Food Science and Biotechnology (2024) 33:191–202
https://doi.org/10.1007/s10068-023-01324-y

RESEARCH ARTICLE

Combined antibacterial effect of 460 nm light-emitting diode illumination and chitosan against *Escherichia coli* O157:H7, *Salmonella* spp. and *Listeria monocytogenes* on fresh-cut melon, and the impact of combined treatment on fruit quality

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Received: 31 August 2022 / Revised: 8 April 2023 / Accepted: 26 April 2023 / Published online: 10 May 2023

Abstract
This study evaluated the combined antibacterial effect of 460 nm LED illumination and chitosan on *Escherichia coli* O157:H7, *Salmonella* spp. and *Listeria monocytogenes* on fresh-cut melon surface and its impact on the quality of melon at a total dose of 2.4 kJ/cm^2 at 4 and 10 °C. Results showed that the antibacterial effect of LED illumination in combination with chitosan (0.5 and 1.0%) was much better than that of LED illumination alone, showing their synergistic effect. Among the pathogens, *L. monocytogenes* was the most susceptible pathogen to LED illumination. Although the color of melons became paler after LED illumination, there was little to no change in ascorbic acid content, total flavonoid content, or antioxidant capacity of the illuminated fruits compared with non-illuminated fruits. Thus, these results suggest that chitosan-mediated 460 nm LED illumination could be applied to inactivate foodborne pathogens on fresh-cut melons during storage at food establishments.

Keywords 460 nm light-emitting diode chitosan · Combined antibacterial effect · Foodborne pathogens · Fresh-cut melon

Introduction

A light-emitting diode (LED) is a semiconductor device that emits light at a very narrow range of wavelengths when an electronic current passes through it (Held, 2009). LEDs have many benefits, such as lower energy consumption and high durability, compared with traditional fluorescent light. Moreover, LEDs can be easily installed in existing systems as their small size can fit most of designs (Hamamoto et al., 2007; Mori et al., 2007). Based on these advantages, LEDs have been broadly applied not only in the electronic industry but also in medical and agricultural fields for a variety of purposes (Held, 2009).

Among the LEDs emitting at various wavelengths, blue LEDs with a peak of 460–465 nm have been well studied for their antibacterial efficacy. For example, our previous studies indicated that 460–465 nm LEDs were able to inactivate various foodborne pathogens in broth or buffer (Ghate et al., 2013; Kumar et al., 2016), orange juice (Ghate et al., 2016), and fresh-cut pineapple (Ghate et al., 2017). In addition, studies have confirmed that the antibacterial effect of 460–465 nm LED could be significantly improved when exogenous photosensitizers such as chlorophyllin and riboflavin were added to the surface of food samples (Josewin et al., 2018; Kim et al., 2021a; 2021b).

The antibacterial mechanism of blue LED can be explained using a Jablonski diagram (Gupta et al., 2013). According to the diagram, an intracellular photosensitizer absorbs light of the wavelengths that match the peaks of its absorption spectrum. After the photon absorption, the photosensitizer enters an excited state and then transfers energy

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to the surrounding oxygen molecules when it returns to the ground state. Consequently, reactive oxygen species (ROS) and superoxide anions are intracellularly produced, which attack key intracellular molecules such as nucleic acids and membrane proteins, leading to bacterial death (Kim and Yuk, 2017). Thus, photosensitizers and oxygen are necessary for the antibacterial effect of blue LEDs. Since very small amounts of intracellular photosensitizers like protoporphyrin compounds exist within bacterial cells, the addition of exogenous photosensitizers could help to improve the antibacterial action of blue LED. However, the major drawback of exogenous photosensitizers such as chlorophyllin and riboflavin is that they alter the color of foods because most photosensitizers have their own colors (Kim et al., 2021a; 2021b). Thus, the use of exogenous photosensitizers to improve the antibacterial effectiveness of blue LEDs may be limited to a variety of foods.

Chitosan is a polycationic polymer, consisting of glucosamine units, that is obtained from crustacean chitin by alkaline deacetylation (Rabea et al., 2003). Chitosan has antibacterial activity against various bacteria including foodborne pathogens and spoilage bacteria (Shahidi et al., 1999). It is believed that the antimicrobial effect of chitosan is mainly due to pronated NH_3^+ groups on the side chain of its glucosamine backbone, which can interact with the negatively charged microbial cell membrane, resulting in increased cell membrane permeability (Shahidi et al., 1999). Unlike exogenous photosensitizers, chitosan is colorless when it is dissolved in water and has been approved as a food additive in several countries such as Korea and the United States (Kong et al., 2010). Thus, water soluble chitosan as a natural antimicrobial could be an alternative to exogenous photosensitizers for blue LED illumination technology.

Although the antibacterial effects of 460 nm LED illumination and chitosan individually have been well studied, to our knowledge, there are no reports on their synergistic antibacterial effect against foodborne pathogens. Thus, the objective of this study was to investigate the antibacterial effect of 460 nm LED illumination in combination with water-soluble chitosan against *Escherichia coli* O157:H7, *Salmonella* spp. and *Listeria monocytogenes* on fresh-cut melons. The effectiveness of chitosan in enhancing the antibacterial effect of 460 nm LED illumination was also compared with that of riboflavin. The color and nutritional quality of treated cut melons were also evaluated to assess whether 460 nm LED illumination in combination with chitosan affected their quality during storage at chilling temperatures. Fresh-cut melon was selected as a model food in this study since this fruit has been associated with several foodborne outbreaks caused by three aforementioned major pathogens. For example, the United States Centers for Disease Control and Prevention (CDC) reported that consumption of pre-cut melons contaminated with *S.* Carrau caused an outbreak in 2019 (CDC, 2019) and cantaloupe melon was also linked to a multistate outbreak caused by *E. coli* O157:H7 and *L. monocytogenes* in 1993 (del Rosario and Beuchat, 1995) and 2011 (CDC, 2011), respectively.

