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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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ABSTRACT

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.

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 (Luksienė 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 (Luksienė 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 (Luksienė 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 (Luksienė 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