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Antibacterial effect of 405 ± 5 nm light emitting diode illumination against *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella* on the surface of fresh-cut mango and its influence on fruit quality

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ABSTRACT

To investigate a potential of 405 ± 5 nm light emitting diode (LED) as a novel technology for food preservation, the antibacterial effect of 405 ± 5 nm LED on *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella* spp. on the surface of fresh-cut mango and its influence on fruit quality were evaluated at different storage temperatures. LED-illumination inactivated 1.0–1.6 log CFU/cm^2 of populations at 4 and 10 °C for 36–48 h (total dose, 2.6–3.5 kJ/cm^2) regardless of bacterial species, while those on non-illuminated mange remained unchanged or slightly increased during storage. At 20 °C for 24 h (total dose, 1.7 kJ/cm^2), non-illuminated *E. coli* O157:H7 and *Salmonella* gradually grew, whereas LED-illumination reduced 1.2 log of *Salmonella* and inhibited the growth of *E. coli* O157:H7. Unlike these, non-illuminated *L. monocytogenes* cells rapidly increased to 7.3 log, while illuminated cells reached 4.6 log, revealing that LED-illumination delayed their growth. There were no significant ($P > 0.05$) differences in color, antioxidant capacity, ascorbic acid, β-carotene, and flavonoid between non-illuminated and illuminated cut mangoes, regardless of storage temperature. These results suggest that 405 ± 5 nm LEDs in combination with chilling temperatures could be applied to preserve fresh-cut fruits without deterioration of physicochemical quality of fruits at food establishments, minimizing the risk of foodborne disease.

1. Introduction

Concerns about bacterial contamination in fresh-cut fruits have greatly increased since fresh-cut fruits became popular because there is no further step to effectively eliminate bacteria in fresh-cut fruit processing. For this reason, numerous salmonellosis outbreaks have been recently associated with consumption of fresh fruits, such as cantaloupe and watermelon. For example, *Salmonella* Newport outbreak in ready-to-eat (RTE) cut watermelons was reported in the United Kingdom (UK) in 2012 (Byrne et al., 2014). In addition, a total of 127 confirmed cases of infection and 33 hospitalizations with *S.* Braenderup were linked to imported mangoes in 15 states of the United States (US) in 2012 (CDC, 2012). Whole cantaloupes were also linked to a *Listeria monocytogenes* outbreak, resulting in 146 illnesses and 32 deaths in the US in 2011 (CDC, 2012). In the US, *Escherichia coli* O157:H7 has been identified as a causative agent for two cantaloupe outbreaks in 1997 and 2004 (Castillo et al., 2014). Therefore, fresh fruits can be a vehicle for pathogenic bacteria, resulting in potentially hazardous food to human.

To keep fresh-cut fruits safe in retail stores, refrigeration as one of preservation technologies has been widely used. However, some foodborne pathogens are able to survive or grow at refrigeration temperature. It is known that mesophilic *E. coli* O157:H7 and *Salmonella* can survive during storage at 5 °C, while psychrotrophic *L. monocytogenes* can grow at 4 °C (Dose, 2001). Thus, an alternative technology in combination with refrigeration should be applied to effectively control foodborne pathogens on fresh-cut fruits without deterioration.

Light emitting diodes (LEDs) of visible wavelengths have recently gained attention as a novel preservation technology due to their antibacterial effect. Previous studies showed that blue LEDs of 405 and 460 nm wavelengths could inactivate various foodborne pathogens, such as *E. coli* O157:H7, *L. monocytogenes*, and *S.* Typhimurium in phosphate buffered saline (PBS) solution or trypticase soy broth (TSB) without the addition of exogenous photosensitizers under refrigerated condition (Ghate et al., 2013; Kim et al., 2015, 2016). Kumar et al. (2015) have demonstrated that 405 nm LED showed the greatest antibacterial effect compared with 460 and 520 nm LEDs. Another previous study has also shown that 405 nm wavelength revealed the greatest antibacterial efficacy on *L. monocytogenes* within the wavelengths ranging from 400 to 500 nm (Endarko et al., 2012). A recent study has reported

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the antimicrobial effect of 460 nm LED against *Salmonella* in orange juice without any additional photosensitizer (Ghate et al., 2016).

Blue LED illumination could be explained by photodynamic inactivation (PDI) that requires photosensitizers, such as intracellular porphyrin compounds naturally produced by some bacteria, and visible lights within 400–430 nm wavelengths under the presence of oxygen (Luksienė and Zukauskas, 2009). Once bacterial cells are exposed to light under existing oxygen, the endogenous porphyrin compounds inside cells absorb the light, followed by being excited. As a result, reactive oxygen species (ROS), such as superoxide ion and singlet oxygen, are produced. The ROS can bring about a cytotoxic effect by interacting with adjacent intracellular components, such as DNA, protein, and lipids, resulting in bacterial death (Luksienė and Zukauskas, 2009).

Although several studies have been published regarding the efficacy of LED in inactivating pathogenic bacteria on fresh produce by adding exogenous photosensitizers (Luksiene and Paskeviciute, 2011), to our knowledge, no information is available on the effectiveness of LED alone and its impact on food quality. Thus, the aims of this study were to evaluate the effectiveness of 405 ± 5 nm LED illumination on *E. coli* O157:H7, *L. monocytogenes*, and *Salmonella* spp. on fresh-cut mango during storage at different temperatures and to investigate physicochemical and nutritional qualities of fresh-cut mangos after long-term LED illumination.

