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Antibacterial effect of light emitting diodes of visible wavelengths on selected foodborne pathogens at different illumination temperatures

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ABSTRACT

The antibacterial effect of light emitting diodes (LEDs) in the visible region (461, 521 and 642 nm) of the electromagnetic spectrum was investigated on *Escherichia coli* O157:H7, *Salmonella typhimurium*, *Listeria monocytogenes* and *Staphylococcus aureus*. The irradiances of the 461, 521 and 642 nm LEDs were 22.1, 16 and 25.4 mW/cm^2, respectively. Bacterial cultures suspended in tryptic soy broth were illuminated by 10-watt LEDs at a distance of 4.5 cm for 7.5 h at 20, 15 and 10 °C. Regardless of the bacterial strains, bacterial inactivation was observed with the range of 4.6–5.2 log CFU/ml at 10 and 15 °C after illumination with the 461 nm LED, while illumination with the 521 nm LED resulted in only 1.0–2.0 log reductions after 7.5 h. On the other hand, no antibacterial effect was observed using the 642 nm LED treatment. The photodynamic inactivation by 461 and 521 nm LEDs was found to be greater at the set temperatures of 10 and 15 °C than at 20 °C. The D-values for the four bacterial strains at 10 and 15 °C after the illumination of 461 nm LED ranged from 1.29 to 1.74 h, indicating that there was no significant difference in the susceptibility of the bacterial strains to the LED illumination between 10 and 15 °C, except for *L. monocytogenes*. Regardless of the illumination temperature, sublethal injury was observed in all bacterial strains during illumination with the 461 and the 521 nm LED and the percentage of injured cells increased as the treatment time increased. Thus, the results show that the antibacterial effect of the LEDs was highly dependent on the wavelength and the illumination temperature. This study suggests the potential of 461 and 521 nm LEDs in combination with chilling to be used as a novel food preservation technology.

1. Introduction

The preservation of raw fruits, vegetables, fish and meat products is of prime importance to the food industry. This is not only because these foods are consumed widely throughout the world as part of a staple diet, but also because they are highly prone to contamination by microorganisms due to their high water activity and rich content of nutrients. The most common preservation method for these raw foods is refrigeration or chilling; however, it does not kill microorganisms, but simply inhibits or retards their growth. Moreover, some pathogenic bacteria such as *Listeria monocytogenes* and *Yersinia enterocolitica*, and other psychrotrophic bacteria can grow at low temperatures, threatening public health and shortening the shelf life of raw foods (Walker et al., 1990; Andersen et al., 1991). Thus, there is a need for the development of another hurdle for these raw foods which will be effective in eliminating or reducing microbial contamination and be environmentally friendly without compromising on the quality of foods and public health.

A light emitting diode (LED) is a semiconductor device that emits visible light when an electric current passes through it. LEDs can emit light within a very narrow wavelength spectrum and can be considered to be of a monochromatic wavelength (Held, 2009). This is an advantage over other traditional visible light sources as they are not able to produce monochromatic wavelengths. LEDs also have several other advantages over traditional visible light sources such as lower energy consumption and high durability. The size of the LED can be made to be very small which would be flexible to fit most designs and be easily implemented into existing systems without requiring special disposal methods at the end of its use (Mori et al., 2007; Hamamoto et al., 2007). For these reasons, LED technology has been widely applied to not only optics and electronics, but also agriculture and medicine.

LEDs bring about an antibacterial effect through a phenomenon known as photodynamic inactivation. Some intracellular molecules known as photosensitizers can produce reactive oxygen species (ROS) once they absorb light, which react with cellular constituents such as lipids, proteins and the DNA to bring about a cytotoxic effect (Luksiene, 2009). Based on this mechanism, the use of LEDs has recently received increased attention and its potential for clinical applications has been

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investigated as a novel technology for bacterial inactivation. Maclean et al. (2010) reported that a 405 nm LED system inactivated methicillin resistant *Staphylococcus aureus* (MRSA) in clinical environments such as the vascular ward, the burns unit and the intensive care unit, and observed reductions between 56 and 90% in the bacterial concentration on frequently touched contact surfaces. The studies conducted by Guffey and Wilborn (2006a; 2006b) tested the effect of LEDs of 405 and 470 nm on the populations of *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Propionibacterium acnes*, and a wavelength dependent inactivation pattern was observed.

Although there have been some studies on using LEDs to produce a 405 nm wavelength for inactivating the bacteria aforementioned, little information is available on the antibacterial effect of other wavelengths. Thus, the objective of this study was to determine if wavelengths above the 405 nm region such as 461, 521 and 642 nm have an antibacterial effect on four common foodborne pathogens, *Escherichia coli* O157:H7, *L. monocytogenes*, *Salmonella typhimurium* and *S. aureus*. To see if temperature affects the inactivation of pathogens by LEDs, three different temperatures were tested during the LED illumination. Testing the effect of temperature on the inactivation would give insights into the potential for using chilling in combination with LEDs as a novel technology for food preservation. The sublethal injury of the surviving cells after the LED illumination was also evaluated in this study.