Materials and methods

Bacterial strains and culture conditions

Four strains of *E. coli* O157:H7 (ATCC 35150, C7927, EDL933, and 13B990), five serotypes of *Salmonella* (*S.* Gaminara BAA-711, *S.* Newport ATCC 6962, *S.* Poona ATCC BAA-1673, *S.* Tennessee ATCC 10722, and *S.* Typhimurium ATCC 14028) and three serotypes of *L. monocyotogenes* (SSA81 1/2a, BAA-839 1/2b, and ATCC 13932 4b) were used in this study. The ATCC and BAA strains of each pathogen were purchased from the American Type Culture Collection (Manassas, VA, USA) and three *E. coli* O157:H7 strains C7927, EDL933, and 13B990) were obtained from Dr Kun-Ho Seo of Konkuk University in the Republic of Korea. One strain of *L. monocytogenes* (SSA81 1/2a) was isolated from smoked salmon (Chau et al., 2017). Each frozen stock culture at − 70 °C was revived in tryptic soy broth (TSB; Becton, Dickinson and Company, Sparks, MD, USA) for 18–24 h at 37 °C. After two consecutive transfers were performed, each culture was adapted to 200 µg/mL of nalidixic acid (Sigma-Aldrich, St. Louis, MO, USA) by consecutive culturing with increasing concentrations of nalidixic acid in TSB with incubation at 37 °C for 18–24 h. All media used in this study were supplemented with 200 µg/mL nalidixic acid to enumerate only inoculated cells on fresh-cut melons. Finally, two consecutive cultures were carried out in 10 mL of TSB supplemented with 200 µg/mL of nalidixic acid at 37 °C for 18–24 h before inoculation on the surface of fresh-cut melons.

Preparation of fresh-cut melon and inoculation

Fresh muskmelons (*Cucumis melo* L.) were purchased from local supermarkets in Jeungpyeong-gun, Korea, and stored at 4 °C prior to use. The melon was washed with tap water to remove debris and existing microorganisms, and then wiped with Kimwipes (Kimtech Science, Kimberly Clark Professional, Roswell, GA, USA) to dry it. The dried melon was peeled and cut into rectangular shape (4 cm × 3 cm × 1.2 cm) weighing ca. 20 g with a sterile knife in a biosafety cabinet (BSC; CHC LAB, Daejeon, Korea) before inoculation.

To prepare the cocktail culture, bacterial strains or serotypes cultured in 10 mL TSB containing nalidixic acid, as described previously, were combined with equal portions of each culture. The cocktail culture was centrifuged (Micro 17TR, Hanil Science Co. Ltd., Daejeon, Korea)

at 4000×*g* for 10 min at 4 °C and washed twice using phosphate-buffered saline (PBS; Biosesang, Seongnam-si, Korea). The resulting pellet was finally suspended in PBS to achieve a final concentration of approximately 10^8 CFU/mL. The suspended cocktail culture was serially diluted in PBS and a 10-µL aliquot of the diluents was inoculated at 10 sites on the surface of cut melons to obtain an inoculum of approximately $10^{4–5}$ CFU/cm^2. Commercial water-soluble chitosan (MW 10,000–100,000) purchased from Biopolytech (Cheonju-si, Korea) was dissolved in distilled water (DW) at concentrations of 0.5 and 1.0% (w/v). As exogenous photosensitizer, 100 µM riboflavin (RBF; Sigma-Aldrich) was also prepared by dissolving it in DW. The prepared chitosan or riboflavin was added to each cocktail culture before inoculation. The inoculated melons with or without chitosan or riboflavin were dried for 30 min in the BSC and were individually wrapped to simulate the conditions found in retail stores (Kim et al., 2017a).

Light emitting diode (LED) illumination

A small-sized LED illumination system was constructed to illuminate a fresh-cut melon. In the system, a high-intensity 460 nm LED (8×8 mm, 9 W, Shenzhen Getian Opto-Electronics Co., Ltd., Shenzhen, Guangdong, China) equipped with heat sink and cooling fan was placed on the top and was enclosed by acrylonitrile butadiene styrene to avoid external light contamination. The irradiance of 460 nm LED (21 ± 2 mW/cm^2) was determined using a Compact Power and Energy Meter Console (PM100D; Thorlabs GmbH, Dachau, Germany). A total dose (kJ/cm^2) applied to each fruit sample during LED illumination was calculated by multiplying irradiance (W/cm^2) and illumination time (s) (Kim et al., 2021a; 2021b).

Each melon inoculated as previously described was positioned in a sterile petri dish (60 mm) and placed under the light source in the LED illumination system. The distance between the LED and fruit and was maintained at 3.4 cm to illuminate the whole fruit. The temperature of the cut melon surface without inoculation was also monitored during LED illumination at 4 and 10 °C, respectively, using a Fluke 5.4 thermocouple thermometer (Everett, WA, USA) to observe the temperature increase induced by LED illumination, which can help to set up non-illuminated controls. The inoculated melons were illuminated using 460 nm LED at each temperature for 32 h (a total dose of 2.4 kJ/cm^2) in an incubator (IL-11-4C, JEIO Tech, Co. Ltd., Daejeon, Korea) and were taken at 8 (0.6 kJ/cm^2), 16 (1.2 kJ/cm^2), 24 (1.8 kJ/cm^2) and 32 h for the analysis of their microbial and chemical quality. Non-illuminated controls with or without chitosan or riboflavin were stored in the dark at 4 and 10 °C.

Bacterial inactivation using 460 nm LED illumination with or without chitosan

At each time interval, the illuminated or non-illuminated melon was taken and transferred into a sterile stomacher bag containing 180 mL of 0.1% peptone water (PW; Becton, Dickinson and Company). After homogenizing in a blender (WiseMix® WES, DAIHAN Scientific, Wonju-si, Korea), the homogenate was serially diluted with 0.1% PW and spread plated on tryptic soy agar (TSA; Becton, Dickinson and Company) containing 200 µg/mL of nalidixic acid and then incubated at 37 °C for 24–48 h. The colonies on the plates were manually enumerated and the results were reported in log CFU/cm^2. To compare the inactivation rate of each bacterial species by LED illumination with or without chitosan or riboflavin, the log number of colonies were plotted against total dose and the inactivation curves were fitted to the Weibull model (Coroller et al., 2006) using the GlnaFit (http://frisbeetool.eu/GInaFit/ginafit.php) program. Delta (δ) values were calculated from each fitted inactivation curve using the GlnaFit program. These values indicate the dose required for the first 90% reduction of the bacterial population.

Color analysis

Color changes in the illuminated or non-illuminated fruits for 32 h were analyzed using a Chroma Meter (CR-300, Konica Minolta Sensing Americas, Inc., Ramsey, NJ, USA) with a Minolta calibration plate No. 18833087 (Y = 91.8, x = 0.3136, y = 3196). The color parameters of L^* (lightness), a^* (red to green), and b^* (yellow to blue) were measured for each melon sample, and these values were also used to calculate the whiteness index (WI) of each fruit using the following equation (Bermúdez-Aguirre and Barbosa-Cánovas, 2013):

$$WI = 100 - \sqrt{(100 - L) + a^2 + b^2}$$

The WI value is useful to quantify the effect of LED illumination on the loss of natural color of melon.