2. Materials and methods

2.1. Bacterial strains and culture conditions

Three strains of *E. coli* O157:H7 (ATCC 35150, C7927, and F12), 3 serotypes of *L. monocytogenes* (1/2a ATCC BAA-679, 1/2b ATCC BAA-839, and 4b ATCC 13932), and 5 serotypes of *Salmonella* (*S.* Agona ATCC BAA-707, *S.* Newport ATCC 6962, *S.* Saintpaul ATCC 9712, *S.* Tennessee ATCC 10722, and *S.* Typhimurium ATCC 14028) were used in this study. All ATCC strains were purchased from the American Type Culture Collection (Manassas, VA, USA) and two *E. coli* O157:H7 strains (C7927 and F12) were obtained from Dr. Kun-Ho Seo of Konkuk University in Republic of Korea. Frozen stock cultures were activated in 10 mL of tryptone soya broth (TSB; Oxoid, Basingstoke, UK) at 37 °C for 18–24 h. All cultures were adapted to 200 μg/mL of nalidixic acid (Sigma-Aldrich, St. Louis, MO, USA) by successive culturing with incremental concentrations of nalidixic acid in TSB to develop antibiotic resistance that allows isolation of inoculated cells from naturally existing microbiota in fresh-cut mangoes. Two consecutive transfers were carried out in 10 mL of TSB supplemented with 200 μg/mL of nalidixic acid at 37 for 18–24 h prior to use.

2.2. Light emitting diode (LED) illumination system

High intensity 405 ± 5 nm LED (8 × 8 mm; Shenzhen, Guangdong, China) was attached to a heat sink and a fan for the dissipation of heat generated during LED illumination. A resistance of 5 Ω was used in the circuit by connecting two 10 Ω resistors in parallel to adjust the intensity of LEDs. The irradiance of 405 ± 5 nm LED at the fruit surface was 20 ± 2 mW/cm^2 that was measured using a Compact power and energy meter console (PM100D; Thorlabs GmbH, Dachau, Germany). Each LED system was set up in an acrylonitrile butadiene system (ABS) housing with open space to prevent overheating of the sample. Two mango samples in a sterile Petri dish (60 mm diameter) were placed in the LED system at a distance of 4.5 cm to illuminate the entire fruit samples (Fig. 1). The temperature on the surface of cut mango was monitored for 8 h with 1 min intervals during LED illumination using a Fluke 5.4 thermocouple thermometer (Everett, WA, USA). The dose received by each sample was calculated using the following equation (Ghate et al., 2013).

$$E = Pt$$

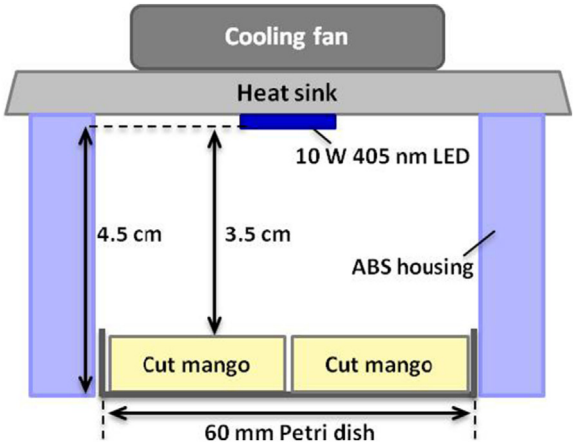

Fig. 1. Schematic diagrams of 405 ± 5 nm LED illumination system.

where E = dose in J/cm^2, P = irradiance in W/cm^2, and t = time in second.

2.3. Preparation of mango and inoculation

Fresh Thailand mangoes were purchased from local supermarkets in Singapore. For each trial, mangoes were washed with tap water, sanitized by spraying with 70% ethanol solution, rinsed three times with sterile deionized water, and finally dried with Kimwipes (Kimtech Science, Kimberly Clark Professional, Roswell, GA, USA). The dried mangoes were peeled and cut into ca. 10 g pieces in the shape of a half-moon (60 mm diameter).

Each cocktail culture of *E. coli* O157:H7, *L. monocytogenes*, or *Salmonella* spp. was prepared by combining equal portions of each strain or serotype, centrifuging at 6000 × *g* for 10 min at 4 °C and washing twice with phosphate buffered saline (PBS; Vivantis Technologies Sdn. Bhd., Malaysia). The cocktail culture (ca. 10^9 CFU/mL) was serially diluted in PBS and a 10-μL aliquot of the diluents (ca. 10^5 CFU/mL) was inoculated at 10 sites on the surface of mangoes to reach a final concentration of ca. 10^3 CFU/cm^2. The inoculated mangoes were dried for 30 min in a biosafety cabinet and were individually packaged with cling wrap to simulate the conditions found in retail stores.

