Food Microbiology 48 (2015) 49–57

Contents lists available at ScienceDirect

Food Microbiology

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

Enhancing the antibacterial effect of 461 and 521 nm light emitting diodes on selected foodborne pathogens in trypticase soy broth by acidic and alkaline pH conditions

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ARTICLE INFO

Article history:
Received 23 May 2014
Received in revised form
26 September 2014
Accepted 24 October 2014
Available online 24 December 2014

Keywords:
Light emitting diode
pH
Antibacterial effect
Foodborne pathogens

ABSTRACT

Light emitting diodes (LEDs) with their antibacterial effect present a novel method for food preservation. This effect may be influenced by environmental conditions such as the pH of the food contaminated by the pathogen. Thus, it is necessary to investigate the influence of pH on the antibacterial effect of LEDs before their application to real food matrices. *Escherichia coli* O157:H7, *Salmonella* Typhimurium and *Listeria monocytogenes* in trypticase soy broth were illuminated using 10-W 461 (22.1 mW/cm^2) and 521 nm (16 mW/cm^2) LEDs at pH values of 4.5, 6.0, 7.3, 8.0 and 9.5 for 7.5 h at 15 °C. Using the 461 nm LEDs, the populations of *E. coli* O157:H7 decreased by 2.1 ± 0.02, 1.2 ± 0.08 and 4.1 ± 0.42 log CFU/ml at pH 4.5, 7.3 and 9.5 respectively, after a dosage of 596.7 J/cm^2. For *L. monocytogenes*, approximately a 5.8 ± 0.03 log reduction was observed after 238.7 J/cm^2 at pH 4.5 using the 461 nm LEDs, while the bacterial concentration was reduced by 1.8 ± 0.01 log at pH 9.5 after 596.7 J/cm^2. Bacterial inactivation using the 521 nm LEDs showed similar trends to the 461 nm LEDs at both acidic and alkaline pH conditions but with lower (1–2 log CFU/ml) reductions after 432 J/cm^2. Lower D-values were observed for *L. monocytogenes* when exposed to LEDs at acidic pH values, while the sensitivity of *E. coli* O157:H7 and *S.* Typhimurium to LED was markedly increased at an alkaline pH. Regardless of the pH at which the cultures were illuminated, the percentage of sublethal injury increased with the treatment time. These results highlight the enhanced antibacterial effect of the 461 nm LED under acidic and alkaline pH conditions, proving its potential to preserve foods as well as to have synergistic effect with acidic and alkaline antimicrobials.

1. Introduction

A light emitting diode (LED) is a semiconductor device that can emit light of a very narrow emission spectrum when there is an electric current flowing through it. LEDs offer several advantages over traditional light sources, such as lower energy consumption and high durability, both important aspects for commercial applications. A further advantage of LEDs is that they can be easily implemented into existing systems as a result of their compact design. They also do not require special disposal methods after their use (Matioli et al., 2012). For these reasons, LEDs have been widely used in the areas of electronics and agriculture (Tamulaitis et al., 2005; Craford, 2005).

LEDs have been known to bring about an antibacterial effect (Luksiene, 2005) due to the phenomenon of photodynamic inactivation. Bacterial cells contain light sensitive compounds known as porphyrins, which absorb light in the visible region of the electromagnetic spectrum (400–430 nm). While returning to the ground state, they collide with compounds of oxygen, transferring this energy to them and leading to the production of reactive oxygen species (ROS). The ROS generated include singlet oxygen, the

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http://dx.doi.org/10.1016/j.fm.2014.10.014
0740-0020/

superoxide anion, the hydroxyl radical and hydrogen peroxide. These ROS react in a cytotoxic manner with various cellular constituents such as lipids, proteins and DNA and ultimately cause cell death (Luksiene, 2005). Based on these findings, LEDs have found applications in the fields of medicine, where light therapy has emerged as a novel means to minimize the use of antibiotics. Several studies have also been carried out involving microbial inactivation, with successful results for bacteria, fungi, yeasts, viruses, and parasites (Lambrechts et al., 2005; Ferro et al., 2006; Lipovsky et al., 2009).

More recently, the use of LEDs has been researched for use in the food industry. LEDs in combination with photosensitizers have emerged as a novel antimicrobial treatment for surface decontamination (Luksiene, 2005). Bacterial inactivation following exposure to visible light ranging from 400 to 470 nm in wavelength without a photosensitizer has also been reported. Maclean et al. (2009) reported that *Pseudomonas aeruginosa* and *Staphylococcus aureus* could be inactivated following exposure to a 405 nm LED array. Similarly, Murdoch et al. (2010) demonstrated that the population of *Campylobacter jejuni* could be effectively reduced by as much as 5.6 log CFU/ml following exposure to a high intensity 405 nm LED light source for 30 min.

Although the antibacterial effect of blue LEDs has been confirmed, the effect of different environmental stresses on their antibacterial effect has not been fully understood. Our previous study showed that the effectiveness of 461 and 521 nm LEDs in inactivating four major foodborne pathogens was highly influenced by the illumination temperature. The antibacterial effect of the LEDs was much higher at lower temperatures (10 and 15 °C) than at room temperature (20 °C) (Ghate et al., 2013). To our knowledge, the effect of pH, another important environmental stress for bacterial growth, on the antibacterial activity of blue LEDs has not yet been studied. Since different foods have different pH values, it was necessary to determine whether the antibacterial effect of LED would be enhanced or reduced under different pH conditions, prior to the application of this technology for food preservation. Hence, the objective of this study was to determine the influence of acidic and alkaline conditions on the effectiveness of LEDs in inactivating selected foodborne pathogens.

