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Effect of various conditions on inactivation of *Escherichia coli* O157:H7, *Salmonella* Typhimurium, and *Listeria monocytogenes* in fresh-cut lettuce using ultraviolet radiation

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ABSTRACT

The effect of various conditions on inactivation of foodborne pathogens and quality of fresh-cut lettuce during ultraviolet (254 nm, UVC) radiation was investigated. Lettuce was inoculated with a cocktail of *Escherichia coli* O157:H7, *Salmonella* Typhimurium, and *Listeria monocytogenes* and treated at different temperatures (4 and 25 °C), distances between sample and lamp (10 and 50 cm), type of exposure (illuminated from one or two sides), UV intensities (1.36 to 6.80 mW/cm^2), and exposure times (0.5 to 10 min), sequentially. UV treatment at 25 °C for 1 min achieved 1.45-, 1.35-, and 2.12-log reductions in surface-inoculated *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes*, respectively, whereas the reduction of these pathogens at 4 °C was 0.31, 0.57, and 1.16 log, respectively. UV radiation was most effective when distance from UV lamp to the sample was minimal (10 cm) and radiation area was maximal (two-sided exposure). All UV intensities significantly ($P < 0.05$) reduced the three pathogens after 10 min exposure, but the effect of treatment was correlated with UV intensity and exposure time. Color values and texture parameters of lettuce subjected to UV treatment under the optimum conditions (25 °C, 10 cm between sample and lamp, two-sided exposure, 6.80 mW/cm^2) were not significantly ($P > 0.05$) different from those of nontreated samples up to 5 min exposure. However, these qualities significantly ($P < 0.05$) changed at prolonged treatment time. These results suggest that UV radiation under optimized conditions could reduce foodborne pathogens without adversely affecting color quality properties of fresh-cut lettuce.

1. Introduction

There has been an increasing demand for minimally processed organic produce, as consumers have taken a profound interest in health and nutrition (Organic Trade Association, 2009). However, the number of outbreaks involving fresh produce has also risen in the last few years (Sivapalasingam et al., 2004). This is because minimally processed fresh produce can readily harbor foodborne pathogens during harvest, storage, and transportation (Beuchat, 1996). Foodborne outbreaks from contaminated fresh-cut produce such as lettuce, spinach, cantaloupe, and others have been increasingly reported to the Center for Disease Control and Prevention (CDC) (Massey et al., 2013).

Lettuce is one of the major foods implicated in fresh produce-associated outbreaks, because it is widely consumed worldwide and also consumed raw due to the popularity of salad bars (United States Department of Agriculture, 2007). In the United States 175 lettuce-associated outbreaks were reported to the Centers for Disease Control and Prevention (CDC) between 1998 and 2011. Reported lettuce-associated outbreaks have been caused mainly by common foodborne pathogens such as *Escherichia coli* O157:H7 and *Salmonella* (Sivapalasingam et al., 2004). A large multistate outbreak of *E. coli* O157:H7 infections associated with lettuce occurred in the United States in November 2011. During this outbreak, a reported 60 persons in 10 states became ill (CDC, 2011). No outbreaks involving *Listeria monocytogenes* on lettuce have been reported; however, the Environmental Protection Agency (EPA) Scientific Advisory Panel identified *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* as pathogens of public health importance on produce (Environmental Protection Agency, 1997).

Surface contamination is the main cause of foodborne illness involving fresh fruits and vegetables (Doyle and Erickson, 2008). Non-thermal rather than thermal processing is preferred for the inactivation of pathogens on fresh produce surfaces. To reduce surface microbial populations and prolong shelf life of fresh produce, many chemical

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sanitizers (Beuchat et al., 2001; Sy et al., 2005; Seymour and Appleton, 2001) have been used, as well as cold storage (Zhuang et al., 1995; Jacxsens et al., 2002). However, as most chemical treatments are controversial due to residues remaining on fresh produce, many countries have restricted or even banned their use (Seymour and Appleton, 2001). In addition, whereas low temperature affects microbial growth rate by increasing lag-phase, it produces minimal reduction (<2 log CFU/g) of pathogens on fresh produce (Jacxsens et al., 2002).

For the above reasons alternative interventions incorporating both high efficacy and operator safety need to be developed. One of these alternatives is ultraviolet (UV) radiation which is subdivided into three regions: short-wave UV (UV-C), medium wave UV (UV-B), and long wave UV (UV-A) (Giese, 1964). Among these, UV-C with wavelengths from 200 to 280 nm is germicidal to microorganisms (Bintsis et al., 2000). The highest germicidal range is between 250 and 260 nm because it is near the peak effectiveness for UV absorption by DNA (Sharma, 1999). Inactivation of microorganisms by UV radiation is mainly caused by DNA damage and formation of pyrimidine dimers in UV-irradiated nucleotide bases. These photoproducts distort the sugar phosphate backbone and prevent proper DNA replication and transcription (Harm, 1980). This has caused UV irradiation to be explored as a non-thermal method for killing pathogens or other spoilage microorganisms on fresh produce. The advantages of UV radiation include that it does not leave a residue, does not change sensory characteristics, and does not involve high cost (Chang et al., 1985).

UV treatment can be used in combination with refrigeration to inactivate foodborne pathogens and simultaneously extend shelf life of fresh produce (Bintsis et al., 2000). Although UV-treatment temperature also influences inactivation of foodborne pathogens, research has been focused on the effect of storage temperature following UV treatment (Lemoine et al., 2007; Gonzalez-Aguilar et al., 2004). Unfortunately, most processors might not appreciate the effects of UV-treatment temperature on the survival of pathogens. Many treatment variables, besides UV-treatment temperature, influence inactivation effects on microorganisms. However, many research studies have been undertaken examining UV treatment conditions individually.

