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Fate of *E. coli* O157:H7, *Salmonella* spp. and potential surrogate bacteria on apricot fruit, following exposure to UV-C light ☆

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ABSTRACT

Some soft fruit, such as tree-ripened apricots, cannot be washed with aqueous sanitizers, due to their innate softness and delicate surfaces. In this study, ultraviolet-C (UV-C) light was investigated for its efficacy in inactivating 4–5 individual strains of *Escherichia coli* O157:H7 and *Salmonella* spp. on apricots, in addition to a four-strain composite of Shiga toxin-negative *E. coli* O157:H7 and a cocktail of three attenuated strains of *Salmonella* Typhimurium and *Salmonella* Typhimurium LT2. Also, the survival of *E. coli* O157:H7 and *Salmonella* spp. after exposure to 74 and 442 mJ/cm^2 of UV-C was evaluated during post-UV storage at 2 and 20 °C. The fruit was spot inoculated and the areas (ca. 1.5 cm^2) of fruit surface with the inoculated bacteria were exposed to UV-C at 7.4 mW/cm^2. *E. coli* O157:H7 and *Salmonella* spp. populations decreased rapidly (1–2 logs) ($P < 0.05$) with increasing UV-C doses of 0 to 74 mJ/cm^2. Further increases in UV-C dosage achieved only limited additional reductions in bacterial populations. Shiga toxin-negative bacteria and attenuated *S.* Typhimurium strains, along with *S.* Typhimurium LT2, responded similarly to corresponding pathogenic *E. coli* O157:H7 and *Salmonella* spp. During storage at 2 or 20 °C, populations of pathogenic *E. coli* O157:H7 and *Salmonella* spp. on untreated fruit decreased slowly; however, populations on fruit treated with 442 mJ/cm^2 decreased rapidly at both temperatures. After 8 days at 20 °C or 21 days at 2 °C, *E. coli* O157:H7 and *Salmonella* spp. populations on UV-C treated fruit were at least 2 log CFU/g lower than on non-treated controls. Our results suggest that surface-inoculated bacteria survived poorly following UV-C treatment of apricots.

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1. Introduction

The recent U.S. Food Safety Modernization Act (Food Safety Act) established minimum standards for the safe production and harvesting of fruits and vegetables, based on known safety risks (US-FDA, United States Food and Drug Administration, 2011). Each registered food processing or packing facility/plant is required to conduct a hazard evaluation to identify "known or reasonably foreseeable hazards," including "biological" hazards. Each registered facility is then required to implement preventive controls or provide assurances that the identified hazards will be significantly minimized or prevented. Currently, almost all fresh fruit and vegetable facilities utilize aqueous sanitizers to wash fresh fruits and vegetables. Chlorine (viz., NaOCl) is the most widely used sanitizing agent for fresh produce despite chlorine's limited efficacy (ca. 1–2 log inactivation) in reducing human pathogens on fresh produce (Beuchat et al., 1998; Herdt and Feng, 2009).

Some fruit growers/packers have marketed high-maturity fruit for years to satisfy consumers' increasing demand for high quality/improved-flavor fresh fruit. The so-called "tree ripe" fruits are usually harvested at greater than normal maturity (Crisosto et al., 1995; Crisosto and Valero, 2008). Because of the softness associated with advanced maturity, these fruit do not withstand the rigors of typical commercial packing lines. Washing with aqueous sanitizers (or just water) damages fruit surfaces and shortens their shelf-life. These advanced maturity fruits (e.g., stone fruit, etc.) are packed on small, labor intensive, hand packing lines without the use of water. Implementation of acceptable Food Safety Act control methods for these types of fruit would be a significant challenge. To meet this challenge, non-aqueous sanitization techniques are needed.

