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Effect of 390 nm Light on Surface Microbes of Cut Carrots

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Authors' contributions

This work was carried out in collaboration among all authors. Author AM, Ph.D scholar, conceptualized and carried out the research work and prepared the manuscript. Author SKC reviewed and edited the manuscript. Author MKT guided through laboratory facilities. Author NK guided throughout the research work. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To reduce the microbial load on cut-carrot surface using 390 nm Light Emitting Diode (LED) illumination
Place and Duration of Study: Agro-Produce Processing Division, ICAR Central Institute of Agricultural Engineering, India between August, 2017 to November, 2019
Methodology: Samples were collected from local market from India and LED treatment was given to samples and microbial analysis was conducted using spread plate method.
Results: The effect of 390 nm LEDs on cut-carrot surface has been evaluated in this study. The effect of dosage was found significant ($p<0.05$) for reducing bacterial and fungal load. Significant bacterial and fungal reduction was observed from 20 min of treatment till 3 h. Maximum inactivation of 28.4 % of bacteria and 24.1 % of fungus were detected at 3 h of exposure. These results demonstrated the potential of 390 nm LEDs as surface decontamination technology for cut-fruits and vegetables.
Conclusion: 390 nm LED illumination can be a suitable and eco-friendly novel technology for surface decontamination of cut fruits and vegetables in the food industry.

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Keywords: Light emitting diode; photodynamic inactivation; surface decontamination; dosage.

1. INTRODUCTION

Carrot (*Daucus carota* L.) is a root vegetable belonging to *Apiaceae* family is more than a versatile orange vegetable [1]. It was firstly used for medical purposes due to its high nutritional value then gradually taken as food [2]. The chemical composition contains 88% water, 7% carbohydrate, 3% fiber, 1% protein, 0.2% fat, and various other nutrients like carotenoids, flavonoids, polyacetylenes, vitamins and minerals [2]. Carrots consumption as fresh salad vegetables or snack item has become more popular after its availability in market was observed as minimally processed prepacked 'cut and peel' vegetable [3]. However, the risk of microbial contamination increases as it is consumed raw [4] and become a vehicle for infectious disease transmission [5]. One of the microbiological measures of food quality is the aerobic plate count of mesophilic microorganisms present in food [6].

In USA, the outbreaks occurred due to carrots were eighteen in number between 2000 and 2007 which was maximum among other vegetables [4]. Washed, unpeeled carrots usually had the highest number of aerobic bacteria [7] and the mould population reported maximum in shredded carrots [5]. To prohibit such outbreaks raw and cut carrots are treated with various disinfection techniques like chlorination, UV treatment, irradiation, pulsed light treatment, gas plasma, high pressure processing, etc. [8,9], however, all of them have some drawbacks like consumer safety concerns, higher costs, changes in product quality, etc.

Light emitting diode is a solid state lighting source that emits light of narrow bandwidth. Now-a-days it is used for microbial inactivation through the process of photodynamic inactivation (PDI) [10,11], PDI requires only three agents i.e. light source (visible), oxygen and photosensitizer. It causes cell destruction when light falls on a photosensitizer present in microbial cells thus making photosensitizer active and producing reactive oxygen species in the presence of oxygen. The reactive oxygen species then gives cytotoxic effect on microbial cells by damaging the DNA, proteins or lipids present inside microbial cell [12].

Violet-blue light has maximum effect on microbial cells [13]. The effect of 395 nm on raw chicken has been observed to reduce by 2.62 $\log_{10}$ CFU/g of *campylobacter spp.* within 5 min [14]. Similarly the antimicrobial effects of other wavelengths have been observed on various fresh products like mango (405 nm), papaya (405 nm), pineapple (460 nm), cucumber (435 nm), and orange juice (460 nm) [11,15-18]. As per our knowledge, limited work has been conducted to study the effect of LEDs' illumination on cut-carrot surface. Hence, the present work has been taken to study the effect of 390 nm for a time period of 10 min to 3 h on cut-carrot surface.

2. MATERIAL AND METHODS

2.1 Raw Material and Sample Preparation

Fresh carrots have been purchased from the local market and kept in polyethylene bags inside refrigerator at 4°C until further analysis. Carrots were taken out from refrigerator and kept inside laminar (sterile environment) for some time to attain room temperature. 2-4 g samples were taken from the carrots and further cut into two halves. Half portion was taken as treated while the other half as control.

