Journal of Food Engineering 196 (2017) 130–138

Contents lists available at ScienceDirect

Journal of Food Engineering

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

Effect of 460 nm light emitting diode illumination on survival of *Salmonella* spp. on fresh-cut pineapples at different irradiances and temperatures

Vinayak Ghate [a], Amit Kumar [a], Min-Jeong Kim [a], Woo-Suk Bang [b], Weibiao Zhou [a, c], Hyun-Gyun Yuk [a, c, *]

[a] Food Science & Technology Programme, Department of Chemistry, National University of Singapore, Science Drive 4, 117543, Singapore
[b] Department of Food and Nutrition, Yeungnam University, 214-1 Dae-dong, Gyeongsan-si, Gyeongsangbuk-do, 712-749, South Korea
[c] National University of Singapore (Suzhou) Research Institute, No. 377 Linquan Street, Suzhou Industrial Park, Suzhou, Jiangsu, 215123, China

ARTICLE INFO

Article history:
Received 30 May 2016
Received in revised form
8 September 2016
Accepted 9 October 2016
Available online 12 October 2016

Keywords:
Light emitting diode
Antibacterial effect
Salmonella
Fresh-cut fruit
Weibull model

ABSTRACT

Blue light emitting diodes (LEDs) have emerged as an intervention against *Salmonella*, which colonizes and grows on fresh-cut fruits. This study evaluated their efficacy on fresh-cut pineapples. Pineapple slices were surface-inoculated with a *Salmonella* cocktail and illuminated with 460 nm LEDs at different irradiances (92, 147.7 and 254.7 mW/cm^2) and temperatures (7, 16 and 25 °C). The resulting differences in the populations of control and illuminated samples were modeled to determine the antibacterial effect. The color of the slices was also measured. Bactericidal action was observed at 7 and 16 °C and growth inhibition at 25 °C. An adapted Weibull model best described the inactivation, with the D values ranging from 15 to 27 kJ/cm^2. Temperature influenced the antibacterial effect but the irradiance had no significant effect ($P \geq 0.05$). Though the illuminated pineapple slices tended to be bleached, this study demonstrated the potential of 460 nm LEDs against *Salmonella* on fresh-cut pineapple slices.

1. Introduction

The perilous ability of *Salmonella* spp. to survive and thrive on fresh-cut fruits has been evident from many studies. Sim et al. (2013) shed light on the ability of a five-strain cocktail of *Salmonella* to grow on fresh-cut dragon fruit at 28 and 12 °C, and to survive at 4 °C. Rezende et al. (2009) showed that *S.* Enteritidis could grow on the surface of persimmon pulp at temperatures of 10, 20 and 30 °C. *S.* Enteritidis has displayed the capacity to grow on melon, watermelon and papaya pulp in the same temperature range (Penteado and Leitao, 2004). Other serotypes of *Salmonella* have also been shown to survive on fresh-cut pineapples at 4 and 12 °C for 21 and 14 days, respectively (Strawn and Danyluk, 2010).

Recent outbreaks have also made evident the susceptibility of fruits to *Salmonella* spp. In 2010, an outbreak of typhoid across two states in the United States (US) was attributed to the consumption of frozen mamey fruit pulp contaminated with *S.* Typhi (CDC, 2010). Consumption of cantaloupe infected with *S.* Typhimurium and *S.* Newport in 2012 hospitalized 94 people and resulted in the death of three (CDC, 2012a). In the same year, mangoes tainted with *S.* Braenderup affected 127 people across 15 states in the US (CDC, 2012b). A study by DeWaal and Bhuiya (2007) has attributed half of the outbreaks resulting from consumption of fresh produce at restaurants and food establishments. There is therefore, an underlying need for a preservation technology that can inactivate or inhibit the growth of *Salmonella* on fresh-cut fruits such as pineapples sold in such establishments.

The application of visible light emitting diodes (LEDs) for bacterial inactivation holds promise in this regard. Visible LEDs have an antibacterial effect due to their ability to trigger photodynamic reactions. When wavelength-specific light is concentrated on a bacterial cell, the endogenous porphyrins help in the formation of reactive oxygen species (ROS) that cause cell death (Luksiene, 2003). As opposed to UV-C light which raises safety concerns and pulsed light which uses lasers, visible LEDs are both safe and energy efficient. Blue LEDs have been proven effective against a variety of foodborne pathogens, including *S.* Typhimurium, in tryptone soya broth (TSB) (Ghate et al., 2013). It has also been shown that an

* Corresponding author. Food Science & Technology Programme, Department of Chemistry, National University of Singapore, Science Drive 4, 117543, Singapore.
E-mail address: chmyukhg@nus.edu.sg (H.-G. Yuk).

http://dx.doi.org/10.1016/j.jfoodeng.2016.10.013
0260-8774/

acidic pH and the presence of citric acid, a predominant acid in many fruits (Hawkins Watts, 2015), significantly aids the bactericidal effect (Ghate et al., 2015a, 2015b). The results of our previous studies thus encourage the application of LEDs for the preservation of fresh-cut fruits, especially those that are significantly acidic such as pineapples, apples, strawberries, mangoes and pears.

Although LEDs have been used previously for food safety applications (Buchovec et al., 2010; Luksiene and Paskeviciute, 2011), these have required the use of an exogenous photosensitizer. Given the ever-increasing consumer resistance to food additives, it is preferable to avoid the use of these exogenous photosensitizers. The current study aimed to evaluate an effectiveness of 460 nm LEDs without an exogenous photosensitizer in eliminating *Salmonella* spp. on pineapple slices at different combinations of irradiance and temperature. Subsequently, the effect of these two factors on the antibacterial action of the LEDs and the color of the slices was studied.