Thus, the overall objective of this study was to comprehensively investigate the effect of UV treatment conditions for inactivating *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* in fresh-cut lettuce.

2. Materials and methods

2.1. Bacterial strains

All bacterial strains, namely, *E. coli* O157:H7 (ATCC 35150, ATCC 43889, ATCC 43890), *S.* Typhimurium (ATCC 19585, ATCC 43971, DT 104), and *L. monocytogenes* (ATCC 7644, ATCC 19114, ATCC 19115) were obtained from the Department of Food and Animal Biotechnology Culture Collection, Seoul National University (Seoul, Korea). Stock cultures were stored at −80 °C in 0.7 ml of tryptic soy broth (TSB; Difco, Becton Dickinson, Sparks, MD, USA) and 0.3 ml of 50% glycerol. For this study and all experiments, working cultures were streaked onto tryptic soy agar (TSA; Difco), incubated at 37 °C for 24 h, and stored at 4 °C.

2.2. Culture preparation

Each strain of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* was cultured in 5 ml TSB at 37 °C for 24 h, harvested by centrifugation at 4000 ×*g* for 20 min at 4 °C and washed three times with buffered peptone water (BPW; Difco). The final pellets were resuspended in BPW, corresponding to approximately 10^8 to 10^9 CFU/g. Resuspended pellets of each strain of all pathogen species were combined to constitute a 3-pathogen mixed culture cocktail.

2.3. Sample inoculation

Iceberg lettuce was purchased at a local grocery store (Seoul, Korea) and stored at 4 °C. Several layers of outer lettuce leaves were removed and discarded. Intact inner leaves were cut into 5 by 5 cm pieces, washed in sterile distilled water, and placed on sterile aluminum foil. For inoculation, 0.1 ml of the culture cocktail was applied as 15 droplets onto the surface of each sample (ca. 1.5 g) with a micropipettor. The inoculated sample pieces were dried for 2 h inside a biosafety hood with the fan running.

2.4. UV treatment system

The UV radiation apparatus consisted of two banks of 5 germicidal emitting lamps (254 nm, G6T5, Sankyo Denki, Japan) each located in the ceiling and bottom of the radiation vessel (Fig. 1). The UV lamps and treatment apparatus were enclosed in an incubator (IL-11, Lab Companion, Daejeon, Korea). The lamps' positions were adjusted to either increase or decrease intensity. UV intensity was measured using a UV-C light meter (RS-232, Lutron, Taipei, Taiwan) placed in the same location as that of lettuce samples. Prior to use, UV lamps were allowed to stabilize by turning them on for at least 15 min.

2.5. UV treatment

Twenty-five grams of inoculated lettuce leaves was placed on a tray which was 50 cm long and 50 cm wide. The tray consisted of a net (0.01 mm filament size and 1 cm mesh) that minimized blockage of UV-C light. In order to optimize UV-treatment conditions, the following experiments were conducted sequentially: for UV treatment at different temperatures, a constant UV intensity (3.40 mW/cm^2) of the emitting lamps was applied to samples for 1 min at 4 °C or 25 °C. Samples were treated at optimum temperature for 1 min at the lamp to tray distances of 10 or 50 cm with five UV lamps. For examining the effect of type of exposure, inoculated lettuce was treated on one or both sides with 2.72 mW/cm^2 intensity for 0.5, 1, 3, 5, or 10 min at optimum temperature and distance between sample and lamp. Four different UV intensities, 1.36, 2.72, 4.08, or 6.80 mW/cm^2 were applied to samples for 0.5, 1, 3, 5, or 10 min at optimum temperature, distance between sample and lamp, and type of exposure.

2.6. Bacterial enumeration

For enumeration of pathogens, each treated 25 g lettuce sample was immediately transferred into a sterile stomacher bag (Labplas Inc., Sainte-Julie, Quebec, Canada) containing 225 ml of BPW and homogenized with a stomacher (EASY MIX, AES Chemunex, Rennes, France) for 2 min. For all food samples, 1 ml aliquots of stomached samples were serially diluted in 9 ml of BPW, and 0.1 ml of sample or diluent was spread-plated onto each selective medium (*E. coli* O157:H7: Sorbitol MacConkey Agar (SMAC), Difco; *S.* Typhimurium: Xylose Lysine Desoxycholate Agar (XLD), Difco; and *L. monocytogenes*: Oxford Agar Base (OAB) with antimicrobic supplement Bacto™, Difco). When low levels of surviving cells were anticipated, 1 ml of undiluted homogenate was equally divided onto four plates of each medium and spread-plated (detection limit, 10 CFU/g). All agar media were incubated at 37 °C for 24–48 h before counting.

2.7. Color and texture measurement

In order to identify quality changes during storage following UV treatments, samples subjected to treatment conditions found to be optimum for pathogen reduction were packed into a ziplock bag and stored at 4 °C and 57% relative humidity for 7 days. Color change of lettuce was measured at 3 random locations 0, 1, 3, 5, and 7 days following treatment using a Minolta colorimeter (model CR300, Minolta

