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Inactivation efficacy of 405 nm light emitting diodes (LEDs) on *Salmonella* Enteritidis at different illumination temperatures

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Abstract

Salmonella Enteritidis is the major cause of foodborne salmonellosis affecting human health. The light emitting diodes (LEDs) is a novel approach to inactivate of the foodborne pathogens. The aim of this study was to determine the antibacterial effect of 405 nm LEDs illumination on *S.* Enteritidis and *S.* Enteritidis PT4. The irradiance of the 405 nm LEDs was 27.7 mW/cm^2. Bacterial cultures suspended in tryptic soy broth were illuminated by 10-watt LEDs at a distance of 4.5 cm for 24 hours at 4 °C, 25 °C and 37 °C. Approximately 7-log reduction in colony forming unit (CFU) counts of both *S.* Enteritidis and *S.* Enteritidis PT4 at each temperature were observed following exposure after 7.5 hours to the LEDs, concluding that temperature did not affect the inactivation of the bacteria. The decimal reduction times (D-values) for the serotypes ranged from 55.78 to 67.88 min at 4, 25 and 37 °C after 405 nm LEDs illumination. No significant difference in D-values was observed among both the serotypes and temperatures, except for *S.* Enteritidis which had lower D-value at 4 °C. The LEDs technology has shown antibacterial efficacy and can be implemented in the food processing for reducing *S.* Enteritidis.

Keywords: LEDs; photodynamic inactivation; *Salmonella* Enteritidis.

Practical Application: Determining the antibacterial activities of 405 nm LEDs with various application times and temperatures on *Salmonella* Enteritidis and *Salmonella* Enteritidis PT4.

1 Introduction

Foodborne pathogens mostly affect children, pregnant women, babies, elderly and susceptible individuals, causing various types of diseases and deaths (Whitney et al., 2015; Wei et al., 2019). *Salmonella* spp. are one of the major foodborne pathogenic bacteria all over the world and have a lot of serotypes which lead to many problems in human and domestic animals (Sariçam & Müştak, 2018; Kuang et al., 2015; Cunha-Neto et al., 2020; Schuh et al., 2020). *Salmonella* Enteritidis is one of the most frequently reported *Salmonella* serotypes from foods of animal origin, especially shelled eggs and poultry (Wuyts et al., 2015; Fardsanei et al., 2017). *Salmonella* infection is generally treated with antibiotics and the antibiotic is proving less effective due to the emergence of food-borne human infections caused by multiple-antibiotic resistant strains of *S.* Enteritidis (e.g., *S.* Enteritidis PT4). The occurrence of *Salmonella* resistant to antibiotics is a major threat to global health care and security, which has accelerated by the intensive use of antibiotics for growth promoters and feed enhancers in animals as well as for treatments of human and animal diseases (Hur et al., 2012; Nathan & Cars, 2014; Karagöz et al., 2021).

New strategies are needed to overcome the antibacterial resistance and to slow down its emergence (Delorme et al., 2020). Light emitting diodes (LEDs) technologies have become increasingly feasible and advantageous in potential applications of the food industry due to its low radiant heat emissions, long life expectancy, flexibility and mechanical robustness (D'Souza et al., 2015). Photodynamic inactivation (PDI), based on the use of photosensitizers activated by an appropriate wavelength light, utilizes the photosensitizer-mediated and light-induced overproduction of reactive oxygen species (ROS) to inactivate the foodborne and food spoilage bacteria (Penha et al., 2017; Hasenleitner & Plaetzer, 2019). A light source of suitable wavelength, photosensitizer and oxygen are the essential components for the PDI (Ghate et al., 2015). Compared to conventional antimicrobials, PDI kills the target microorganisms irrespective of their resistance to antimicrobials without occurring resistance and any toxic substances (Jiang et al., 2014). In previous studies, the efficacy of light with a wavelength range of 400 to 450 nm against microorganisms with and without adding exogenous photosensitizer has been reported (Murdoch et al., 2012; Kumar et al., 2015; Liang et al., 2015). Porphyrins, present in the bacterial cell walls, act as endogenous photosensitizers within the bacterial cells (Nitzan & Kauffman, 1999). Porphyrins absorb light energy and enter an excited energy state. The excited porphyrin may form light-excited ROS by reacting directly with molecular oxygen