2.2 LED Illumination

10 W LED of 390 nm (LED Engin, San Jose, California, USA) were used for illumination. Samples were kept at a distance of 4 cm from LED and illuminated at an irradiance of 25 W/m^2 for 10 min, 20 min, 30 min, 60 min, 120 min, and 180 min. The dosage was measured as a product of irradiance and time:

$$E = P \times t \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

Where, E = Dosage, J/ cm^2
P = Irradiance, W/cm^2
t = Time, s

2.3 Microbial Analysis

Prepared plate count agar (PCA) and potato dextrose agar (PDA) media were poured into sterile disposable petri plates. Carrot samples (control and treated) were diluted in distilled water (10 % w/v) and shaked using vortex test-tube shaker for two minutes for proper homogenization of samples. The samples were then serially diluted (10 % v/v) up to fifth dilution. 0.1 ml sample from each dilution was taken and spread plated into prepared media plates (PCA and PDA). The plates were then kept in incubator at 25°C [19] for fungal count and 37°C [20] for bacterial count. Colonies were counted after 48 h of incubation.

$$Microbial\ count\ \left(\frac{CFU}{g}\right) = \frac{No.of\ colonies \times dilution\ factor}{vol.of\ sample\ plated\ (ml)} \times \frac{vol.\ of\ DW\ for\ dilution\ (ml)}{wt.of\ sample\ taken\ (g\ )} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(2)$$

2.4 Statistical Analysis

Completely randomized design (CRD) was used as experimental design. All experiments were conducted in triplicates. One way ANOVA was performed to see the effect of time or dosage on microbes at confidence interval of 95% (P<.05). To find the significant difference between treatments Tukey's HSD test was performed. SAS software (SAS ver. 9.3, USA) was used to carry out the statistical analysis.

3. RESULTS

3.1 Effect of Dosage on Bacterial Count

The effect of dosage on bacterial count was found significant (P<.05) with R^2 = 0.94 as shown in ANOVA (Table 1). The bacterial load of control and 10 min, 20 min, 30 min, 1 h, 2 h and 3 h treated samples were 6.341±0.122, 5.865±0.147, 4.886±0.106, 5.387±0.232, 4.813±0.121, 4.767±0.115 and 4.542±0.320 log10 CFU/g, respectively. The log reductions of 0.47 to 1.80 were obtained across full range of treatments (10 min - 3 h). It was observed that the bacterial reduction was non-significant for first 10 min of treatment as dosage level was lower (1.5J/cm^2) (Fig. 1). As dosage level increased by increasing the treatment time, the bacterial inactivation became significant up to 30 min. On further increasing the treatment time to 1 to 3 h, the antibacterial effect became non-significant with respect to treatment time of 30 min. The bacterial count showed a polynomial trend of third order (R^2=0.97).

3.2 Effect of Dosage on Fungal Count

The initial fungal count was 5.27±0.03 log_{10} CFU/g which decreased by increasing dosage as shown in Fig. 2. The ANOVA (Table 2) was found significant at P<.05 (R^2=0.89). The fungal load of the LED treated samples at 10 min, 20 min, 30 min, 1 h, 2h and 3 h were 4.757, 4.317, 4.124, 4.079, 3.999 and 3.998 log_{10} CFU/g respectively. Treatment time of 10 min presented a non-significant (P>.05) reduction in fungal count, while 20 min of treatment time have shown significant (P<.05) reduction in fungal count. Maximum log reduction of 1.27 was observed after 3 h of LED treatment. 30 min of treatment time have shown a reduction of 1.14 log_{10} CFU/g, which was non-significant compared to further treatments of 1 h (1.18), 2 h (1.27) and 3 h (1.27). The fungal count followed a third order polynomial trend (R^2=0.94).

4. DISCUSSION

The practical application of LEDs has been observed in various fields like agriculture, medicine and animal husbandry [21-23]. However in the field of food industry significant research is needed. This study has been taken as an attempt to check whether LEDs can be used as a surface decontamination technology for fresh fruits and vegetables. The non-significant antimicrobial effect of 390 nm LED has been observed at 10 min. This might be due to the lower dosage levels as dosage is the important factor for microbial inactivation [24]. At lower dosage level the ROS formed might be inadequate for oxidizing the lipids present in the microbial cells after attacking the DNA molecules adjacent to the porphyrin compounds existing in cytoplasm [15]. As the dosage level further increased the inactivation of microbes became significant. It is assumed that microbial inactivation by visible light occurs through ROS production [25,14]. As also explained by [18] in the visible range, the porphyrins present in the microbial cells are believed to act as endogenous photosensitizers that absorb light. The porphyrins collide with oxygen molecules on their way back to the ground state after subsequent excitation by this fraction of the LEDs' emission spectrum and transform some of these molecules into ROS. Because of their nucleophilic existence and extremely short half-life, the ROS quickly destroys the lipids, proteins, and nucleic acids inside the cell, causing its death.