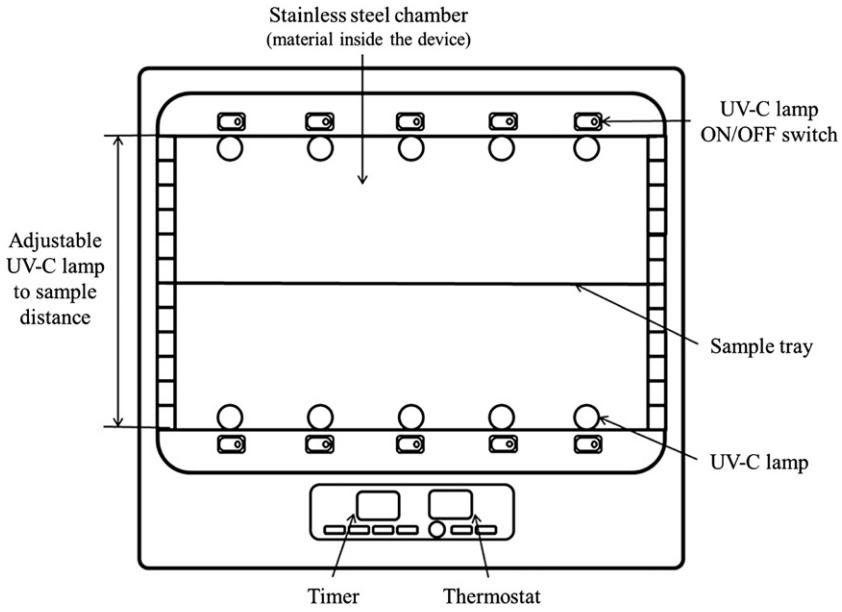

Fig. 1. Schematic diagram of UV radiation apparatus at Seoul National University (Seoul, Korea).

Co., Osaka, Japan) and expressed as L*, a*, and b* values. L*, a*, and b* values indicate color lightness, redness, and yellowness of the sample, respectively.

Changes in texture of UV-treated lettuce were evaluated with a Brookfield texture analyzer (model CT3-10k, Brookfield Engineering Laboratories, Inc., Middleboro, MA, USA) with a blade set probe. Three stacked samples (5 by 5 cm) were placed onto the press holder, and a blade was moved down at 2 mm/s. Maximum shear force was recorded using TexturePro CT software (version 1.2, Brookfield Engineering Laboratories, Inc.). The peak force required to shear the samples was referred as a measure of hardness. These experiments were replicated three times.

2.8. Statistical analysis

All experiments were replicated three times with duplicate samples. Data were analyzed by Statistical Analysis System (SAS Institute, Cary, NC, USA) for analysis of variance and Duncan's multiple range tests to determine significant differences ($P < 0.05$).

3. Results

3.1. Effect of UV-treatment temperature

The survival of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* on fresh-cut lettuce following 1 min of UV treatment is shown in Fig. 2. For *E. coli* O157:H7, there was a significant reduction ($P < 0.05$) of 0.31 log in microbial levels at 4 °C. Also, treatment at 25 °C significantly reduced ($P < 0.05$) levels of this pathogen by 1.45 log CFU/g. The reduction of *E. coli* O157:H7 at 25 °C was more effective compared to 4 °C. Reduction patterns of *S.* Typhimurium and *L. monocytogenes* on fresh-cut lettuce were similar to those of *E. coli* O157:H7. After 1 min, levels of *S.* Typhimurium were reduced by 0.57 and 1.35 log CFU/g when treated at 4 °C and 25 °C, respectively. Reductions of *L. monocytogenes* were 1.16 and 2.12 log CFU/g at 4 °C and 25 °C, respectively.

3.2. Effect of distance between sample and UV lamp

Fig. 3 shows surviving populations of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* treated with five UV lamps at distances of 10 and 50 cm between sample and UV lamp. Both treatments significantly decreased ($P < 0.05$) populations of those pathogens, but the 10 cm distance yielded about a 1 log greater reduction compared to 50 cm. In all pathogens, there were significant differences ($P < 0.05$) of reduction between the two exposure distances.

3.3. Effect of type of UV exposure

Populations of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* on fresh-cut lettuce surfaces treated with one-sided and two-sided UV at 2.72 mW/cm^2 are shown in Table 1. Compared with one-sided treatments, two-sided treatments produced significantly greater population decreases ($P < 0.05$) of *E. coli* O157:H7, resulting in additional reductions of 0.83, 0.56, 0.73, 0.71, and 0.52 log for 0.5, 1, 3, 5, and 10 min treatments, respectively. The patterns of inactivation for *S.* Typhimurium and *L. monocytogenes* were similar to those of *E. coli* O157:H7.

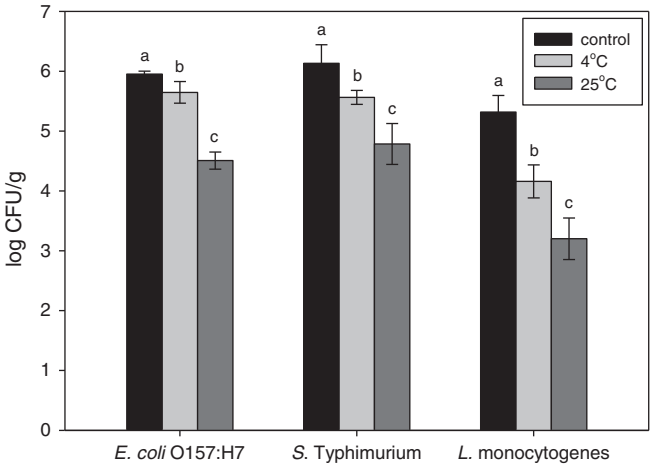

Fig. 2. Surviving populations of *Escherichia coli* O157:H7, *Salmonella* Typhimurium, and *Listeria monocytogenes* on lettuce following UV treatment with single sided exposure at 4 °C or 25 °C, 50 cm distance between sample and lamp, 3.40 mW/cm^2 for 1 min. The results are means from three experiments, and error bars indicate standard errors. † Different letters between treatments within the same pathogen indicate significant differences ($P < 0.05$).