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(Afonso et al., 1999). The ROS is responsible for cytotoxic activity and bacterial cell death (Luksiene, 2003). In studies on LED light wavelength for bacterial inactivation, it has been reported that 405 nm LEDs proved more effective (Guffey & Wilborn, 2006; Murdoch et al., 2012). The purpose of the present study was to investigate the antibacterial activity of 405 nm LEDs application on *S.* Enteritidis and *S.* Enteritidis PT4 at different temperatures and incubation times.

2 Material and methods

2.1 Bacterial strains

American Type Culture Collection (ATCC) standard *S.* Enteritidis (ATCC 13076) and National Collection of Type Cultures Standard *S.* Enteritidis PT4 (NCTC 13349) were used in this study.

2.2 Light Emitting Diodes (LEDs) illumination system

LED light application was conducted by a LEDs system designed for this study with some modifications of the system developed by Ghate et al. (2013). Construction components of the system were produced by using a 3D printer (Rigid3D Zero2) from acrylonitrile butadiene styrene (ABS) thermoplastic material. The system consists of a LEDs light source (10 W 405 nm, Shenzen, China), a heat sink and cooling fan assembly to avoid overheating (Figure 1). The circuit was connected to a 5 Ω resistor to avoid over-currents.

2.3 Measuring applied light dose

Light dose measurements for the prepared LEDs system were carried out with a photovoltaic power meter (Thorlabs PM100D/S130C sensor, USA) with parameters in Table 1. The radiant fluency occurring according to the experimental groups was calculated according to the following calculation method (Aurum & Nguyen, 2019). In the current study, 405 nm LEDs light dose was measured as 27.7 mW/cm^2.

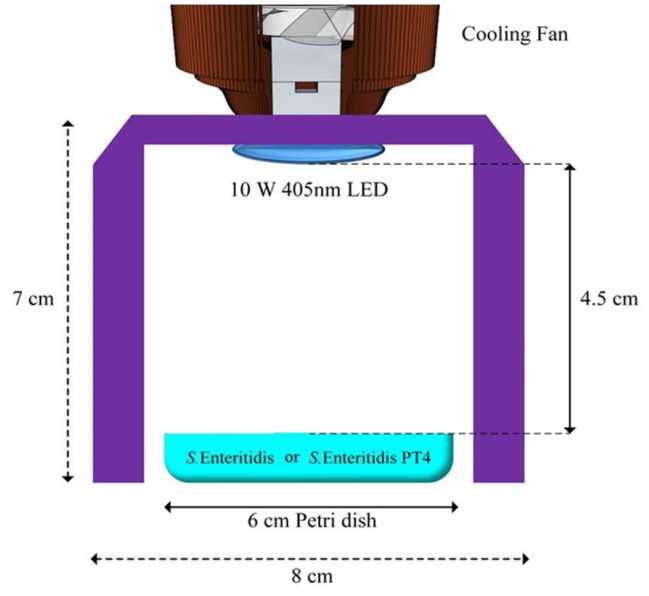

Figure 1. The cross-sectional diagram of the LED illumination system.

2.4 Bacterial inactivation via LED illumination

The bacterial inactivation effects of the system for each bacterium were tested at different temperatures (4, 25 and 37 °C) and incubation times (1.5, 3, 7.5 and 24 hours). *S.* Enteritidis and *S.* Enteritidis PT4 were enriched in 10 mL sterile tryptic soy broth (TSB, Oxoid, CM0876) at 37 °C for 24 hours. The bacterial density was adjusted spectrophotometrically to 0.5 McFarland with 0.1% peptone water (PS, Oxoid, CM0009) and 1 mL of the bacteria culture was transferred into a tube containing 9 mL TSB. Ten mL of bacterial suspension was transferred into sterile petri dish and the dish was placed in LEDs illumination system. The LEDs illumination system was started and transferred to an incubator (Nuve, ES120). Each bacterial suspension was illuminated with 405 nm LEDs at 4, 25 and 37 °C and 0.1 mL of sample was taken at 1.5, 3, 7.5 and 24 hours of the incubation periods. Serial dilutions of the samples were prepared in 0.1% PS, and cultivated on tryptone soy agar (TSA) and incubated for 24-48 h at 37 °C. After incubation, the population of bacterial cells were given as CFU/mL (colony-forming units per milliliter) (Ghate et al., 2013). The study was carried out in triplicate.

