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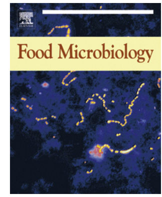

405 ± 5 nm light emitting diode illumination causes photodynamic inactivation of *Salmonella* spp. on fresh-cut papaya without deterioration

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This study evaluated the antibacterial effect of 405 ± 5 nm light emitting diode (LED) illumination against four *Salmonella* serovars on fresh-cut papaya and on fruit quality at various storage temperatures. To determine the antibacterial mechanism of LED illumination at 0.9 kJ/cm^2, oxidative damage to DNA and membrane lipids of *Salmonella* in phosphate-buffered saline solution was measured. The populations of *Salmonella* on cut fruits were significantly ($P < 0.05$) reduced by 0.3–1.3 log CFU/cm^2 at chilling temperatures following LED illumination for 36–48 h (1.3–1.7 kJ/cm^2). However, at room temperature, bacterial populations increased rapidly to 6.3–7.0 log CFU/cm^2 following LED illumination for 24 h (0.9 kJ/cm^2), which was approximately 1.0 log lower than the number of colonies on non-illuminated fruits. Levels of bacterial DNA oxidation significantly increased, whereas lipid peroxidation in bacterial membrane was not observed, suggesting that DNA oxidation contributes to photodynamic inactivation by LED illumination. LED illumination did not adversely affect the physicochemical and nutritional qualities of cut papaya, regardless of storage temperature. These results indicate that a food chiller equipped with 405 ± 5 nm LEDs can preserve fresh-cut papayas in retail stores without deterioration, minimizing the risk of salmonellosis.

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1. Introduction

Markets for refrigerated fresh-cut fruit products have dramatically increased in recent years due to consumer demand for fresh, convenient, additive-free, and minimally processed fruits that are nutritious and safe (James and Nagramsak, 2011). However, ready-to-eat (RTE) fresh-cut fruits can be easily exposed to unhygienic environmental conditions during processing, such as peeling and cutting, leading to cross-contamination with pathogenic bacteria from raw fruits or equipment. Moreover, these microorganisms on fresh-cut fruits can grow due to temperature fluctuations during storage at food establishments (Raybaudi-Massilia et al., 2013; Sim et al., 2013). These factors might contribute to outbreaks caused by consumption of RTE fresh-cut fruits (CDC, 2011).

Papaya is one of the most popular fresh-cut fruit products worldwide, especially in Southeast Asia, due to its large size and high nutrient contents, but it perishes easily after harvest and during storage. Fresh-cut papayas have been linked to *Salmonella* outbreaks in Australia (2006) (Gibbs et al., 2009) and Singapore (1996) (Ooi et al., 1997). In the former case, the papaya was washed using unhygienic river water prior to sale, resulting in contamination with *Salmonella* (Gibbs et al., 2009). In addition, a total of 106 confirmed cases with *S.* Agona linked to whole and fresh imported papayas have been reported from 25 states of the United States (US) (Raybaudi-Massilia et al., 2013; CDC, 2011). Thus, the implementation of proper preservation technologies in the fresh fruit supply chain is necessary to minimize the risk of salmonellosis.

Refrigeration is one of the most widely used preservation technologies to extend the shelf life of fresh-cut fruits and ensure food safety. However, pathogenic bacteria, including *Salmonella*

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spp., are capable of surviving refrigeration conditions. For this reason, refrigeration cannot be used alone as a preservation technology to ensure the safety of fresh-cut fruits. Previous studies have demonstrated the antibacterial efficacy of antimicrobial packaging film or natural antimicrobials combined with refrigeration. For example, Sangsuwan et al. (2008) reported that chitosan/methyl cellulose film at 10 °C inactivated 1.3 log and 2.9 log of *E. coli* on the surface on fresh-cut cantaloupe when applied for 24 and 48 h, respectively. Another study demonstrated that carvacrol, an essential oil, combined with refrigeration inhibited the growth of natural microbiota on the surface of fresh-cut honeydew surface for 2 and 5 days at 8 and 4 °C, respectively (Roller and Seedhar, 2002). However, consumers require additive-free or additive-reduced foods regardless of whether the food additives are naturally or artificially originated. Thus, a secondary antimicrobial measure without any food preservatives under refrigeration should be developed to effectively control the growth of pathogenic bacteria on the surface of fresh-cut and RTE fruits.

Ultraviolet (UV) light has been developed for surface decontamination; however, it has some limitations as an food preservation technology such as harmful effects on human and decolorization in certain products at high doses (Maclean et al., 2009; Kim et al., 2016). On the other hand, light emitting diode (LED) with visible wavelength has been recognized as an alternative technology to UV light since it is an environmentally friendly and safe technology for humans despite of its less antibacterial efficacy than UV light (Lukšienė and Zukauskas, 2009; Maclean et al., 2009). For this reason, LED technology has recently received attention in the field of food microbiology due to its antibacterial effect on foodborne pathogens. The antibacterial effect of 400 nm LED has been reported to be effective against *Listeria monocytogenes* and *S.* Typhimurium with the addition of exogenous photosensitizers of chlorophyllin or 5-aminolevulinic acid (ALA) in buffered solution (Lukšienė et al., 2013). In our previous studies, 405 and 461 nm LEDs without additional photosensitizers have demonstrated antibacterial effects against various foodborne pathogens in phosphate buffered saline solution (PBS) and trypticase soy broth (TSB), resulting in 1–5 log reduction after LED illumination for 7.5 h (Ghate et al., 2013; Kim et al., 2015; 2016; Kumar et al., 2015).

Visible light inactivation, called photodynamic inactivation (PDI), is a non-thermal photophysical and photochemical reaction that requires visible light, particularly in the 400–430 nm wavelength range, and photosensitizers such as porphyrin molecules in the presence of oxygen (Lukšienė and Zukauskas, 2009; Dai et al., 2012). Intracellular photosensitizer molecules absorb photons of visible light during LED illumination, and then the molecules are excited. While returning to the ground state, they transfer energy to oxygen molecules, resulting in the production of reactive oxygen species (ROS) such as superoxide anion, hydrogen peroxide, hydroxyl radicals, and singlet oxygen. The ROS may attack cellular components, such as DNA, lipids, and proteins, resulting in bacterial death (Lukšienė and Zukauskas, 2009; Dai et al., 2012).