2. Materials and methods

2.1. Bacterial cultures

Three major foodborne pathogens were selected for this study. *Listeria monocytogenes* 1/2a (BAA-679) and *Salmonella* Typhimurium (ATCC 14028) were obtained from the American Type Culture Collection (ATCC; Manassas, VA, USA). *Escherichia coli* O157:H7 (EDL 933) was obtained from Dr. Henry Mok at the Department of Biological Sciences at the National University of Singapore. The frozen stock cultures were revived in 10 ml of sterile trypticase soy broth (TSB; Oxoid, Basingstoke, UK) at 37 °C for 24 h with at least two consecutive transfers prior to use. Each bacterial strain was also adapted to 100 μg/ml nalidixic acid (Sigma, St Louis, MO, USA). This adaptation was done with stepwise increments of nalidixic acid.

2.2. Light emitting diode (LED) illumination system

The characteristics of the LEDs and the LED illumination system were as described in Ghate et al. (2013). Ten watt LEDs (12.5 mm diameter) with peak wavelengths of 461 (22.1 mW/cm^2) and 521 nm (16.0 mW/cm^2) were attached to a heat sink and a cooling fan to dissipate the heat and minimize thermal damage to the LED. The distance between the light source and the bacterial suspension was chosen as 4.5 cm, the distance at which the wavefront from the LED illuminated an area equal to the sample surface area. The irradiances were also measured at the same distance using a Thorlabs laser power and an energy meter console (PM100D) attached with a photodiode power sensor (S130C) (Newton, New Jersey, USA). Each LED was mounted onto a plastic acrylonitrile butadiene styrene (ABS) housing, and the entire equipment was housed in a temperature-controlled incubator (Zhicheng ZSD-A1160A, Zhicheng Analytical Instruments Manufacturing Co. Ltd., Shanghai, China).

2.3. Bacterial inactivation by LED illumination

The bacterial cultures were serially diluted from an initial population on the order 10^9 CFU/ml to the order 10^7 CFU/ml using 0.1% peptone water. The culture was finally transferred into 9 ml of TSB, in order to simulate the nutritional qualities of a food matrix. The bacterial suspension was placed in a sterile glass petri dish (60 mm diameter) inside the LED illumination system. The pH of the TSB was preadjusted to either acidic (pH 4.5 and 6.0) or alkaline (pH 8.0 and 9.5) values using 1 N hydrochloric acid (HCl; Merck, Darmstadt, Germany) or 1 N sodium hydroxide (NaOH; Schedelco, Singapore) respectively, and each pH value was measured using a pH meter (Mettler-Toledo S220, Greifensee, Switzerland). The pH of the TSB without HCl and NaOH was 7.3. At each pH, the bacterial suspension was illuminated with LEDs for 3–7.5 h at 15 °C with sampling at six equally spaced data points. For the control samples, the same set-up was used in the absence of the LEDs at a temperature of 20 °C in order to take into account the temperature increase of about 5 °C due to the illumination (Ghate et al., 2013).

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, t = time in sec.

The number of survivors (log CFU/ml) was plotted against the illumination time. Survival data at pH 4.5 and 9.5 was fitted to the Weibull model (Peleg, 1999; Huang, 2009, 2014). The decimal reduction times (D-values) were then calculated using the standard Weibull equation given below:

$$y(t) = y_0 - (t/D)^{\alpha}$$

where y is the bacterial population in log CFU/ml after time t, y_0 is the initial bacterial population and α is a parameter in the equation.

2.4. Determination of sublethal injury

The sublethal injury to the bacterial cultures after LED treatment under different pH conditions was determined by comparing the colony counts on trypticase soy agar (TSA; Oxoid) and TSA containing sodium chloride (Unichem Ltd, Doha, Qatar). The concentration of sodium chloride used for determining the sublethal injury for each strain was selected as the highest concentration which did not bring about the death of healthy, intact cells. This concentration was determined to be 3% (w/v) for *E. coli* O157:H7 and *S.* Typhimurium and 4% for *L. monocytogenes*. The percentage of sublethal injury to a culture was calculated using the equation (Yuk et al., 2010):

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

2.5. Enumeration

At each sampling interval, a 0.1-mL aliquot from the illuminated bacterial suspension or the controls was serially diluted with 0.1% peptone water and pour plated onto sterile TSA supplemented with 100 μg/ml nalidixic acid. The plates were then incubated at 37 °C for 24–48 h, followed by counting of the colonies using a colony counter (Rocker Scientific Co. Ltd., Taipei, Taiwan). The number of viable cells was reported in $\log_{10}$ CFU/ml. The detection limit was 10 CFU/ml.

2.6. Statistical analysis

Mean values of the data were obtained from three independent trials with duplicate plating for each LED treatment. Bacterial log reductions were expressed as mean ± standard deviation. One-way analysis of variance (ANOVA) analysis using the IBM SPSS statistical software (version 17.0; SPSS Inc., IBM Corporation, Armonk, NY, USA) at a confidence interval of 95% ($P < 0.05$) was performed to determine the statistically differences among the mean values.