2.5 Statistical analysis

The results are documented as mean ± standard deviations. The mean values were obtained from triplicate trials. Significant differences in the results were calculated at the 95% confidence interval by using a one-way analysis of variance ANOVA (Version 21.0; SPSS Inc., IBM Corporation, Armonk, New York, USA). The decimal reduction times (D-values) were calculated from a plot of the survived *S.* Enteritidis and *S.* Enteritidis PT4 (log10) versus time. The D-values were determined by using survival curve at each temperature by taking the negative inverse of the relevant s values (slope) of each bacterium using Microsoft Excel (Microsoft, USA).

3 Results and discussion

Foodborne pathogens are causing a great number of diseases which is a major international problem. Food preservation has been essential activity, serving the purpose of prolonging the shelf life of products, preventing deterioration and foodborne illness and ensuring a safer food supply. Chemical preservatives, heat treatments such as pasteurization and physical methods such as

Table 1. Program parameters during measurement.

Resolution	Medium
Wavelength	405 nm
Bandwith	Low
Autorange	On
Ranges	100 mW/cm^2
Shaper	Circular
Diameter	9.50 mm
Profile	Flat

dehydration and irradiation are used to ensure microbial safety in food industry (Gonzalez & Barrett, 2010; Guimarães et al., 2019; Torres et al., 2020). PDI of pathogens using a LEDs-based illumination is a novel approach to inactivate foodborne pathogens and antibacterial effect of LEDs varies depending upon the wavelength and the bacteria species (Ghate et al., 2013; Alves et al., 2015). Previous studies have shown that LEDs between 405-420 nm exhibited high antibacterial activity against a wide range of bacteria including *Salmonella* spp., *Bacillus cereus*, *Listeria monocytogenes*, and *Staphylococcus aureus*, methicillin resistant *S. aureus* (MRSA), *S. epidermidis*, *Clostridium perfringens*, *Streptococcus pyogenes*, *Acinetobacter baumannii*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Escherichia coli* by damaging physically to bacterial cell membrane without affecting exposed mammalian cells (MacLean et al., 2009, 2014; Kim et al., 2015; Du et al., 2020). Since endogenous bacterial photosensitizer such as porphyrins have highest wavelength sensitivity at 405 nm, the bacteria-inoculated dishes were illuminated under 405 nm LEDs (27.7 mW/cm^2) for 24 hours at 4, 25 and 37 °C in this study. Figure 2 presents the inactivation curve of illumination with the 405 nm on *S.* Enteritidis and *S.* Enteritidis PT4 during 24 hours. The bacterial populations in untreated control groups increased approximately 2.0 to 2.5 log after 7.5-24 hours of incubation at 25 and 37 °C (Table 2). After 24 hours of incubation at 4 °C, while no significant bacterial population change in *S.* Enteritidis PT4 occurred in the control group, a small reduction (approx. 0.5 log) in population of *S.* Enteritidis was observed. 405 nm LEDs illumination achieved complete bacterial inactivation in both bacteria after 7.5 hours of incubation at all temperature parameters (Table 2). Bacterial population declines after 24 hours of incubation were similar to those of 7.5 hours of incubation. Under the illumination of the 405 nm LEDs, the populations of *S.* Enteritidis decreased by 3.44, 1.05 and 4.37 log CFU/mL, respectively and the decreases in *S.* Enteritidis PT4 were 1.19, 3.73 and 2.60 log CFU/mL at 4, 25 and 37 °C, respectively after 3 hours. Also, 405 nm LEDs illumination also resulted in significant reductions in both bacterial populations after 1.5 at all temperature parameters, except for *S.* Enteritidis at 4 °C. After 3 hours of illumination, a significant reduction in CFU counts of *S.* Enteritidis and *S.* Enteritidis PT4 was observed at 37 °C and at 25 and 37 °C, respectively (Table 3).