To the best of our knowledge, no study has been conducted to explore the effectiveness of 405 ± 5 nm LED on the inhibition of bacterial growth on fresh-cut fruits without exogenous photosensitizers. Therefore, the objective of this study was to assess the potential of 405 ± 5 nm LED in inhibiting or eliminating *Salmonella* spp. on fresh-cut papaya at different storage temperatures. The physicochemical and nutritional qualities of illuminated fruits were also analyzed to determine whether long-term exposure of fruits to LED illumination influences food quality. Lastly, the extent of oxidative damage to the bacterial membrane and DNA was investigated to elucidate the antibacterial mechanism of LED illumination.

2. Materials and methods

2.1. Bacterial strains and culture conditions

The four *Salmonella enterica* serovars used in this study, *S.* Agona (BAA-707) (SA), *S.* Newport (ATCC 6962) (SN), *S.* Saintpaul (ATCC 9712) (SS), and *S.* Typhimurium (ATCC 14028) (ST), were purchased from the American Type Culture Collection (ATCC; Manassas, VA, USA). Frozen stock cultures stored at −70 °C were revived in 10 mL of sterile tryptone soya broth (TSB; Oxoid, Basingstoke, UK) at 37 °C for 24 h. All *Salmonella* 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 10 mL of TSB to isolate inoculated *Salmonella* cells from background microbiota in fresh-cut papaya. The working cultures at a stationary phase were prepared by incubation in 10 mL of TSB supplemented with 200 μg/mL of nalidixic acid at 37 °C for 18–24 h with two consecutive transfers.

2.2. Light emitting diode (LED) source and illumination system

The 405 ± 5 nm LED (8 × 8 mm) used in this study was purchased from Shenzhen Getian Opto-Electronics Co., Ltd. (Shenzhen, Guangdong, China). To minimize heat transfer to samples and to protect the LED from its own heat, a cooling fan and a heat sink were attached to the LED. To illuminate fresh-cut papaya, two fruit samples in a sterile Petri dish (60 mm diameter) were placed directly below the LED at a distance of 4.5 cm. The irradiance of 405 ± 5 LED on the fruit surface was 10 ± 1 mW/cm^2 as measured by a compact power and energy meter console (PM100D; Thorlabs GmbH, Dachau, Germany). To determine the antibacterial properties of the LED, a bacterial suspension in a sterile Petri dish (35 mm diameter) was placed directly below the LED at a distance of 2.3 cm and the irradiance was 35 ± 3 mW/cm^2 at the surface of the bacterial suspension.

Temperatures of the cut fruit surface and bacterial suspension were monitored using a Fluke 5.4 thermocouple thermometer (Everett, WA, USA) during LED illumination. The dose obtained from each sample was calculated by the following equation (Maclean et al., 2009):

$$E = Pt$$

where E = dose (energy density) in J/cm^2, P = Irradiance (power density) in W/cm^2, and t = time in sec.

2.3. Preparation of fresh-cut papaya

Fresh papayas were purchased from a local supermarket in Singapore. Papayas were washed with tap water, surface-sterilized with 30% (v/v) commercial bleach (0.9 ± 0.05% (v/w) sodium hypochlorite) for 30 min, rinsed three times with sterile deionized water, and dried with Kimwipes (Kimtech Science, Kimberly Clark Professional, Roswell, GA, USA). The dried papayas were peeled aseptically and cut into approximately 10 g slices in a semicircle shape (60 mm diameter) in a biosafety cabinet.

2.4. Inoculation on fresh-cut papaya

One mL of each *Salmonella* serovar adapted to nalidixic acid was centrifuged at 6000*g* for 10 min at 4 °C and washed twice with 1 mL of sterilized phosphate buffered saline (PBS; Vivantis Technologies Sdn Bhd, Malaysia). The resultant pellet was resuspended in 1 mL of PBS and diluted to approximately 10^5 CFU/mL in PBS. A 10-μL aliquot of the diluents was spot-inoculated at 10 sites on the fruit

surface to reach a final concentration of approximately 10^3 CFU/cm^2, and the inoculated fruits were dried for 30 min in a biosafety cabinet. Then, the fruits were individually packed with absolutely transparent cling wrap (approximately 90 × 90 mm) to simulate packaging conditions of cut fruit in retail stores, which can prevent dehydration of cut fruit during storage. Our preliminary study showed that there was no significant difference in the intensity of 405 ± 5 LED when the cling wrap was used to pack the fruits (data now shown), indicating the cling wrap did not interfere the antibacterial effect of 405 ± 5 LED.

2.5. LED illumination on fresh-cut papaya

The inoculated cut papayas were placed directly into the LED system as previously described, and were illuminated by 405 ± 5 nm LED at set temperatures of 4, 10, or 20 °C, for 24–48 h (a total dose of 0.9–1.7 kJ/cm^2) in a temperature controlled incubator (MIR-154, Panasonic Healthcare Co., Ltd., Osaka, Japan). A non-illuminated control sample was also placed in an incubator under dark conditions. For duplicate sampling, two fruits were illuminated simultaneously.

Illuminated or non-illuminated fruits were taken at selected time intervals, immediately transferred into sterile stomacher bags including 90 mL of 0.1% (v/w) peptone water, and homogenized for 2 min using a paddle blender (Silver Masticator, IUL Instruments GmbH, Königswinter, Germany). The homogenized samples were serially diluted with 0.1% peptone water if necessary, and the diluents were plated onto tryptone soya agar (TSA; Oxoid) supplemented with 200 μg/mL nalidixic acid, followed by incubation at 37 °C for 24–48 h. The number of colonies was enumerated manually with a colony counter (Rocker Scientific Co. Ltd., Taipei, Taiwan) and reported in log CFU/cm^2.

2.6. Analysis of cellular lipid peroxidation

A thiobarbituric acid reaction substance (TBARS) assay was performed to analyze oxidative damage to membrane lipids in *S*. Agona and *S*. Typhimurium, which were chosen as model microorganisms, by 405 ± 5 nm LED illumination. Malondialdehyde (MDA) is produced naturally by lipid peroxidation and is a reliable indicator of lipid peroxidation (Joshi et al., 2011).