2. Materials and methods

2.1. Preparation of Salmonella cocktail

A cocktail of five serovars of *Salmonella enterica* – Gaminara (BAA 711), Montevideo (BAA 710), Newport (ATCC 6962), Saintpaul (ATCC 9712) and Typhimurium (ATCC 14028), was used in this study. All the cultures were obtained in their lyophilized form from the American Type Culture Collection (ATCC; Manassas, VA, USA). The cultures were revived by inoculation into 10 ml of sterile TSB (Oxoid, Basingstoke, UK) at 37 °C for 24 h. At least two transfers were performed consecutively before the bacterial cells were used for experiments. Immediately before an experiment, each strain was washed twice with 0.1% peptone water by centrifugation (4229*g* for 10 min each time) at 4 °C. The strains were then mixed in equal volumes to yield the *Salmonella* cocktail to be used for inoculation.

2.2. Inoculation on pineapple slices

Pineapples of the 'South African' variety were purchased from a local supermarket in Singapore. After washing the pineapples with water and cutting off the skin, the pineapple flesh was divided into cubes of 5 cm × 2.5 cm × 0.5 cm, with each cube weighing an average of 10 g. After diluting the *Salmonella* cocktail to 6 log CFU/ml, it was spot-inoculated onto the surface of each cube by placing five droplets of 20 μl each at different points on the surface. This resulted in a population density on the cube of approximately 4–5 log CFU/g. The cubes were then left to dry for 90 min to allow attachment of the inoculum onto the pulp. The inoculation and drying steps were performed in a biosafety cabinet (Esco Class II, Type A2, E-Series, Esco Micro Pvt. Ltd., Singapore). Subsequently, prior to illumination, the slices were wrapped skin-tight in food-grade polyethylene, which simulated the actual storage condition found in food establishments. The polyethylene was observed to be almost completely transparent to the LED light, as evidenced by the near identical irradiances measured at the surface of the radiometer detector with or without the polyethylene wrapped around it. Throughout the illumination period, only the inoculated surface of the cube was exposed to the LED light.

2.3. LED illumination assembly

LEDs with a peak wavelength of 460 nm (emission spectrum: 455–465 nm) and a power of 10 W each were procured from Shenzhen Getian Opto-electronics Co. Ltd. (Shenzhen, Guangdong, China). To dissipate the heat emanating from the LEDs and prevent consequent damage to them, each LED was thermally glued to a heat sink that was electrically connected to a cooling fan. With the help of acrylonitrile butadiene styrene (ABS) blocks, an assembly was constructed accommodating the LED at the top and the inoculated samples at the base (Ghate et al., 2013).

The light intensity was calculated as irradiance (P) at the surface of the sample, measured using a portable radiometer (UVATA, Shanghai, China). Subsequently, the dose *E* delivered after a time was determined with the help of the following equation (Maclean et al., 2009):

$$E = Pt$$

where the units of *E, P* and *t* were J/cm^2, W/cm^2 and sec, respectively.

2.4. Control of temperature and irradiance

All the assemblies were stationed inside a temperature controlled incubator (Zhicheng ZSD-A1160A, Zhicheng Analytical Instruments Manufacturing Co. Ltd., Shanghai, China). The temperature of the incubator was set at 7, 16 or 25 °C. The temperature of the illuminated samples was recorded using a Fluke 54 Thermocouple Thermometer (Everett, WA, USA) at 1-min intervals. The thermocouple tip was placed on the inoculated surface. It was observed that the temperature of LED illuminated cut-fruit surface was higher than the incubator temperature due to a heating effect produced by the light. Based on this temperature rise, the incubator temperature for the control samples was adjusted to a commensurately higher value. Meanwhile, the irradiance was controlled by changing the distance between the LEDs and the samples. The distances were set at 4.5, 3.5 and 2.5 cm to achieve irradiances of 92.0, 147.7 or 254.7 mW/cm^2 respectively on the pineapple surface. Illumination times were correspondingly adjusted to 24, 13.91 and 8.66 h to deliver the same dose of 7950 J/cm^2 to the slices.

2.5. Bacterial inactivation and enumeration

After every 1325 J/cm^2, two 10 g samples of the cut-pineapple were separately placed in separate sterile bags containing 90 ml of 0.1% peptone water and homogenized for 1 min in a blender (iUL Classic Panoramic Masticator, IUL, Barcelona, Spain). One ml of the homogenized mixture from each bag was pipetted out and diluted 10-fold as necessary to be spread-plated on brilliant green agar (Oxoid). Following incubation at 37 °C for 24 h, colonies were manually counted using a colony counter (Rocker Scientific Co. Ltd., Taipei, Taiwan). Control samples were placed in the dark and treated in the same manner as the illuminated samples post sampling. The populations of the control and the illuminated samples in log CFU/g were plotted against the dosage (J/cm^2). The difference in counts between the control and illuminated sample was also plotted on the same figure as the 'antibacterial effect' (log CFU/g).

2.6. Mathematical modeling of antibacterial effect

Five growth models were used to mathematically describe the increase of antibacterial effect. These five models were the following:

Linear model: $y = mx$, where y is the antibacterial effect in log CFU/g, x is the dose in kJ/cm^2 and m is the rate of antibacterial effect in (log CFU/g)/(kJ/cm^2).

Weibull model: This was an adaptation of the Weibull model described in Peleg (1999) and Huang (2009). As per this adaptation, $y = (x/D)^{\alpha}$, where the parameter D (log CFU/g) is the