at the low wavelength, 266 nm, and with increasing wavelength, DNA damage diminished. GN showed over 90% DNA damage at 266 nm compared to the positive control but GP experienced less than 90% DNA damage at the same wavelength.

(a)


Percentage (%)
100
80
60
40
20
0
93.93
78.13
69.04
59.20
266
270
275
279
Wavelength (nm)

(b)


Percentage (%)
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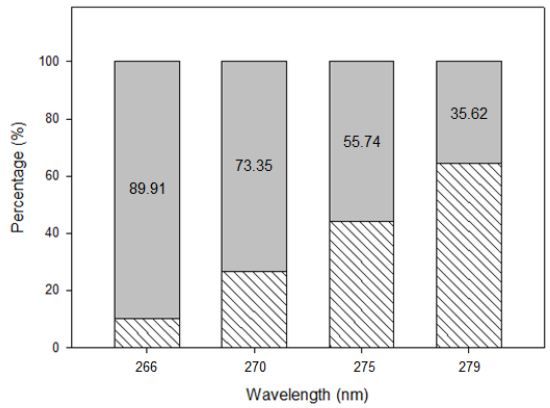
80
60
40
20
0
92.82
86.96
80.17
66.78
266
270
275
279
Wavelength (nm)

(c)



(d)

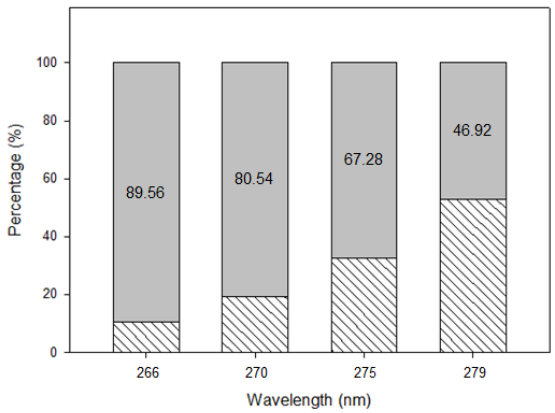


Fig. 4. Percent DNA damage following 1 mJ/cm^2 of UVC-LED irradiation. ▨ indicates Hoechst 33258 uptake percentage of treated samples and ▭ represents DNA percent damage obtained by subtracting Hoechst 33258 uptake percentage of treated samples from that of the positive control (100% value). (a) *E. coli* O157:H7, (b) *Salmonella* spp., (c) *L. monocytogenes*, and (d) *S. aureus*. The number expressed on the block indicates percent of DNA damage (%) for each wavelength.

IV. DISCUSSION

Ultraviolet-C (UVC) is widely used for the surface sterilization of many foods including fruits, vegetables, processed foods as well as equipment. UVC irradiation doses of 0.60-6.0 kJ/m^2 achieved 2.3 to 3.5 log CFU/fruit reduction of *E. coli* O157:H7 and 2.15 to 3.1 log CFU/fruit reduction of *Salmonella* on the grape tomato surfaces (Mukhopadhyay et al., 2014). *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* on fresh-cut lettuce were inactivated by more than 4 log after 10 min exposure to a UV-lamp at 6.80 mW/cm^2 (Kim et al., 2013). In a pulsed UV system in which 3800 V of input was used to generate 1.27 J/cm^2 per pulse for a lamp with a frequency of three pulses per sec, *L. monocytogenes* inoculated onto unpackaged white American cheese slices (9 by 9 cm) was reduced by 1.1 to 3.08 log CFU/cm^2 at a distance of 13 and 8 cm at intervals from 5 to 40 seconds (Can et al., 2014). Another promising disinfection method of cheese, PHI technology, consisting of a combination effect of plasmas, 16.65 mJ/cm^2 UV-lamp irradiation, ozone, and hydrogen peroxide, decontaminated *L. monocytogenes* on sliced American cheese by slightly more than 2 log CFU/sample after 5 min of treatment (Saini et al., 2014). As shown by these earlier studies, UVC is obviously an effective sterilization technology

available to the food industry and potentially useful for pasteurizing cheese using UV-LEDs as a highly competitive and promising novel intervention.