3. Results

The illumination of *E. coli* O157:H7 using the 461 nm LED brought about average log reductions of 2.1 ± 0.02, 1.2 ± 0.11, 1.2 ± 0.02, 1.7 ± 0.21 and 4.1 ± 0.33 log CFU/ml at pH values of 4.5, 6.0, 7.3, 8.0 and 9.5, respectively, after a dosage of 596.7 J/cm^2 (Fig. 1). The 521 nm LED illumination reduced the level of *E. coli* O157:H7 by less than 1 log unit after the maximum dosage of 432 J/cm^2 at all the pH values tested. The average log reductions using the 521 nm were significantly ($P < 0.05$) lower than those using 461 nm at the same dosage. For example, the population of *E. coli* O157:H7 was reduced by only 0.9 ± 0.05 log CFU/ml after illumination with a dosage of 432 J/cm^2 by 521 nm LEDs at pH 9.5, but this reduction was as high as 1.9 ± 0.09 log CFU/ml after illumination with the 461 nm LEDs using a lower dosage (358.02 J/cm^2) at the same pH.

For *S.* Typhimurium, treatment with 461 nm at pH 8.0 and 9.5 reduced the bacterial populations by 1.8 ± 0.03 and 4.7 ± 0.50 log units, respectively, after the maximum dosage of 596.7 J/cm^2. This was significantly higher compared to 0.4 ± 0.06 and 2.0 ± 0.02 log reductions obtained at pH values of 6.0 and 4.5, respectively, using

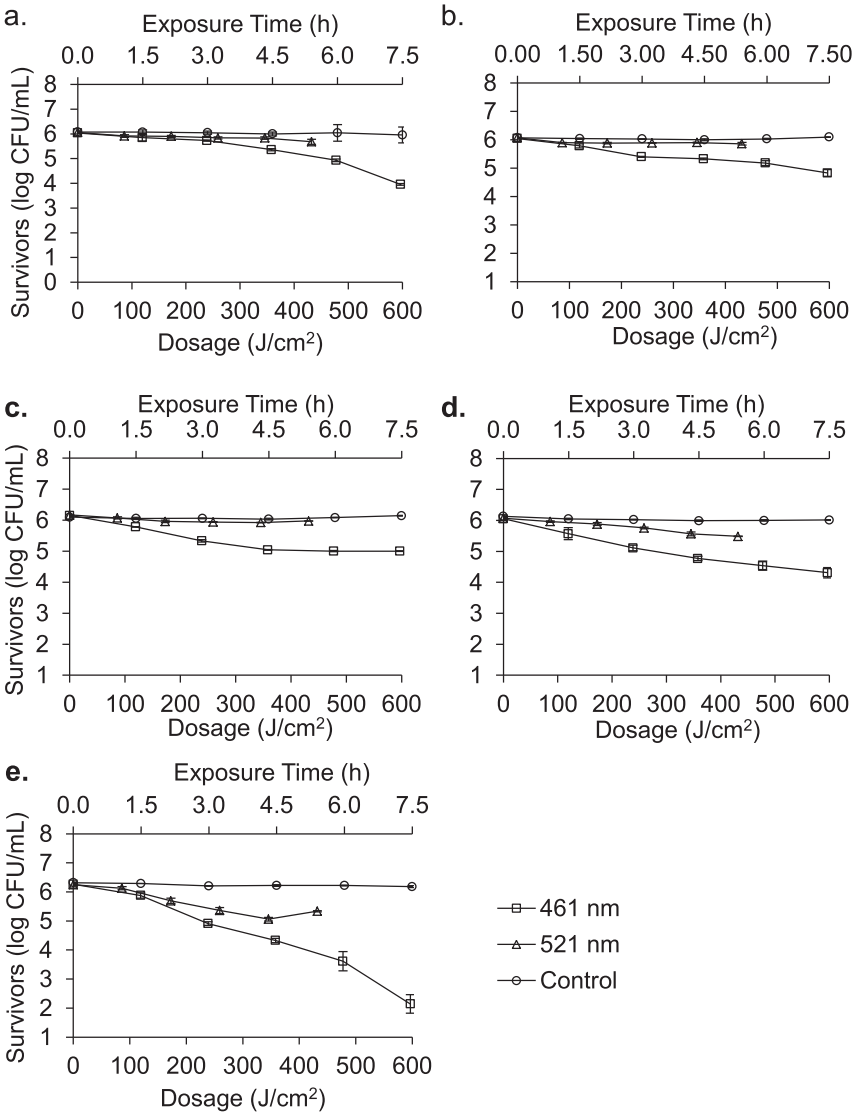

Fig. 1. Inactivation of *Escherichia coli* O157:H7 on exposure to 461 nm and 521 nm LEDs at 15 °C for 7.5 h at pH 4.5 (a), 6.0 (b), 7.3 (c), 8.0 (d) and 9.5 (e). The 461 nm and 521 nm results are to be read off the bottom axis while the results for the control are to be read off the top axis. Each data point represents three independent trials with duplicate plating examined for each experiment. Error bars were drawn based on standard deviations of results.

the same dosage (Fig. 2). The inactivation at all the pH values tested except pH 6.0 was significantly higher ($P < 0.05$) than that at the near neutral pH of 7.3. Using the 521 nm LED, the average reductions after the maximum dosage of 432 J/cm^2 at pH values of 4.5 and 6.0 were less than 1 log, while that at 8.0 and 9.5 was 1 log unit. There was no significant ($P > 0.05$) reduction at pH 7.3. The average log reductions at pH 6.0 and pH 7.3 were also not significantly different. The untreated control showed no significant change in the bacterial populations during storage after 7.5 h.