Several studies reported that 405 nm LEDs illuminations are more effective on *Salmonella* spp. at low temperatures. Josewin et al. (2018) showed that LEDs illumination with 405 nm for 48 hours

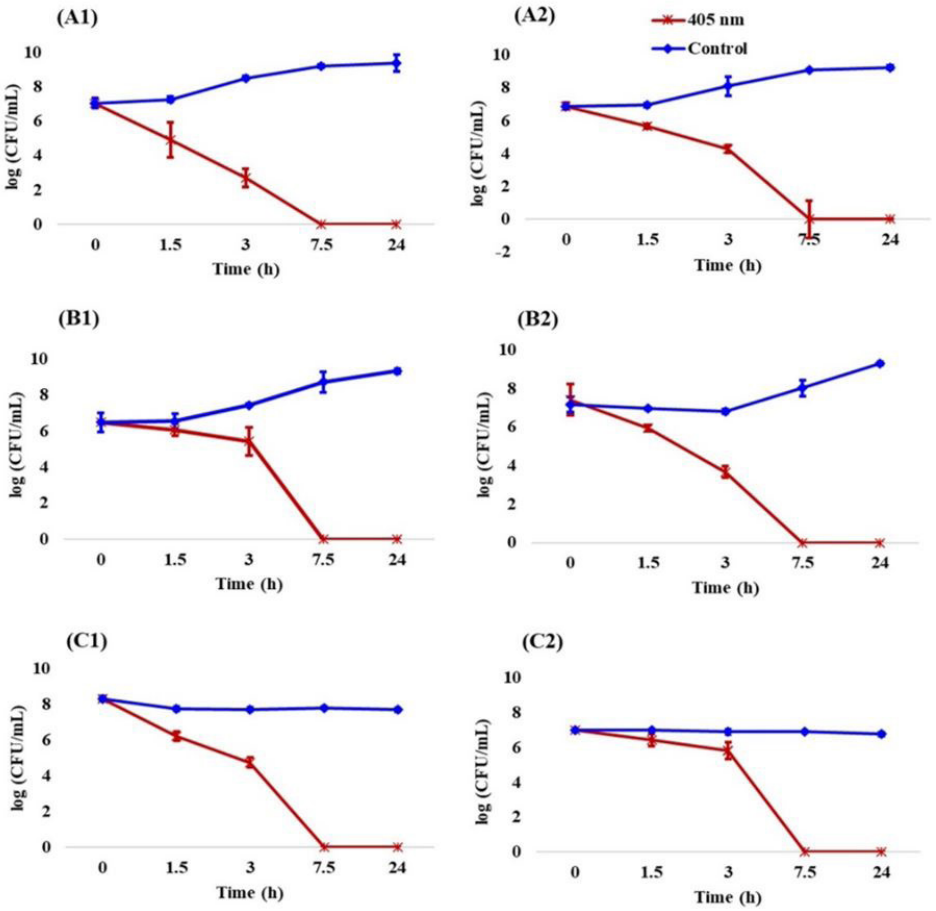

Figure 2. Inactivation curves for the *Salmonella* Enteritidis (A1, B1 and C1) and *Salmonella* Enteritidis PT4 (A2, B2 and B3) at 4, 25 and 37°C, respectively.

Table 2. Comparison of photoinactivation results for *S.* Enteritidis and *S.* Enteritidis PT4 by 405 nm LEDs illumination at each temperature for 24 hours.