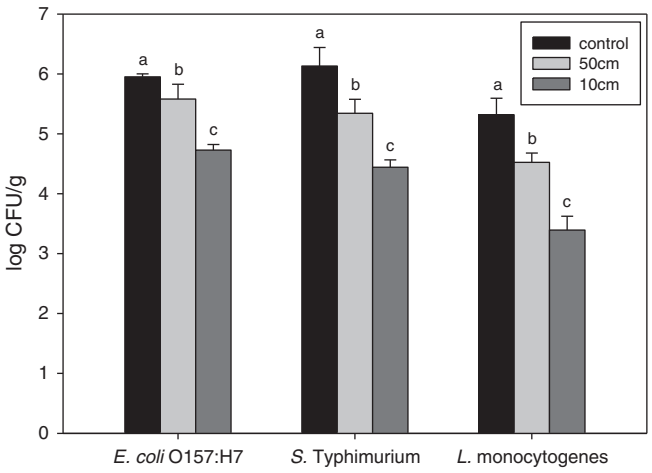

Fig. 3. Surviving populations of *Escherichia coli* O157:H7, *Salmonella* Typhimurium, and *Listeria monocytogenes* after UV-C treatment with single sided exposure of five UV lamps at 10 or 50 cm distance between sample and lamp, 25 °C for 1 min. The results are means from three experiments, and error bars indicate standard errors. † Different letters between treatments within the same pathogen indicate significant differences ($P < 0.05$).

3.4. Effect of UV intensity and exposure time

As UV intensity increased from 1.36 to 6.80 mW/cm^2, populations of the three pathogens experienced greater reduction (Fig. 4). Surviving cells of these pathogens were reduced by >4 log after 10 min exposure when treated with 6.80 mW/cm^2. At 4.08 mW/cm^2, levels of *E. coli* O157:H7 were reduced by 2.01 and 4.03 log CFU/g after 0.5 min and 10 min, respectively. The number of *S.* Typhimurium cells experienced significant reductions of 1.63 log CFU/g after 0.5 min treatment and 3.78 log reduction after 10 min treatment. For *L. monocytogenes*, the reduction was 1.53 log CFU/g after 0.5 min and 4.25 log CFU/g after 10 min. At 2.72 mW/cm^2, reduction patterns of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* were similar. However, UV treatment with 1.36 mW/cm^2 reduced the three pathogens by <3 log after 10 min. These results indicate that higher UV intensity and longer exposure time increase the inactivation of pathogenic bacteria in fresh-cut lettuce.

3.5. Quality changes of UV treated lettuce

Color values of lettuce after UV treatment under optimized conditions (25 °C, 10 cm distance between sample and lamp, two-sided exposure, 6.80 mW/cm^2) are summarized in Table 2. There were no significant ($P > 0.05$) differences in L*, a*, b* values of color measurements for up to 5 min (20.40 kJ/m^2), but there was a significant difference at prolonged treatment time during the entire storage interval. At the end of UV treatment (10 min, 40.80 kJ/m^2), the L* values greatly decreased while a* and b* values significantly ($P < 0.05$) increased. These values changed greatly as storage time increased. Table 3 shows the texture parameters of lettuce following UV treatment. The overall change patterns of texture were similar to those of color. As the treatment time and dose increased, maximum shear loads of UV treated samples were not significantly different ($P > 0.05$) from those of non-treated samples and then significantly ($P < 0.05$) decreased when the treatment time exceeded 5 min (20.40 kJ/m^2).

4. Discussion

In the present study, *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* in fresh-cut lettuce were significantly reduced by UV treatment. Numerous studies have investigated the effect of UV radiation on fresh produce (Gardner and Shama, 2000). Irradiation of fresh vegetables and fruits such as onions, sweet potatoes, tomatoes, strawberries, and peaches has been reported to result in the reduction of postharvest decay (Lu et al., 1987; Stevens et al., 1998, 1999; Maharaj et al., 1999; Marquenie et al., 2002). Although UV irradiation cannot control pathogens internalized through stomata, breaks, and so on due to low penetration, it has been emerging as an alternative for reducing surface contamination. As a potential sterilization technique, more work needs to be documented on the effectiveness of UV radiation for inactivating foodborne pathogens. The effect of UV treatment on foods is affected by UV-treatment temperature, distance between sample and lamp, type of exposure, UV intensity, exposure time, and a few other treatment variables (Halmann and Platzner, 1966; Cha et al., 2010; Allende et al., 2006; Yaun et al., 2004). However, these conditions are typically studied not collectively but individually. Thus, there is a need to investigate the efficacy of various integrated conditions on inactivating pathogenic microorganisms to maximize the effectiveness of UV radiation.

Most of the research on the relationship between UV radiation and temperature has focused on the effect of storage temperature following UV treatment. Lemoine et al. (2007) concluded that UV radiation reduced tissue damage as well as microbial growth of minimally processed broccoli during storage at 4 °C. Similar results were observed in peaches (Gonzalez-Aguilar et al., 2004). Besides post-UV radiation storage temperature, UV-treatment temperature is one of the key conditions. Halmann and Platzner (1966) reported that absorption of light in the far-ultraviolet region by liquid water was dependent upon temperature. In the present study, the reduction of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* in fresh-cut lettuce at 25 °C was significantly higher ($P < 0.05$) than at 4 °C. This is in agreement with the effect of irradiation temperature on inactivation of pathogens. Thayer and Boyd (1995) reasoned that bacterial resistance to gamma radiation was higher at low temperatures due to a decrease in the interactions with radiolytic products of water, which are responsible for

Table 1
Survival of *Escherichia coli* O157:H7, *Salmonella* Typhimurium, and *Listeria monocytogenes* on lettuce after UV treatment with single or double sided exposure at 25 °C, 10 cm distance between sample and lamp, 2.72 mW/cm^2[a].