Ultraviolet light (UV) is a nonthermal/non-aqueous intervention technology that employs physical light energy of a specific wavelength to inactivate microorganisms. The germicidal effects of UV irradiation (viz., UV-C between 200 and 290 nm) are a result of the introduction of DNA mutations (i.e., thymine dimers) to the bacterial cells induced by the absorption of UV light. The FDA has approved the use of UV light at a wavelength of 254 nm as a disinfectant to treat food (US-

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FDA, United States Food and Drug Administration, 2000). There are more than 400 UV units in commercial use for the pasteurization of apple cider/juice (R. Worobo, personal communication, 2010); however, research on UV treatment of fruits and vegetables regarding inactivation of human pathogens is very limited. Very few studies have investigated the inactivation of human pathogens on stone fruit by UV-C.

The bactericidal effect of UV-C irradiation on cultures of *Salmonella* spp. or *Escherichia coli* O157:H7, inoculated on the surface of red delicious apples, green leaf lettuce and tomatoes, has been investigated (Yaun et al., 2004). *E. coli* O157:H7 inoculated on apples experienced the highest reductions (ca. 3.3 logs) after UV-C irradiation at 24 mW/cm^2 (Yaun et al., 2004). Smaller log reductions were observed on tomatoes inoculated with *Salmonella* (2.19 logs) and on leaf lettuce inoculated with either *Salmonella* spp. or *E. coli* O157:H7 (2.65 and 2.79 logs, respectively). Bialka and Dimirci (2008), using pulsed UV-C, found that *E. coli* O157:H7 and *Salmonella* populations inoculated on raspberries were reduced by 3.9 and 3.4 logs at 72 and 59.2 kJ/m^2, respectively. The maximum reductions obtained on the surfaces of strawberries were 2.1 and 2.8 log CFU/g at 25.7 and 34.2 kJ/m^2, respectively. Similar reductions of pathogens were observed on blueberries (Bialka and Dimirci, 2007), and there was no observable damage to the fruit at these UV doses. Even though there have been studies dealing with UV-C effects on shelf-life and spoilage of peach (Lu et al., 1991; Stevens et al., 1998), the impact of UV-C on pathogenic bacteria inoculated on soft fruit has not been studied. Furthermore, the fate of bacteria following UV-C treatments on these fruits has not been investigated during post-UV storage of fruit.

Using pathogenic bacteria in a laboratory setting offers useful information about the effectiveness of the intervention technology. However, actual human pathogens cannot be used in a food processing establishment for a validation study due to the difficulties of eliminating all pathogens after use. Non-pathogenic surrogate bacteria, with similar responses to specific food processes as the pathogenic bacteria, would offer food processors opportunities to validate a process in-plant, without the use of the actual pathogens. Various non-pathogenic bacteria have been used as surrogates in studies of various intervention technologies (Gurtler et al., 2010; Marshall et al., 2005). There were also studies regarding UV inactivation of surrogate bacteria for *E. coli* O157:H7 in apple cider (Donahue et al., 2004). However, suitable surrogates have not been evaluated for use in studies of response of fruits and vegetables to UV-C treatment.

The objectives of the present study were to investigate the reduction of *E. coli* O157:H7 and *Salmonella* spp. on apricot fruit by UV-C treatment, and to evaluate the survival of *E. coli* O157:H7 and *Salmonella* spp. during post-treatment storage at two different temperatures. In addition, the response of Shiga toxin-negative *E. coli* O157:H7 and attenuated *Salmonella* spp. to UV-C was examined.

2. Materials and methods

2.1. Source of materials

Apricots were obtained from packers in California or South America. Nalidixic acid, rifampicin and ampicillin were purchased from Sigma-Aldrich (St. Louis, MO). Media for growing bacteria were from Difco (Becton Dickinson, Franklin Lakes, NJ).