Ascorbic acid content

Ascorbic acid in the fresh, illuminated, and non-illuminated melon samples was extracted and analyzed based on the method described by Spilimbergo et al. (2013) with a slight modification. The fruit sample weighing 5 g was homogenized with 10 mL of 2.5% meta-phosphoric acid (Sigma-Aldrich) using a blender (KM-230MC, Kitchenart co., Ltd., Seoul, Korea) for 1 min and the homogenate was incubated for 45 min at room temperature, followed by centrifugation

(Micro 17TR) at 3000×*g* for 2 min at 4 °C. The supernatant was filtered using Advantec No. 1 filter paper (Advantec Toyo Kaisha, Ltd., Tokyo, Japan) and stored at 4 °C prior to analysis.

A stock solution for the analysis of ascorbic acid content was freshly prepared by dissolving 12.5 mg of 2,6-dichloroindophenol sodium salt (Sigma-Aldrich) in 50 mL of deionized (DI) water containing 1 mg of sodium bicarbonate ($NaHCO_3$; Sigma-Aldrich). A 150-µL of the filtered supernatant was mixed with 100 µL of stock solution and transferred to a 96-well plate in duplicate. The mixture was measured at 515 nm using the Microplate Spectrophotometer (BioTek instrument Inc., Winooski, VT, USA) and the ascorbic acid content of the fruit was calculated based on a standard curve established using 0–7.5 mg/100 mL L-ascorbic acid (Sigma-Aldrich). Ascorbic acid content was expressed as mg ascorbic acid/100 g FW (fresh weight).

Antioxidant capacity and total flavonoid content assays

The antioxidant capacity and total flavonoid content of fresh, illuminated or non-illuminated cut-melon samples were analyzed according to the method described by González-Aguilar et al. (2007) with a slight modification. After illumination or storage under dark condition for 32 h, a 10 g fruit sample was added to 25 mL of 80% methanol with 0.5% sodium bisulfate (Sigma-Aldrich), and homogenized for 1 min using a blender (KM-230MC). The homogenate was sonicated (DH.D400H, Daihan Scientific co. Ltd., Wonju-si, Korea) for 60 min and then centrifuged (Micro 17TR) at 3000×*g* for 10 min at room temperature. The supernatant was filtered using a filter paper (No. 1, Advantec Toyo Kaisha, Ltd.) and then was stored at − 20 °C prior to 2,2-diphenyl-1-picrylhydrazyl (DPPH) and total flavonoid content assays.

Fresh stock solution of DPPH (Sigma-Aldrich) was prepared by dissolving 2.82 mg of DPPH in 10 mL of methanol (99.5%) prior to each experiment. A 30-µL sample of filtered supernatant as described above was mixed with 420 µL of the DPPH stock solution and then incubated for 30 min at room temperature in the dark, and then 200 µL of the mixture was transferred to a 96-well plate and the absorbance was measured at 515 nm using an Epoch 2 Microplate Spectrophotometer (BioTek instrument Inc.). The percentage of antioxidant capacity of the fresh, illuminated, and non-illuminated fruit samples was calculated with the following equation:

$$\text{Radical scavenging activity}(\%) = [1 - \frac{(Abs_{sample} - Abs_{blank})}{Abs_{control}}] \times 100$$

For the analysis of total flavonoid content, a 50-µL sample of filtered supernatant was transferred to a 1.5 mL tube containing 200 µL of DI water and 15 µL of 5% sodium nitrite (Sigma-Aldrich). After mixing, the solution was equilibrated for 5 min and then 15 µL of 10% aluminum chloride (Sigma-Aldrich) and 100 µL of 1 M sodium hydroxide (Sigma-Aldrich) were added into the mixture. To adjust the final volume (500 µL), 120 µL of DI water was added to the mixture, and 200 µL of the mixture was added to a 96-well plate. Absorbance of the mixture was determined at 415 nm using the Microplate Spectrophotometer (BioTek instrument Inc.). The total flavonoid content of the fruit samples was calculated based on the standard curve which was constructed with 0–10 mg of quercetin (Sigma-Aldrich), and thus the total flavonoid content of fruit sample was expressed as mg QE (quercetin equivalents)/100 g FW (Kim et al., 2017a).

Statistical analysis

All experiments were independently performed in triplicate with duplicate sampling ($n = 6$). All data were analyzed using one-way analysis of variance (ANOVA), followed by Tukey's test using IBM SPSS statistical software (version 25.0; SPSS Inc., IBM Co., Armonk, NY, USA). The difference among mean values was considered significant when the *P*-value was less than 0.05 ($P < 0.05$).

Results and discussion

Temperature change in the fresh-cut melon surface during LED illumination

To decide the storage temperature of the non-illuminated control in the dark, changes in temperatures of fresh-cut melon surface at 4 and 10 °C for ideal refrigerated and temperature abuse conditions, respectively, were observed during 460 nm LED illumination for 180 min at 1-min intervals. Results showed that the fruit temperatures rapidly increased up to 7.5 and 16 °C during illumination, revealing a 3.5 and 6 °C rise over the set temperatures of 4 and 10 °C, respectively (data not shown). Thus, the illuminated fruits were stored at 0.5 and 4 °C to match the same temperature conditions with the non-illuminated controls at 4 and 10 °C, respectively, which is critical because temperature is one of the key extrinsic factors influencing bacterial growth or inactivation during LED illumination. Previous studies also showed that 460 nm LED illumination increased the surface temperatures of pineapple (Ghate et al., 2017) and salmon (Josewin et al., 2018), indicating that the temperature rise is highly dependent on LED intensity. Thus, the adjustment of temperature conditions for blue LED used in this study was necessary because its intensity was different from those used in previous studies.

Bacterial inactivation by LED illumination with or without chitosan

The antibacterial effect of 460 nm LED illumination in combination with chitosan against *E. coli* O157:H7, *Salmonella* spp. and *L. monocytogenes* on fresh-cut melons was evaluated. Riboflavin, an exogenous photosensitizer, was used in this study to compare its antibacterial activity with that of chitosan, since a recent study showed that riboflavin was more effective photosensitizer than 5-aminolevulinic acid and chlorognic acid for bacterial inactivation by 460 nm LED illumination (Kim et al., 2021a).

The *E. coli* O157:H7 strain remained unaffected in non-illuminated controls, chitosan, and riboflavin treatments alone for 32 h, regardless of storage temperatures (Fig. 1). The exposure to 460 nm LED alone at both temperatures significantly ($P < 0.05$) inactivated approximately 0.8–0.9 log CFU/cm^2 of bacteria, which was statistically similar to the effect of the combined treatment of LED illumination and riboflavin. On the other hand, *E. coli* O157:H7 on fresh-cut melons were significantly reduced by 2.2–2.5 log CFU/cm^2 at 4 °C and 1.1–1.7 log CFU/cm^2 at 10 °C on exposure to LED illumination in combination with chitosan at 2.4 kJ/cm^2.