The population of *L. monocytogenes* treated with 461 nm LED was reduced below detectable levels with dosages of 238.7 and 358.0 J/cm^2 at pH 4.5 and 6.0, respectively (Fig. 3). On the other hand, treatment with the 461 nm LED at neutral and alkaline conditions after a dosage of 596.7 J/cm^2 reduced the population by less than 2 log units. With the 521 nm LED, 1.1 ± 0.04 and 1.7 ± 0.32 log reductions were observed at pH 6.0 and 4.5, respectively, with a dosage of 432 J/cm^2. The bacterial inactivation using 521 nm LED did not significantly ($P > 0.05$) differ when the pH was changed from a neutral to an alkaline range. Irrespective to the pH of medium, the level of *L. monocytogenes* remained unchanged when the cells were stored at 20 °C without LED treatment for 7.5 h.

Table 1 compares the susceptibility of the three pathogens to the 461 nm LED under the acidic as well as alkaline extremes by their decimal reduction times (D-values). From these values, it can be observed that *L. monocytogenes*, the Gram-positive bacterium, was rendered more susceptible to the LED illumination under acidic conditions as compared to alkaline conditions. This was in stark contrast to the two Gram-negative bacteria, which were more susceptible to the LED illumination under alkaline conditions than under acidic conditions.

The sublethal injury of *E. coli* O157:H7 at pH 7.3 after treatment with 461 nm and a dosage of 596.7 J/cm^2 was 82.9%, significantly ($P < 0.05$) increasing to 91.9% at pH 4.5 (Fig. 4). At pH 9.5, the percentage of sublethally injured cells increased significantly ($P < 0.05$) to 94.9%. The percentage of sublethally injured cells using the 521 nm LED was 19.4% at pH 7.3, and it significantly ($P < 0.05$) increased to 41.5% at pH 4.5 and 73.2% at pH 9.5. In general, the degree of sublethal injury was significantly lower ($P < 0.05$) with the 521 nm as compared to the 461 nm LED. For the control samples, the percentage of bacterial injury after 7.5 h was less than 20% during the incubation without LED treatment, regardless of the pH conditions.

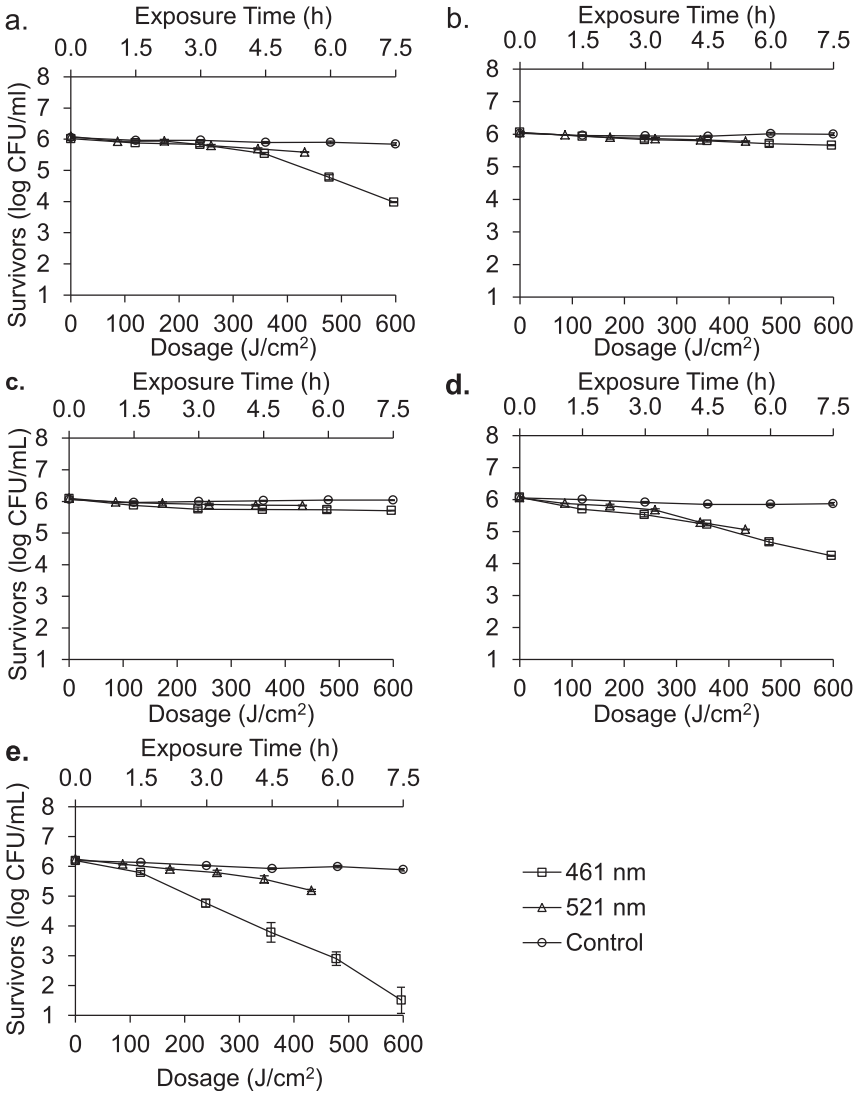

Fig. 2. Inactivation of *Salmonella* Typhimurium on exposure to 461 nm and 521 nm LEDs at 15 °C for 7.5 h at pH 4.5 (a), 6.0 (b), 7.3 (c), 8.0 (d) and 9.5 (e).

