ORIGINAL

Vegetable Surface Sterilization System Using UVA Light-Emitting Diodes

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Abstract : Surface sterilization of fresh produce has been needed in the food manufacturing/processing industry. Here we report a UVA-LED (Ultra Violet A-Light Emitting Diode) system for surface sterilization that is safe, efficacious, low cost, and apparently harmless to fresh produce. To test the system, *Escherichia coli* strain DH5α was spot-inoculated onto vegetable tissues, and treated under UVA-LED. Tissues were homogenized and bacteria quantified by colony-forming assay. Possible effects of UVA-LED on vegetable quality were evaluated by HPLC. Tissue weight changes were checked after treatment at 4˚C, 15˚C, and 30˚C. Bacterial inactivation by UVA-LED radiation was observed after a 10 min treatment and increased with increasing time of irradiation. The log survival ratio reached -3.23 after a 90 min treatment. Bacterial cells surviving treatment grew slowly compared to non-irradiated control cells. Cabbage tissue lost weight over time after treatment, and weight loss increased with increasing incubation temperature, but there was no difference between losses by UVA-LED treated and control tissues at any temperature tested. In addition, no differences of Vitamin C content in cabbage tissue were detected by HPLC after UVA-LED treatment. These results suggest that UVA-LED treatment has great potential for vegetable surface sterilization in the food manufacturing/processing industry. J. Med. Invest. 61 : 285-290, August, 2014

Keywords **:** *UVA, LED, vegetable, surface sterilization*

1. INTRODUCTION

The food manufacturing/processing industry needs a surface sterilization method that eliminates pathogenic microorganisms but does not influence the taste or smell of food. Freshly harvested fruits and vegetables are considered high risk in terms of food safety (1-3). Because they may contain contaminating microorganisms at levels varying between 3 and 7 log units, depending on the season, freshness, and production region. Recently, a number of severe disease outbreaks have been traced to pathogens associated with fresh-cut fruits and vegetables that were processed under less than sanitary conditions (4-6). It has been reported that fresh produce (most frequently lettuce and sprouts) is

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Received for publication January 7, 2014 ; accepted February 4, 2014.

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the fourth highest cause of all food-borne diseases since 1990 in the US (7). In Europe, 4.3% of all food-borne disease outbreaks reported between 1992 and 1999 were associated with fresh fruits and vegetables (8). It is well known that disinfection is one of the most critical processing steps in fresh-cut vegetable production, affecting the quality, safety and shelf-life of the end product.

Sodium hypochlorite (NaClO) is the most widely used disinfectant in the food industry, despite the increasing availability of alternatives. Sodium hypochlorite has excellent cleaning action and fulfills many other requirements desired of a surface disinfectant. However, recent outbreaks associated with pathogen contamination in fresh-cut vegetables after chlorine treatment have raised concerns about its efficacy. Moreover, chlorine treatment poses environmental and health risks due to its reactions with natural organic matter that result in formation of carcinogenic halogenated disinfection by-products (DBP) like trihalomethanes (THMs) and haloacetic acids (HAAs) (9, 10). Thus, there is a need for alternative methods to disinfect fresh-cut vegetables, in both the organic and the conventional food industries.

The primary aim of this study was to develop a new surface sterilization system for vegetables based on use of UVA-LED, which has been previously proposed as an effective and safe food disinfection method. The potential of ultraviolet radiation to affect fresh vegetable quality was also investigated.

2. MATERIAL AND METHODS

2.1 Experimental device and sterilization system

We used a high-power UVA-LED (manufactured by Nichia Corporation, wavelength 365 nm, output 12.5×10^2 W/m^2) to construct a sterilization device ; nine UVA-LEDs were connected in series to a DC [direct-current] power supply (PAS40-9, Kikusui Electronics Corp.). Voltage and current of the circuit were set at constant 40.5 V and 0.5 A. UV light intensity was measured by an UV meter (UIT-250 ; Ushio Corp. Tokyo, Japan). Experiments were conducted after the temperature of the experimental chamber (see below) had stabilized at 4°C. The distance between the UVA-LED and the floor upon which vegetable tissue rested varied among experiments ; the distance to the surface of the vegetable was 50 ± 10 mm. Dimensions of the UVA-LED experimental device and its characteristics are shown in Fig. 1.