	Temp.	Treatment	Initial	1.5 h	3 h	7.5 h	24 h	P
			x ± SD	x ± SD	x ± SD	x ± SD	x ± SD	
S. Enteritidis	4 °C	Control	8.19 ± 0.14^{a}	7.77 ± 0.12^{bx}	7.72 ± 0.10^{bx}	7.81 ± 0.06^{bx}	7.69 ± 0.09^{bx}	*
		LED	8.19 ± 0.14^{a}	6.22 ± 0.27^{by}	4.75 ± 0.29^{cy}	ND^{dy}	ND^{dy}	*
		P		*	*	*	*	
	25 °C	Control	6.48 ± 0.52^{c}	6.55 ± 0.42^{cx}	7.44 ± 0.05^{bx}	8.71 ± 0.59^{ax}	9.33 ± 0.14ax	*
		LED	6.48 ± 0.52^{a}	6.06 ± 0.33^{aby}	5.43 ± 0.77^{by}	ND^{cy}	ND^{cy}	*
		P		*	*	*	*	
	37 °C	Control	7.06 ± 0,28^{c}	7.28 ± 0.15^{cx}	8.53 ± 0.12^{bx}	9.23 ± 0.09^{ax}	9.40 ± 0.48^{ax}	*
		LED	7.06 ± 0,28^{a}	4.92 ± 1.00^{by}	2.69 ± 0,53^{cy}	ND^{dy}	ND^{dy}	*
		P		*	*	*	*	
S. Enteritidis PT4	4 °C	Control	7.01 ± 0.01	7.02 ± 0.08^{x}	6.92 ± 0.20^{x}	6.90 ± 0.09^{x}	6.77 ± 0.15^{x}	
		LED	7.01 ± 0.01^{a}	6.42 ± 0,35^{by}	5.82 ± 0,47^{cy}	ND^{dy}	ND^{dy}	*
		P		*	*	*	*	
	25 °C	Control	7.18 ± 0.40^{c}	6.96 ± 0.05^{cx}	6.82 ± 0.12^{cx}	8.03 ± 0.40^{bx}	9.29 ± 0.07^{ax}	*
		LED	7.41 ± 0.81^{a}	5.94 ± 0.17^{by}	3.68 ± 0.31^{cy}	ND^{dy}	ND^{dy}	*
		P		*	*	*	*	
	37 °C	Control	6.89 ± 0.13^{c}	6.95 ± 0.10^{cx}	8.11 ± 0.58^{bx}	9.10 ± 0.04^{ax}	9.25 ± 0.13^{ax}	*
		LED	6.89 ± 0.13^{a}	5.66 ± 0.21^{by}	4.29 ± 1.15^{cy}	ND^{dy}	ND^{dy}	*
		P		*	*	*	*	

Data (log CFU/mL) was expressed as mean ± standard deviation (x ± SD). Different superscripts within a column (x,y) and a row (a,b,c) indicate that the means are significantly (*P<0.05) different from each other. ND: Non detected (<2 log CFU/mL).

Table 3. Comparison of photoinactivation results in the LEDs illumination among temperatures for each bacterium for 24 hours.

	Temp.	1.5 h	3 h	7.5 h	24 h
		x ± SD	x ± SD	x ± SD	x ± SD
S. Enteritidis	4 °C	6.06 ± 0.33	5.43 ± 0.77^{x}	ND	ND
	25° C	6.22 ± 0.27	4.75 ± 0.29^{x}	ND	ND
	37 °C	4.92 ± 1.00	2.69 ± 0.53^{y}	ND	ND
	P		*	*	*
S. Enteritidis PT4	4 °C	6.42 ± 0.35^{x}	5.82 ± 0.47^{x}	ND	ND
	25 °C	5.94 ± 0.17^{xy}	3.68 ± 0.31^{y}	ND	ND
	37 °C	5.66 ± 0.21^{y}	4.29 ± 1.15^{y}	ND	ND
	P	*	*		

Data (log CFU/mL) was expressed as mean ± standard deviation (x ± SD). Different superscripts within a column (x,y,z) indicate that the means are significantly (*P<0.05) different from each other. ND: Non detected (<2 log CFU/mL).