2.2. Efficacy of UV-C in inactivating pathogenic E. coli O1 57:H7 and Salmonella spp.

2.2.1. Bacterial strains

Five strains of *E. coli* O157:H7 were used: RM 6535 (lettuce), RM 7386 (Romaine lettuce), O6FOO475 (spinach outbreak), RM 1484 (apple juice), and *E. coli* O157:H7 Sakai (sprouts). A cocktail of four *Salmonella* spp. cultures was used: *S.* Newport H1275 (sprout), *S.* Montevideo G4639 (raw tomato), *S.* Stanley HO588 (sprout) and *S.* Saintpaul 02-517-1 (cantaloupe). *Salmonella* spp. and *E. coli* O157:H7 were made resistant to nalidixic acid and rifampicin by successive transfers of the bacteria in Tryptic Soy Broth (TSB) containing increasing concentration of antibiotics to a final level of 100 μg/mL during a ca. 10-day period.

Strains of *Salmonella* spp. and *E. coli* O157:H7 were grown separately in 10 mL TSB at 37 °C with 100 μg/mL of either nalidixic acid (for *Salmonella*) or rifampicin (for *E. coli* O157:H7) for 24 h. After centrifuging, pellets were washed with 10 mL peptone water. Cultures of each representative pathogen type were aseptically combined to produce a cocktail of the four or five strains.

2.2.2. Inoculation of apricots

Apricots were spot-inoculated onto the cheek area of the fruit with 100 μL aliquots (i.e., 10 droplets) of the strain cocktails of *Salmonella* or *E. coli* O157:H7 using a micropipette. The area (ca. 1.5 cm^2) of fruit with the inocula was marked with an indelible pen for easy recovery of the pathogens following treatments. Inoculation levels on fruit were ca. 10^7–10^8 CFU/fruit. Fruit was dried for 2–3 h in a biohood. There were 3 fruits for each treatment, and experiments were repeated three times.

2.2.3. UV-C treatment

Inoculated apricots were placed into a custom-built UV treatment chamber containing two 0.61-m 55 W UV-C emitting bulbs (SaniLIGHTTM, Atlantic Ultraviolet, White Plains, NY). All fruits were positioned with the inoculated surfaces facing upwards towards the UV-C tubes so that the inoculated areas on fruit surface received full UV exposure. The treatment times were 0 (control), 2, 5, 10, 20, 40, 60, 90, 120 and 180 s (UV-C intensity, ca. 7.4 mW/cm^2). UV-C light was stabilized by turning it on for 10 min before treating the apricots. UV treatment was performed at ambient temperature (~23 °C). The distance between the UV-C light bulbs and the surface of the apricots was approximately 12.5 cm. The UVC (at 254-nm wavelength) intensity at the same distance as the fruit was measured using a UVX-25 radiometer (UVP Inc., Upland, CA, USA), and the UV dose was calculated with the following equation: UV dose (mJ/cm^2) = irradiance (mW/cm^2) × exposure time (s).

2.2.4. Recovery of Salmonella and E. coli O157:H7

After UV-C treatment, the fruit skin with the inoculum was excised using a pair of surface-sterilized scissors. The skins given the same treatment time were combined (total weight: ~3 g) and placed into 80 mL stomacher bags. Twenty milliliters of buffered peptone water (pH = 7.1) was added to each bag and homogenized using a stomacher circulator (Panoramic™, Neutec Group Inc., Farmingdale, NY) for 1.5 min.

2.2.5. Enumeration of Salmonella and E. coli O157:H7

Fifty microliter aliquots of each of the samples were spiral-plated onto two Tryptic Soy Agar (TSA) plates containing 100 μg/mL nalidixic acid (TSAN) and two xylose lysine tergitol-4 (XLT-4) plates for *Salmonella*, as well as two TSA plates containing 100 μg/mL rifampicin (TSAR) and two Sorbitol MacConkey Agar (SMAC) plates for *E. coli* O157:H7. Plates were incubated at 37 °C for 24 h before colonies were counted. Injured cells were estimated by comparing the difference in cell populations between non-selective and selective media (Wu, 2008; Gurtler et al., 2010) as follows:

$$\text{Injured cells}(\%) = (P_{UV-TSA} - P_{UV-S})/P_{UV-TSA} \times 100 - (P_{NUV-TSA} - P_{NUV-S})/P_{NUV} \times 100,$$

where $P_{UV\text{-}TSA}$ is the population of bacteria treated with UV-C and enumerated on TSA; $P_{UV\text{-}S}$ is the population of bacteria treated with UV-C and enumerated on a selective medium;

$P_{NUV\text{-}TSA}$ is the population of bacteria not treated with UV-C and enumerated on TSA; and $P_{NUV\text{-}S}$ is the population of bacteria not treated with UV-C and enumerated on a selective medium.