UVC emitted from LEDs is an emerging technology offering an alternative to mercury lamps to compensate for their limitations. There have been several studies involving UV-LEDs but comparison of sterilization efficacy of UV-LEDs with different wavelengths in the UVC region has barely been studied before. One of the major strengths of UV-LED technology is that it can be configured to emit a specific wavelength. The inactivation ability of UV-lamps has been evaluated only at a wavelength of 254 nm since it can only generate a peak wavelength of 254 nm. Therefore, an actual evaluation and comparison of disinfection efficacy of UVC with different wavelengths is needed at this time.

In this study, I investigated germicidal effects of UVC-LEDs at wavelengths of 266, 270, 275, and 279 nm, and a UV-lamp at 254 nm was applied to the pathogens at an intensity similar to those of UV-LEDs. UV-lamps emit a considerably high irradiation intensity of light at natural condition which leads to a high inactivation effect. However, according to my research, UV-lamps showed much lower sterilization capacity for all three pathogens than UV-LEDs when applied at same intensity. It was assumed that this result was due to differences in irradiation characteristics between UV-lamps and UV-LEDs. UV-lamps radiate light from a point

source which disperses in every direction, the intensity with distance following a classic inverse square relationship. But light from UV-LEDs converge at one point vertically. That is, UV-lamps scatter light over a large area and thus actual irradiation strength impinging on the target area may only be a small fraction of what was emitted. On the other hand, UV-LED light, rather than radiating in all directions, proceeds in a linear fashion without much loss of light intensity due to spreading. Thus, I postulate that LED light is concentrated onto the target area and is thereby more efficacious than a UV-lamp.

The UV-LED experiments were performed at an intensity of 4 $\mu W/cm^2$; therefore, I covered the UV-lamp with polypropylene films to adjust its intensity to be almost equivalent to that of the UV-LED. UV-LEDs are still under development and the output power of erstwhile UV-LEDs are relatively low, so it was necessary to lower the UV-lamp intensity for an exact comparison under the same conditions. Raising the radiation intensity of UV-LEDs to that of UV-lamps is difficult with current technology and this is a technical challenge that needs to be solved.

Among UV-LEDs of different wavelengths, 266 and 270 nm LEDs achieved more pathogen reductions than those of longer wavelengths, but these differences were not so critical. Other studies also showed a similar tendency. Chevremont et al. (2012) treated mesophilic bacteria, fecal

enterococci, and coliforms in effluent with UVA and UVC-LED for 60 seconds. There were only less than one log reductions and the inactivation efficacy of 254 and 280 nm were not significantly different. In my study, sterilization efficacy was more related to dose than to wavelength. UV-LEDs achieved more than 5 log reductions of *E. coli* O157:H7 after 0.5 mJ/cm^2 and *S.* Typhimurium after 0.7 mJ/cm^2, and in the case of *L. monocytogenes,* they achieved over 5 log reductions after 0.7 mJ/cm^2 only at 266 and 270 nm. The inactivation level of *L. monocytogenes* was relatively less than those of *E. coli* O157:H7 or *S.* Typhimurium because *L. monocytogenes* is a GP and the other two pathogens are GN. Thus, I evaluated the bactericidal effect with regard to 4 wavelengths and different categories of microorganisms (GN and GP) and manifested the major inactivation mechanism of UVC-LEDs.

Inactivation within bacterial groups showed a similar tendency, as higher doses of irradiation resulted in higher reduction levels. However, quite different levels of UVC-susceptibility were observed in that GN had the lower level of resistance against UVC-LEDs than GP. There are some factors that affect resistance, such as cell wall thickness, cell size, photoproducts generated by irradiation, and DNA repair ability (Lopez-Malo and Palou, 2005; Tran and Farid, 2004). Also, the physiological state of microorganisms determines the degree of UVC sensitivity (Bucheli-Witschel et al., 2010; Wassmann et al., 2011). UV causes physical electron movements and