Treatment time (min)	Population ($\log_{10}$ CFU/g) by organism					
	E. coli O157:H7		*S.* Typhimurium		*L. monocytogenes*	
	One side	Both sides	One side	Both sides	One side	Both sides
0	7.57 ± 0.71 Aa	7.57 ± 0.71 Aa	7.37 ± 0.43 Aa	7.37 ± 0.43 Aa	6.36 ± 0.32 Aa	6.36 ± 0.32 Aa
0.5	7.02 ± 0.50 Aa	6.19 ± 0.32 Bb	6.66 ± 0.30 Ba	5.84 ± 0.17 Bb	5.92 ± 0.33 Aa	5.13 ± 0.39 Bb
1	6.67 ± 0.30 Ba	6.11 ± 0.22 Bb	6.38 ± 0.12 Ba	5.54 ± 0.29 Bb	5.53 ± 0.10 Ba	4.92 ± 0.27 Bb
3	6.51 ± 0.42 Ba	5.78 ± 0.44 Ba	6.17 ± 0.19 Ca	4.78 ± 0.28 Cb	5.36 ± 0.21 Ba	4.56 ± 0.24 Cb
5	6.38 ± 0.12 Ba	5.67 ± 0.30 Cb	5.88 ± 0.36 Ca	4.67 ± 0.19 Cb	4.87 ± 0.34 Ca	4.19 ± 0.35 Cb
10	6.08 ± 0.26 Ca	5.56 ± 0.42 Cb	5.32 ± 0.19 Da	4.45 ± 0.39 Cb	4.80 ± 0.34 Ca	4.21 ± 0.39 Cb

Means with the same uppercase letter in the same column are not significantly different ($P > 0.05$). Means with the same lowercase letter in the same row are not significantly different ($P > 0.05$).
[a] Means ± standard deviations from three replications.

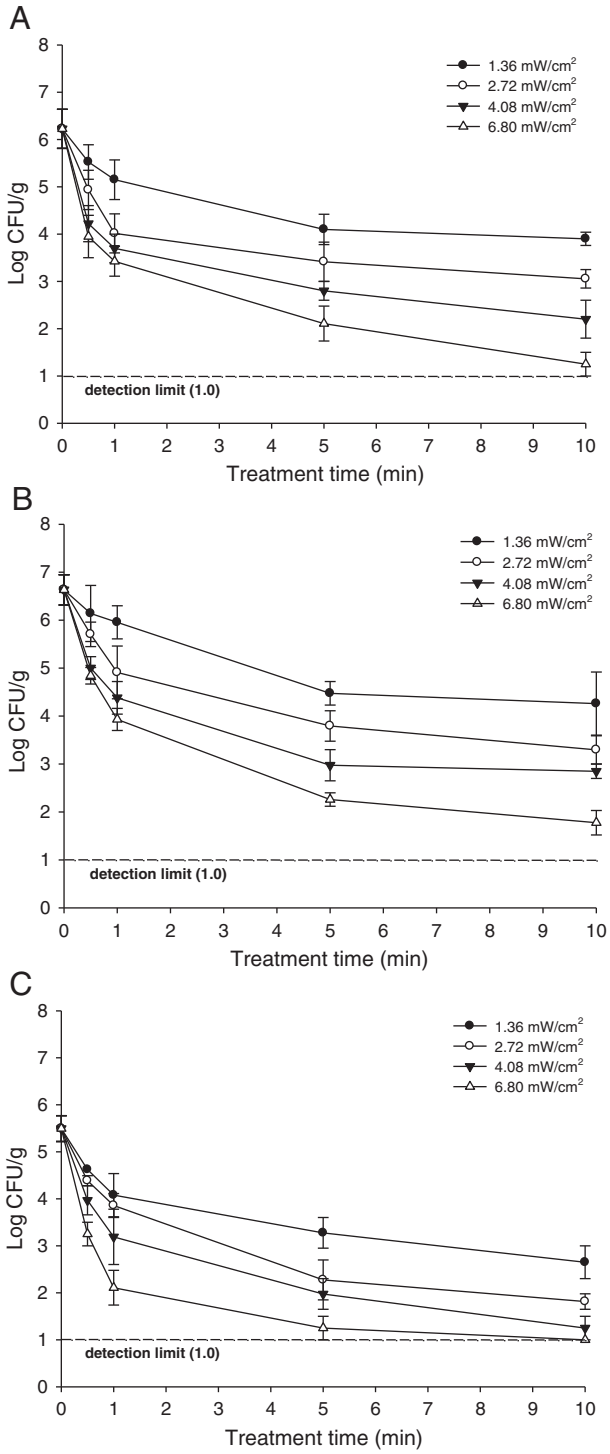

Fig. 4. Survival curves for *Escherichia coli* O157:H7 (A), *Salmonella* Typhimurium (B), and *Listeria monocytogenes* (C) on lettuce subjected to UV with double sided exposure at 1.36 (●), 2.72 (○), 4.08(▼), or 6.80(△) mW/cm^2, 25 °C, 10 cm distance between sample and lamp. The results are means from three experiments, and error bars indicate standard errors.

cellular inactivation. It can apply to temperature-dependent UV radiation, because photochemical reactions can occur as a direct result of UV radiation energy (Koutchma, 2009).