The calculation deducted the injury that occurred in the non-treated samples. Therefore the injury was solely due to the UV-C treatment.

2.3. UV-C sensitivity of attenuated E. coli O157:H7 and S. Typhimurium

A cocktail of 4 strains of Shiga toxin-negative *E. coli* O157:H7 included 6980-2, 6982-2, CV267 and ATCC 700728. ATCC# 700728 (strain BDMS T4169), purchased from American Type Culture Collection (Manassas, VA, USA), does not have *stx*1 and *stx*2 genes but has the single base mismatch at +93 in the *uid*A gene, characteristic of *E. coli* O157:H7 (Moyne et al., 2011).

Four strains of *S.* Typhimurium were used in the study including three attenuated strains of *S.* Typhimurium (χ 3985, χ 4096 and χ 8089) and one strain of non-pathogenic *S.* Typhimurium (ATCC# 700720, LT2 strain). The LT2 strain was classified by ATCC as biosafety level (BSL) 1 when it was purchased; however, the strain has recently been re-classified by ATCC as a BSL 2 bacterium. The nature of attenuated *Salmonella* strains has been previously described (Gurtler et al., 2010; Yun et al., 2013).

Individual *S.* Typhimurium strains (if not already resistant to nalidixic acid) were made nalidixic acid-resistant by successive transfers into TSB with increasing concentrations of nalidixic acid to a final concentration of 100 μg/mL over 10 days. ATCC# 700728 was made ampicillin resistant by a $CaCl_2$ shock method (Sambrook et al., 1989).

The procedures for growing bacterial cultures, as well as inoculation and recovery were the same as described above. Inoculated apricots were treated with UV for 0 (control), 2, 5, 10, 20, 30 and 60 s at UV intensity of ca. 7.4 mW/cm^2 at an ambient temperature (ca. 23 °C). Aliquots (100 μL) of each of the dilutions (10^0 to 10^5) of recovered bacteria were spread-plated on TSA containing 100 μg/mL nalidixic acid (TSAN) and XLT-4 (for *Salmonella*) as well as TSA containing 100 μg/mL ampicillin (TSAA) and SMAC (for *E. coli* O157:H7). Plates were incubated for 24 h at 37 °C and colonies were counted.

2.4. Determination of survival and growth of pathogens during post-UV storage

Cocktails of the four pathogenic *Salmonella* spp. and five *E. coli* O157:H7 strains mentioned earlier were prepared and used to inoculate apricots as described above. Inoculated apricots were treated with UV-C for 0 (control), 10, and 60 s (corresponding UV-C doses of 0, 74 and 442 mJ/cm^2). Following treatment, fruit was stored at 2 °C for 21 days as well as at 20 °C for 8 days. Populations of *Salmonella* spp. and *E. coli* O157:H7 were recovered from apricots on days 1, 3, and 8 at 20 °C and on days 1, 3, 8, 11 and 21 at 2 °C during storage, followed by enumeration on two TSA plates containing 100 μg/mL nalidixic acid (TSAN) and two XLT-4 plates for *Salmonella*, as well as two TSA plates containing 100 μg/mL rifampicin (TSAR) and two SMAC plates for *E. coli* O157:H7. Colonies on plates were counted after incubating for 24 h at 37 °C.