destroys DNA bonds. UV induces the formation of photoproducts due to the direct absorption of photons by pyrimidine and purine nucleic acid bases (Lopez-Malo and Palou, 2005). Photoproducts lead to structural distortion in DNA and interrupt RNA transcription and DNA replication, finally causing cell mutagenesis or death. The major photoproducts caused by UV are cyclobutane pyrimidine dimers (CPDs) and pyrimidine 6-4 pyrimidone photoproducts (6-4pps) (Friedberg et al., 2006). GP are generally more resistant to UV than GN. This was demonstrated by the study of S Beauchamp et al. (Beauchamp and Lacroix, 2012) which reported that *L. monocytogenes* produced 35% less CPDs and 10% less 6-4 PPs than *E. coli* during a UV-lamp irradiation dose of more than 3 J/cm^2. This low production of UV photoproducts indicates greater resistance of GP. Also, after *L. monocytogenes*, *Salmonella* is more resistant to UV than *E. coli* (Rowan et al., 1999).

The inactivation effect of UV-LEDs on pathogens itself is very meaningful, but every sterilization method may show very different results when applied to food. Through my experiments, I learned that UV-lamps showed a greatly lowered germicidal effect than UV-LEDs at almost the same intensity through media experiments, and actual application of UV-LEDs to a food matrix has never been implemented before. So I decided to apply the UV-LEDs of 266, 270, 275, and 279 nm to inoculated sliced

cheese. To inactivate pathogens on sliced cheese, much higher irradiation doses were needed compared to microbiological media. Reduction of pathogen populations on sliced cheese showed a similar tendency to those of media experiments including the minor differences within various wavelengths, and 3 to 4 log reductions were achieved after exposure at 3 mJ/cm^2.

As for injured cells, non-selective TSA or SPRAB agar was used because stressed sub-populations are viable but not culturable in the presence of selective agents. They do have metabolic activity and can be resuscitated under the proper conditions but cannot be recovered or detected on typical selective media (Ueckert et al., 1995). *E. coli* O157:H7 and *L. monocytogenes* did not produce sublethally injured cells (Table 2), but *S.* Typhimurium after 0.5 and 0.7 mJ/cm^2 exposure yielded about one log of injured cells at all wavelengths evaluated in my study. Choi et al. (2015) investigated sublethally injured cells on cherry tomatoes inoculated with *S.* Typhimurium after 2 to 10 kJ/m^2 treatment with an UV-lamp, and injured cells increased from 60.73 to 93.14% as irradiation dose increased. Also, there were no differences in *L. monocytogenes* population estimates in sterile distilled water between samples enumerated on MOX and TSAYE ($P > 0.05$) after 12.4 mJ/cm^2 UV-lamp exposure which indicates no sublethal injury occurred due to UV exposure (McKinney et al., 2009). Though previous

studies about UV-induced injured cells are not especially numerous, my results proved that UVC scarcely generates injured cells but at some doses, sublethally damaged cells can form in *E. coli* O157:H7 and *Salmonella* spp. However, the selective action of sodium desoxycholate in XLD is so powerful, there is tendency to underestimate actual live cell counts on this medium. Therefore, injured cells in XLD are not thought to be significant. And it seems that a little injury was observed at *E. coli* O157:H7 and *Salmonella* spp. because of not sufficient irradiation at low dose.

In order to investigate the inactivation mechanism of this new technology, UVC-LED, fluorescence dyes were used in this study. Interaction with fluorescence dyes indicates the physiological status of cells under certain conditions, so that I can surmise how an inactivating method affects cell properties such as membrane integrity, membrane potential, enzyme activity, metabolic performance, DNA damage and so on. By using Propidium Iodide (PI) and carboxyfluorescein diacetate (cFDA) with flow cytometry analysis, two bacterial cell properties - membrane integrity and activity of intracellular esterase - were determined after ultrasound treatment (Li et al., 2016). As the treatment progressed, cell properties shifted to greater PI and lower cFDA values which meant that not only cell integrity but also enzyme activity was degraded. Also, Schenk et al (2011) reported that UV-lamps had an effect on non-pathogenic bacteria relative to cell morphological change, membrane

integrity, and enzyme activity by using PI and cFDA with flow cytometric analysis. Physiological changes in *E. coli* during orange juice clarification were investigated with PI and Bis-(1,3-Dibutylbarbituric Acid) Trimethine Oxonol ($DiBAC_4(3)$) (Anvarian et al., 2016).