Fifty mL of *Salmonella* culture at a stationary phase in TSB were centrifuged at 8000*g* for 10 min at 4 °C and washed twice with 5 mL of PBS. The resultant pellet was resuspended in 2 mL of PBS to provide a final population of approximately 10^{11} CFU/mL for appropriate determination of cellular lipid peroxidation (Carré et al., 2014). A 2-mL bacterial suspension was illuminated to 405 ± 5 nm at set temperature of 4 °C for 7 h (a total dose of 0.9 kJ/cm^2) in the incubator. A bacterial suspension incubated for 7 h without LED illumination served as the non-illuminated control.

To quantify lipid peroxidation, LED-illuminated and non-illuminated bacterial cells were collected and centrifuged at 9000*g* for 10 min. The resultant pellet was resuspended in 100 μL of PBS containing 5 μL of 100 × butylated hydroxytoluene (BHT; Sigma-Aldrich) to prevent further oxidation and subsequently added to 200 μL of 5% sodium dodecyl sulfate (SDS) lysis buffer (Sigma-Aldrich). After incubation of the mixture for 30 min at room temperature, 900 μL of TBA reagent freshly prepared (5.2 mg TBA per 1 mL of 10% trichloroacetic acid, TCA; Sigma-Aldrich) were added to the mixture and incubated at 95 °C for 1 h, followed by cooling to room temperature in an ice bath for 10 min before a final centrifugation at 3000*g* for 15 min. Afterwards, the supernatant (200 μL) was transferred in duplicate to a 96-well plate and measured at 532 nm using a Synergy HT multi-detection Microplate reader (Bio-Tek Instruments Inc., Winooski, VT, USA). The degree of peroxidation of cellular lipids was calculated based on a standard curve between 0 and 62.5 μM MDA (Merck, Darmstadt, Germany). Results were expressed in nM MDA equivalents per 10^{11} cells.

2.7. Analysis of DNA oxidation

A 2-mL bacterial suspension containing an initial population of approximately 10^9 CFU/mL was prepared as previously described and illuminated at set temperature of 4 °C for 7 h (a total dose of 0.9 kJ/cm^2). LED-illuminated and non-illuminated samples were collected and centrifuged at 9000*g* for 10 min at 4 °C. The resultant pellet was used for DNA extraction with a GeneJET Genomic DNA Purification Kit (Thermo Scientific, Waltham, MA, USA) according to the manufacturer's instructions. The purified DNA was dissolved in 100 μL of elution buffer. The concentration of purified DNA was measured using a BioDrop Touch Duo Spectrophotometer (BioDrop, Cambridge, UK).

The degree of oxidation of purified DNA was measured using an OxiSelect™ Oxidative DNA Damage ELISA kit (Cell Biolabs, San Diego, CA, USA) according to the manufacturer's instructions with slight modifications. Briefly, the purified DNA (2–5 μg) was converted into single-stranded DNA by incubation at 95 °C for 5 min and immediately chilled in an ice bath. Then, the denatured DNA sample was incubated with 6 units of nuclease P1 (Wako, Osaka, Japan) in 20 mM of sodium acetate buffer (pH 5.2) (Sigma- Aldrich) at 37 °C for 2 h. After incubation, 2 units of *E. coli* alkaline phosphatase (Takara Bio Inc., Shiga, Japan) and 10% volume of 10 × alkaline phosphatase buffer (Takara Bio Inc.) were added to the DNA sample and incubated at 37 °C for 1 h. The reaction mixture was centrifuged at 6000*g* for 5 min and the supernatant was used for 8-hydroxydeoxyguanosine (8-OHdG) quantification as described in the assay protocol. The resulting sample was measured at 450 nm using the microplate reader. The amount of 8-OHdG was quantified using a standard curve (0–20 ng/mL) and expressed as ng 8-OHdG per mg DNA.

2.8. Color analysis

To determine the effect of 405 ± 5 nm LED illumination on the color of fresh-cut papaya, the color of illuminated fruits was measured using a reflectance spectrometer (CM-3500d; Konica Minolta Sensing Inc., Osaka, Japan) by set to a D65 illuminant at 10° observation. The parameters of color distribution consisted of L^* (lightness, black = 0, white = 100), a^* (red > 0, green < 0), and b^* (yellow > 0, blue < 0). The $L^*a^*b^*$ values obtained from papaya samples were used to determine the color difference (ΔE) between LED-illuminated fruits and non-illuminated control fruits using the following equation (Mohammadi et al., 2008).

$$\Delta E = \sqrt{(L_t - L_0)^2 + (a_t - a_0)^2 + (b_t - b_0)^2}$$

2.9. Antioxidant capacity

To determine antioxidant capacity, LED-illuminated and non-illuminated samples were extracted according to González-Aguilar et al. (2007) with slight modifications. LED-illuminated and non-illuminated fruits (ca. 10 g) were homogenized in 25 mL of 80% methanol containing 0.5% sodium bisulfate (Sigma-Aldrich) and were sonicated for 60 min at room temperature by adding ice to inhibit temperature increase. After sonication, the homogenates were centrifuged at 3000*g* for 10 min at room temperature. The

supernatant was filtered using Whatman™ No. 1 filter paper (GE Healthcare, Little Chalfont, Buckinghamshire, UK). The filtered extracts were stored at −20 °C prior to analysis of antioxidant capacity and total flavonoid content.

A 20-μL extract aliquot was added to 280 μL of 2,2-diphenyl-1-picrylhydrazyl (DPPH; Sigma-Aldrich) solution freshly prepared by dissolving 1.42 mg of DPPH in 10 mL of methanol. Afterwards, the mixture was incubated for 30 min at room temperature in darkness. Then, 200 μL of the mixture was transferred in duplicate to a 96-well plate and measured at 515 nm using the microplate reader. Antioxidant capacity was calculated on the basis of a standard curve between 0 and 800 μM Trolox (Sigma-Aldrich). Results were expressed in mg Trolox equivalents (TE)/100 g fresh weight (FW).