2.4. LED illumination

Two inoculated or uninoculated fruits were placed in the LED illumination system and were exposed to 405 ± 5 nm LED at 4, 10, or 20 °C, for 24–48 h (a total dose of 1.7–3.5 kJ/cm^2) in a temperature-controlled incubator (MIR-154, Panasonic Healthcare Co., Ltd., Osaka, Japan). Non-illuminated control fruits were also placed in the incubator without LED illumination (dark condition). LED-illuminated and non-illuminated fruits were taken at selected time intervals, promptly transferred into a sterile stomacher bag containing 90 mL of 0.1% (w/v) peptone water (PW; Oxoid), and homogenized for 2 min using a paddle blender (Silver Masticator, IUL Instruments GmbH, Königswinter, Germany). After serial dilution if necessary, the diluents were pour-plated onto tryptone soya agar (TSA; Oxoid) supplemented with 200 μg/mL of nalidixic acid and then incubated at 37 °C for 24–48 h. The number of colonies was manually enumerated with a colony counter (Rocker Scientific Co. Ltd., Taipei, Taiwan) and was expressed in log CFU/cm^2.

2.5. Modified Weibull model for bacterial inactivation kinetics

The modified Weibull model was applied to compare the bacterial susceptibility to 405 ± 5 nm LED illumination. The model is useful for fitting various bacterial inactivation curves, such as convex, concave, and linear curves (Kim et al., 2015, 2016; Kumar et al., 2015). The

model parameters consist of α (scale) and β (shape) in the following equation (Bialka et al., 2008).

$$\log_{10}\left(\frac{N}{N_0}\right) = -\frac{1}{2.303}\left(\frac{t}{\alpha}\right)^{\beta}$$

where N_0 and N are the microbial population before and after LED illumination (CFU/cm^2), and t is the exposure time (h) to 405 ± 5 nm LED. Based on α and β parameters, the reliable life (t_R) was calculated using the following equation (Bialka et al., 2008)

$$t_R = \alpha\,(2.303)^{\frac{1}{\beta}}$$

The t_R value signifies the time required for a 90% reduction of the bacterial population, which is similar to the concept of the D-value for first-order inactivation kinetics (Bialka et al., 2008). The t_R values were analyzed using Origin 9.0 software (OriginLab Co., Northampton, MA, USA).

2.6. Color analysis

Color of illuminated or non-illuminated fruit samples was measured with a reflectance spectrometer (CM-3500d; Konica Minolta Sensing Inc., Osaka, Japan) with a D65 illuminant and an observation angle of 10°. The color parameters of L^* (lightness) and b^* (yellow to blue) were measured through reflectance values. The values were used to calculate the yellow index (YI) using the following equation (Anyasi et al., 2015).

$$\mathrm{YI} = \frac{142.86 \times b^*}{L^*}$$

YI is useful to quantify the effects of degradation processes such as exposure to light (Anyasi et al., 2015).

2.7. DPPH assay

To monitor the change in antioxidant capacity of fresh-cut mangoes, illuminated or non-illuminated fruit samples were analyzed by the method of González-Aguilar et al. (2007a) with some modifications. A 10 g sample was homogenized with 25 mL of 80% methanol including 0.5% sodium bisulfate (Sigma-Aldrich) for 1 min using a paddle blender, sonicated for 60 min at room temperature by adding ice to avoid increasing the temperature, and then centrifuged at 3000 ×g for 10 min at room temperature. The supernatant was filtered with Whatman™ No. 1 filter paper (GE Healthcare, Little Chalfont, Buckinghamshire, UK) and then was stored at −20 °C prior to analysis of antioxidant capacity and total flavonoid contents.

The stock solution was freshly prepared by dissolving 2.82 mg of 2,2-diphenyl-1-picrylhydrazyl (DPPH; Sigma-Aldrich) in 10 mL of methanol. A 30-μL extract was mixed with 420 μL of the DPPH solution, followed by incubation for 30 min at room temperature in the dark. A 200-μL mixture was transferred to a 96-well plate in duplicate and measured at 515 nm using a Synergy HT multi-detection microplate reader (Bio-Tek Instruments Inc., Winooski, VT, USA). A standard curve was established between 0 and 20.0 mg/100 mL Trolox (Sigma-Aldrich). Antioxidant capacity was expressed in mg Trolox equivalents (TE)/100 g fresh weight (FW).

2.8. Total flavonoid content

A 50-μL extract of fruits was mixed with 200 μL of deionized H_2O and 15 μL of 5% sodium nitrite (Sigma-Aldrich) and then equilibrated for 5 min, followed by adding 15 μL of 10% aluminum chloride (Sigma-Aldrich) into the mixture. After equilibrium for 5 min, 100 μL of 1 M sodium hydroxide (Sigma-Aldrich) was added. The final volume was adjusted to 500 μL with deionized H_2O and then 200 μL of the mixture was transferred in a 96-well plate in duplicate. The mixture was measured at 415 nm using the microplate reader. The total flavonoid content was calculated on the basis of a standard curve between 0 and 7.6 mg/100 mL Quercetin (Sigma-Aldrich) and reported in mg Quercetin equivalents (QE)/100 g FW.

2.9. Ascorbic acid content

Fruit extracts were prepared using the method described by Barros et al. (2007) and Spilimbergo et al. (2013) with some modifications. A 5 g fruit sample was mixed with 10 mL of 2.5% meta-phosphoric acid (Sigma-Aldrich). The mixture was incubated for 45 min at room temperature and then centrifuged at 3000 ×g for 2 min. The supernatant was filtered using Whatman™ No. 1 filter and then kept at 4 °C prior to use.

