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Effect of light emitting diode treatment on inactivation of *Escherichia coli* in milk

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ABSTRACT

The effect of blue monochromatic light emitting diode (LED) light treatment on inactivation of *Escherichia coli* ATCC 25922 in milk was investigated in the present study. Wave length (405 nm–460 nm), temperature (5 °C–15 °C), and treatment time (0 min–90 min) were considered as the independent variables and log reduction of *E. coli* and overall colour change of the treated milk were measured as the dependent variables. It was observed that the maximum microbial reduction was achieved at higher temperatures and lower wavelengths. The optimized values of wavelength, temperature, and treatment time corresponding to log reduction value of five (pasteurization), and minimum overall colour change were 406 nm, 13.8 °C, and 37.83 min, respectively. There was no significant difference ($P > 0.05$) in physico-chemical properties of the LED treated milk in comparison to untreated samples. The shelf-lives of the LED treated milk samples, packaged aseptically in low density polyethylene (LDPE) pouches and stored at 37 °C and 4 °C were 19 h and 9 days, respectively. The data obtained in the research are expected to form a platform for development of a continuous LED based pasteurization system.

1. Introduction

Food safety and quality management is witnessing increased attention due to consumers' awareness towards safe, minimally processed, fresh, additive or preservative free, convenient, and balanced diets (Ali, 2013). Different non-thermal technologies including high pressure processing, pulsed electric field, ozone, irradiation, and dense phase carbon dioxide have proven to be more efficient and beneficial in this regard (Cullen, Tiwari, & Vasilis, 2012; Da-Wen Sun, 2005; Prokopov & Tanchev, 2007). Exposure of light has shown to inactivate microorganisms to a large extent (Rahman, 2007; Oms-Oliu, Martín-Belloso, Soliva-Fortuny, 2010). Recent studies have investigated the potential of microbial inactivation by ultraviolet light in the range of 240–280 nm wavelength which damages the DNA of the microbes (Forney & Pierson, 2004; Guerrero-Beltrn & Barbosa-Canovas, 2004; Kim, Silva, & Chen, 2002; Warriner, Kolstad, Rumsby, & Waites, 2002; Wallner-Pendleton, Sumner, Froning, & Stetson, 1994; Wong, Linton, & Gerrard, 1998).

LED is a semiconductor that emits visible light when an electric current passes through it (Schubert, 2003). LEDs can emit light within a very narrow wavelength spectrum and can be considered as a monochromatic wavelength (Held, 2009). This is an advantage over traditional visible light sources which are not able to produce monochromatic wavelengths. LEDs have advantages like low energy consumption and high durability (D'Souza, Yuk, Khoo, & Zhou, 2015). The size of a LED can be made very small and can be easily implemented into existing systems without requiring any special disposal technique at the end of its use (Hamamoto et al., 2007; Mori et al., 2007). Now, LED has been widely applied to optics, electronics and medicine.

LED lights in the range 400–480 nm (blue) induce phototoxic effect on microbial cells. The photosensitization of bacteria is caused due to the photosensitizing metabolites present in the bacterial cells (Durantini, 2006; Luksiene, 2003). Specific wavelengths of LEDs bring about an antibacterial effect through a phenomenon known as photodynamic inactivation (PDI) in microorganisms. This is due to the presence of endogenous

Abbreviations: ml, milli litre; AOAC, Association of Official Analytical Chemists; IS, Indian Standards; BIS, Bureau of Indian Standards; nm, nano meter (10^{-9} m); W, Watt; mA, milli ampere; mm, millimeter; h, hour; min, minute.

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photosensitizers such as porphyrins, flavins, cytochromes, and NADH which absorb light and get excited. Absorption of photons results in excitation of endogenous porphyrins, resulting in the release of reactive oxygen species (ROSs) which degrade cellular components such as lipids, proteins and DNA to bring about a cytotoxic effect. The ROSs which includes hydroxyl ions, peroxides, superoxide, and singlet oxygen are the main tool of eradication of the bacterial cell (Bumah, Masson-Meyers, Cashin, & Enwemeka, 2013; Guffey & Wilborn, 2006a). The photo-eradicating effect of blue light on bacterial cells causes inactivation by chemical modification and cleavage of nucleic acids present in the microbial cell. Concurrently, it has a great impact on proteins, cell membranes, and other cellular materials. Based on this mechanism, use of LEDs has recently received increased attention and its potential especially in clinical and food applications has been investigated as a novel technology for bacterial inactivation (Wu et al., 2007; ANSES, 2013; Guffey & Wilborn, 2006b; Mori et al., 2007; Maclean, Scott, MacGregor, & Anderson, 2009; Bumah et al., 2013).