2.2. Bacterial strain and preparation

Escherichia coli strain DH5α, purchased from Takara Bio Inc. (Otsu, Japan), was cultured in Luria-Bertani (LB) broth (1% tryptone, 1% NaCl, 0.5% yeast extract) at 37°C for 18 h. Cells were collected by centrifugation (5000 g, 10 min, 4°C), washed three times with sterile phosphate-buffered saline (PBS, pH 7.4) and suspended in PBS at 10^4 CFU ml^{-1}. An aliquot (100 µl) of the bacterial suspension was spot inoculated on the surface of vegetable tissue and irradiated with UV light. The entire experiment was carried out in a Biosafety Level 2 Laboratory.

2.3. Vegetable preparation

Vegetables (lettuce and cabbage) used in this study were purchased at a local supermarket and immediately transported to the laboratory for experiments. Small pieces (3-4 cm square) of vegetable tissue were cut and one gram samples were used for experiments. After UVA irradiation, tissue was homogenized in 9 ml Buffer Peptone Water (BPW, 0.1% peptone in PBS) and 100 µl of the suspension was diluted appropriately before plating on LB agar medium to estimate numbers of bacterial cells.

2.4. UVA-LED irradiation

The UVA-LED equipment was contained within a Carbon-Graphite covered acrylic box (90 × 90 × 200 mm) inside an incubator. During irradiation for various time periods, the temperature in the box was maintained at constant 4°C ; control samples were kept in a completely dark environment at the same temperature for the same periods of time. The distance from the UVA-LED device to the surface of the vegetable tissue sample was set at 50 ± 10 mm.

2.5. Determination of bacterial inactivation level

After UV irradiation and tissue homogenization, bacterial suspensions were diluted appropriately, plated on LB agar medium and incubated at 37°C for 18 h. After incubation, the numbers of colonies were counted and log survival ratios were calculated using the following equation :

Log survival ratio = log (N_t /N_0)

N_t is the colony count of the UV irradiated sample, and N_0 is the colony count of the sample before UV irradiation.

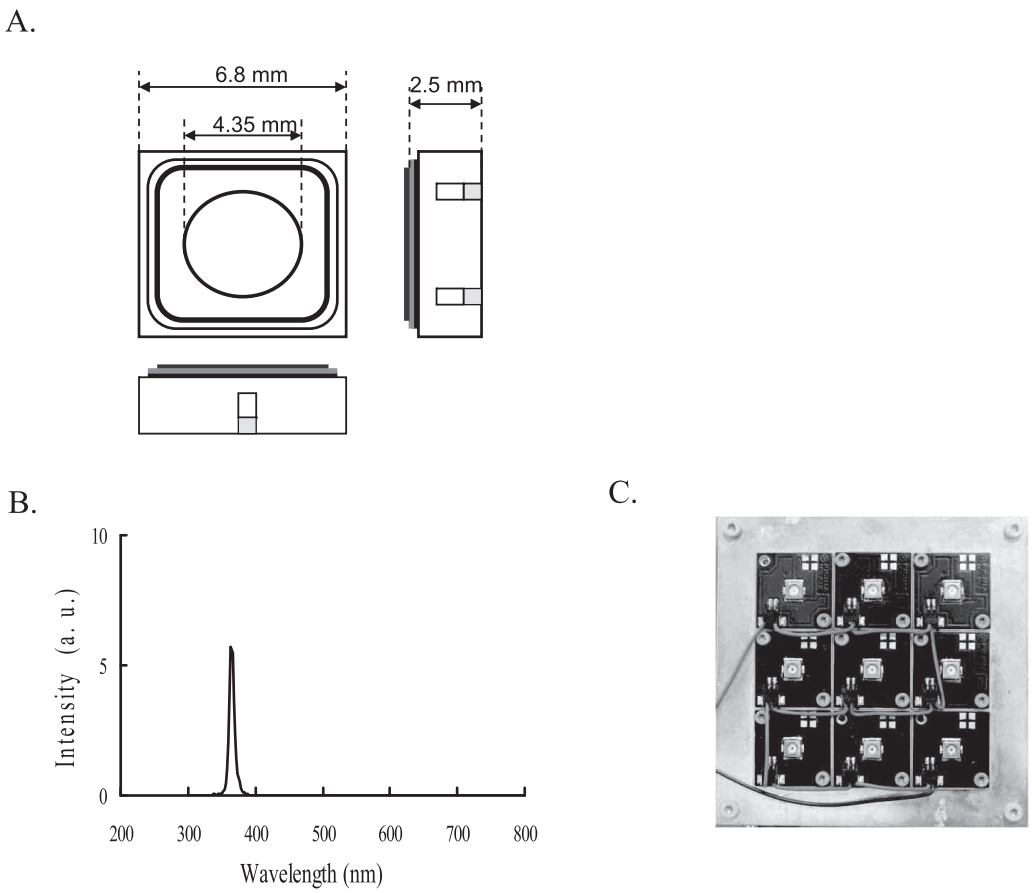

Fig. 1 Parameters of the UVA LED experimental device. A : Dimensions of the UVA-LED. B : Radiation characteristic. C : Complete UVA-LED experimental system.