2.10. Total flavonoid content

Flavonoids were extracted as described above. A 50-μL extract aliquot was added to 200 μL of deionized H_2O and 15 μL of 5% sodium nitrite ($NaNO_2$; Sigma-Aldrich). After equilibration for 5 min, 15 μL of 10% aluminum chloride ($AlCl_3$; Sigma-Aldrich) were added and equilibrated for 5 min, followed by addition of 100 μL of 1 M sodium hydroxide (NaOH). The final volume was adjusted to 500 μL with deionized H_2O. A 200-μL mixture was loaded in a 96-well plate in duplicate, followed by reading at 415 nm using the microplate reader. A standard curve was prepared in the concentration range of 0–250 μM Quercetin (Sigma-Aldrich). The total flavonoid content was expressed in mg Quercetin equivalents (QE)/100 g FW.

2.11. Ascorbic acid

Ascorbic acid was extracted as described by Barros et al. (2007) and Spilimbergo et al. (2013) with slight modifications. Fruit samples (ca. 5 g) were added to 10 mL of 2.5% *meta*-phosphoric acid (Sigma-Aldrich), incubated for 45 min at room temperature, and centrifuged at 3000*g* for 2 min. The supernatant was filtered using Whatman™ No. 1 filter paper and stored at 4 °C until analysis.

A stock solution (0.025% DCIP) was freshly prepared by dissolving 12.5 mg 2,6-dichloroindophenol (DCIP) sodium salt (Sigma-Aldrich) in 50 mL of deionized H_2O containing 1 mg of sodium bicarbonate ($NaHCO_3$; Sigma-Aldrich). A 120-μL aliquot of the extract was mixed with 80 μL of stock solution in a 96-well plate and the mixture was measured at 515 nm using the microplate reader (Barros et al., 2007; Freed, 1966). A standard curve was prepared using 250–700 μM L-Ascorbic acid (Sigma-Aldrich). The concentration of ascorbic acid was calculated and expressed as mg ascorbic acid/100 g FW.

2.12. β-carotene and lycopene

Preparation of fruit samples for β-carotene and lycopene measurements was performed according to the methods of Barros et al. (2007) and Nagata and Yamashita (1992) with slight modifications. Illuminated or non-illuminated fruit samples (ca. 5 g) in 50-mL Falcon tubes were vigorously shaken with 10 mL of acetone-hexane mixture (4:6, v/v) for 2 min. The mixture was centrifuged for 2 min at 3000*g* and filtered through Whatman™ No. 1 filter paper. The extracts were kept in 4 °C until analysis.

Extracts (200 μL) were added in duplicate to a 96-well plate and measured at 663, 645, 505, and 453 nm using the microplate reader. Results were expressed as mg β-carotene/100 g FW or mg lycopene/100 g FW and calculated using the following equations (Nagata and Yamashita, 1992):

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

$$\text{Lycopene (mg/100 g)} = -0.0458A_{663} + 0.204A_{645} + 0.372A_{505} - 0.0806A_{453}$$

2.13. Statistical analysis

All experiments were performed independently in triplicate with duplicate samples or plates (n = 6). All data expressed by mean ± standard deviation were analyzed by independent-sample *t*-test ($P < 0.05$) when comparing two values or by analysis of variance (ANOVA) with least significant difference (LSD, $P < 0.05$) post-hoc test when comparing more than two values using SPSS statistical software (version 17.0; SPSS Inc., IBM Co., Armonk, NY, USA).

3. Results

3.1. Temperature change of fruit surface and PBS during LED illumination

To measure the heating effect of LED illumination, temperatures of the surface of fresh-cut papaya and PBS were monitored during the 405 ± 5 nm LED illumination. Results show that LED illumination increased the temperatures of the fruit surface and PBS by approximately 3.2 and 5.6 °C, respectively, within 1 h, when incubator temperatures were set at 4 °C (data not shown). The variance in temperature increase might be explained by different LED intensities, which were adjusted by the distance between the bacterial suspension or the fruit surface and the LED source, indicating that lower LED intensities result in less variance in temperature. Similar temperature increases were also observed at 10 and 20 °C (data not shown). Thus, non-illuminated control experiments were performed at 3.2 and 5.6 °C higher than illuminated samples for fruits and PBS, respectively, to eliminate the influence of temperature increase on bacterial inactivation or bacterial growth by LED illumination.

3.2. Behavior of *Salmonella* spp. on fruit surface during LED illumination

The behaviors of *S*. Agona, *S*. Newport, *S*. Saintpaul, and *S*. Typhimurium on the surface of fresh-cut papaya were monitored during 405 ± 5 nm LED illumination at different storage temperatures. At 4 °C (actual surface temperature of 7.2 °C), populations of all *Salmonella* serovars were significantly ($P < 0.05$) reduced by 1.0–1.2 log CFU/cm^2 during LED illumination (a total dose of 1.7 kJ/cm^2), whereas no significant ($P \geq 0.05$) reduction or increase was observed in cell populations on non-illuminated fruits for 48 h at 7.2 °C (Fig. 1).

Non-illuminated cell populations on fruits gradually increased to 4.6, 4.3, 4.0, and 3.8 log CFU/cm^2 for *S*. Agona, *S*. Newport, *S*. Saintpaul, and *S*. Typhimurium, respectively, for 36 h at 13.2 °C compared to 4 °C (Fig. 2). In contrast, LED illumination resulted in 0.3-, 0.6-, and 1.3-log reductions for *S*. Newport, *S*. Saintpaul, and *S*. Typhimurium, respectively, while LED-illumination inhibited the growth of *S*. Agona cells for 36 h (a total dose of 1.3 kJ/cm^2) at 10 °C.