To date, there have been no studies on the inactivation of foodborne pathogens in fresh produce by UV radiation relative to the distance between sample and UV lamp. However, other researchers have conducted studies into the effect of distance on UV radiation. Cha et al. (2010) reported that attenuation of UV intensity in sea water rose as distance from UV lamps increased. This result was similar to our data which showed that reduction of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* at a distance of 10 cm was 1 log greater than achieved at 50 cm. In addition, the effect of type of exposure was observed. There were significant differences ($P < 0.05$) in the inactivation of these pathogens when one-sided and two-sided UV treatments were compared. The latter was more effective than the former, although significant reductions were observed in both treatments. In a study performed by Allende et al. (2006), UV radiation was applied to both sides of fresh-cut lettuce to obtain microbial reductions by using short exposure times and low radiation doses. In short, we found the shorter the distance between sample and UV lamp, and the larger the radiation exposure area at the same UV intensity, the more effective the UV radiation.

UV intensity, a fundamental property of UV light, is another important condition for UV disinfection. We determined that increasing UV intensity and exposure time correlated with increased pathogen reductions in fresh-cut lettuce. These results are in agreement with the equation $D = I \cdot t$ where D = applied dose, I = applied intensity and t = exposure time. Thus, UV dose is directly proportional to the product of UV intensity and exposure time (Environmental Protection Agency, 2006). Moreover, Rice and Ewell (2001) reasoned that high UV intensity over a short period of time provides the same germicidal effect as a lower UV intensity at a proportionally longer period of time. This effect was confirmed by our data which showed similar levels of inactivation with a given UV dose (8.16 kJ/m^2) of 1.36 mW/cm^2 for 10 min, and 2.72 mW/cm^2 for 5 min.

The average bacterial concentrations of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* inoculated on the exposed surfaces of fresh-cut lettuce were 6 log CFU/g. A high-inoculum concentration was used not only to enumerate surviving bacteria easily but also to clearly differentiate the effects of various conditions of UV radiation. Beuchat et al. (2004) concluded that reductions were generally higher as the inoculum level increased. Therefore, in order to guarantee the safety of fresh-cut produce after UV treatment, further investigations to study minimum effective UV doses are required.

In this study, the most effective conditions for pathogen inactivation by UV radiation in fresh-cut lettuce were 25 °C, 10 cm distance between sample and lamp, two-sided exposure, and 6.80 mW/cm^2. Hunter color parameters (L*, a*, and b*) and maximum shear loads were used to describe color and texture changes of lettuce treated under optimized treatment conditions. For up to 5 min equivalent to 20.40 kJ/m^2 of treatment, there were no significant differences ($P > 0.05$) between UV treated and non-treated lettuce among all stored samples. However, both the color and texture properties were adversely affected at the final part of the treatment (10 min, 40.80 kJ/m^2). It has been proposed that overexposure to UV light causes fresh produce to become discolored and softened (Allende et al., 2006; Fonseca and Rushing, 2006). Harvested commodities may tolerate doses up to 20 kJ/m^2 without showing external damage (Stevens et al., 1997). Therefore, our results suggest 5 min as an ideal time of UV treatment under optimized conditions, since it led to very similar pathogen reduction compared to 10 min, but without degrading the sensory properties of lettuce.

Our results indicated that proper UV radiation can be effectively used to inactivate *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* in fresh-cut lettuce without affecting color quality changes. The effects of inactivation are dependent on applied temperature, distance between sample and lamp, direction of lamp, UV intensity and exposure time. With a fuller understanding of the influence of these conditions on inactivating pathogens, UV radiation could be effectively applied to control foodborne pathogens in fresh-cut produce over conventional decontamination methods.

Acknowledgments

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Table 2
Color values of UV treated lettuce stored at 4 °C for 7 days under optimized conditions (25 °C, 10 cm between sample and lamp, two-sided exposure, 6.80 mW/cm)[a].

Parameter[b]	Treatment time (min)	Dose (kJ/m^2)	Storage time (day)				
			0	1	3	5	7
L*	0	0	78.05 ± 1.65 a	78.09 ± 1.36 a	75.53 ± 0.66 a	71.50 ± 0.61 a	71.28 ± 1.08 a
	0.5	2.04	78.51 ± 0.66 a	77.80 ± 0.55 a	76.13 ± 0.76 a	71.38 ± 0.94 a	70.62 ± 0.82 a
	1	4.08	77.74 ± 0.84 a	78.85 ± 1.82 a	76.12 ± 0.60 a	71.78 ± 0.83 a	71.49 ± 1.26 a
	5	20.40	77.12 ± 0.46 a	78.65 ± 1.44 a	75.57 ± 1.04 a	71.24 ± 0.51 a	71.00 ± 0.21 a
	10	40.80	65.58 ± 1.20 b	66.01 ± 0.38 b	61.57 ± 0.64 b	61.45 ± 0.16 b	62.03 ± 0.47 b
a*	0	0	−3.68 ± 0.35 a	−3.42 ± 0.15 b	−3.76 ± 0.25 b	−2.76 ± 0.43 b	−2.64 ± 0.25 b
	0.5	2.04	−3.65 ± 0.23 a	−3.38 ± 0.14 b	−3.65 ± 0.53 b	−2.81 ± 0.11 b	−2.40 ± 0.32 b
	1	4.08	−3.56 ± 0.13 a	−3.22 ± 0.22 b	−3.57 ± 0.25 b	−2.46 ± 0.39 b	−2.49 ± 0.35 b
	5	20.40	−3.55 ± 0.15 a	−3.35 ± 0.16 b	−3.51 ± 0.42 b	−2.46 ± 0.23 b	−2.42 ± 0.29 b
	10	40.80	−3.75 ± 0.32 a	−2.41 ± 0.18 a	−2.31 ± 0.25 a	−0.81 ± 0.29 a	−0.81 ± 0.55 a
b*	0	0	9.29 ± 0.74 b	9.33 ± 0.57 b	11.29 ± 0.78 b	11.26 ± 0.40 b	14.93 ± 0.41 b
	0.5	2.04	9.71 ± 0.12 b	9.47 ± 0.21 b	10.95 ± 0.66 b	11.28 ± 0.33 b	15.25 ± 0.67 b
	1	4.08	9.60 ± 0.21 b	9.46 ± 0.49 b	11.29 ± 0.34 b	11.30 ± 0.37 b	15.22 ± 0.78 b
	5	20.40	9.25 ± 0.68 b	9.55 ± 0.17 b	11.55 ± 0.38 b	11.06 ± 0.77 b	15.09 ± 0.89 b
	10	40.80	11.81 ± 0.76 a	11.94 ± 0.77 a	15.36 ± 0.23 a	20.65 ± 0.31 a	22.19 ± 0.26 a