Ascorbic acid content was determined using the method described in Barros et al. (2007) and Freed (1966) with some modifications. A stock solution (0.025% DCIP) was freshly prepared by dissolving 12.5 mg of 2,6-dichloroindophenol sodium salt (DCIP; Sigma-Aldrich) in 50 mL of deionized H_2O with 1 mg of sodium bicarbonate (Sigma-Aldrich). A 150-μL extract was added to 100 μL of the 0.025% DCIP in a 96-well plate in duplicate and measured at 515 nm using the microplate reader. The concentration of ascorbic acid was calculated based on a standard curve in the range of 4.4–12.3 mg/100 mL L-Ascorbic acid (Sigma-Aldrich) and expressed as mg ascorbic acid/100 g FW.

2.10. β-carotene content

Fruit extracts were carried out according to the method of Barros et al. (2007) and Nagata and Yamashita (1992) with some modifications. A 5 g fruit sample was vigorously shaken in a 50-mL Falcon tube containing 10 mL of an acetone-hexane mixture (4:6, v/v) for 2 min. The mixture was centrifuged at 3000 ×g for 2 min at room temperature and then filtered with Whatman™ No. 1 filter. The filtered extracts were kept at 4 °C prior to use.

A 200-μL extract was loaded in a 96-well plate in duplicate and then its absorbance was measured at 663, 645, 505, and 453 nm using the microplate reader. The concentration of β-carotene was calculated using the following equation (Nagata and Yamashita, 1992) and reported as mg β-carotene/100 g FW.

$$\beta\text{−carotene (mg/100 g)} = 0.216\,A_{663} - 1.22\,A_{645} - 0.304\,A_{505} + 0.452\,A_{453}$$

2.11. Statistical analysis

Each experiment was independently performed in triplicate with duplicate samples (n = 6). All data were expressed as mean ± standard deviation and were analyzed using analysis of variance (ANOVA) and least significant difference (LSD) to compare any significant difference ($P \le 0.05$) between samples using IBM SPSS statistical software (version 20; SPSS Inc., IBM Co., Armonk, NY, USA).

3. Results

Because 405 ± 5 nm LED illumination could possibly have a heating effect, the surface temperature of fresh-cut mango was monitored for 8 h with 1 min intervals during LED illumination to select the temperature condition for the control experiment. The results show temperature increase of about 3.2 °C from the incubator set temperature within 1 h, regardless of set temperature (Fig. 2). Thus, non-illuminated control fruits were stored at 7.2, 13.2, and 23.2 °C for set temperatures of 4, 10, and 20 °C, respectively, to eliminate the effect of temperature

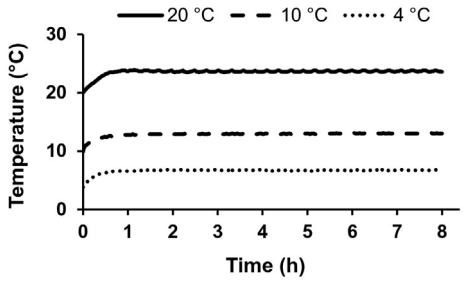

Fig. 2. Temperature profile on the surface of fresh-cut mango during 405 ± 5 nm LED illumination at set temperatures of 4, 10, and 20 °C, respectively.

difference on the bacterial inactivation or growth between LED illumination and non-illumination.

The antibacterial effect of 405 ± 5 nm LED illumination on *E. coli* O157:H7, *L. monocytogenes*, and *Salmonella* spp. on cut mango was evaluated at set temperatures of 4 and 10 °C for 48 and 36 h, respectively (Figs. 3 and 4). Regardless of bacterial species, the number of bacterial cells on non-illuminated mangoes remained unchanged during storage, with the exception for *E. coli* O157:H7 and *L. monocytogenes*. For example, the number of *E. coli* O157:H7 cells on non-illuminated mango was statistically significantly ($P \le 0.05$) decreased of 0.2 log CFU/cm^2 after 48 h at set temperature of 4 °C, whereas the population of *L. monocytogenes* on non-illuminated mango significantly ($P \le 0.05$) increased to 4.1 log CFU/cm^2 at set temperature of 10 °C. On the other hand, LED illumination significantly reduced the populations of the bacterial pathogens to <1.6 log CFU/cm^2 for 36–48 h (a total dose of 2.6–3.5 kJ/cm^2). In particular, LED illumination inactivated *E. coli* O157:H7 and *Salmonella* spp. to below the detection limit (1.0 log) after 36 h at 4 and 10 °C.

Under the room temperature condition, *E. coli* O157:H7, *L. monocytogenes*, and *Salmonella* spp. on non-illuminated mangoes grew to 4.6, 7.3, and 4.3 log CFU/cm^2, respectively, within 24 h (Fig. 5). The final populations of LED illuminated cells were significantly ($P \le 0.05$) lower than those of non-illuminated cells, indicating the antibacterial effect of LED illumination during storage at room temperature; however, its effectiveness was species dependent. For example, the growth of *E. coli* O157:H7 was completely inhibited and the number of *Salmonella* cells significantly decreased to 1.2 log CFU/cm^2 during LED illumination for 24 h (a total dose of 1.7 kJ/cm^2), whereas the population of *L. monocytogenes* was inhibited during LED exposure during the first 12 h of storage, but thereafter, gradually increased to 4.6 log CFU/cm^2 at the end of the LED illumination.