At set temperature of 20 °C for 24 h (a total dose of 0.9 kJ/cm^2), non-illuminated *S*. Agona, *S*. Newport, *S*. Saintpaul, and *S*. Typhimurium cells rapidly grew to 8.1, 7.5, 8.2, and 8.4 log CFU/cm^2,

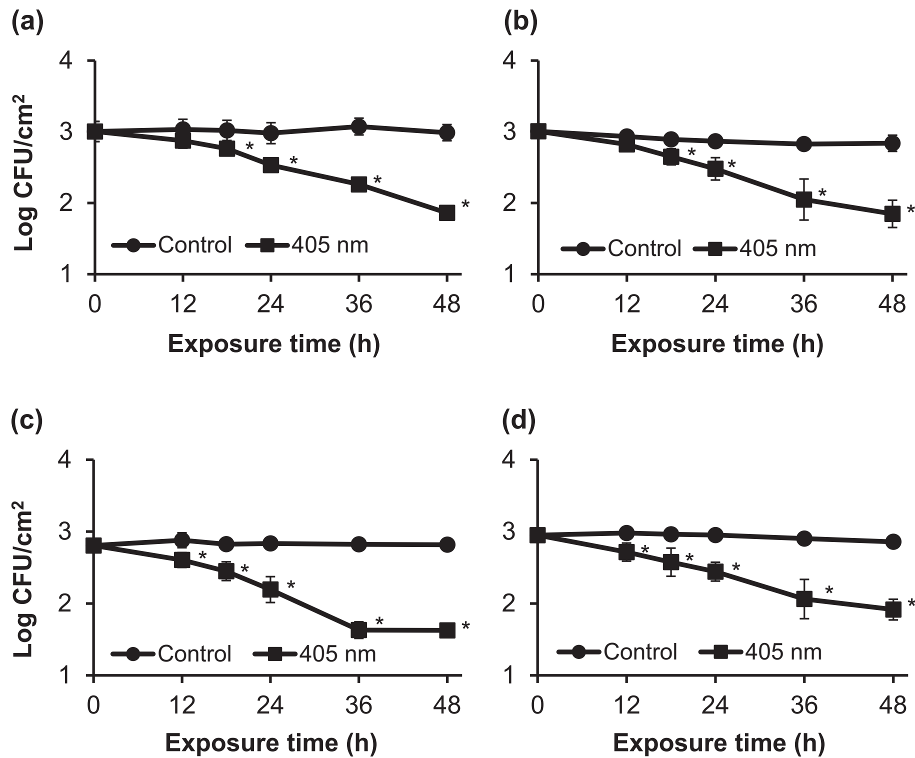

Fig. 1. Effects of 405 ± 5 nm LED illumination on survival of *S.* Agona (a), *S.* Newport (b), *S.* Saintpaul (c), and *S.* Typhimurium (d) on the surface of fresh-cut papaya at 4 °C (actual temperature of 7.2 °C). Asterisk (*) indicates significant ($P < 0.05$) difference between LED-illuminated and non-illuminated bacterial cell counts.

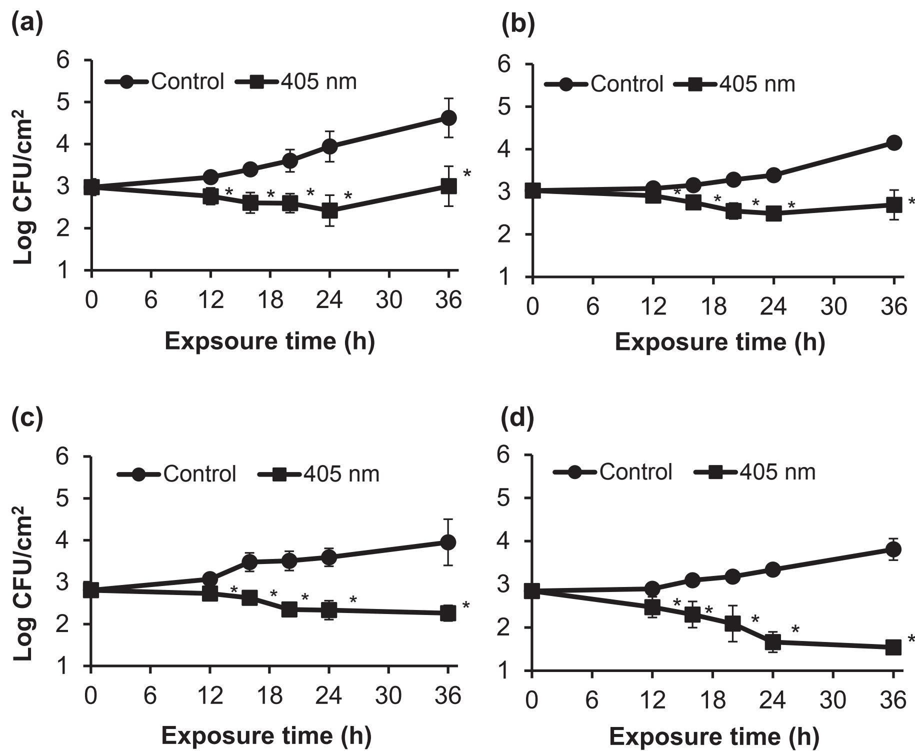

Fig. 2. Effects of 405 ± 5 nm LED illumination on survival of *S.* Agona (a), *S.* Newport (b), *S.* Saintpaul (c), and *S.* Typhimurium (d) on the surface of fresh-cut papaya at 10 °C (actual temperature of 13.2 °C).

while the number of LED-illuminated cells reached 6.7, 6.3, 7.0, and 6.8 log CFU/cm^2 during the same period, revealing LED illumination delayed the growth of *Salmonella* cells on fresh-cut papaya at room temperature (Fig. 3).