There were no significant ($P \geq 0.05$) reductions in the population of *Salmonella* spp. on the fruits in the absence of LED illumination or upon single treatment of riboflavin or chitosan at both storage temperatures for 32 h (Fig. 2). LED illumination alone at 2.4 kJ/cm^2 resulted in approximately 1.0 log reduction, while LED illumination in combination with 0.5% chitosan, 1.0% chitosan and 100 μM riboflavin decreased the populations of *Salmonella* spp. by 2.0, 2.5,

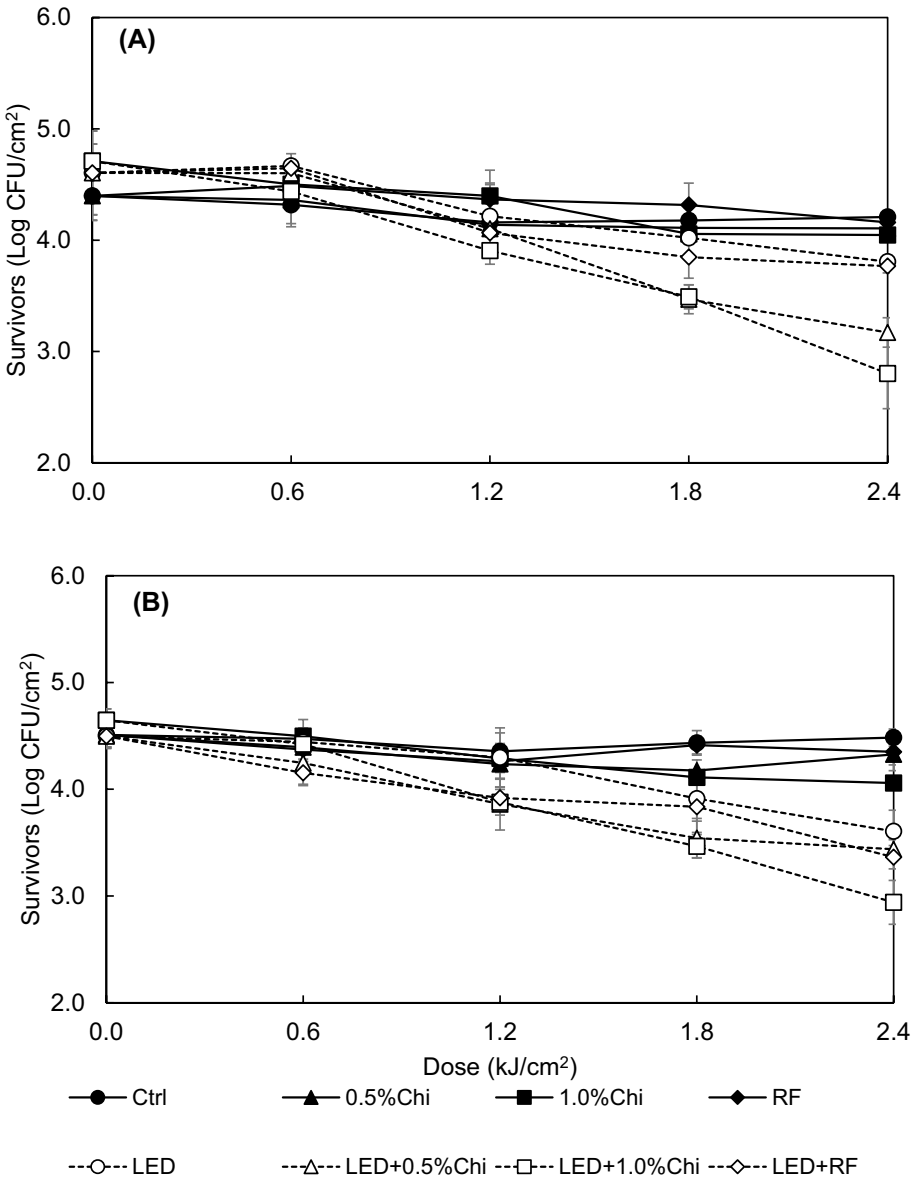

Fig. 1 Survival of *Escherichia coli* O157:H7 on the surface of fresh-cut melons under the dark condition (control, Ctrl) and, during 460 nm LED illumination alone (LED), LED in combination with or without chitosan (0.5%Chi or 1.0%Chi), or 100 μM riboflavin (RF) at 4 (**A**) and 10 °C (**B**). The error bars reflect the standard deviation at each data point