2.6. Bacterial growth rate after UVA-LED irradiation

Colony sizes of bacteria from control and treated samples were measured 24 and 48 h after plating. To determine bacterial growth rates, colonies after 24 h were transferred by toothpick to LB solution and incubated at 37°C overnight (12-15 h). Cells were collected by centrifugation (5000 g, 10 min, 4°C), washed three times with sterilized PBS, and suspended in PBS at 10^8 CFU ml^{-1}. Bacterial suspensions (5 µl each) of each sample were inoculated into 20 ml LB solution and incubated at 37°C (BioShaker, BR-23UM, TAITEC Corporation, Japan). Optical Density (OD) at 600 nm was measured every hour until the stationary phase.

2.7. Evaluation of vegetable quality

Weight of cabbage was measured before and after UVA irradiation. Control experiments were performed at the same temperature in the dark. High performance liquid chromatography (HPLC) was used to evaluate cabbage components with or without UVA irradiation (Lichrospher column, flow rate 1 ml/min, sensitivity 0.16, UV 220 nm, solvent 60% CH_3CN). Vitamine C solution was added into the cabbage homogenization as a positive control. The NO_2/NO_3 assay was performed using a commercial kit (Griess Reagent Kit, DOJINDO, Japan).

2.8. Statistical analysis

Each datum in this study represents the average value of four replicates ± standard deviations. Statistical significance was calculated by paired and unpaired *t*-tests. In all cases, a p value of < 0.05 was considered significant.

3. RESULTS

3.1. The bactericidal effect of UVA-LED

Inoculated fresh-cut cabbage tissue was irradiated with UVA for 90 minutes, homogenized, diluted appropriately and bacterial levels were determined. Log survival ratio reductions of -0.41, -1.87 and -3.23 log cfu/g were observed after 30, 60 or 90 minutes

UVA irradiation (Fig. 2). The same experiments were performed using fresh-cut lettuce, with similar results (data not shown). The data indicate that the UVA-LED system can inactivate *E. coli* on the surfaces of cabbage and lettuce leaves, and suggest that the method may be appropriate for use with other vegetables as well.

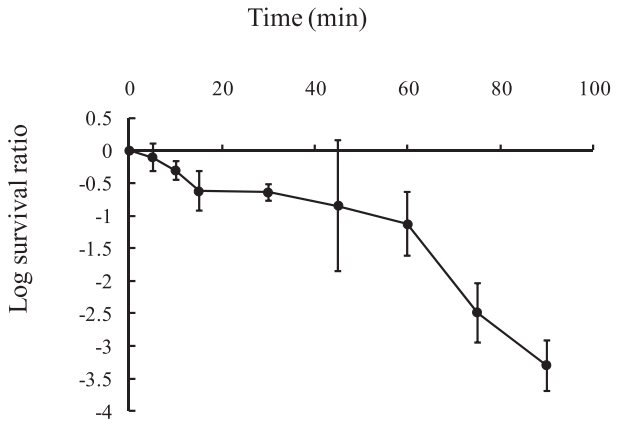

Fig. 2. Log survival ratio of Escherichia coli in inoculated fresh-cut cabbage after UVA irradiation. Each point represents the mean (n=4)± standard deviation.