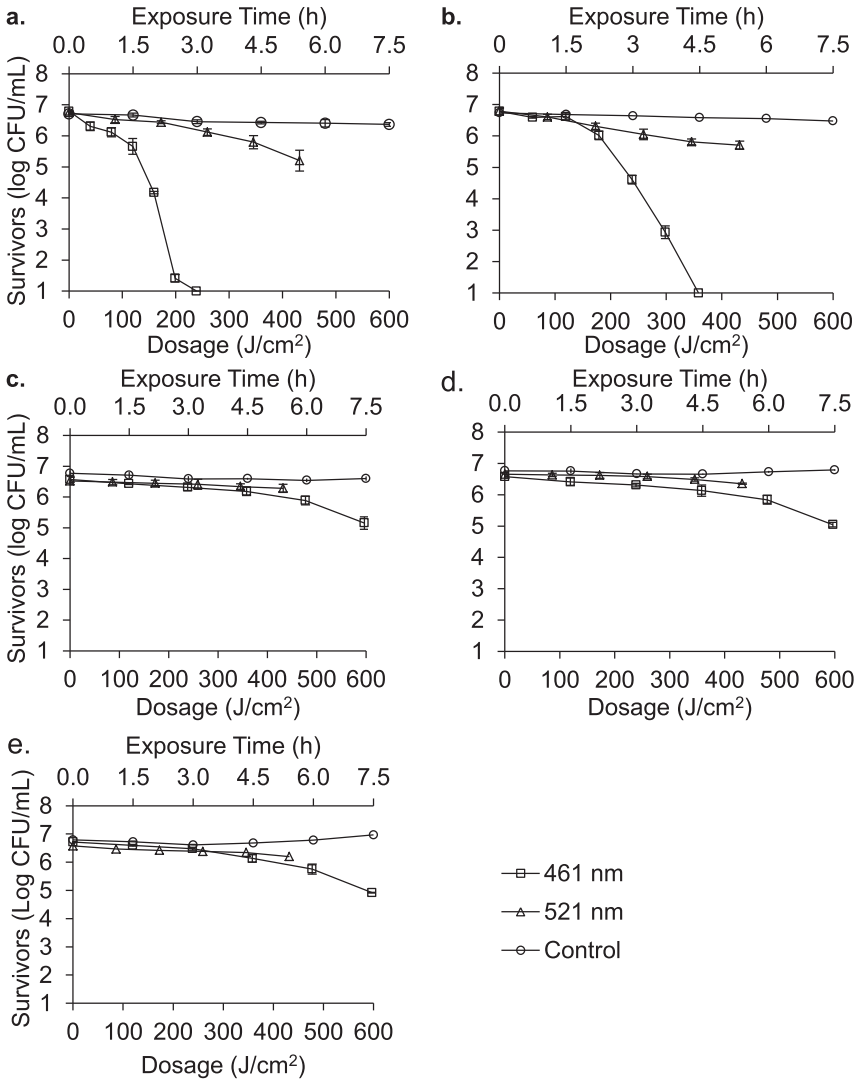

Fig. 3. Inactivation of *Listeria monocytogenes* on exposure to 461 nm and 521 nm LEDs at 15 °C for 7.5 h at pH 4.5 (a), 6.0 (b), 7.3 (c), 8.0 (d) and 9.5 (e).

After a dosage of 596.7 J/cm^2 with the 461 nm LED, the sublethal injury to the *S.* Typhimurium culture at pH 6.0 and pH 7.3 was 35.9% and 55.9%, respectively (Fig. 5). This was consistent with the low inactivation results at pH 6.0 and 7.3 in Fig. 2. The percentage of sublethally injured cells significantly ($P < 0.05$) increased to 72.5% at pH 4.5 and 94.2% at pH 9.5, corresponding well with the log reductions observed under these conditions. Exposure to 521 nm also significantly ($P < 0.05$) increased the sublethal injury from 13.1% at pH 7.3 to 51.6% at pH 9.5. In the case of *L. monocytogenes*, the sublethal injury with the 461 nm LED illumination at pH 7.3, 8.0 and 9.5 was 97.0, 98.6 and 95.0% after 7.5 h, respectively, revealing that they were not significantly ($P > 0.05$) different (Fig. 6). The sublethal injury using the 521 nm LEDs significantly ($P < 0.05$) increased when the pH was changed from neutral to acidic at a dose of 432 J/cm^2. There was no significant difference ($P > 0.05$) in the degree of injury between the pH 7.3 and the alkaline values.

Table 1
Comparison[a] of the decimal reduction times (D-values) in hours of the three food-borne pathogens at pH values of 4.5 and 9.5 using 461 nm LED at 15 °C.

Bacterial strain	pH 4.5	pH 9.5
E. coli O157:H7	5.75 ± 0.21[a]	2.82 ± 0.11[a]
S. Typhimurium	5.74 ± 0.09[a]	2.38 ± 0.36[a]
L. monocytogenes	1.46 ± 0.44[b]	6.04 ± 0.26[b]

[a] All measurements were done in triplicate with replication, and all values are means ± standard deviation. Different superscripts within a column ([a,b]) indicate that the means significantly ($P < 0.05$) differed from each other.

4. Discussion

Nalidixic acid adapted cells were prepared and used in this study to facilitate the extension of this study to real food matrices in the future. Our preliminary studies showed that the sensitivities of non-adapted and adapted cells to the LED illumination were not significantly ($P > 0.05$) different (data not shown).