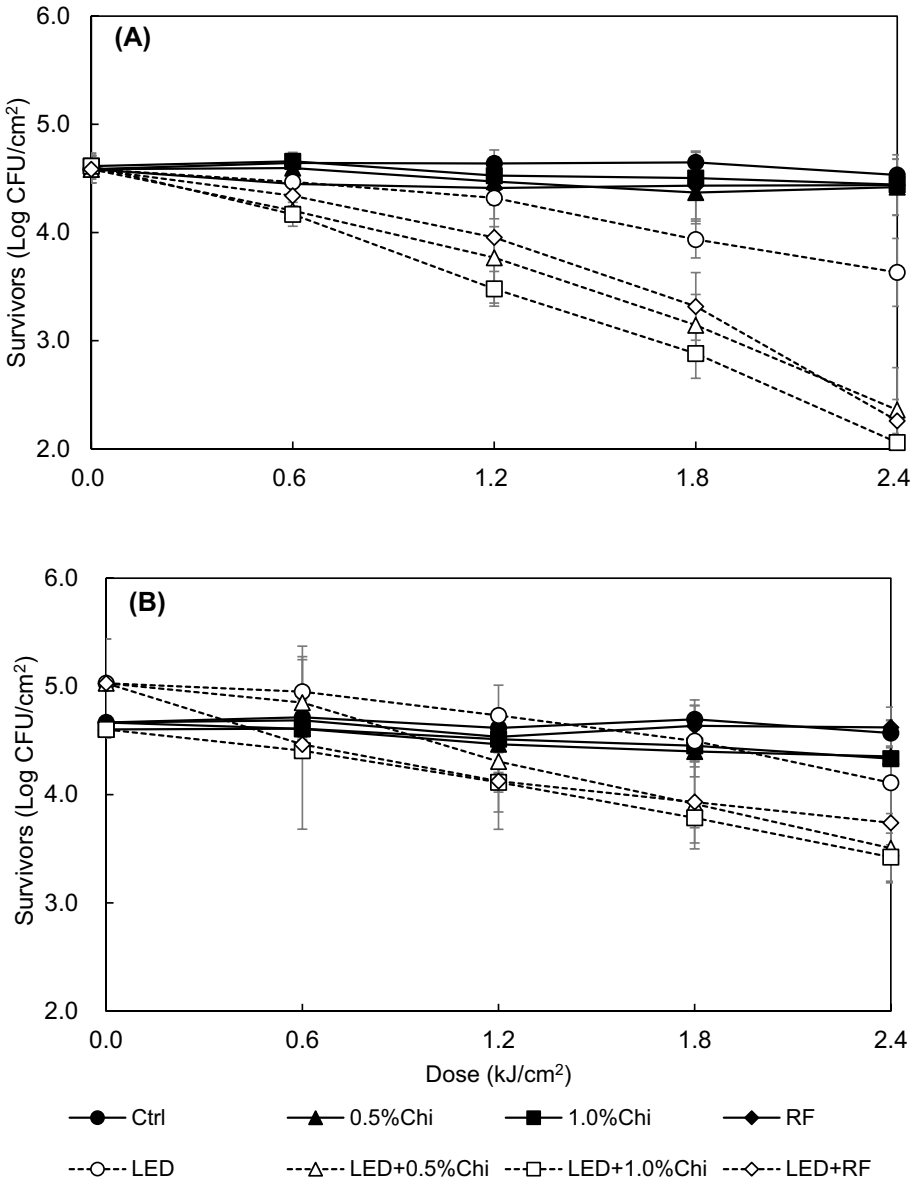

Fig. 2 Survival of *Salmonella* spp. on the surface of fresh-cut melons under the dark condition (control, Ctrl) and, during 460 nm LED alone (LED), LED in combination with or without chitosan (0.5%Chi or 1.0%Chi), or 100 μM riboflavin (RF) at 4 (**A**) and 10 °C (**B**)

and 2.3 log CFU/cm^2, respectively, when compared with the initial populations at 4 °C. An insignificant difference in the bacterial reduction was observed between riboflavin and chitosan with LED illumination. Similar to *E. coli* O157:H7, the degree of bacterial inactivation by LED illumination in combination with chitosan and riboflavin was apparently reduced at 10 °C.

The initial populations of *L. monocytogenes* on the surface of fruits remained unchanged when they were stored in the dark and treated with riboflavin, whereas with chitosan treatment alone they were significantly ($P < 0.05$) reduced by 0.5 log CFU/cm^2 for 32 h regardless of storage temperatures (Fig. 3). Unlike the two pathogens, the populations of *L. monocytogenes* on the fruits rapidly decreased below undetectable levels upon LED illumination with or without chitosan and riboflavin, with 3.5 log reductions at both illumination temperatures. In particular, undetectable levels were achieved by LED illumination with and without riboflavin at 2.4 kJ/cm^2, while only 0.6–0.8 kJ/cm^2 was required when the fruits were illuminated with 460 nm LED in combination with chitosan. These results indicate that 460 nm LED illumination in combination with chitosan was more effective in eliminating these bacterial pathogens on fresh-cut melon than LED illumination with and without riboflavin, although the degree of difference in bacterial inactivation among the treatments varied with bacterial pathogen.

The present results of 460 nm LED illumination alone are consistent with the studies of Ghate et al. (2016), who reported that 460 nm LED illumination resulted in significant reductions of 2.0–3.0 logs in *Salmonella* populations

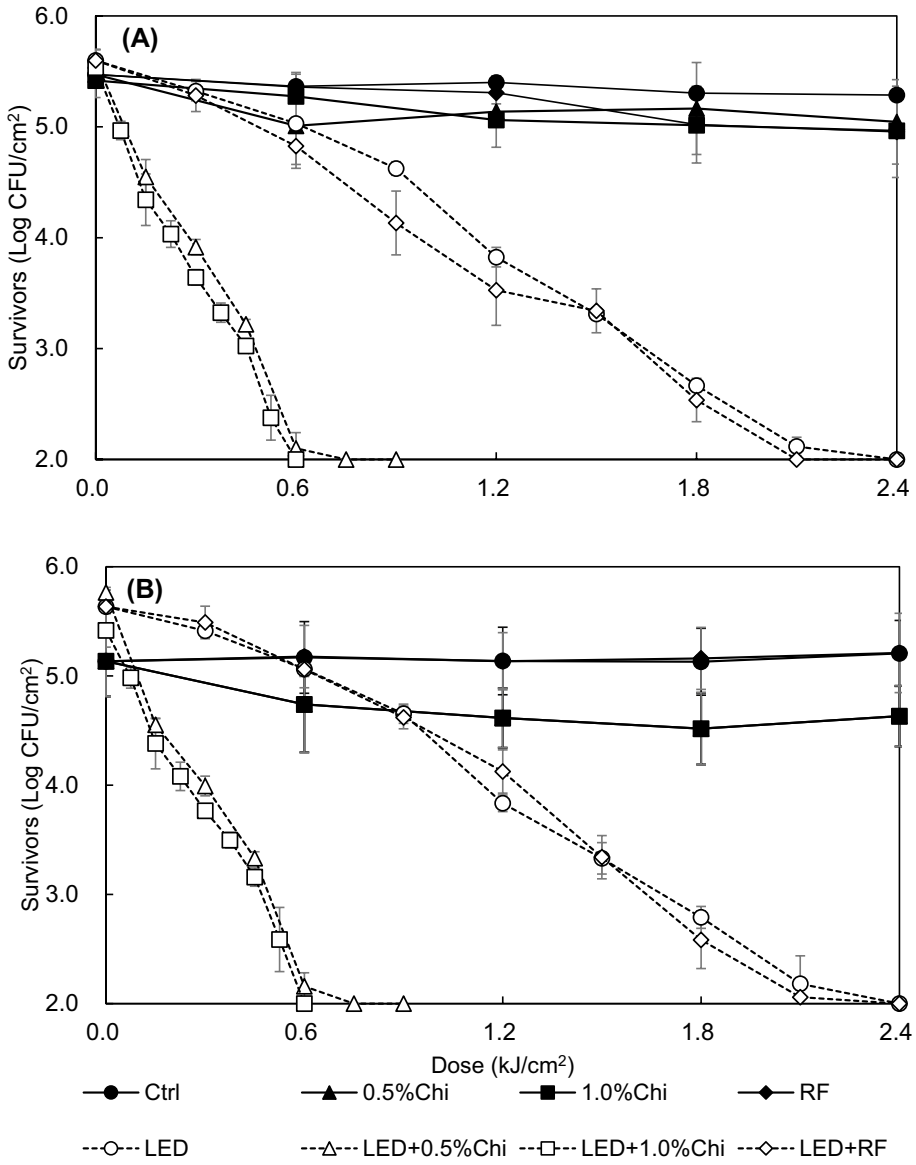

Fig. 3 Survival of *Listeria monocytogenes* on the surface of fresh-cut melons under the dark condition (control, Ctrl) and, during 460 nm LED alone (LED), LED in combination with or without chitosan (0.5%Chi or 1.0%Chi), or 100 μM riboflavin (RF) at 4 (**A**) and 10 °C (**B**)

in orange juice, demonstrating that its antibacterial effect was highly influenced by the intensity of LED and storage temperatures. Unlike these results, there was no reduction in the population of *L. monocytogenes* on smoked salmon filets, regardless of the LED illumination intensity (Josewin et al., 2018). A possible explanation for the contradictory results of these studies might be due to the different nature of food matrices such as the pH and nutrient availability. Foods like melon and orange juice have lower pH or less nutrient availability than salmon fillet, which make the bacterial cells more susceptible to LED illumination.