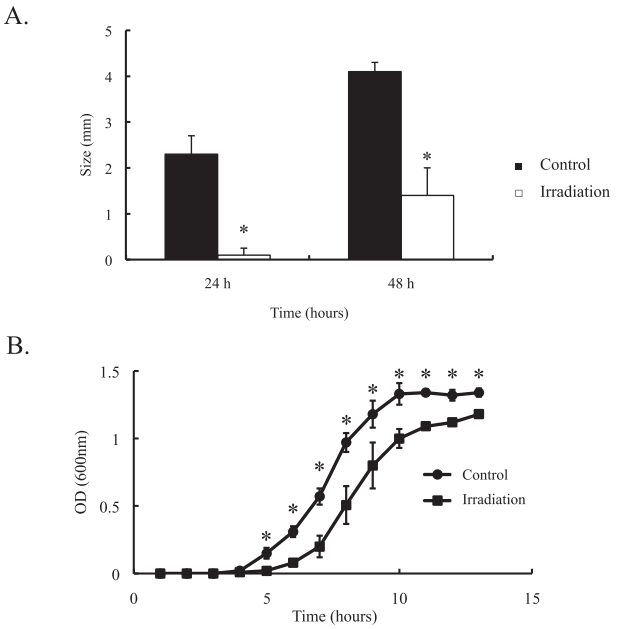

Fig. 3 UVA irradiation caused decreased bacterial growth rate. A : Colony diameters after 90 minute UVA irradiation (closed column) were smaller than those of control samples open column) after 24 and 48 h incubation. Each point represents the mean (n=4)± standard deviation. $p < 0.01$ (*) versus non-irradiated control. B : UVA irradiation delayed the bacterial growth curve. Circle indicate no UVA irradiation. Square indicate UVA irradiation. Each point represents the mean (n=4)± standard deviation. $p < 0.01$ (*) versus UVA irradiation.

3.2. Bacterial growth rate is reduced by UVA irradiation

Colonies were found to be clearly smaller after UVA irradiation compared with controls. As part of the colony-forming assay, sizes of colonies from both control and UVA treated samples were measured (Fig. 3A). After 24 and 48 h incubation, colony diameters following 90 minute UVA irradiation were 0.11 and 1.40 mm, whereas control measurements were 2.34 and 3.96 mm. Colonies from each sample were transferred by toothpick to liquid LB. OD_{600} was measured every hour until stationary phase was reached the next day (Fig. 3B). After UVA irradiation bacterial growth was slower compared with growth of control cells. This indicates that UVA irradiation has both a lethal effect (disinfection of vegetable surfaces) and an inhibitory effect (growth rate reduction of surviving bacteria). Together, these two effects may offer advantages to the food industry compared with other surface sterilization methods.

3.3. Vegetable quality is not affected by UVA irradiation

After harvest vegetable cell metabolism continues and provides protection against infection by moulds, bacteria and other microorganisms. Of the criteria to evaluate vegetable quality, Vitamin C and nitrite/nitrate content and percentage weight loss are most important. Cabbage weights were measured before and after UVA irradiation. Control experiments were performed at the same temperature (4°C) in the dark. There was ~5% weight loss for both irradiated and non-irradiated samples (Fig. 4A). When the same experiment was performed at 15°C and 30°C, weight loss increased with increasing temperature but again no significant differences between treated and control samples were detected (data not shown). HPLC analysis (Fig. 4B) showed similar traces for both UVA irradiated and control samples, suggesting no effect of UVA on cabbage content. Positive control suggested the highest peak of the graph for HPLC indicated the Vitamin C content in cabbage. The content of Vitamin C was not decreased by the UVA irradiation. Furthermore nitrite/nitrate contents were not detected after UVA irradiation in this experimental level (data not shown). We conclude that UVA-LED irradiation does not affect vegetable quality.