In my study, there were significantly different PI uptake percentages between 266 nm and 279 nm peak wavelengths for all two microorganism types. PI uptake percentage values at 279 nm were 5-8 times greater than those at 266 nm, and the results were analogous through all types of microorganisms. It is well known that protein has a peak absorbance of approximately 280 nm light due to aromatic amino acids such as Tryptophan, Tyrosine, and by using this simple characteristic, the absorbance value at 280 nm is one of the methods that determines protein concentration (Pace et al., 1995; Stoscheck, 1990). Light with an approximately 280 nm peak wavelength can be absorbed by proteins and transform them into an excitation state leading to their destruction. Therefore, higher peak wavelength irradiation induced a greater level of membrane protein vulnerability, so that membrane integrity deteriorated by means of DNA nick formation. Furthermore, reactive oxygen species (ROS) such as the superoxide anion ($\cdot O_2^-$), hydrogen peroxide (H_2O_2), and the hydroxyl ion (OH^-) generated by UVC irradiation can easily cause heavy stress to aggravated membrane proteins (Scharffetter-Kochanek et al., 1997).

Interestingly, GP showed a much higher PI uptake percentage than did GN. GN showed a maximum 8% PI uptake, while over 15% PI uptake was observed in GP. This indicates that there was twice the membrane damage in GP than in GN. These differences in PI uptake are thought to be attributed to the degree of susceptibility of microorganisms' specific organelles to ROS. ROS, except superoxide anion radicals ($\cdot O_2^-$), can penetrate the bacterial cell envelope and cause several types of damage to microorganism organelles. For different types of microorganisms, cellular components differ in their vulnerability to ROS, following this damage sequence: phospholipids > peptidoglycan in GP; lipopolysaccharides (LPS) > phospholipids > peptidoglycan in GN (Bogdan et al., 2015). Such different susceptibilities in cellular components were clearly related to membrane potential and membrane integrity of UVC-LED treated microorganisms. Because GP bacterial cell walls are composed of only peptidoglycan and phospholipid layers, membrane integrity was easily overcame by ROS so that PI could penetrate into cytosol and bind to DNA. However, because GN have outer membrane obstacles including LPS and glycoproteins, the degree of membrane damage in GN was reduced compared with GP, which induces lower fluorescence values.

Membrane potential forms a homeostasis activity which maintains different concentrations of ions inside and outside of the cell, and only active

cells can generate differences in membrane potential (Disdale and Lloyd, 1995). In fact, reduced membrane potential does not indicate cell death, but is associated with decreasing cell activity; when cell activity declines, membrane depolarization occurs and charged ions can cross the membrane without hindrance. Therefore, membrane potential is strongly related to membrane damage, because membrane depolarization develops prior to structural membrane damage. That is, the membrane potential value, which is indicated by lipophilic dyes ($DiBAC_4(3)$ in the present study), has to be higher than the membrane damage value measured by PI uptake (Diaz et al., 2010). In this manner, Table 5 shows that $DiBAC_4(3)$ accumulation values showed a similar trend to that of PI uptake (Table 6); i.e., $DiBAC_4(3)$ values increased with higher peak wavelengths, and depolarization in GP was higher than for GN.

DNA damage by UVC-LED treatment was measured by using Hoechst 33258. This fluorescence dye binds to minor grooves of the Adenine-Thymine rich region in double-stranded DNA (dsDNA) (Gavathiotis et al., 2000). Therefore, Hoechst 33258 was selected to measure several DNA photo-damage mechanisms such as i) pyrimidine dimer formation which induces dsDNA conformation deterioration (kinking, twisting) (Park et al., 2002), ii) oxidation of DNA bases by ROS which induces DNA breakage (Cadet and Wagner, 2013), iii) dsDNA breakage into single-stranded DNA