Chitosan has been well studied for its antimicrobial effect against various microorganisms including spoilage-inducing and pathogenic bacteria, molds, and yeasts (Shahidi et al., 1999). In this study, both 0.5 and 1.0% chitosan solutions did not induce significant reductions in the bacterial pathogens on fresh-cut melon, except for *L. monocytogenes* at 1.0% chitosan treatment. Similar to the present results, 0.5–1.0% chitosan solutions did not cause any reductions of *E. coli* and *S. Typhimurium* on chicken filets during storage at 4 °C for 9 days (El-Khawas et al., 2020); however, significant reductions were observed in *L. monocytogenes* on vacuum-packed pork loin for 28 days at 4 °C when 1.0 and 2.0% chitosan coatings were used (Serio et al., 2018). Furthermore, Ibañez-Peinado et al. (2020) reported that 1.0% chitosan in acetic acid significantly reduced *E. coli*, *S. Typhimurium,* and *L. monocytogenes* in TSB for 49 h. These different results might be due to the differences in matrix of interest used for chitosan treatment, indicating less effectiveness when chitosan was used to treat solid foods than liquid ones.

The present results indicate that chitosan-mediated 460 nm LED illumination might be a synergistic effect

because this combined treatment was much effective in inactivating these bacterial pathogens than the sum of the single treatment. Similarly, a previous study by Tsai et al. (2011) indicated that 635 nm LED illumination in combination with chitosan (0.025–0.1%) in the presence of exogenous photosensitizers, such as hematoporphyrin dihydrochloride and toluidine blue O, completely inactivated *Staphylococcus aureus* and *Pseudomonas aeruginosa* in PBS, respectively, which was more effective than photosensitizer-mediated LED illumination. This might be due to the different antibacterial mechanism of LED illumination and chitosan. Chitosan attacks the bacterial membrane mainly through the interaction of its positively charged side chains with negatively charged phospholipids of the cell membrane (Shahidi et al., 1999). Contrastingly, bacterial inactivation using blue LED illumination was achieved by transferring light energy from intracellular photosensitizer to oxygen nearby, followed by the generation of ROS, which attack intracellular components such as DNA and proteins (Dai et al., 2012; Luksienė and Zukauskas, 2009). Thus, it can be speculated that chitosan and 460 nm LED illumination could simultaneously attack cell membranes and intracellular components, leading to bacterial death.

The inactivation rate of each bacterial pathogen using LED illumination with or without riboflavin or chitosan was also calculated using the Weibull model based on the inactivation curve (Table 1). Regardless of illumination temperature and bacterial species, there was no significant ($P \geq 0.05$) difference in delta (δ) values between 0.5 and 1.0% chitosan during LED illumination, except for *E. coli* O157:H7 at 10 °C which had lower δ value at 1.0% chitosan than that at 0.5% chitosan. In addition, the inactivation rates of *E. coli* O157:H7 and *Salmonella* spp. during LED illumination in combination with chitosan were statistically similar to those when riboflavin was added. On the other hand, 460 nm LED illumination in combination with chitosan significantly ($P < 0.05$) decreased δ values of *L. monocytogenes* about 3.0–3.5 folds more than LED illumination with or without riboflavin. These results indicate that the addition of chitosan was similar or more effective than riboflavin in augmenting the antibacterial effect of LED illumination, although the effect varied with bacterial species.

Furthermore, the data show that the δ values of *L. monocytogenes* on fresh cut melons were 0.2–0.7 kJ/cm^2, whereas those of *E. coli* O157:H7 and *Salmonella* spp. were 1.0–2.6 and 1.1–3.2 kJ/cm^2, showing that Gram-positive pathogen was more vulnerable to 460 nm LED illumination than Gram-negative pathogens, irrespective of whether riboflavin or chitosan was present. These results are similar to a previous study reporting that Gram-positive bacteria, such as *Staphylococcus* spp., were more susceptible to 405 mm LED illumination than Gram-negative bacteria such as *E. coli* and *P. aeruginosa* (Maclean et al., 2009). In contrast to these results, no such trend was observed when *E. coli* O157:H7, *L. monocytogenes*, and *Salmonella* spp. in TSB or on fresh-cut mango were illuminated by 461 nm LED (Ghate et al., 2013) or 405 nm LED (Kim et al., 2017b), revealing that the Gram-nature of bacteria did not appear to have a significant impact on their susceptibilities to LED illumination. The discrepancy of these results indicates that the sensitivity of bacterial strains to blue LED illumination might be influenced not only by their Gram-nature but also by other factors such as bacterial strains, the intensity and wavelength of the LED applied, and matrix of interest (Ghate et al., 2019).

Change in color of fresh-cut melon by LED illumination in combination with chitosan

It is necessary to determine the impact of 460 nm LED illumination with or without chitosan on the color change of fresh-cut melons, because this technology may be applicable to the preservation of cut fruits at food establishments. There were no significant ($P \geq 0.05$) differences in L^* values among fresh, non-illuminated, and LED-illuminated fruits with or without chitosan and riboflavin, with a range of 62.6–71.2 (Table 2). However, the a^* and b^* values of LED-illuminated fruits were significantly ($P < 0.05$) reduced compared with that of fresh and non-illuminated fruits in

Table 1 Comparison[1] of delta (δ) values (kJ/cm^2) of *E. coli* O157:H7, *Salmonella* spp. and *Listeria monocytogenes* during 460 nm LED illumination in combination with or without chitosan (0.5% and 1.0%) and riboflavin (100 μM) at 4 and 10 °C