To compare the bacterial susceptibility to LED illumination, the reliable life (t_R) was calculated based on log reductions at 4 and 10 °C using scale (α) and shape (β) parameters of the Weibull model (Table 1). The α value can be used as a measure of bacterial resistance to LED illumination and is consistent in the mean of the distribution accounting for bacterial inactivation time (h), while β values indicate an efficacy on predicted inactivation rate (Bialka et al., 2008; Kim et al., 2015, 2016). At 4 °C, all three bacterial species had β value of <1, which means a higher inactivation rate at a lower dose of LED illumination. *Salmonella* cells at 10 °C had significantly higher β value of 2.1 compared to those of other bacterial species, meaning that the rate of inactivation increased with increasing light dose (Bialka et al., 2008; Kim et al., 2015, 2016). *Salmonella* had also the highest α and t_R values at 10 °C among the three species. The three species showed statistically similar ($P > 0.05$) sensitivity to LED illumination at 4 °C based on the t_R values, although the mean t_R value of *E. coli* O157:H7 was lower. By contrast, the t_R value of *Salmonella* at 10 °C was 1.7–1.9 times higher than those of *E. coli* O157:H7 and *L. monocytogenes*, indicating that *Salmonella* was the most resistant pathogen to 405 ± 5 nm LED illumination under temperature abuse conditions. However, the number of surviving *Salmonella* cells was similar to *E. coli* O157:H7 and *L. monocytogenes* at the end of illumination (Fig. 4).

To determine the impact of long-term LED illumination on physicochemical and nutritional qualities of cut fruits, the color,

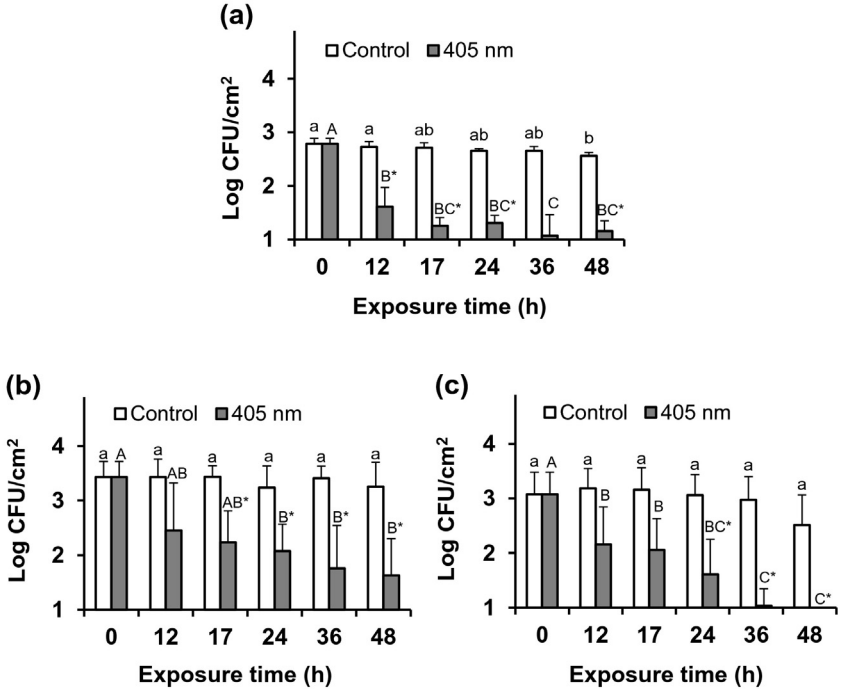

Fig. 3. Survival of *E. coli* O157:H7 (a), *L. monocytogenes* (b), and *Salmonella* spp. (c) on the surface of fresh-cut mango at set temperature of 4 °C (actual temperature of 7.2 °C) by 405 ± 5 nm LED illumination. Asterisk (*) indicates significant ($P \le 0.05$) difference between LED-illuminated and non-illuminated bacterial cell counts. Different letters (A–C or a–c) in the same bar are significantly ($P \le 0.05$) different one another.