2. Materials and methods

2.1. Bacterial cultures

The bacterial strains used in this study were *E. coli* O157:H7 (EDL 933), *S. typhimurium* (ATCC 14028), *L. monocytogenes* (BAA-679) and *S. aureus* (ATCC 6538). *E. coli* O157:H7 was obtained from Dr. Henry Mok at the Department of Biological Sciences at the National University of Singapore. The other bacterial strains were purchased from the American Type Culture Collection (ATCC; Manassas, Virginia, USA). To obtain working cultures from the frozen stock, vials were thawed at room temperature, and a 0.1-ml portion of the thawed culture was transferred into a test tube containing 10 ml of sterile tryptic soy broth (TSB; Oxoid, Basingstoke, UK) followed by incubation at 37 °C under static conditions for 24 h. Two consecutive 24-h transfers were made before the culture was used for further experiments, and a daily transfer was performed to maintain cell viability prior to use.

2.2. Characterization of light emitting diodes (LEDs)

High intensity LEDs (10 W) of three different colors (blue, green and red) were purchased from a local lighting shop specializing in LEDs (Angel LED Lighting, Singapore). Emission spectra were measured using the Oriel spectrometer, MS275 and Oriel enhanced UV photodiode (Oriel Instruments, Connecticut, USA). The irradiance of each LED was measured using a Thorlabs laser power and energy meter console (PM100D) attached to a photodiode power sensor (S130C) (Newton, New Jersey, USA). The irradiance measurement equipment was used in order to determine the light intensity to which the bacterial suspension was being subjected during the exposure period. The dosage received by each bacterial sample was calculated using the 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. LED illumination system

In order to protect each LED from excessive current, a resistance of 5 Ω was used in the circuit, achieved by connecting two 10 Ω resistors in parallel with each other. The LED was attached to a heat sink with a cooling fan to dissipate the heat generated. Each LED system was set up in an acrylonitrile butadiene styrene (ABS) housing for the illumination to prevent the entry of external light. A 10-ml aliquot of the bacterial suspension (1.2 cm depth) in a sterile glass Petri dish (60 mm diameter) was placed directly below the LED system at a distance of 4.5 cm from it (Fig. 1). The temperature of the bacterial suspension during the illumination was also monitored using the Fluke 54 thermocouple thermometer (Everett, Washington, USA) at one minute intervals.

2.4. Bacterial inactivation by LED illumination

The bacterial cultures were serially diluted to an initial population of approximately 10^6 CFU/ml using 0.1% peptone water, with the final transfer taking place in TSB. Ten milliliters of the bacterial suspension in a glass Petri dish was placed in the LED housing chamber as described above. The entire assembly was placed inside a temperature controlled incubator to ensure that the sample was exposed to a constant temperature during the LED illumination. The glass door of the incubator was covered using black paper throughout the experiment to minimize the effect of environmental light such as sunlight and domestic light. An aliquot of 0.1 ml was withdrawn every 1.5 h until 7.5 h, serially diluted with 0.1% peptone water and pour plated onto tryptic soy agar (TSA; Oxoid). Plates were incubated at 37 °C for 24–48 h, followed by manual counting of the colonies. The populations of the bacterial cells were expressed in log CFU/ml.

2.5. Sublethal injury test

For the sublethal injury test, the aliquot withdrawn from the Petri dish at each interval was plated onto TSA supplemented with sodium chloride (NaCl), after appropriate dilution. After incubation for 24–48 h at 37 °C, the colonies were enumerated and the sub-lethal injury was calculated using the following equation:

$$\text{Sublethal injury } (\%) = \left[\left(1 - \frac{\text{Colonies on TSA} + \text{NaCl}}{\text{Colonies on TSA}}\right) \times 100\right].$$

The concentration of NaCl to be used for each strain was determined as the maximum concentration that did not affect the growth of healthy untreated cells (Ukuku et al., 2008).

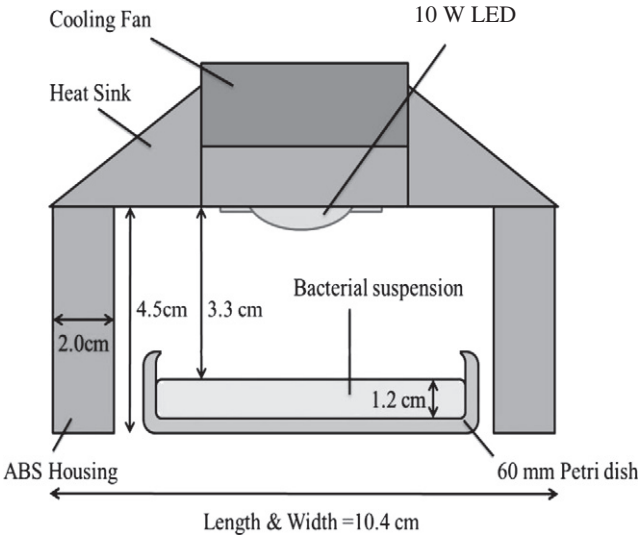

Fig. 1. The cross sectional diagram of the LED illumination system.