In the present study, 461 nm LEDs were found to be more effective than 521 nm LEDs at the same dosage for the inactivation of the three pathogens under all the pH conditions tested. This finding was similar to that of our previous study (Ghate et al., 2013), which had 461 nm LEDs being much more effective in producing an antibacterial effect as compared to LEDs of wavelengths 521 and 642 nm. This might be due to the fact that the porphyrins, which absorb in the region 400–430 nm, have a greater portion of their absorption spectrum coinciding with the emission spectrum of the

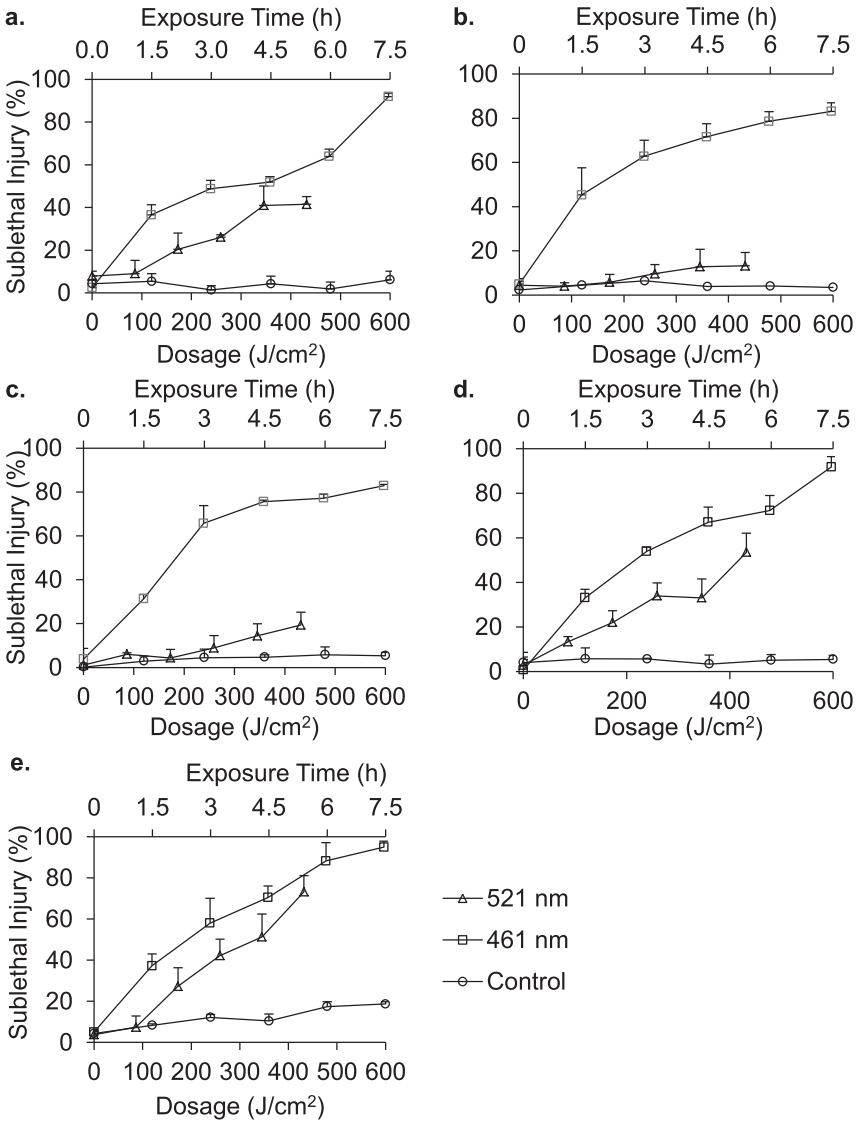

Fig. 4. Percent injury to the *E. coli* O157:H7 culture during the exposure to 461 and 521 nm for 7.5 h at pH 4.5 (a), 6.0 (b), 7.3 (c), 8.0 (d) and 9.5 (e). The control sets were conducted in the absence of an LED. The 461 nm and 521 nm results are to be read off the bottom axis while the results for the control are to be read off the top axis. Each data point represents three independent trials with duplicate plating examined for each experiment. Error bars were drawn based on standard deviations of results.

461 nm LEDs than the 521 nm LEDs. The wavelength-dependence of the bacterial inactivation was also demonstrated in a previous study (Guffey and Wilborn, 2006) which showed that a 405 nm LED treatment decreased the population of *S. aureus* by 87.9%, whereas there was only a 62% reduction in the bacterial population when a 470 nm LED was used for the illumination. These results indicate that the antibacterial effect of LEDs relied on their wavelengths such that a lower wavelength in the visible region of the electromagnetic spectrum was more effective.

The present results showed Gram-negative *E. coli* O157:H7 and *S.* Typhimurium and Gram-positive *L. monocytogenes* markedly differed in their susceptibility to LED illumination, depending on the pH of the suspension medium. These results are similar to those of a study conducted by Schäfer et al. (2007) investigating the influence of pH on *E. coli* and *Deinococcus radiodurans* subjected to photodynamic therapy using a xenon arc lamp. This lamp was fitted with a filter which produced visible light wavelengths between 400 and 600 nm. It was demonstrated that the survival of *E. coli* was drastically reduced under photodynamic therapy at pH 4.5 and pH 9.5 in comparison to the survival at pH 7.0 at a temperature of 25 °C. In addition, a pH of 9.5 was found to be more effective than pH 4.5 in inactivating the bacteria. For Gram-positive *D. radiodurans*, inactivation occurred rapidly, and cell viability at pH 4.5 after illumination was too low for the measurement of the photodynamic effect. At pH 9.5, the inactivation of *D. radiodurans* was found to be only slightly lower than at pH 7.0.