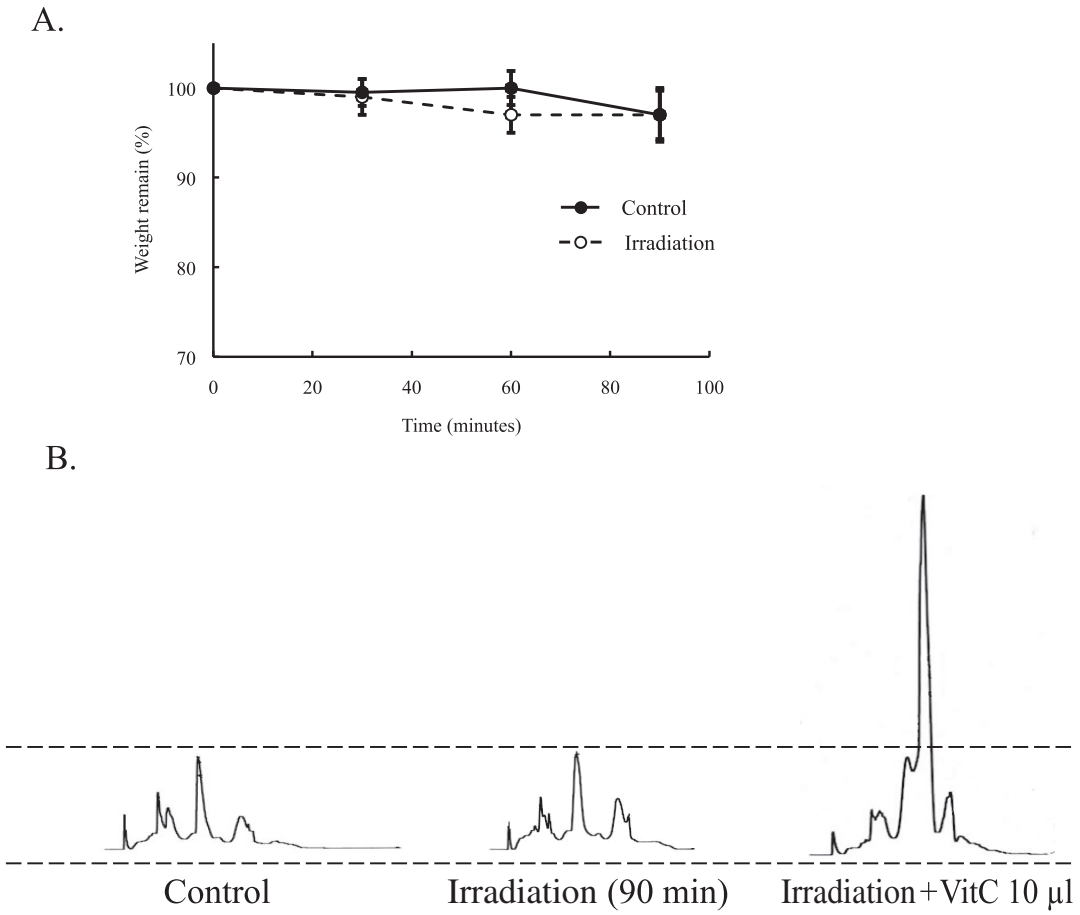

Fig. 4 Evaluation of cabbage quality after UVA irradiation. A : Percentage sample weight remaining after UVA irradiation (open circle) or no radiation (closed circle). Every sample kept in 4°C. Each point represents the mean (n=4)± standard deviation. B : HPLC results for control, UVA treated cabbage samples for 90 min, and UVA treated cabbage samples for 90 min pulls vitamin C (10 µl).

4. DISCUSSION

Vegetable surface disinfection efficacy depends on both the type of produce and its physicochemical properties. The effectiveness of chlorine and chlorine based derivatives has been established in practice over the past 30 years (11, 12) and decontamination of fresh-cut produce by chlorinated water washing is widespread throughout the fresh produce industry. Without chlorine-based technology, there probably would not be a market for fresh-cut salads and vegetables. In an industry survey, ~76% of respondents reported use of hypochlorite, although many important aspects of chlorine chemistry (*e.g.* effect of chlorine on pH) were not included in the study (13). Although chlorine use has been beneficial for preservation of fresh produce, its very success has led to over-use in industrialized countries ; many reports have documented risks associated with chlorine use in the food industry. The authority of food regulatory agencies is needed to ensure proper use of chlorine on food (14-16).

Development of the new UVA-LED sterilization equipment reported in this study was based on the water sterilization system we reported previously (17-19). Over -3.0 log reduction of bacterial populations was observed after UVA irradiation for 90 minutes (6.75×10^2 J/m^2), a result consistent with the bactericidal effect we have seen on vegetable surfaces. Moreover, any bacterial cells surviving UVA irradiation appeared debilitated, since their subsequent growth rates were substantially reduced.

Side effects of UVA irradiation appear minimal, since we did not observe any change in cabbage tissue weight or its content of Vitamin C or nitrite/nitrate ; these criteria are considered to be the most relevant for estimation of fresh vegetable and fruit quality. The apparent health of fresh produce after UVA irradiation suggests the treated tissues may retain their natural resistance to infection by moulds, bacteria, and other microorganisms during storage. Thus, the UVA-LED technology is potentially useful for application in the food manufacturing/processing industry.

ACKNOWLEDGMENTS

This project was partly supported by JSPS Grants-in-Aid for Scientific Research/Scientific Research on Priority Areas, and JST A-STEP (Japan Science and Technology Agency Adaptable and Seamless Technology transfer Program through target-driven R&D).

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