Milk is highly nutritious. However, it is extremely perishable and prone to contamination by various pathogenic and spoilage microorganisms. The most common microorganisms for contamination of milk are *Escherichia coli O157:H7*, *Staphylococcus aureus*, *Streptococcus agalactiae*, *Str. Dysgalactiae* and *Str. Uberis* (Walstra, Geurts, Noomen, Jellema, & Van Boekel, 2008). Microorganisms principally involved in spoilage of milk are psychotropic in nature. To ensure the safety of milk and milk products, the dairy industries subject milk to thermal pasteurization. Some psychrotrophs can survive pasteurization temperatures and grow at refrigeration temperatures, which can cause spoilage (Burton, 1988; Robinso, 2002). Also, thermal treatment can adversely affect the organoleptic properties and nutritional values of milk. Consumers' demand for milk with desirable sensory and nutritional quality has encouraged researchers to focus on non-thermal techniques for pasteurization of milk. Therefore, it is necessary to provide appropriate treatment in order to inactivate these spoilage and pathogenic microorganisms in milk (Mansel, 2010; Rojek, Hill, & Griffiths, 1995).

In food processing operations, temperature produces the most detrimental effect in foods. High or improper temperature develops cooked flavor and taste, results improper inactivation of enzymes and microorganisms, and creates hot and cold spots in food products (Cullen et al., 2012; Da-Wen Sun, 2005). However, growing consumers demand for microbiologically safe, nutritious, minimally processed, additive/chemical free, and convenient foods has insisted to develop non-thermal technologies in food processing. In this regard, the present paper discusses the potential of LED, a novel non-thermal technology, for application in the dairy industry. Although Aponiene and Luksiene (2015), Maclean et al. (2009), Dai et al. (2012) and Kim et al. (2013) have evaluated the antimicrobial efficacy of LED based monochromatic light on foodborne pathogens, it is the first work aiming at microbial inactivation in a food matrix. The specific objective of the research is to evaluate the effect of blue LED light (405 nm–460 nm) on inactivation of *E. coli* ATCC 25922, a surrogate of pathogenic *E. coli* O157:H7 in milk and to determine the shelf-life of the LED treated milk based on a qualitative assessment.

2. Materials and methods

2.1. Raw material

UHT sterilized skim milk (<0.5% fat) of 500 ml pouches, were procured from the local supermarket in Thanjavur, India. The milk was stored at 4±1 °C before use. Composite sampling method was used to collect samples for treatment from different milk pouches.

2.2. Analysis of milk quality

The milk was analyzed for its quality characteristics before and after LED treatment. Moisture content was measured by a digital moisture analyzer (Mettler LJ16; India). Fat content was determined by Gerber method (IS: 1224, 1997). Carbohydrate was measured by Lane and Eynon method (Ranganna, 2001). Protein content was analyzed using standard Kjeldahl method as prescribed in AOAC (1997). Total nitrogen percentage in milk was calculated by using Eq. (1). Protein content was estimated by multiplying total percent of nitrogen by 6.38 as nitrogen to protein conversion factor (BIS, 1981). Ash content was determined as per IS: 5162 (1980). Titrable acidity (% lactic acid) was determined by Lane-Eynon method as described in BIS (1981). pH was measured by using a digital pH meter (Systronic, India). Viscosity of milk was measured using a rotational Brookefield viscometer (Brookfield Engineering Laboratories, Inc., USA). The color values (L, a, b) were measured using a Hunter Lab Colorimeter (Color Quest XE, Hunter Lab, USA).

$$\%\,\text{Nitrogen} = \frac{(\text{volume of titre (acid)} - \text{volume of blank}) * \text{Normality of acid} * 89.32}{\text{weight of sample in grams}} \quad (1)$$

2.3. Experimental set-up

A batch type LED set-up was developed in the present study. Based on the literature, the wavelength of LED lights was selected between 405 nm and 460 nm, since the range has the maximum bacterial effect on food borne pathogens (Guffey & Wilborn, 2006a, 2006b). The LED lights of three different wavelengths i.e., 405, 433, and 460 nm were manufactured by M/s. E-Mac Opto Electronics Pvt. Ltd, Mumbai, India based on the requirement for this research. All lights were of 10 W and 50 mA. A sample holder of 125 × 115 × 40 mm (length x breadth x depth) was fabricated with food grade stainless steel (SS-304) of 2 mm thickness. A line diagram of the set-up is shown in Fig. 2. The heat sink was attached at the top of the light source for removal of any heat rise due to LED lights. The inner side of the sample holder was spray coated with white paint in order to increase its reflectivity. The thickness of the sample was 7 mm. The distance between the sample and the light source was 30 mm. A K-type thermocouple (Model no: KMTIN-040) was used to record the temperatures in the study. Three thermocouples randomly positioned in the center and at the edges were used for monitoring the temperature variation due to LED light. Milk temperature was recorded once in every 5s using a Data Logger (DT 800 Model, Thermo Fisher Scientific, Mumbai).

2.4. Experimental design

In the study, wavelength (X_1), temperature (X_2), and treatment time (X_3) were considered as independent variables (X_i). Based on the literature studies, the ranges (minimum and maximum) of X_1,