Temperature (°C)	Species	LED	LED + riboflavin	LED + 0.5% chitosan	LED + 1.0% chitosan
4	*E. coli* O157:H7	$2.6 \pm 0.7^{B,b}$	$2.5 \pm 0.6^{B,b}$	$1.7 \pm 0.4^{AB,bc}$	$1.0 \pm 0.1^{A,a}$
	Salmonella spp.	$3.2 \pm 1.3^{B,b}$	$1.6 \pm 0.3^{A,ab}$	$1.4 \pm 0.1^{A,b}$	$1.1 \pm 0.1^{A,b}$
	L. monocytogenes	$0.6 \pm 0.2^{B,a}$	$0.6 \pm 0.2^{B,a}$	$0.2 \pm 0.0^{A,a}$	$0.2 \pm 0.0^{A,a}$
10	*E. coli* O157:H7	$2.5 \pm 0.1^{B,b}$	$2.3 \pm 0.2^{B,b}$	$2.1 \pm 0.1^{B,c}$	$1.6 \pm 0.0^{A,b}$
	Salmonella spp.	$2.6 \pm 0.4^{B,b}$	$1.3 \pm 0.6^{A,ab}$	$1.7 \pm 0.2^{AB,bc}$	$2.2 \pm 0.3^{AB,c}$
	L. monocytogenes	$0.7 \pm 0.2^{BC,a}$	$0.7 \pm 0.3^{C,a}$	$0.2 \pm 0.0^{A,a}$	$0.2 \pm 0.0^{A,a}$

[1]Different superscripts within a raw (A–C) at the same bacterial species and temperature or within column (a–c) at the same temperature indicate that the means are significantly ($P < 0.05$) different from each other

Table 2 Comparison[1] of color change and whiteness index (WI) of fresh fruit, non-illuminated control, 460 nm LED illuminated in combination with or without chitosan (0.5% and 1.0%) and riboflavin (100 μM) at 4 and 10 °C

Temperature (°C)	Dose (kJ/cm^2)	Sample	L^* (Lightness)	a^* (Redness)	b^* (Yellowness)	Whiteness index (WI)
	0.0	Fresh	69.0 ± 1.4^{a}	-8.4 ± 0.6^{bc}	24.0 ± 2.4^{a}	74.0 ± 2.4^{a}
4	0.0	Non-illuminated control	68.7 ± 0.5^{a}	-8.4 ± 0.1^{bc}	19.8 ± 0.2^{a}	77.8 ± 0.2^{a}
		0.5% chitosan	66.4 ± 2.8^{a}	-10.2 ± 0.8^{c}	22.9 ± 2.0^{a}	74.2 ± 1.6^{a}
		1.0% chitosan	62.6 ± 4.7^{a}	-9.0 ± 1.3^{bc}	21.4 ± 0.5^{a}	76.0 ± 0.9^{a}
		Riboflavin	65.5 ± 1.0^{a}	-8.1 ± 0.3^{b}	20.3 ± 0.5^{a}	77.4 ± 0.4^{a}
	2.4	LED	69.7 ± 2.4^{a}	-0.5 ± 0.0^{a}	3.0 ± 0.0^{b}	93.7 ± 0.2^{b}
		LED + 0.5% chitosan	71.2 ± 2.4^{a}	-0.5 ± 0.1^{a}	3.5 ± 0.2^{b}	93.6 ± 0.1^{b}
		LED + 1.0% chitosan	69.4 ± 2.4^{a}	-0.6 ± 0.1^{a}	3.9 ± 0.5^{b}	93.2 ± 0.2^{b}
		LED + riboflavin	67.3 ± 1.3^{a}	-0.5 ± 0.0^{a}	2.6 ± 0.2^{b}	93.7 ± 0.1^{b}
10	0.0	Non-illuminated control	67.6 ± 3.9^{a}	-8.0 ± 0.4^{b}	22.7 ± 1.5^{a}	75.3 ± 1.1^{a}
		0.5% chitosan	67.0 ± 3.6^{a}	-8.9 ± 0.4^{bc}	22.5 ± 2.6^{a}	75.1 ± 2.1^{a}
		1.0% chitosan	66.2 ± 2.2^{a}	-8.7 ± 0.5^{bc}	21.8 ± 1.8^{a}	75.8 ± 1.6^{a}
		Riboflavin	65.7 ± 3.6^{a}	-8.3 ± 0.6^{bc}	20.3 ± 1.4^{a}	77.3 ± 1.0^{a}
	2.4	LED	65.9 ± 2.5^{a}	-0.5 ± 0.1^{a}	3.4 ± 0.5^{b}	93.2 ± 0.4^{b}
		LED + 0.5% chitosan	67.5 ± 0.5^{a}	-0.5 ± 0.1^{a}	3.9 ± 1.2^{b}	93.0 ± 0.7^{b}
		LED + 1.0% chitosan	68.3 ± 1.7^{a}	-0.5 ± 0.0^{a}	4.1 ± 0.2^{b}	93.0 ± 0.0^{b}
		LED + riboflavin	69.2 ± 1.5^{a}	-0.5 ± 0.1^{a}	4.0 ± 0.3^{b}	93.1 ± 0.3^{b}

[1]Different superscripts within a column (a–c) at the same temperature indicate that the means are significantly ($P < 0.05$) different from each other

the dark. Since the equation for whiteness index (WI) of fruit color was calculated based on these values, the WI also significantly ($P < 0.05$) increased when the surface of the fruits was illuminated regardless of the presence of chitosan or riboflavin, indicating that the addition of riboflavin or chitosan did not influence the color change of fruit surface during LED illumination.

Similar to the present results, a previous study by Ghate et al. (2017) reported that a change in color of fresh-cut pineapple occurred under 461 nm LED illumination at 8.0 kJ/cm^2, demonstrating that the degree of color change of the fruits was highly dependent on the intensity of LED and illumination temperatures. Contrastingly, there was no significant difference in the WI of smoked salmon between 460 nm LED illumination (2.4 kJ/cm^2) and the non-illuminated control, but the WI increased when the salmon was illuminated with LED in combination with 100 μM riboflavin (Josewin et al., 2018). These contradictory results might be due to the nature of the pigments present in the food matrix. The main pigments of flesh of melon and pineapple are chlorophyll and β-carotene, which absorb light at wavelengths of 400–500 nm (Guidi et al., 2017), leading to photodegradation of these pigments under 460–465 nm LED illumination, while astaxanthin, which is a main pigment of salmon, is not sensitive to the light (Alfnes et al., 2006). Change in the color of smoked salmon during LED illumination with riboflavin might be attributed to ROS generated from the photo-reaction of upon LED exposure (Josewin et al., 2018). Thus, these results indicate that considering the irradiance of LED, illumination temperature, and the nature of pigments in food matrix is critical for the application of 460 nm LED illumination in combination with chitosan on fresh-cut fruits. Since the color change of fresh-cut melon under 460 nm LED illumination is critical for consumers' preference, further research is necessary on minimizing the color change by controlling the intensity of LED and illumination time.