Values followed by the same letters within the column per parameter are not significantly different ($P > 0.05$).
[a] Means ± standard deviations from three replications.
[b] Color parameters are L* (lightness), a* (redness), b* (yellowness).

Table 3
Maximum load values for texture of lettuce stored at 4 °C for 7 days following UV treatment under optimized conditions (25 °C, 10 cm between sample and lamp, two-sided exposure, 6.80 mW/cm)[a].

Treatment time (min)	Dose (kJ/m^2)	Maximum load (N)[b]				
		Storage time (day)				
		0	1	3	5	7
0	0	53.23 ± 4.44 a	51.11 ± 5.16 ab	54.86 ± 3.86 a	52.55 ± 2.62 a	55.21 ± 5.50 a
0.5	2.04	52.32 ± 3.47 a	49.77 ± 1.24 ab	53.36 ± 2.49 a	51.76 ± 3.84 a	53.08 ± 2.12 ab
1	4.08	50.35 ± 1.88 a	52.03 ± 5.00 a	52.67 ± 2.58 a	49.75 ± 5.06 ab	53.73 ± 2.30 ab
5	20.40	48.86 ± 2.22 a	52.52 ± 3.66 a	52.50 ± 1.88 a	53.23 ± 5.11 a	53.60 ± 3.80 ab
10	40.80	40.22 ± 2.29 b	43.93 ± 3.79 b	43.74 ± 4.19 b	42.81 ± 4.34 b	47.70 ± 1.68 b

Values followed by the same letters within the column per parameter are not significantly different ($P > 0.05$).
[a] Means ± standard deviations from three replications.
[b] Maximum load is load at rupture point.

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References

Allende, A., McEvoy, J.L., Luo, Y., Artes, F., Wang, C.Y., 2006. Effectiveness of two-sided UV-C treatments in inhibiting natural microflora and extending the shelf-life of minimally processed 'red oak leaf' lettuce. Food Microbiology 23, 241–249.

Beuchat, L.R., 1996. Pathogenic microorganisms associated with fresh produce. Journal of Food Protection 59, 204–216.

Beuchat, L.R., Harris, L.J., Ward, T.E., Kajs, T.M., 2001. Development of a proposed standard method for assessing the efficacy of fresh produce sanitizers. Journal of Food Protection 64, 1103–1109.

Beuchat, L.R., Adler, B.B., Lang, M.M., 2004. Efficacy of chlorine and a peroxyacetic acid sanitizer in killing *Listeria monocytogenes* on iceberg and romaine lettuce using simulated commercial processing conditions. Journal of Food Protection 67, 1238–1242.

Bintsis, T., Litopoulou-Tzanetaki, E., Robinson, R.K., 2000. Existing and potential applications of ultraviolet light in the food industry — a critical review. Journal of the Science of Food and Agriculture 80, 637–645.

Centers for Disease Control and Prevention, 2011. Investigation announcement: multistate outbreak of *E. coli* O157:H7 infections linked to romaine lettuce. Available at http://www.cdc.gov/ecoli/2011/ecoliO157/romainelettuce/120711/index.html.

Cha, S.W., Park, D.W., Choi, Y.K., Cheon, S.G., Kil, G.S., 2010. Optimum layout of UV light source for disinfection efficacy improvement. Proceedings of the 36th Korean Society of Marine Engineering Fall Conference, pp. 399–400.

Chang, J.C.H., Ossoff, S.F., Lobe, D.C., Dorfman, M.H., Dumais, C.M., Qualls, R.G., Johnson, J.D., 1985. UV inactivation of pathogenic and indicator microorganisms. Applied and Environmental Microbiology 49, 1361–1365.

Doyle, M.P., Erickson, M.C., 2008. Summer meeting 2007—the problems with fresh produce: an overview. Journal of Applied Microbiology 105, 317–330.

Environmental Protection Agency, 1997. Final report. A set of scientific issues being considered by the agency in connection with the efficacy testing issues concerning public health antimicrobial pesticides. Available at http://www.epa.gov/scipoly/sap/meetings/1997/september/finalsep.htm#3.

Environmental Protection Agency, 2006. Ultraviolet disinfection guidance manual for the final long term 2 enhanced surface water treatment rule. Available at http://www.epa.gov/ogwdw/disinfection/lt2/pdfs/guide_lt2_uvguidance.pdf.

Fonseca, J.M., Rushing, J.W., 2006. Effect of ultraviolet-C light on quality and microbial population of fresh-cut watermelon. Postharvest Biology and Technology 40, 256–261.

Gardner, D.W.M., Shama, G., 2000. Modeling UV-induced inactivation of microorganisms on surfaces. Journal of Food Protection 63, 63–70.

Giese, A.C., 1964. Studies on ultraviolet radiation action upon animal cells. In: Giese, A.C. (Ed.), Photophysiology, 2. Academic Press, New York, pp. 203–245.