2.5. Scanning electron microscopy (SEM)

Fruit was inoculated with a cocktail of five strains of *E. coli* O157:H7. After UV-C treatment, fruit skins (ca. 1 cm^2 in area and 1 mm in thickness) were excised with a sterile stainless steel razor blade and immersed in ca. 20 mL of a 2.5% glutaraldehyde — 0.1 M imidazole buffer solution (pH 7.2). Samples were then washed in 0.1 M imidazole buffer, and dehydrated by immersing in 20 mL ethanol solutions (50%, 80%, absolute ethanol). The samples were then fractured in liquid nitrogen with the aid of a liquid nitrogen cooled razor blade. Mounted samples were sputter-coated with gold using a Scancoat Six Sputter Coater (BOC Edwards, MA, USA) and digitally imaged using a model Quanta 200 FEG scanning electron microscope (FEI Co., Inc., Hillsboro, OR., USA) operated in the high vacuum and secondary electron imaging mode.

2.6. Experimental design and statistical analysis

Experiments were repeated three times. The experimental design for the storage study was a completely randomized block design with three blocks. One batch of fruit from California was broken into three independent groups (blocks), and each group was further divided into eight subgroups. Each subgroup had 3 fruit placed on a rectangular plastic container. The fruit in the container (6 fruits each container) was spot-inoculated with bacteria as described earlier (Section 2.4), treated with UV-C and stored at two different temperatures. On each of the four sampling days, three fruits for each UV-C dose/storage temperature/replicate were taken out for bacterial analysis. Data were subjected to the General Linear Model (GLM) procedure using SAS ver. 9.12 (SAS Institute, Raleigh, NC, USA). When the microbial population fell below the detection limit, a random number between 0 and detection limit was generated using the SAS program prior to being subjected to statistical analysis. The effects of UV dose and storage duration were analyzed using GLM, and treatment differences were compared using the least significant difference (LSD) method at $P = 0.05$. The nonlinear regression and curve fitting were conducted using Sigmaplot, Window Version 11.0 (Systat Software Inc. Chicago, IL, USA).

3. Results and discussion

3.1. Efficacy of UV-C for inactivating pathogenic E. coli O1 57:H7 and Salmonella spp.

The population of *E. coli* O157:H7 decreased with increasing UV-C dose (Fig. 1). As UV dose increased from 0 to 74 mJ/cm^2, there was a rapid decrease in *E. coli* O157:H7 population. The population continued to decrease linearly with further increases in UV dose, but at a much slower rate. Approximately 1.0 and 1.2 log CFU/fruit reductions of *E. coli* O157:H7 were achieved after treatments with 15 and 74 mJ/cm^2 (2 and 10 s) of UV-C, respectively, while increasing the UV-C dose from 74 to 1326 mJ/cm^2 only achieved an additional ca. 1 log reduction. At the dose of 442 mJ/cm^2, approximately 1.9 log reduction of *E. coli* was achieved. Comparisons of data for *E. coli* O157:H7 enumerated on TSA vs. SMAC show that cells injured by UV-C represented less than 3% of the total population of bacterial cells recovered from the apricots treated with UV-C, suggesting that almost all cells affected by UV-C were actually inactivated, rather than simply being injured.

The response of *Salmonella* spp. to different doses of UV-C was similar to that of *E. coli* O157:H7, showing a rapid decrease in population at the low range (0 to 74 mJ/cm^2) of UV-C dose, followed by a slower linear decrease at higher doses (Fig. 1). Approximately 0.9 and 1.5 log CFU/fruit reductions of *Salmonella* spp. were achieved after being treated with 15 and 74 mJ/cm^2 of UV-C, respectively. As doses increased from 74 to 1326 mJ/cm^2, only 1 additional log reduction was achieved. At 442 mJ/cm^2, population of *Salmonella* spp. was reduced by 1.8 log CFU/fruit. Less than 4% of cells were injured with treatment doses from 15 to 884 mJ/cm^2, while UV-C at 1326 mJ/cm^2 injured 9% of cells.