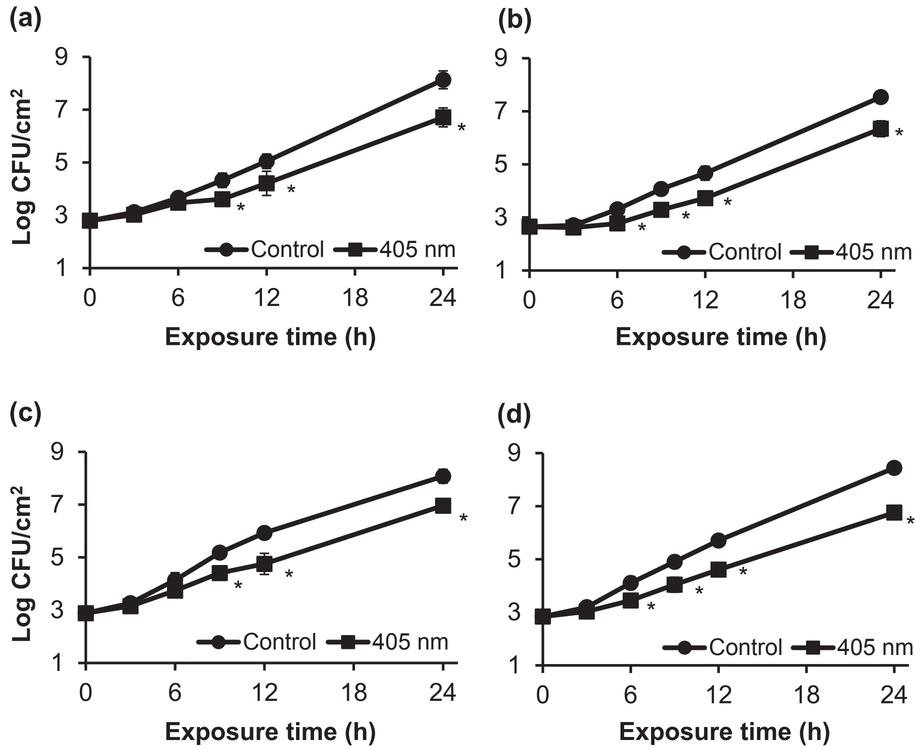

Fig. 3. Effects of 405 ± 5 nm LED illumination on survival of *S.* Agona (a), *S.* Newport (b), *S.* Saintpaul (c), and *S.* Typhimurium (d) on the surface of fresh-cut papaya at 20 °C (actual temperature of 23.2 °C).

3.3. Oxidative damage to membrane lipid and DNA in Salmonella cells

The degrees of lipid peroxidation and DNA oxidation were analyzed to determine whether the ROS generated by LED illumination oxidize these two cellular components. The populations of *S.* Agona and *S.* Typhimurium were similarly inactivated by 2.0–2.7 log CFU/mL at different initial population densities of 10^{11} and 10^{9} CFU/mL for lipid peroxidation and DNA oxidation, respectively, at 0.9 kJ/cm^2 (for 7 h) at 4 °C (actual temperature of 9.6 °C) (Figs. 4a and 5a), whereas no reduction was observed in non-illuminated cells. There was no significant ($P \geq 0.05$) difference in lipid peroxidation between non-illuminated controls and illuminated cells, regardless of serotype (Fig 4b). For DNA oxidation, the levels of 8-OHdG in illuminated *S.* Agona and *S.* Typhimurium were 1.7–1.8 times higher than those of non-illuminated control cells, indicating that ROS might preferentially oxidize DNA rather than lipids in cell membrane (Fig. 5b).

3.4. Changes in color of cut papaya by LED illumination

Changes in color of fresh-cut papaya illuminated by 405 ± 5 nm LED were measured to determine if LED illumination negatively influences fruit quality. As shown in Table 1, no significant ($P \geq 0.05$) differences were observed between color differences (ΔE), although the mean ΔE values of LED-illuminated fruits were higher than those of non-illuminated fruits. However, changes in $L^*a^*b^*$ values were observed in both non-illuminated and LED-

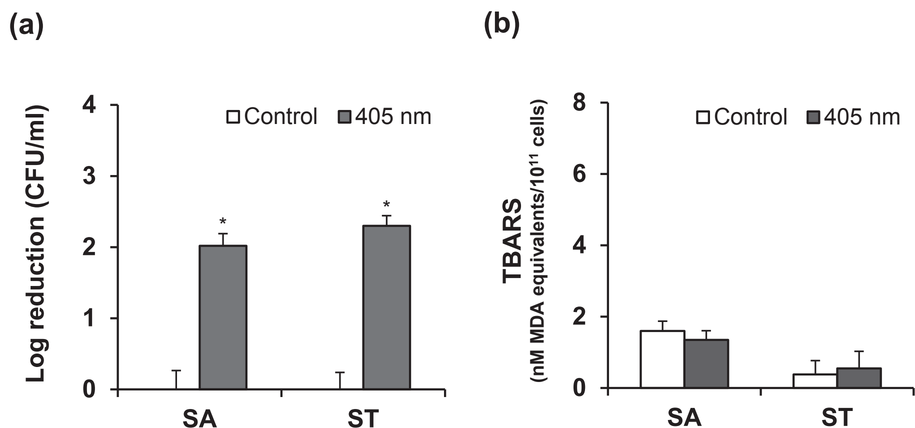

Fig. 4. Log reduction (a) in *S.* Agoan (SA) and *S.* Typhimurium (ST) and lipid peroxidation (b) by 405 ± 5 nm LED illumination at 4 °C for 7 h (a total dose of 0.9 kJ/cm^2) in phosphate buffered saline (PBS).

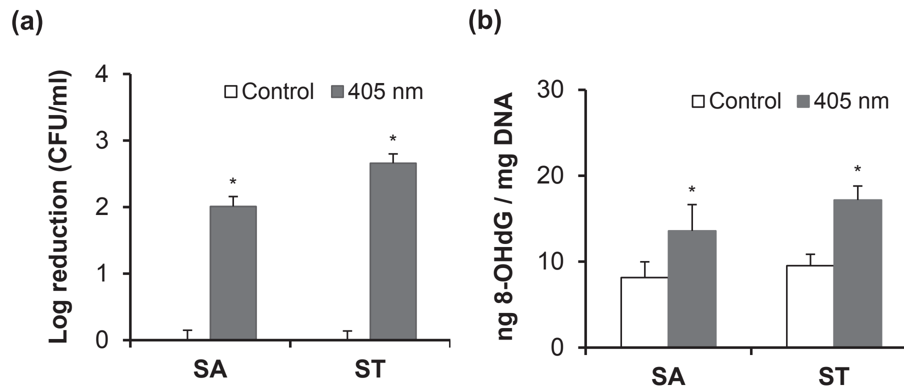

Fig. 5. Log reduction (a) in *S.* Agoan (SA) and *S.* Typhimurium (ST) and the level of 8-hydroxydeoxyguanosine (8-OHdG) (b) by 405 ± 5 nm LED illumination at 4 °C for 7 h (a total dose of 0.9 kJ/cm^2) in PBS.

Table 1
Color changes in fresh-cut papaya treated with 405 ± 5 nm LED illumination.