(ssDNA) (Santos et al., 2013). In many research studies, there has been a concomitant result that GP have more resistance to UV treatment than GN (Hijnen et al., 2006; Lopez-Malo and Palou, 2005; Koutchma, 2009; Anderson et al., 2000). My investigation has shown that different levels of reduction among microorganism groups were clearly attributed to DNA damage generated by UVC-LED treatment. GN showed high susceptibility to UV treatment (Fig. 3a, 3b) and the fluorescent value of Hoechst was determined to be over 90% at 266 nm (Fig. 4a, 4b); the DNA damage percentage was determined to be 90% that of the positive control when treated with a conventional 254 nm UV-lamp with a dose of approximately 250 mJ/cm^2. Even though there were changes in membrane integrity and membrane depolarization, tendencies in microorganism reduction seemed to be highly related to DNA damage. A profound association between quantitative analysis of overall DNA destruction and microorganism resistance against UVC-LED treatment could be demonstrated by my research.

In conclusion, UV-LED is an innovative and effective technology substituting UV-lamp to decontaminate foodborne pathogens on agar media and sliced cheese. The efficacy of UV-LEDs for inactivating bacteria was validated, and UVC-susceptibility followed the sequence of GN > GP. By using quantitative methods for measuring membrane integrity, membrane

potential, and DNA damage caused by UV-LED treatment, the primary factors affecting inactivation levels of bacteria were DNA damage which was primarily responsible for the trend of bacterial death.

V. REFERENCES

Anderson JG, Rowan NJ, MacGregor SJ, Fouracre RA, Faris O. 2000. Inactivation of food-borne enteropathogenic bacteria and spoilage fungi using pulsed-light. IEEE Transactions on Plasma Science **28:**83-88.

Anvarian AHP, Smith MP, Overton TW. 2016. The effect of orange juice clarification on the physiology of *Escherichia coli*; growth-based and flow cytometric analysis. International Journal of Food Microbiology **219:**38-43.

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. Radiation Physics and Chemistry **81:**1193-1197.

Bintsis T, Litopoulou-Tzanetaki E, Robinson RK. 2000. Existing and potential applications of ultraviolet light in the food industry - a

critical review. Journal of Science of Food and agriculture **80:**637-645.

Bogdan J, Zaraynska J, Plawinska-Czarnak J. 2015. Comparison of infectious agents susceptibility to photocatalytic effects of nanosized titanium and zinc oxides: A practical approach. Nanoscale Research Letters **10:**309-323.

Bolton JR, Linden KG. 2003. Standardization of methods for fluence (UV dose) determination in bench-scale UV experiments. Journal of Environmental Engineering **129:**209-215.

Bucheli-Witschel M, Bassin C, Egli T. 2010. UV-C inactivation in *Escherichia coli* is affected by growth conditions preceding irradiation, in particular by the specific growth rate. Journal of Applied Microbiology **109:**1733-1744.

Cadet J, Wagner JR. 2013. DNA base damage by reactive oxygen species, oxidizing agents, and UV radiation. Cold Spring Harbor perspectives in biology **5:**a012559.

Can FO, Demirci A, Puri VM, Gourama H. 2014. Decontamination of Hard Cheeses by Pulsed UV Light. Journal of Food Protection **77:**1723-1731.

Chevremont AC, Farnet AM, Coulomb B, Boudenne JL. 2012. Effect of coupled UV-A and UV-C LEDs on both microbiological and chemical pollution of urban wastewaters. Science of the Total Environment **426:**304-310.

Choi DS, Park SH, Choi SR, Kim JS, Chun HH. 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 packaging and shelf life **3:**19-30.

da Silveira MG, San Romao MV, Loureiro-Dias MC, Rombouts FM, Abee T. 2002. Flow Cytometric Assessment of Membrane Integrity of Ethanol-Stressed *Oenococcus oeni* Cells. Applied and Environmental Microbiology **68:**6087-6093.

Diaz M, Herrero M, Garcia LA, Quiros C. 2010. Application of flow cytometry to industrial microbial bioprocesses. Biochemical Engineering Journal **48:**385-407.

Disdale MG, Lloyd D. 1995. Yeast vitality during cider fermentation: Two approaches to the measurement of membrane potential. Journal of the Institute of Brewing **101:**453-458.