Gonzalez-Aguilar, G., Wang, C.Y., Buta, G.J., 2004. UV-C irradiation reduces breakdown and chilling injury of peaches during cold storage. Journal of the Science of Food and Agriculture 84, 415–422.

Halmann, M., Platzner, I., 1966. Temperature dependence of absorption of liquid water in the far-ultraviolet region. The Journal of Physical Chemistry 70, 580–581.

Harm, W., 1980. Biological Effects of Ultraviolet Radiation. Cambridge University Press, Cambridge.

Jacxsens, L., Devlieghere, F., Debevere, J., 2002. Temperature dependence of shelf-life as affected by microbial proliferation and sensory quality of equilibrium modified atmosphere packaged fresh produce. Postharvest Biology and Technology 26, 59–73.

Koutchma, T., 2009. Advances in ultraviolet light technology for non-thermal processing of liquid foods. Food and Bioprocess Technology 2, 138–155.

Lemoine, M.L., Civello, P.M., Martinez, G.A., Chaves, A.R., 2007. Influence of postharvest UV-C treatment on refrigerated storage of minimally processed broccoli (*Brassica oleracea* var. *Italica*). Journal of the Science of Food and Agriculture 87, 1132–1139.

Lu, J.Y., Stevens, C., Yakubu, P., Loretan, P.A., 1987. Gamma, electron beam and ultraviolet radiation on control of storage rots and quality of Walla Walla onions. Journal of Food Processing and Preservation 12, 53–62.

Maharaj, R., Arul, J., Nadeau, P., 1999. Effect of photochemical treatment in the preservation of fresh tomato (*Lycopersicon esculentum* cv. Capello) by delaying senescence. Postharvest Biology and Technology 15, 13–23.

Marquenie, D., Michiels, C.W., Geeraerd, A.H., Schenk, A., Soontjens, C., Van Impe, J.F., Nicolai, B.M., 2002. Using survival analysis to investigate the effect of UV-C and heat treatment on storage rot of strawberry and sweet cherry. International Journal of Food Microbiology 73, 187–196.

Massey, L.M., Hettiarachchy, N.S., Martin, E.M., Ricke, S.C., 2013. Electrostatic spray of food-grade organic acids and plant extract to reduce *Escherichia coli* O157:H7 on fresh-cut cantaloupe cubes. Journal of Food Safety 33, 71–78.

Organic Trade Association, 2009. The organic trade association's 2009 organic industry survey. Available at http://www.ota.com/pics/documents/01a_OTAExecutiveSummary.pdf.

Rice, J.K., Ewell, M., 2001. Examination of peak power dependence in the UV inactivation of bacterial spores. Applied and Environmental Microbiology 67, 5830–5832.

Seymour, I.J., Appleton, H., 2001. Foodborne viruses and fresh produce. Journal of Applied Microbiology 91, 759–773.

Sharma, G., 1999. Ultraviolet light. In: Robinson, R.K., Batt, C., Patel, P. (Eds.), Encyclopedia of Food Microbiology, 3. Academic Press, London, pp. 2208–2214.

Sivapalasingam, S., Friedman, C.R., Cohen, L., Tauxe, R.V., 2004. Fresh produce: a growing cause of outbreaks of foodborne illness in the United States, 1973 through 1997. Journal of Food Protection 67, 2342–2353.

Stevens, C., Khan, V.A., Lu, J.Y., Wilson, C.L., Pusey, P.L., Igwegbe, E.C.K., Kabwe, K., Mafolo, Y., Liu, J., Chalutz, E., Droby, S., 1997. Integration of ultraviolet (UV-C) light with yeast treatment for control of postharvest storage rots of fruits and vegetables. Biological Control 10, 98–103.

Stevens, C., Khan, V.A., Lu, J.Y., Wilson, C.L., Pusey, P.L., Kabwe, M.K., Igwegbe, E.C.K., Chalutz, E., Droby, S., 1998. The germicidal and hormetic effects of UV-C light on reducing brown rot disease and yeast microflora of peaches. Crop Protection 17, 75–84.

Stevens, C., Khan, V.A., Lu, J.Y., Wilson, C.L., Chalutz, E., Droby, S., Kabwe, M.K., Haung, Z., Adeyeye, O., Pusey, L.P., Tang, A.Y.A., 1999. Induced resistance of sweetpotato to Fusarium root rot by UV-C hormesis. Crop Protection 18, 463–470.

Sy, K.V., Murray, M.B., Harrison, M.D., Beuchat, L.R., 2005. Evaluation of gaseous chlorine dioxide as a sanitizer for killing *Salmonella*, *Escherichia coli* O157:H7, *Listeria monocytogenes*, and yeasts and molds on fresh and fresh-cut produce. Journal of Food Protection 68, 1176–1187.

Thayer, D.W., Boyd, G., 1995. Radiation sensitivity of *Listeria monocytogenes* on beef as affected by temperature. Journal of Food Science 60, 237–240.

United States Department of Agriculture, 2007. Amber Waves. Behavioral Factors Influence Food Choices. Available at http://webarchives.cdlib.org/sw1vh5dg3r/http://ers.usda.gov/AmberWaves/June07/PDF/AW_June07.pdf.

Yaun, B.R., Sumner, S.S., Eifert, J.D., Marcy, J.E., 2004. Inhibition of pathogens on fresh produce by ultraviolet energy. International Journal of Food Microbiology 90, 1–8.

Zhuang, R.Y., Beuchat, L.R., Angulo, F.J., 1995. Fate of *Salmonella montevideo* on and in raw tomatoes as affected by temperature and treatment with chlorine. Applied and Environmental Microbiology 61, 2127–2131.