Temperature (°C)	Time (h)	Dose (kJ/cm^2)	Sample	L^*	a^*	b^*	Color difference (ΔE)
20	0	0	control	47.9 ± 4.9^a	24.0 ± 6.2^a	33.9 ± 3.8^a	
	24	0	dark	30.4 ± 2.0^b	10.3 ± 2.1^b	13.6 ± 1.8^b	30.3 ± 2.5^a
		0.86	405 nm	29.8 ± 1.3^b	9.5 ± 1.2^b	11.1 ± 3.2^b	32.9 ± 1.5^a
10	0	0	control	48.8 ± 3.5^a	29.9 ± 5.0^a	34.1 ± 5.3^a	
	36	0	dark	29.4 ± 2.2^b	13.5 ± 0.6^b	13.7 ± 1.7^b	33.3 ± 4.1^a
		1.30	405 nm	33.0 ± 4.7^b	12.7 ± 2.0^b	8.7 ± 3.9^b	36.9 ± 8.9^a
4	0	0	control	48.1 ± 1.7^a	26.8 ± 0.4^a	35.2 ± 0.9^a	
	48	0	dark	35.8 ± 3.9^b	16.5 ± 2.5^b	16.1 ± 4.7^b	25.1 ± 6.1^a
		1.73	405 nm	35.6 ± 7.3^b	15.4 ± 4.0^b	10.0 ± 5.1^b	30.8 ± 8.2^a

Different letters within the same column at the same storage temperature differ significantly (n = 6; $P < 0.05$).

illuminated cut fruits during storage compared to fresh-cut fruit at 0 h, probably owing to cut processing and ripening.

3.5. Effect of LED illumination on antioxidant capacity, flavonoid content and nutrients of fruit

Table 2 shows the changes in antioxidant capacity and contents of flavonoids, ascorbic acid, β-carotene, and lycopene between LED-illuminated and non-illuminated fruits during storage at different temperatures. There were no significant ($P \geq 0.05$) changes in the antioxidant capacity of cut fruits, irrespective of LED illumination, whereas the total flavonoid content in all illuminated fruits was 1.5–1.9 times higher than that of fruit at 0 h. However, the total flavonoid content in LED-illuminated fruits was found to increase significantly ($P < 0.05$) during storage at 20 and 4 °C, compared to non-illuminated fruits.

No significant changes in the levels of ascorbic acid, β-carotene, and lycopene were observed between LED-illuminated and non-illuminated fruits. On the other hand, higher contents of β-carotene and lycopene were found in LED-illuminated and non-illuminated fruits at 20 °C, whereas a lower content of ascorbic acid was observed in LED-illuminated fruits at 4 °C. Overall, 405 ± 5 nm LED illumination did not negatively impact the physicochemical and nutritional qualities of fresh-cut papaya.

4. Discussion

Our previous findings revealing the antibacterial effect of 405 ± 5 nm LED against *S.* Typhimurium in PBS (Kim et al., 2016) prompted us to design this study to demonstrate the effectiveness of LED illumination on the inactivation of *Salmonella* on fresh-cut papaya at different storage temperatures as well as its effect on fruit quality and to evaluate the potential of LED as viable fresh-cut fruit preservation technology. Amounts of cellular lipid and DNA oxidation were also determined for improved understanding of the antibacterial mechanism of LED illumination. The different storage

Table 2
Effects of 405 ± 5 nm LED illumination on antioxidant capacity and contents of ascorbic acid, total flavonoid, β-carotene, and lycopene in fresh-cut papaya at varying storage temperatures.

Storage temperature (°C)	Time (h)	Sample	Antioxidant capacity (mg TE/100 g FW)	Flavonoids (mg QE/100 g FW)	Ascorbic acid (mg/100 g FW)	β-carotene (mg/100 g FW)	Lycopene (mg/100 g FW)
20	0	Control	45.4 ± 3.4^a	1.9 ± 0.3^a	18.0 ± 1.9^a	0.29 ± 0.1^a	0.34 ± 0.2^a
	24	Dark	45.9 ± 3.4^a	2.7 ± 0.4^a	17.8 ± 1.6^a	0.48 ± 0.1^b	0.74 ± 0.2^{ab}
		405 nm	41.9 ± 5.5^a	3.5 ± 0.5^b	17.5 ± 2.7^a	0.50 ± 0.0^b	0.90 ± 0.3^b
10	0	Control	39.1 ± 6.0^a	1.9 ± 0.3^a	16.9 ± 2.8^a	0.29 ± 0.1^a	0.34 ± 0.2^a
	36	Dark	37.0 ± 4.8^a	3.1 ± 0.9^b	17.2 ± 1.2^a	0.36 ± 0.2^a	0.64 ± 0.1^{ab}
		405 nm	34.6 ± 4.0^a	3.4 ± 0.5^b	16.2 ± 1.5^a	0.30 ± 0.1^a	0.75 ± 0.1^b
4	0	Control	45.3 ± 3.4^a	1.9 ± 0.3^a	18.0 ± 1.9^a	0.29 ± 0.1^a	0.34 ± 0.2^a
	48	Dark	46.1 ± 1.4^a	1.9 ± 0.1^a	14.6 ± 3.4^{ab}	0.37 ± 0.2^a	0.52 ± 0.1^{ab}
		405 nm	44.2 ± 3.2^a	2.8 ± 0.6^b	13.3 ± 0.8^b	0.38 ± 0.2^a	0.64 ± 0.1^b

Different letters within the same column at the same storage temperature differ significantly (n = 6; $P < 0.05$).

conditions at 4, 10, and 20 °C were selected in this study to simulate the ideal refrigeration temperature, temperature fluctuation in retail stores, and room temperature in tropical countries or in summer, respectively.

Four *Salmonella* serotypes on the fresh-cut fruits were inactivated or their growth was effectively inhibited during the 405 ± 5 nm LED illumination at 4 and 10 °C, whereas the effectiveness of LED illumination was not apparent at 20 °C. Similar to these results, a previous study demonstrated that *S*. Typhimurium in TSB was more susceptible to 461 nm LED at 10 and 15 °C than at 20 °C (Ghate et al., 2013). Inactivation of *Salmonella* by LED illumination at chilling temperatures might be due to the inhibition of their oxidative stress response, enzymatically removing ROS (Beales, 2004). Another possible explanation could be a decrease in capacity to transport solutes into cells by altering membrane fluidity of the lipid bilayer at lower temperatures, consequently inhibiting energy yield metabolism, which could make cells more sensitive to LED illumination (Beales, 2004). Unlike chilling temperatures, both LED-illuminated and non-illuminated *Salmonella* cells rapidly grew on the fruit surface at 20 °C, although LED illumination initially delayed the growth of *Salmonella* on cut fruits. These results indicate that LED-illuminated cells likely require an acclimatization period to new environments such as LED illumination (Dickson et al., 1992). Once the LED-illuminated cells adapt to the conditions, their growth rate might be similar to that of non-illuminated cells. Based on the results obtained in this study, storage temperature plays an important role in controlling *Salmonella* on fresh-cut fruit during 405 ± 5 nm LED illumination. Contrarily, Ghate et al. (2016) reported that the antibacterial efficacy of 460 nm LED on *Salmonella* cells in orange juice was enhanced at higher temperatures, indicating that food matrix might be another factor impacting efficacy.