Changes in nutritional values by LED illumination in combination with chitosan

It is known that melon contains ascorbic acid as the main vitamin source and an antioxidant that benefits human health (Fenech et al., 2019). However, ascorbic acid is also known to be sensitive to light exposure since it absorbs light at wavelengths between 220 and 330 nm (Aguilar et al., 2019). Thus, the impact of 460 nm LED illumination with or without chitosan and riboflavin on the ascorbic acid content, total flavonoid content, and antioxidant capacity of fresh-cut melon was evaluated in this study (Table 3). Fresh fruit without LED exposure or stored under dark condition at different temperatures served as a fresh control of whether the changes in these nutritional values occurred naturally.

The results showed that ascorbic acid content of fresh, non-illuminated, and illuminated fruits were 3.1, 2.5–3.4, and 2.5–4.1 mg, respectively, regardless of storage,

Table 3 Effect[1] of 460 nm LED illumination in combination with or without chitosan (0.5% and 1.0%) and riboflavin (100 μM) on antioxidant capacity and, contents of ascorbic acid and flavonoid of fresh-cut melon after storage at 4 and 10 °C

Temperature (°C)	Dose (kJ/cm^2)	Sample	Ascorbic acid content (mg/100 g FW)	Total flavonoid content (mg QE/100 g FW)	Antioxidant capacity (%)
	0.0	Fresh	3.1 ± 0.4^{ab}	8.4 ± 0.9^{a}	11.2 ± 0.6^{ab}
4	0.0	Non-illuminated control	2.5 ± 0.3^{b}	6.3 ± 0.3^{a}	11.8 ± 0.3^{ab}
		0.5% chitosan	2.7 ± 0.4^{ab}	7.5 ± 1.6^{a}	7.6 ± 0.7^{b}
		1.0% chitosan	3.0 ± 0.7^{ab}	6.4 ± 1.4^{a}	10.2 ± 0.7^{ab}
		Riboflavin	3.4 ± 0.5^{ab}	6.5 ± 1.5^{a}	9.1 ± 0.6^{ab}
	2.4	LED	2.5 ± 0.2^{b}	7.1 ± 0.2^{a}	11.9 ± 0.6^{ab}
		LED + 0.5% chitosan	2.4 ± 0.2^{b}	7.7 ± 1.9^{a}	10.5 ± 2.3^{ab}
		LED + 1.0% chitosan	2.5 ± 0.2^{b}	7.7 ± 2.3^{a}	12.8 ± 2.2^{a}
		LED + riboflavin	4.1 ± 0.7^{a}	8.5 ± 0.8^{a}	13.2 ± 1.9^{a}
10	0.0	Non-illuminated control	4.3 ± 0.5^{a}	6.7 ± 1.0^{a}	13.6 ± 2.6^{a}
		0.5% chitosan	2.8 ± 0.5^{a}	7.6 ± 1.5^{a}	8.3 ± 2.2^{a}
		1.0% chitosan	3.3 ± 1.2^{a}	5.7 ± 1.4^{a}	9.4 ± 2.3^{a}
		Riboflavin	3.8 ± 0.8^{a}	8.2 ± 1.6^{a}	11.6 ± 1.0^{a}
	2.4	LED	2.7 ± 0.1^{a}	6.8 ± 0.5^{a}	14.0 ± 2.8^{a}
		LED + 0.5% chitosan	2.6 ± 0.3^{a}	6.1 ± 2.1^{a}	10.7 ± 2.4^{a}
		LED + 1.0% chitosan	2.7 ± 0.5^{a}	8.1 ± 0.6^{a}	13.4 ± 2.9^{a}
		LED + riboflavin	4.0 ± 0.7^{a}	7.3 ± 1.4^{a}	16.3 ± 3.4^{a}

[1]Different superscripts within a column (a, b) at the same temperature indicate that the means are significantly ($P < 0.05$) different from each other

temperature, or the presence of chitosan or riboflavin. Although there were some exceptional cases such as LED illumination combined with 0.5% chitosan and 100 μM riboflavin at 4 °C, most cases showed no significant ($P \geq 0.05$) differences in the ascorbic acid content of fresh, non-illuminated, and illuminated fruits. Irrespective of the presence of chitosan and riboflavin, the total flavonoid contents of non-illuminated and LED illuminated fruits ranged from 5.2 to 8.5 mg, which is similar to the value obtained for fresh controls, with no significant differences. Similar to ascorbic acid content, the antioxidant capacity of the illuminated fruits in most cases did not differ from those of non-illuminated and fresh fruits, which might be attributed to the absence of a significant change in ascorbic acid and total flavonoid contents as the main antioxidant materials in melon. Thus, these results reveal that 460 nm LED illumination on fresh-cut fruits might not influence the nutrient values of the fruits.

It is known that ascorbic acid in fruit juice could be degraded by 250 nm ultra violet (UV) irradiation because of its absorption spectrum, as previously described (Aguilar et al. 2019). However, the wavelength range of the 460 nm LED used in this study was between 400 and 530 nm (Ghate et al., 2013), and thus ascorbic acid in melon is unlikely to be photodegraded by this LED illumination. Another possibility is that the light from the 460 nm LED illumination could not penetrate the fruits because of their thickness, and thus it could not impact the nutrition values of the fruits. The present results are similar to those of previous studies that showed that long-term illumination of 405 nm LED on fresh-cut papaya (Kim et al., 2017a) and mango (Kim et al., 2017b) did not affect the ascorbic acid content, total flavonoid content, or antioxidant capacity of the fruit, although the peak wavelength of that LED was different from that of the LED used in this study.

In conclusion, the present results showed that the 460 nm LED illumination in combination with chitosan was more effective in eliminating *E. coli* O157:H7, *Salmonella* spp., and *L. monocytogenes* on fresh-cut melons at chilling temperatures than either LED illumination alone or LED illumination combined with riboflavin. The data on inactivation kinetics revealed that *L. monocytogenes* was most susceptible to 460 nm LED illumination in combination with chitosan among the pathogens tested in this study. Furthermore, the nutritional values of fresh-cut melon were not influenced by long-term LED illumination. However, the color of the fruits changed, and this requires further research to optimize the LED illumination conditions to minimize the color change. Therefore, this study suggests that 460 nm LED illumination technology with the addition of chitosan could be applied to control foodborne pathogens on fresh-cut melons in retail stores as a novel fruit preservation technology.

Acknowledgements This work was supported by the National Research Foundation of Korea (NRF) Grant funded by the Korea government (MSIT) (No. 2019R1F1A1060624).

Declarations

Conflict of interest The authors declare no conflict of interest.

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