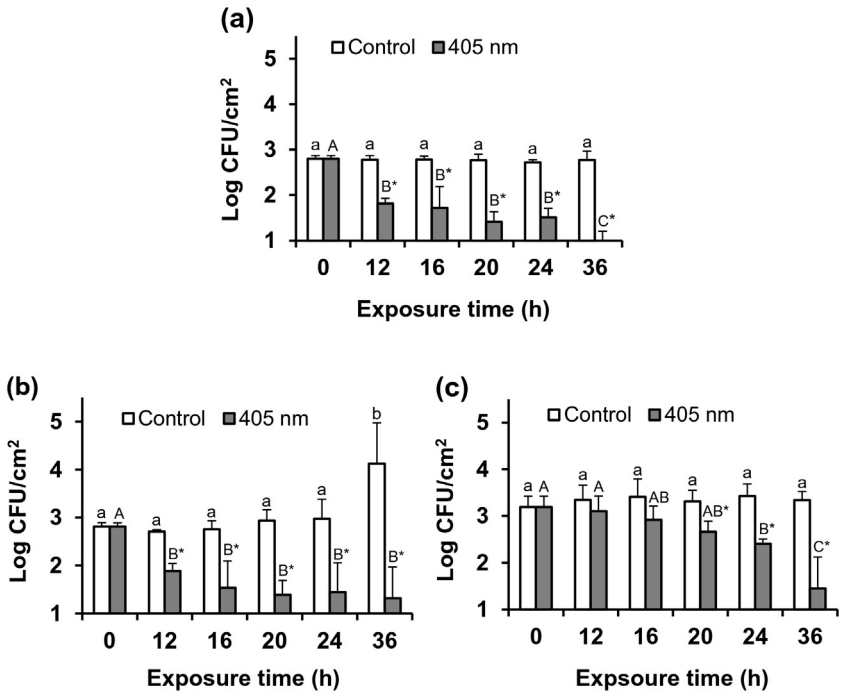

Fig. 4. Survival of *E. coli* O157:H7 (a), *L. monocytogenes* (b), and *Salmonella* spp. (c) on the surface of fresh-cut mango at set temperature of 10 °C (actual temperature of 13.2 °C) by 405 ± 5 nm LED illumination. Different letters $^{(A–C \text{ or } a–c)}$ in the same bar are significantly ($P \leq 0.05$) different one another.

antioxidant capacity, ascorbic acid, β-carotene, and flavonoid were measured. No statistically significant ($P > 0.05$) difference in yellow index (YI) was observed between non-illuminated and illuminated fruits for 24–48 h, regardless of storage temperature (Fig. 6). For nutritional qualities, the results show no significant ($P > 0.05$) differences in the levels of ascorbic acid, β-carotene, and flavonoid between non-illuminated and illuminated cut fruits at all storage temperatures (Table 2).

4. Discussion

LED illumination at 405 ± 5 nm has recently demonstrated its antibacterial effect against various foodborne pathogens without any additional photosensitizer in PBS (Kim et al., 2015, 2016; Kumar et al., 2015). To evaluate the effectiveness of LED illumination on the preservation of fresh-cut fruits, *E. coli* O157:H7, *L. monocytogenes*, and *Salmonella* spp. on the surface of fresh-cut mango were illuminated at different storage

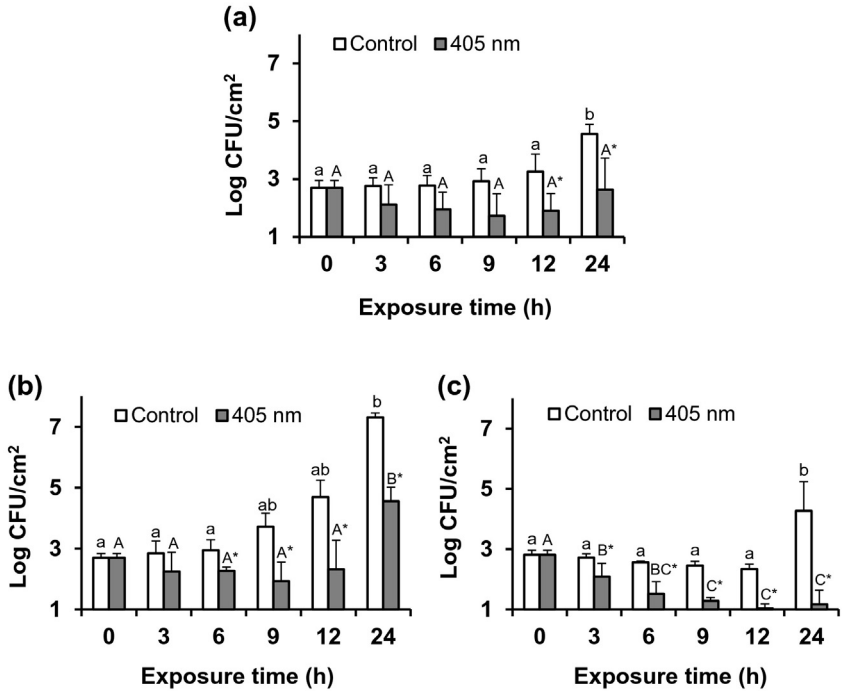

Fig. 5. Survival of *E. coli* O157:H7 (a), *L. monocytogenes* (b), and *Salmonella* spp. (c) on the surface of fresh-cut mango at set temperature of 20 °C (actual temperature of 23.2 °C) by 405 ± 5 nm LED illumination. Different letters $^{(A–C \text{ or } a–c)}$ in the same bar are significantly ($P \leq 0.05$) different one another.

Table 1
Comparison of the t_R-values of *E. coli* O157:H7, *L. monocytogenes*, and *Salmonella* spp. by 405 ± 5 nm LED illumination at refrigerated conditions using Weibull model parameters.

Temperature	Species	α (h)	β	t_R (h)	R^2
4 °C	*E. coli* O157:H7	1.36 ± 0.37^{aA}	0.38 ± 0.25^{aA}	6.85 ± 5.94^{aA}	0.95 ± 0.03
	L. monocytogenes	1.92 ± 1.50^{aA}	0.46 ± 0.12^{aA}	10.02 ± 6.23^{aA}	0.94 ± 0.05
	Salmonella	3.65 ± 2.43^{aA}	0.64 ± 0.23^{aA}	12.54 ± 4.24^{aA}	0.96 ± 0.04
10 °C	*E. coli* O157:H7	4.46 ± 1.25^{aA}	0.69 ± 0.12^{aA}	14.85 ± 1.48^{aA}	0.91 ± 0.07
	L. monocytogenes	4.61 ± 2.72^{aA}	0.79 ± 0.25^{aA}	13.21 ± 3.58^{aA}	0.96 ± 0.03
	Salmonella	17.24 ± 1.84^{bB}	2.10 ± 0.11^{bB}	25.63 ± 2.36^{bB}	0.95 ± 0.05

Lowercase letters $^{(a–b)}$ in the same column at the same temperature and uppercase letters $^{(A–B)}$ in the same column within the same species differ significantly (n = 6; $P \le 0.05$).

temperatures of 4, 10, and 20 °C which simulated ideal refrigeration, temperature abuse in a retail store, and room temperature conditions, respectively.