Populations of *E. coli* O157:H7 and *Salmonella* spp. could be expressed as a function of UV-C dose, using the following exponential decay function with R^2 values higher than 0.95:

$$p = a * e^{-bx} + c * e^{-dx}$$

where p is the population of bacteria (log CFU/fruit), x is UV-C dose (mJ/cm^2), and a, b, c and d are regression coefficients. Bialka et al. (2008) found that survival curves of *E. coli* O157:H7 and *Salmonella*

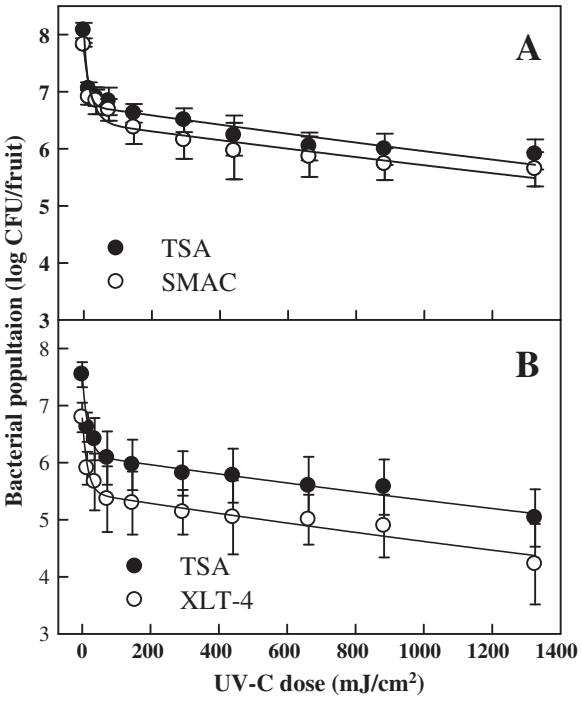

Fig. 1. Populations of toxigenic *E. coli* O157:H7 (A) and *Salmonella* spp. (B) on apricots treated with different doses UV-C. *E. coli* was enumerated on TSA and SMAC while *Salmonella* spp. was enumerated on TSA and XLT-4. Vertical bars represent standard deviations.

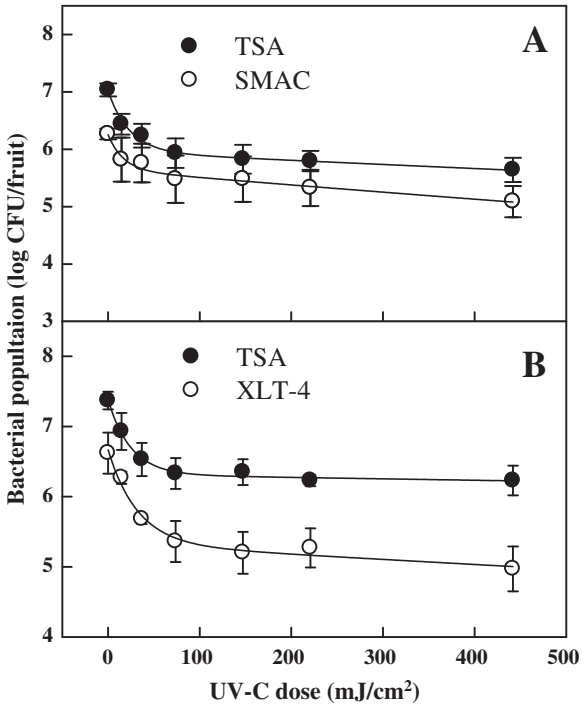

Fig. 2. Populations of Shiga toxin-negative *E. coli* O157:H7 (A) and attenuated *Salmonella* Typhimurium (B) on apricots treated with different doses of UV-C. *E. coli* was enumerated on TSA and SMAC while *Salmonella* was enumerated on TSA and XLT-4. Vertical bars represent standard deviations.

enterica on blueberries and strawberries, following UV-C treatment, were not linear and the Weibull model better described the curves with correlation coefficients of 0.83–0.99. Our data could also be described by the Weibull model with the doses required to achieve 90% reduction of *E. coli* O157:H7 to be 18 and 29 mJ/cm^2 on TSA and SMAC, respectively (data not shown). The doses required to reduce *Salmonella* population by 90% were estimated to be 17 and 22 mJ/cm^2 on TSA and XLT-4, respectively.