Dume B. 2006. LEDs Move into the Ultraviolet. Physics World, Bristol, UK. http://physicsworld.com/cws/article/news/24926. Retrieved June. 24, 2015.

Fornander LH, Wu L, Billeter M, Lincoln P, Norden B. 2013. Minor-groove binding drugs: Where is the second Hoechst 33258 molecule?. The Journal of Physical Chemistry B **117:**5820-5830.

Friedberg EC, Walker GC, Siede W, Wood RD. 2006. DNA repair and mutagenesis, 2nd ed. ASM Press, Herndon, Washington.

Gavathiotis E, Sharman GJ, Searle MS. 2000. Sequence-dependent variation in DNA minor groove width dictates orientational preference of Hoechst 33258 in A-tract recognition: solution NMR structure of the 2:1 complex with d(CTTTTGCAAAAG)$_2$. Nucleic Acid Research **28:**728-735.

Guerrero-Beltrán JA, Barbosa-Cánovas GV. 2004. Review: Advantages and Limitations on Processing Foods by UV Light. Food Science and Technology International **10:**137-147.

Ha JW, Kang DH. 2013. Simultaneous near-infrared radiant heating and UV radiation for inactivating *Escherichia coli* O157: H7 and *Salmonella enterica* serovar Typhimurium in powdered red pepper (*Capsicum annuum* L.). Applied and Environmental Microbiology **79:**6568-6575.

Ha JW, Kang DH. 2014. Synergistic bactericidal effect of simultaneous near-infrared radiant heating and UV radiation against *Cronobacter sakazakii* in powdered infant formula. Applied and Environmental Microbiology **80:**1858-1863.

Hijnen WAM, Beerendonk EF, Medema GF. 2006. Inactivation credit of UV radiation for viruses, bacteria and protozoan (oo)cysts in water: A review. Water Research **40:**3-22.

Kalisvaart BF. 2004. Re-use of wastewater: preventing the recovery of pathogens by using medium-pressure UV lamp technology. Water Science and Technology **50**:337-344.

Kim YH, Jeong SG, Back KH, Park KH, Chung MS, Kang DH. 2013. Effect of various conditions on inactivation of *Escherichia coli* O157:H7, *Salmonella* Typhimurium, and *Listeria monocytogenes* in fresh-cut lettuce using ultraviolet radiation. International Journal of Food Microbiology **166:**349-355.

Koutchma T. 2009. Advances in ultraviolet light technology for non-thermal processing of liquid foods. Food Bioprocess Technology **2:**138-155.

Lee SY, Kang DH. 2001. Suitability of Overlay Method for Recovery of Heat-Injured *Listeria monocytogenes* and *Salmonella* Typhimurium. Food science and Biotechnology **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. Applied and Environmental Microbiology **82:**1828-1837.

Lloyd D, Salgado LEJ, Turner MP, Suller MTE, Murray D. 2002. Cycles of mitochondrial energization driven by the ultradian clock in a continuous culture of *Saccharomyces cerevisiae*. Microbiology **148:**3715-3724.

Lopez-Malo A, Palou E. 2005. Novel food processing technologies. CRC Press, Boca Raton, FL.

McKinney J, Williams RC, Boardman GD, Eifert JD, Sumner SS. 2009. Dose of UV Light Required To Inactivate *Listeria monocytogenes* in Distilled Water, Fresh Brine, and Spent Brine. Journal of Food Protection **72:**2144-2150.

Mori M, Hamamoto A, Takahashi A, Nakano M, Wakikawa N, Tachibana S, Ikehara T, Nakaya Y, Akutagawa M, Kinouchi Y.

2007. Development of a new water sterilization device with a 365 nm UV-LED. Med Bio Eng Comput **45:**1237-1241.

Mukhopadhyay S, Ukuku DO, Juneja V, Fan X. 2014. Effects of UV-C treatment on inactivation of *Salmonella* and *Escherichia coli* O157:H7 on tomato surface and steam scars, native microbial loads, and quality of grape tomatoes. Food Control **44:**110-117.