Results showed that 405 ± 5 nm LED illumination at chilling temperatures could inactivate all bacterial species tested in this study. On the other hand, only growth inhibition or delay was observed at room temperature during LED illumination, except for *Salmonella*, indicating that the effect of LED illumination was highly species dependent. Similarly, a previous study reported that 461 nm LED illumination brought about the inactivation of *E. coli* O157:H7, *L. monocytogenes* and *S.* Typhimurium in TSB at 10 and 15 °C, but only growth inhibition at 20 °C (Ghate et al., 2013). These results indicate that the antibacterial effect of blue LED could be enhanced at lower temperatures. This might be associated with the inability to transport sugars inside cells by changing the fluidity of bacterial lipid membrane at chilling temperatures, resulting in the restriction of energy uptake and consequently increase in cellular susceptibility to LED illumination (Beales, 2004). Another possible reason might be the inactivity of cellular defense systems such as superoxide dismutase and catalase at lower temperatures, resulting in failure to remove intracellular ROS (Beales, 2004).

The fruit surface pH could also affect bacterial behaviors on fresh-cut mango during storage. The pH of mango is to 3.8–4.2 and its major organic acid is citric acid, resulting in the inability to support the growth in non-illuminated bacterial populations dependent on the storage temperatures (González-Aguilar et al., 2000; Ma et al., 2016). The lower pH of mango could give a synergistic effect on the bacterial inactivation by LED illumination at refrigeration conditions. The results conducted by Ghate et al. (2015a, 2015b) reported that *E. coli* O157:H7, *L. monocytogenes*, and *S.* Typhimurium were more susceptible to 460 nm LED illumination in TSB when applied to pH of 4.5 or citric acid, than those at neutral pH of 7.3 at 15 °C. Similar to the present results, a previous study demonstrated that 460 nm LED illumination showed the better efficacy in inactivating *Salmonella* cells in orange juice, containing citric acid as a primary organic acid at room temperature than at chilling temperatures (Ghate et al., 2016). Thus, the pH of food matrix could be another critical factor affecting the efficacy of blue LED illumination in preserving cut fruit.

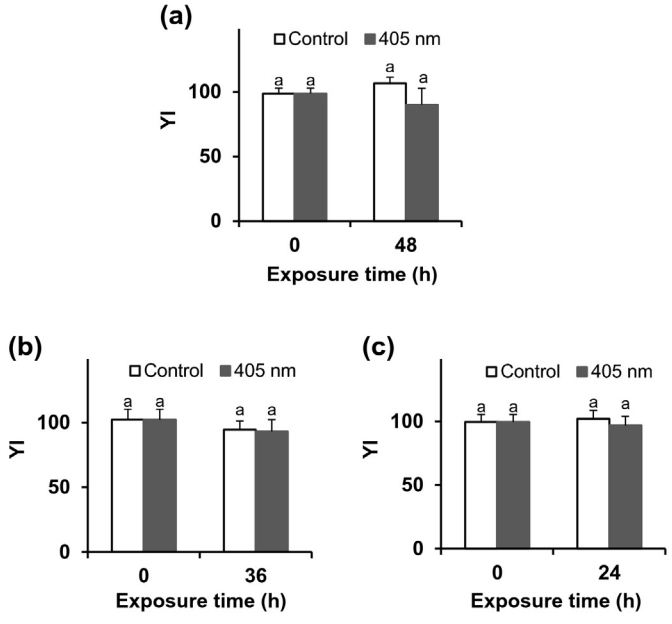

Fig. 6. Comparison of yellowness index (YI) between 405 ± 5 nm LED illuminated and non-illuminated mangoes at different temperatures of 4 °C (a), 10 °C (b), or 20 °C (c). A letter $^{(a)}$ in the same bar did not differ significantly (n = 6; $P > 0.05$).

In this study, both non-illuminated and illuminated *L. monocytogenes* on mango surface grew, while only non-illuminated *Salmonella* and *E. coli* O157:H7cells grew during storage at 20 °C. This is because generally Gram-negative bacteria (pH range, 4.5–9.0) are more sensitive to low pH of food matrix than Gram-positive bacteria (pH range, 4.0–8.5) (Ray and Bhunia, 2013). For this reason, illuminated *L. monocytogenes* cells also could adapt to acidic conditions and grow during LED illumination at room temperature, although LED illumination caused longer lag time to *L. monocytogenes* cells. Unlike bacterial behavior at 20 °C during LED illumination, there was no significant difference in the bacterial susceptibility to 405 ± 5 nm LED illumination among the three species at two chilling temperatures, except for *Salmonella* spp. at 10 °C that was more resistant to LED illumination compared to *E. coli* O157:H7 and *L. monocytogenes*. Our previous study demonstrated that *S.* Typhimurium was more susceptible to LED illumination than *E. coli* O157:H7 in PBS at 4 °C (Kim et al., 2016). Ghate et al. (2013) reported the difference in the bacterial susceptibility to 461 nm LED in TSB, revealing that *L. monocytogenes* was more sensitive to 461 nm LED at 10 °C than *E. coli* O157:H7, but there was no difference between *L. monocytogenes* and *S.* Typhimurium. The other studies conducted by Ghate et al. (2015a, 2015b) demonstrated that *L. monocytogenes* was more sensitive to blue LED illumination at pH 4.5 (adjusted by HCl) at 15 °C than *E. coli* O157:H7 and *S.* Typhimurium, whereas the bacterial susceptibility to the same LED illumination at pH 4.5 (adjusted by citric, lactic and malic acids) varied by the type of added organic acid. These discrepant results could be due to the difference in the LED illumination conditions, such as illumination temperatures, light wavelength, acidulants, and food matrix, indicating that the bacterial susceptibility to LED illumination might be influenced by these parameters.