A probable reason behind such a difference in susceptibility to the 461 nm LED illumination based on pH conditions might lie in the difference in the cell wall structure of Gram-negative and -positive bacteria. The cell wall of Gram-positive bacteria consists of a cytoplasmic membrane and a thick peptidoglycan layer (30 nm) (Mendonca et al., 1994). The cell wall of Gram-negative bacteria, on the other hand, consists of an inner membrane, a thin peptidoglycan layer (2–3 nm) and a relatively impermeable outer membrane consisting of lipopolysaccharides in its outer leaflet and phospholipids in its inner leaflet (Mendonca et al., 1994). The hydrogen ions generated as a result of the dissociation of a strong acid such as HCl in the external environment of the bacterial cells are almost impermeable to the outer membrane, thus protecting the cell wall of Gram-negative bacteria at a relatively low

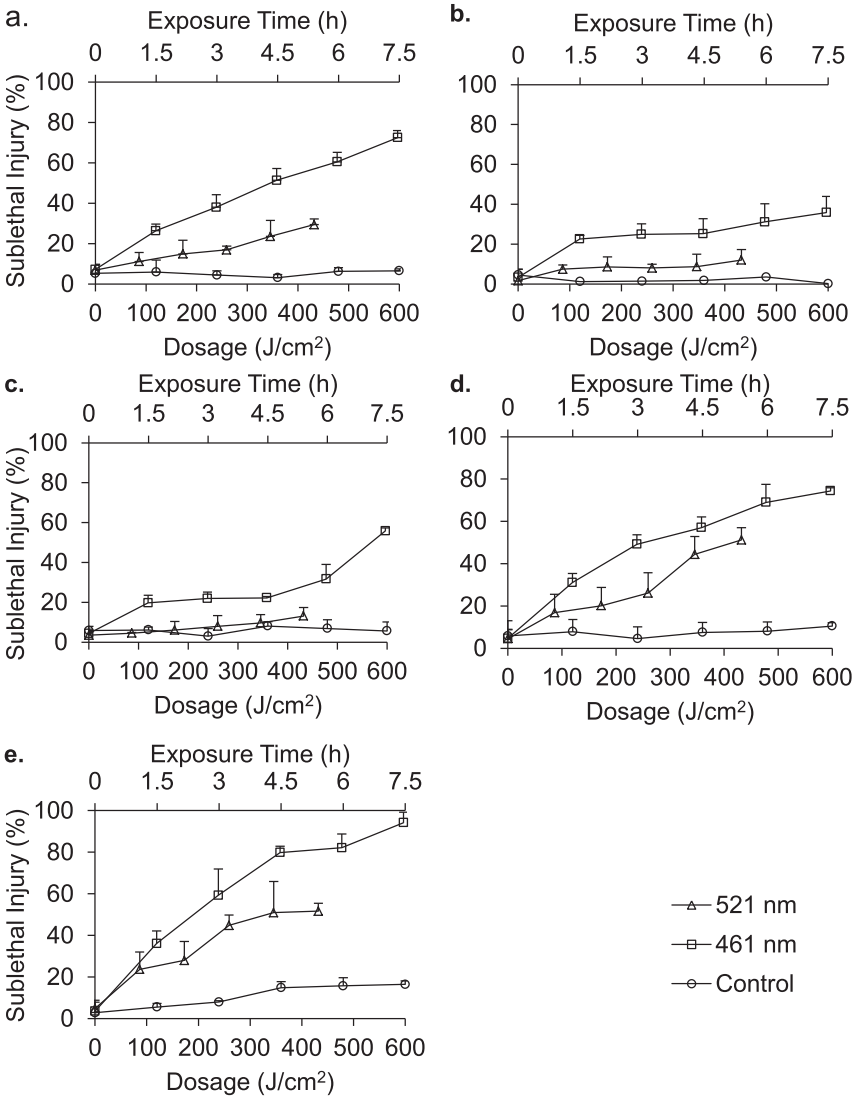

Fig. 5. Percent injury to the *S.* Typhimurium culture during the exposure to 461 and 521 nm for 7.5 h at pH 4.5 (a), 6.0 (b), 7.3 (c), 8.0 (d) and 9.5 (e).

pH. On the other hand, the cell wall of Gram-positive bacteria is not protected by an outer membrane and therefore it might be more sensitive to the hydrogen ions in the external environment compared to Gram-negative bacteria. This also renders the Gram-positive pathogens more susceptible to a combination of acid stress and photodynamic action than Gram-negative bacteria. This could explain why *L. monocytogenes* had the extremely low D-values at pH 4.5 compared to those of *E. coli* O157:H7 and *S.* Typhimurium.

Meanwhile, the enhanced sensitivity of *E. coli* O157:H7 and *S.* Typhimurium to the 461 nm LED illumination at alkaline pH conditions might be due the weakened cell membranes by the solubilization of proteins and the saponification of the membrane lipids by hydroxyl ions (Mendonca et al., 1994). Such a weakened cell membrane by high pH may be easily damaged by the attack of the ROS generated from the photodynamic action of the LED illumination. Another possible explanation could be the alteration of bacterial membrane lipid composition in response to environmental pH. It was reported that the lowering of pH using HCl resulted in an increase in the proportion of saturated fatty acids in the membrane, while an increase in the pH using NaOH led to an increase in the proportion of unsaturated fatty acids in the lipid composition of the cell membrane of *E. coli* O157:H7 (Yuk and Marshall, 2004). Unsaturated fatty acids may be more susceptible to ROS than their saturated counterparts. Hence, a higher proportion of unsaturated fatty acids might enhance the effectiveness of the LED treatment, which relies on the cytotoxic effect of the produced ROS. However, this phenomenon cannot explain why *L. monocytogenes* was more resistant to the LED illumination under the alkaline condition since a similar response is also observed in Gram-positive bacteria (Cotter and Hill, 2003). Thus, further study is needed to elucidate the role of bacterial stress response to the LED illumination.