It is generally known that the antibacterial mechanism of blue LEDs is due to the generation of ROS by the reaction of light, endogenous porphyrin compounds, and oxygen, as previously described. Thus, it is hypothesized that ROS may oxidize guanine residues in genomic DNA and fatty acids in bacterial membranes, resulting in the production of oxidized derivatives such as 8-OHdG and MDA, respectively (Joshi et al., 2011; Sies and Menck, 1992). To test this hypothesis, oxidative damage to bacterial membrane lipids and DNA were measured in this study by analyzing the content of MDA and 8-OHdG in *S*. Agona and *S*. Typhimurium. The results showed there was no lipid peroxidation in both serotypes on LED illumination, whereas LED illumination significantly increased levels of 8-OHdG compared to non-illuminated cells. Similar to this study, Hamamoto et al. (2007) reported that oxidative DNA damage in *S*. Typhimurium was observed by illumination with 365 nm LED. Another study has shown that illumination at 407 nm with cationic tetra-meso (*N*-methylpyridyl) porphine resulted in genomic DNA damage to *Escherichia coli* (Salmon-Divon et al., 2004). Contrary to our findings, a previous study demonstrated phospholipid oxidation in *E. coli* and *Staphylococcus warneri* on illumination with light wavelengths between 380 and 700 nm in the presence of a tricationic porphyrin derivative for 90 min (21.6 J/cm^2) in PBS (Alves et al., 2013). This discrepancy in bacterial lipid peroxidation findings between the present and previous studies might be explained by the presence of an exogenous photosensitizer. It is well established that different cellular components may be targeted by ROS based on the cellular localization of the photosensitizer (Alves et al., 2014). Further, it is speculated that the bacterial cell wall is a major target of ROS generated from exogenous photosensitizers when they bind to the cell membrane (Alves et al., 2014). Protoporphyrin IX produced by the addition of 5-aminolevulinic acid (5-ALA), for example, which has a higher affinity for bacterial membrane phospholipids, could cause damage to the bacterial cell membrane by ROS generated from the photosensitizer (Alves et al., 2014). By contrast, the lack of lipid peroxidation observed in the present study might be attributed to the small amount of intracellularly generated ROS from low levels of endogenous porphyrin compounds in *Salmonella* cells by LED illumination (Kumar et al., 2015). Thus, the amount of ROS might be insufficient to oxidize lipids generally located in bacterial membrane after attacking DNA molecules adjacent to porphyrin compounds in the cytoplasm. However, our previous study demonstrated that LED illumination increased membrane permeability under similar conditions to those reported herein (Kim et al., 2016). Thus, further research is necessary to elucidate the detailed antibacterial mechanism of 405 ± 5 nm LED illumination at both molecular and physiological levels.

The impact of 405 ± 5 nm LED illumination on the quality of cut fruit was evaluated in this study, as physicochemical and nutritional quality parameters such as color and ascorbic acid are known to be sensitive to light. The results showed that all quality parameters tested in this study were well preserved during LED illumination. Moreover, LED illumination had a positive effect on the total flavonoid content in cut papaya at 4 and 20 °C. Similar to our results, Hong et al. (2015) reported that 440 nm LED illumination resulted in higher contents of flavonoid and β-carotene in various plants and fruits compared to the controls in dark. Another previous study also showed a positive effect of blue light on Chinese bayberry, resulting in accumulation of anthocyanin compared to non-illuminated controls (Shi et al., 2014). These positive effects of LEDs on fruit quality might be attributable to the stimulation effect of light on the production of primary and secondary metabolites, which are involved in defense against ROS generated during LED illumination (Darko et al., 2014). In contrast, mature green tomatoes had lower lycopene content during blue LED illumination for 7 days compared to red LED- and non-illuminated fruits, but after blue light illumination, they gradually changed in color, ripened, and accumulated lycopene during storage, revealing inhibition of the ripening process and consequently long-term shelf life (Dhakal and Baek, 2014). Together, these data indicate that the influence of LEDs on fruit quality varies by fruit and ripening conditions. However, the detailed mechanism regulating nutritional quality of fruits by LEDs has yet to be established.

5. Conclusions

This is the first study to evaluate the efficacy of 405 ± 5 nm LED in controlling *Salmonella* spp. on fresh-cut papaya at different storage temperatures. Results demonstrated that LED illumination was able to inactivate *Salmonella* cells on cut fruit at chilling temperatures, but was not effective at room temperature. Our findings confirmed that bacterial genomic DNA can be oxidized, contributing to pathogen inactivation and antimicrobial properties, while bacterial membrane lipid peroxidation is unlikely to happen by ROS generated from LED illumination. Furthermore, no significant differences were found in color, antioxidant capacity, or flavonoid, ascorbic acid, β-carotene, and lycopene content between LED-illuminated and non-illuminated fruits at all temperatures, except at 4 and 20 °C, wherein significantly higher flavonoid content was detected in illuminated fruits. Thus, this study proposes that 405 ± 5 nm LED, combined with refrigeration conditions, could be useful to control *Salmonella* on fresh-cut fruits without any deterioration, helping to reduce the risk of salmonellosis. However, further study is needed to evaluate the efficacy of 405 ± 5 nm LED against other foodborne pathogens and natural microbiota as well as on other food matrices, as the behavior of bacterial pathogens may vary according to differences in types and contents of bioactive compounds and nutrition in foods. In addition, the LED system

needs to be improved to effectively inactivate microorganism on both sides of cut fruits.

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