Pace CN, Vajdos F, Fee L, Grimsley G, Gray T. 1995. How to measure and predict the molar absorption coefficient of protein. Protein Science **4:**2411-2423.

Park H, Zhang K, Ren Y, Nadji S, Sinha N, Taylor J-S, Kang C. 2002. Crystal structure of a DNA decamer containing a *cis-syn* thymine dimer. PNAS **99:**15965-15970.

Park IK, Kang DH. 2013. Effect of electropermeabilization by ohmic heating for inactivation of *Escherichia coli* O157:H7, *Salmonella enterica* Serovar Typhimurium, and *Listeria monocytogenes* in buffered peptone water and apple Juice. Applied and Environmental Microbiology **79:**7122-7129.

Pjura PE, Grzeskowiak K, Dickerson RE. 1987. Binding of Hoechst 33258 to the Minor Groove of *B*-DNA. Journal of Molecular Biology **197:**257-271.

Rhee MS, Lee SY, Hiller VN, McCurdy SM, Kang DH. 2003. Evaluation of Consumer-Style Cooking Methods for Reduction of *Escherichia coli* O157:H7 in Ground Beef. Journal of Food Protection **66:**1030-1034.

Rottenberg H. 1979. The Measurement of Membrane Potential and ΔpH in Cells, Organelles, and Vesicles. Methods in Enzymology **55:**547-569.

Rowan NJ, MacGregor SJ, Anderson JG, Fouracre RA, McIlvaney L, Farish O. 1999. Pulsed-Light Inactivation of Food-Related Microorganisms. Applied and Environmental Microbiology **65:**1312-1315.

Saini JK, Marsden JL, Getty KJK, Fung DYC. 2014. Advanced Oxidation Technology with Photohydroionization as a Surface

Treatment for Controlling *Listeria monocytogenes* on Stainless Steel Surfaces and Ready-to-Eat Cheese and Turkey. Foodborne Pathogens and Disease **11:**295-300.

Sanchez E, Garcia S, Heredia N. 2010. Extracts of Edible and Medicinal Plants Damage Membranes of *Vibrio Cholerae*. Applied and Environmental Microbiology **76:**6888-6894.

Santos AL, Oliveira V, Baptista I, Henriques I, Gomes NCM, Almeida A, Correia A, Cunha A. 2013. Wavelength dependence of biological damage induced by UV radiation on bacteria. Archives of Microbiology **195:**63-74.

Scharffetter-Kochanek K, Wlaschek M, Brenneisen P, Schauen M, Blaudschun R, Wenk J. 1997. UV-induced Reactive Oxygen Species in Photocarcinogenesis and Photoaging. Biological Chemistry **378:**1247-1258.

Schenk M, Raffellini S, Guerrero S, Blanco GA, Alzamora SM. 2011. Inactivation of *Escherichia coli, Listeria innocua* and

Saccharomyces cerevisiae by UV-C light: Study of cell injury by flow cytometry. LWT – Food science and Technology **44:**191-198.

Sharma G. 2000. Ultraviolet light. Encyclopedia of Food Microbiology **3:** 2208-2214.

Shin JY, Kim SJ, Kim DK, Kang DH. 2016. Fundamental Characteristics of Deep-UV Light-Emitting Diodes and Their Application To Control Foodborne Pathogens. Applied and Environmental Microbiology **82:**2-10.

Siva F, Ferreira S, Queiroz JA, Domingues FC. 2011. Coriander (*Coriandrum sativum* L.) essential oil: its antibacterial activity and mode of action evaluated by flow cytometry. Journal of Medical Microbiology **60:**1479-1486.

Stoscheck CM. 1990. Quantitation of protein. Methods in Enzymology **182:**50-68.

Tran MTT, Farid M. 2004. Ultraviolet treatment of orange juice. Innovative Food Science and Emerging Technologies **5:**495-502.

Ueckert J, Breeuwer P, Abee T, Stephens P, Nebe von Caron G, Ter Steeg PF. 1995. Flow cytometry applications in physiological study and detection of foodborne microorganisms. International Journal of Food Microbiology **28:**317-326.