The determination of sublethal injury to the bacterial culture is of great importance to microbial food safety as it screens for cells that may not be detected on supplemented or selective agar. These cells may recover when conditions turn favorable during storage, causing foodborne diseases (Yuk et al., 2010). Injury testing also justifies the bacterial inactivation achieved and prevents an overestimation of the treatment lethality. In this study, a direct relationship was observed between sublethal injury inflicted on the bacterial cultures and the inactivation experienced by them. In general, an increase in the sublethal injury correlated with an increase in inactivation for all the four pathogens treated with the

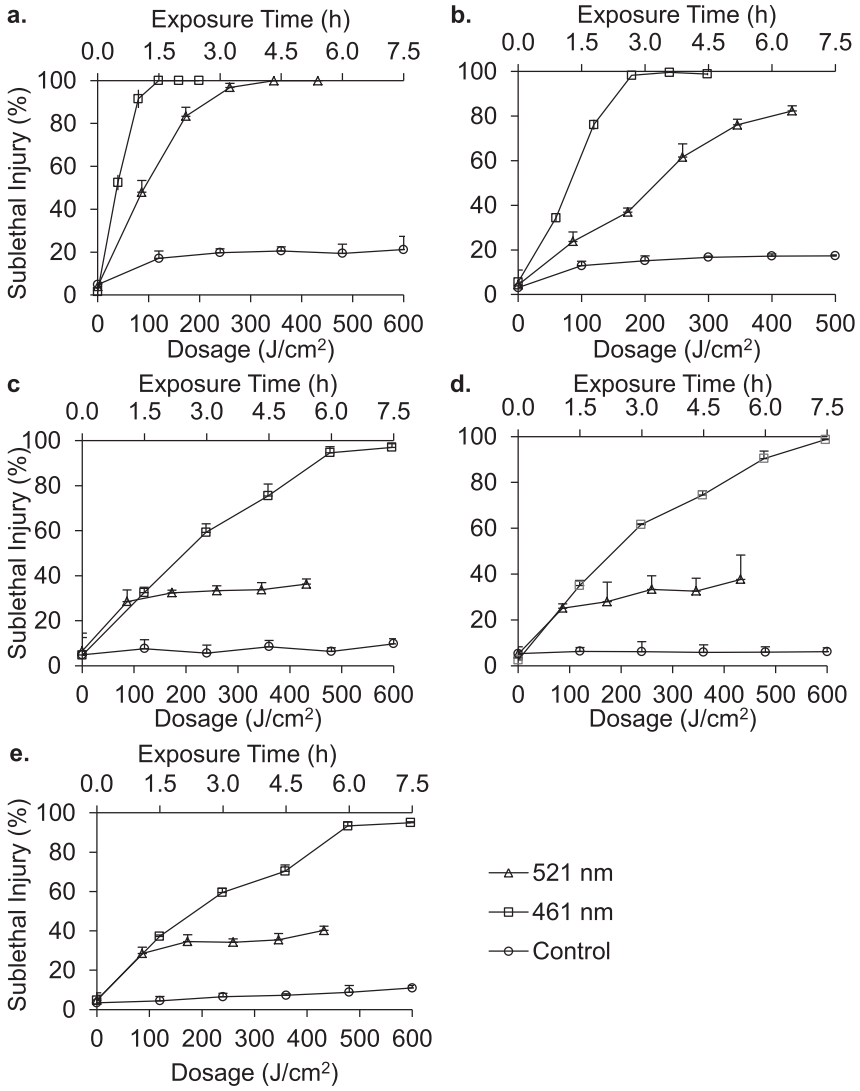

Fig. 6. Percent injury to the *L. monocytogenes* culture during the exposure to 461 and 521 nm for 7.5 h at pH 4.5 (a), 6.0 (b), 7.3 (c), 8.0 (d) and 9.5 (e).

LEDs. A similar observation was found in a study conducted by Yuk et al. (2010) who investigated the inactivation and injury of supercritical carbon dioxide processing on *E. coli* K12 in apple cider at various temperatures.

Besides acidic and alkaline conditions, LED illumination at a near neutral pH of 7.3 also caused 1–2 log reductions in the populations of the selected foodborne pathogens. A high degree of sublethal injury was also observed after illumination at this pH. This meant that most of the surviving cells were injured, but this injury did not result in bacterial death. These results are different to those obtained in our previous study (Ghate et al., 2013) where this injury translated into bacterial death and resulted in a greater inactivation (4 – 5 log). No explanation is currently available for this difference, although much effort has been made. Accordingly, further studies on the effect of LEDs are required to explain this variation in their antibacterial effect.

5. Conclusion

This is the first study to show that the antibacterial effect of 461 nm LED is highly influenced by the pH of suspension medium. Higher bacterial inactivation was achieved at acidic and alkaline pH conditions during the 461 nm LED illumination compared to neutral pH. In particular, acidic conditions were more detrimental than alkaline conditions for *L. monocytogenes*, while inactivation was more effective at alkaline conditions than acidic conditions for *E. coli* O157:H7 and *S.* Typhimurium. Besides the influence of pH, it was found that the illumination with 461 nm LED was more effective in eliminating pathogens than that with the 521 nm LED. Thus, this study demonstrates the potential of 461 nm LEDs in not only preserving acidic foods but also in exerting a combined effect with alkaline antimicrobials.

Acknowledgments

This research was funded by the A*STAR Nutrition and Food Science grant (SERC 112-177-0035).

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