reduced the population of *Salmonella* spp. (*S.* Typhimurium ATCC 14028, *S.* Newport ATCC 6962 and *S.* Poona BAA 1673) by 2.3 log CFU/cm^2 at 4 °C, while a significant reduction was observed until 8 hours of illumination after which it grew significantly slower to non-illuminated controls at 20 °C. Kim et al. (2017) demonstrated that while 405 nm LEDs illumination significantly reduced *Salmonella* spp. (*S.* Agona, *S.* Newport, *S.* Saintpaul, and *S.* Typhimurium) population on the surface of fresh-cut papaya by 0.3-1.3 log CFU/cm^2 at 4 °C for 36-48 hours (1.3-1.7 kJ/cm^2), it delayed the growth of *Salmonella* spp. at 20 °C for 24 hours (Kim et al., 2017). A previous study reported that *S.* Typhimurium was more susceptible at lower temperatures (10 and 15 °C) than at 20 °C (Ghate et al., 2013). Contrary, a paper reported that a significant inactivation of *S.* Typhimurium with 405 nm LEDs was achieved (0.6 log) only at 25 °C and not at 4 or 10 °C, concluding that higher temperature resulted in enhanced inactivation (Kumar et al., 2015). Interestingly, we did not observe a similar trend for the temperature-dependent inactivation of *S.* Enteritidis and *S.* Enteritidis PT4 in 405 nm LEDs illumination.

D-values for the serotypes ranged from 55.78 to 67.88 min at 4, 25 and 37 °C after 405 nm LED illumination in this study (Table 4). No significant difference in D-values was observed among both the serotypes and temperatures (at 4, 25 and 37 °C), except for *S.* Enteritidis which had lower D-value at 4 °C, concluding that there was no significant difference in the susceptibility of the serotypes to the LEDs illumination between 25 and 37 °C. Similarly, Ghate et al. (2013) reported that there was no significant difference in D-values (1.40 to 1.44 h) of *S.* Typhimurium between 10 and 15 °C.

When the bacteria were exposed to LEDs illumination with 405 nm, they behaved in a similar manner at 4, 25 and 37 °C in this study. Approximately 7-log reduction in CFU counts

Table 4. Comparison of the decimal reduction times of the bacteria using 405 nm LEDs illumination at 4, 25 and 37 °C.

Pathogens	D value			
	4 °C	25 °C	37 °C	*P*
S. Enteritidis	55.78 ± 1.19^{by}	66.54 ± 4.01^{a}	67.88 ± 3.98^{a}	*
S. Enteritidis PT4	61.62 ± 1.17^{x}	60.92 ± 4.11	64.96 ± 1.44	
P	*			

D values were expressed as x ± SD (minutes). Different superscripts within a column (x,y) and a row (a,b) indicate that the means are significantly (*P<0.05) different from each other.

observed following exposure between 7.5 and 24 hours to the LEDs with an irradiance of 27.7 mW/cm^2. According to the inactivation results of both *S.* Enteritidis and *S.* Enteritidis PT4 at each temperature, the inactivation trend for these bacteria was similar, resulting that temperature did not affect the inactivation of the bacteria. Also, no difference was observed between resistant (PT4) and non-resistant bacteria in LEDs inactivation, indicating that the bacteria-inactivating effect of the LEDs is independent of bacterial resistance. The high level of photoinactivation of *S.* Enteritidis is likely due to the presence of high levels of endogenous porphyrins which are bacterial photosensitizer and have highest wavelength sensitivity at 405 nm.

PDI is a new and important microbial inactivation application effective on bacteria, yeast-mold, virus and parasites (Alves et al., 2015). In addition to the strong antimicrobial effects of the 405 nm LEDs light wavelength, it can be used in microbial inactivation systems since it has no harmful effects compared to UV light (MacLean et al., 2014). Various studies conducted to determine the effects of LEDs illumination on inactivation of foodborne pathogens showed that the potential applications on hot dogs and milk for photoinactivations of *L. monocytogenes* and *E. coli*, respectively, by visible light (Meurer & Lancaster, 2016; Srimagal et al., 2016). Similarly, Ghate et al. (2019) has been reported that LEDs application to paperboard cartons reduced the risk of *L.* monocytogenes under refrigeration. In the present study, we have observed that the LEDs illumination with 405 nm causes strong bacterial inactivation under refrigerator condition (at 4 °C). The LEDs illumination may be developed for use in the control of *S.* Enteritidis under this condition.

4 Conclusion

This study showed that the LEDs illumination system caused a significant photoinactivation of *S.* Enteritidis in a short time at three different temperatures. It may be further studied on photo-inactivation of *S.* Enteritidis in foods such as poultry meat, egg and egg products.

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