Wassmann M, Moeller R, Reitz G, Rettberg P. 2011. Growth phase-dependent UV-C resistance of *Bacillus subtilis*: data from a short-term evolution experiment. Archives of Microbiology **193:**823-832.

VI. 국문초록

자외선은 살균 산업에서 널리 사용되는 기술로, 일반적으로 램프 타입으로 사용되고 있다. 하지만 자외선 램프는 냉장 온도에서는 출력이 낮고, 최대의 출력을 내기 위해서는 일정 시간이 필요하며, 수은 노출의 위험 등이 있다. 이의 대안으로서 개발된 UV-LED 는 자외선 램프가 오직 254 nm 의 파장만 낼 수 있는 데 반해 원하는 특정 파장으로 제작할 수 있다. 본 연구에서는 파장에 따른 UV-LED 의 저감화 효과를 알아보았고, 자외선 램프와의 살균 효율을 비교해보았다. 우선 고체 배지 실험에서는, *E. coli* O157:H7, *S.* Typhimurium, *L. monocytogenes* 가 접종된 선택배지에 266, 270, 275, 279 nm 의 UV-LED 를 0.1, 0.2, 0.5, 0.7 mJ/cm^2 의 조사량으로 처리하였다. 빛의 세기는 약 4 μW/cm^2 였고, 자외선 램프의 세기를 UV-LED 와 비슷한 수준으로 맞추기 위해서 자외선 램프에 폴리프로필렌 필름을 씌워 빛의 세기를 조정하였다. 또한 실제 식품인 슬라이스 치즈에도 UV-LED 를 적용하여 1, 2, 3 mJ/cm^2 로 처리하였다. 실험 결과, 같은 조사량 처리에서 UV-LED 가 자외선 램프에 비해 훨씬 높은 저감화 효율을 나타내었다.

배지 실험에서는 266, 270 nm 와 같은 낮은 파장의 UV-LED 처리가 높은 파장보다 큰 저감화를 나타냈지만 파장별로 큰 차이는 없었다. 슬라이스 치즈에는 3 mJ/cm^2 처리 후 접종한 모든 균에서 인저드 셀 발생 없이 4-5 로그의 저감화가 일어났다.

균의 특성별로 보이는 저감화 경향의 가장 주요한 요인을 알아보기 위하여 그람 음성 균과 그람 양성 균으로 세균의 그룹을 나누어 실험을 진행하였다. 대표적 식중독 균인 *E. coli* O157:H7, *Salmonella* spp., *L. monocytogenes*, *S. aureus* 를 선택배지와 비선택배지에 도말하여 파장별 UV-LED 를 조사하였을 때 어떠한 경향성이 나타나는지 확인하였다. 실험 결과, 조사량이 증가할수록 저감화 정도가 증가하는 것은 같았지만, 그람 양성 균이 그람 음성 균에 비해 자외선에 대해 더욱 높은 저항성을 나타내었다. 또한 PI, $DiBAC_4(3)$, Hoechst 33258 의 형광성 염색제를 사용하여 UV-LED 의 조사로 인한 세포막과 DNA 의 손상을 조사하였다. 모든 세균에서 파장이 증가할수록 막의 포텐셜을 나타내는 $DiBAC_4(3)$ 값이 증가하였고, 막의 실질적 손상을 나타내는 PI 값도 같은 경향성을 나타내었다. 그러나 Hoechst 33258 로 측정한 DNA 손상은 파장이 감소할수록 증가하여 막 손상의 결과와 반대로

나타났다. 저감화 결과의 경향성과 비교해볼 때, 이 경향과 일치하는 DNA 손상이 자외선으로 인한 미생물 저감화의 주요 요인으로 확인되었고, 이를 통해 자외선의 조사로 막 손상이 수반되지만 막 손상보다는 DNA 손상이 저감화의 가장 주요한 요인이라는 것을 정량적으로 검증하였다.

주요어: 자외선 조사, UVC, 파장, 식중독 균, 슬라이스 치즈, Light Emitting Diodes (LED), 그람 음성 균, 그람 양성 균

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