After 30 min the inactivation rates of microbes decreases. Initially they inactivated at higher rate, then the rate of inactivation decreases. The non-significant reduction in microbial population compared to 30 min treatment might be due to the lower levels of porphyrins available inside microbial cells. There might be another possible reason that microbial cells require an adjustment period to LED illumination [26]. Similar results were obtained for microbial reduction in mango at higher exposure time of 17 to 48 h [15].

Table 1. One-way ANOVA table showing effect of treatment time on bacterial count

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	7.92430827	1.32071804	39.88	<.0001
Error	14	0.46367203	0.03311943		
Corrected Total	20	8.38798029			

Table 2. One-way ANOVA table showing effect of treatment time on fungal count

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	4.13043599	0.68840600	19.47	<.0001
Error	14	0.49490771	0.03535055		
Corrected Total	20	4.62534370			

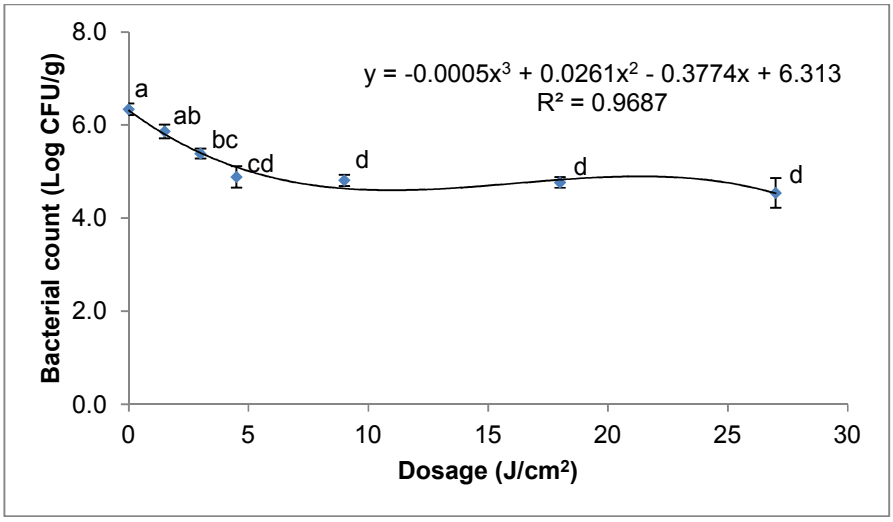

Fig. 1. Effect of dosage on bacterial count

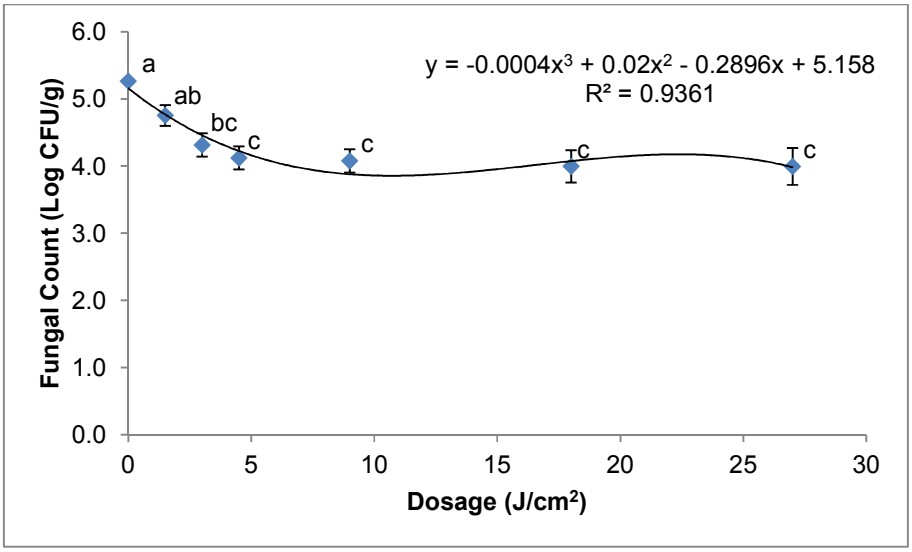

Fig. 2. Effect of dosage on fungal count

5. CONCLUSION

The study presented an insight, how the better reduction of surface contamination in cut carrots can be achieved using the 390 nm LED illumination. The effect of the treatment time varying from 10 min to 3 h on the bacterial and fungal population was determined. Maximum bacterial and fungal reduction of 1.8 log CFU/g and 1.25 log CFU/g, respectively were achieved. 390 nm LED illumination can be a suitable and eco-friendly novel technology for surface decontamination of cut fruits and vegetables in the food industry.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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