Changes in physicochemical and nutritional qualities of fresh-cut mangoes by LED illumination were also evaluated since some of quality parameters, such as ascorbic acid and β-carotene, are susceptible to light. In this study, 405 ± 5 nm LED illumination preserved color as well as antioxidant capacity and contents of ascorbic acid, β-carotene, and flavonoid of mangoes compared to non-illuminated control fruits, indicating that the long-term illumination of LED did not influence cut fruit quality. Unlike the present results, a previous study conducted by Hong et al. (2015) showed that light exposure at 440 nm in diverse fruits had a more positive effect on flavonoid and β-carotene content than those of controls in the dark. The different results between the present study and previous studies could be due to different storage period. For example, fruit qualities of mangoes, such as antioxidant capacity and flavonoid, were not changed immediately after the treatment of

Table 2
Effect of 405 ± 5 nm LED illumination on antioxidant capacity and contents of ascorbic acid, β-carotene, and flavonoid of fresh-cut mangoes after storage at different temperatures.

Quality parameters	Storage temperature	Control (0 h) mango	Non-illuminated mango	LED illuminated mango
Antioxidant activity	4 °C	75.75 ± 12.04[a]	78.84 ± 6.02[a]	79.00 ± 15.12[a]
(mg TE/100 g FW)	10 °C	79.21 ± 13.22[a]	96.58 ± 16.83[a]	90.74 ± 26.56[a]
	20 °C	74.91 ± 10.78[a]	77.88 ± 13.30[a]	82.39 ± 11.51[a]
Ascorbic acid	4 °C	10.65 ± 1.59[a]	7.87 ± 2.11[a]	7.74 ± 2.98[a]
(mg/100 g FW)	10 °C	10.65 ± 1.59[a]	8.26 ± 3.93[a]	7.97 ± 2.50[a]
	20 °C	10.65 ± 1.59[a]	9.11 ± 2.09[a]	9.28 ± 1.64[a]
β-carotene	4 °C	0.79 ± 0.14[a]	0.58 ± 0.16[a]	0.61 ± 0.11[a]
(mg/100 g FW)	10 °C	0.96 ± 0.25[a]	0.96 ± 0.25[a]	0.77 ± 0.26[a]
	20 °C	0.79 ± 0.14[a]	0.57 ± 0.19[a]	0.56 ± 0.14[a]
Flavonoids	4 °C	9.00 ± 1.32[a]	11.46 ± 3.06[a]	10.94 ± 3.89[a]
(mg QE/100 g FW)	10 °C	7.62 ± 1.07[a]	8.77 ± 3.35[a]	7.67 ± 1.16[a]
	20 °C	9.00 ± 1.82[a]	9.71 ± 3.16[a]	10.58 ± 1.44[a]

A letter [(a)] in a row at same storage temperature did not differ significantly (n = 6; $P > 0.05$).

UV-C or pulsed light, but significant changes were only observed after storage at 3 days (González-Aguilar et al., 2007a, 2007b; Charles et al., 2013). On the other hand, mature green tomatoes with blue light illumination for 7 days had a lower amount of lycopene than that of non-illuminated controls; however, the tomatoes after illumination increasingly changed in accumulation of lycopene, color, and ripening during storage (Dhakal and Baek, 2014). Thus, blue light illumination could inhibit the ripening process of the tomatoes and eventually extend the shelf life. For these advantages, using light technology in the agriculture area is of special interest, but the detailed mechanism related to nutritional qualities of fruits is still unknown.

In conclusion, 405 ± 5 nm LED illumination effectively inactivated 97–99% of *E. coli* O157:H7, *L. monocytogenes*, and *Salmonella* spp. on the mango surface at chilling temperatures, but was less effective to *L. monocytogenes* and *E. coli* O157:H7 at room temperature. Furthermore, quality parameters of cut mango were not influenced by long-term LED illumination, irrespective of storage temperature. Thus, this study demonstrates the potential of 405 ± 5 nm LEDs to be applied at chilling temperatures to control foodborne pathogens on fresh-cut mangoes without deterioration of physicochemical quality of fruits in retail stores or industry as a novel food preservation technology. These LEDs could replace the fluorescent lights in fruit display showcase and storage room with dual functions as light and control measure. Further study is needed to evaluate sensory attributes of cut fruits as well as investigate the effectiveness of 405 ± 5 nm LED on various food matrices against natural microbiota, including molds and yeasts. Furthermore, a scale-up study is essential for commercial food applications under realistic operation conditions.

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