International Journal of Food Microbiology 244 (2017) 82–89

Contents lists available at ScienceDirect

International Journal of Food Microbiology

journal homepage: www.elsevier.com/locate/ijfoodmicro

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

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ARTICLE INFO

Article history:
Received 6 September 2016
Received in revised form 20 December 2016
Accepted 29 December 2016
Available online 30 December 2016

Keywords:
405 nm light emitting diode
Photodynamic inactivation
Foodborne pathogens
Mango
Quality parameters

ABSTRACT

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

1. Introduction

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

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

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

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http://dx.doi.org/10.1016/j.ijfoodmicro.2016.12.023
0168-1605/

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

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

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

2. Materials and methods

2.1. Bacterial strains and culture conditions

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

2.2. Light emitting diode (LED) illumination system

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

$$E = Pt$$

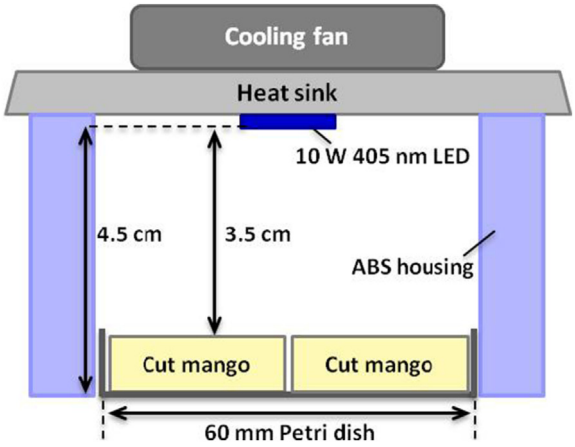

Fig. 1. Schematic diagrams of 405 ± 5 nm LED illumination system.

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

2.3. Preparation of mango and inoculation

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

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

2.4. LED illumination

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

2.5. Modified Weibull model for bacterial inactivation kinetics

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