Our present results suggest that UV-C could reduce population of *E. coli* O157:H7 and *Salmonella* spp. by 0.9–2.5 log CFU/fruit, the two genera of bacteria showing a similar response to UV-C. Allende et al. (2006) reported that, in an in vitro test of various bacterial strains, *E. coli*, was inhibited with a UV-C dose of 30 J m^{-2}, while *S.* Typhimurium was among the most resistant strains, requiring a UV-C dose of 85 J m^{-2} to completely inhibit growth. The effectiveness of UV-C in inactivating bacteria depends on UV-C intensity, structure, and topography of the surface of the food product (Gardner and Shama, 2000).

3.2. UV-C sensitivity of attenuated E. coli O1 57:H7 and S. Typhimurium

Showing a similar response to treatment as that of pathogenic *E. coli* O157:H7, Shiga toxin-negative *E. coli* O157:H7 was inactivated by 0.6 and 1.1 logs/fruit (on TSA media) after UV-C treatments at doses of 15 and 74 mJ/cm^2, respectively (Fig. 2). Increasing doses from 74 to 442 mJ/cm^2 only produced an additional 0.3–0.4 log reduction of the bacterium, and the injured cells in UV-C-treated samples represented less than 8% of total cell population.

The population of attenuated *S.* Typhimurium decreased as UV dose increased from 0 to 74 mJ/cm^2 (0 to 10 s) (Fig. 2). Further increases in UV doses did not increase the reduction of *S.* Typhimurium. The population of *Salmonella*, when assessed on XLT-4 agar, was reduced by 0.4 and 1.3 log/fruit after 15 to 74 mJ/cm^2 UV-C treatment. Similar reductions (0.4 and 1.1 logs) were achieved when assessed on TSA. The cells injured by UV-C represented a small percentage ($\leq$10%) of total population of *Salmonella*.

3.3. Scanning electron microscopy of E. coli O157:H7 on apricot surface

Trichomes with different lengths, stomata and cuticle waxes were observed on apricot surfaces (Fig. 3). Wax deposition on the surface of apricots formed ridging and cracks (gaps). *E. coli* O157:H7 was not found on the trichomes. Instead, the bacteria were attached on the epidermic cuticle around trichome bases. Some bacteria were found inside the stomata, and many were located in the cracks/gaps between the ridging of cuticle wax. The results suggest that UV-C light may be blocked by the surface structures on the apricot surface, preventing UV-C exposure of all bacteria. Our results suggested that some bacteria may be internalized in stomata and other protective areas, which may render UV-C less effective as UV-C with low penetration ability cannot reach to the bacteria. Previously studies on other types of fresh produce have suggested that pathogens may be internalized through various portals of entry including stomata, lenticels, trichomes, wounds, area of decay and stem scars (Ryser et al., 2009). The internalization minimizes pathogen exposure to chemical sanitizers and reduces the effectiveness of the sanitizers.

Our results show that all bacteria tested exhibited exponential decay curves in response to increasing UV-C doses (i.e. rapid decreases in population at lower UV-C range [0–74 mJ/cm^2]), and slow decreases at higher UV-C dose ranges. Murakami et al. (2006) found that the *E. coli* K12 inactivation in malate buffer containing 5 g/mL of suspended solids could be interpreted as being made up of two linear plots; the slope for the first linear plot (lower dose range, 0–13 mJ/cm^2) was 0.24 log reduction per each mJ/cm^2, while the slope of the second linear plot (higher than 13 mJ/cm^2) was 0.06. This phenomenon may be explained as the exposure of two groups of the same bacteria to the UV-C dose, where the first group of bacteria was located at a more exposed attachment site and received a higher dose of UV-C. The second group of bacteria, located in more protected sites such as the interior of a biofilm, experienced a shadowing effect, resulting in their receiving lower doses of radiation. This shielding could also occur due to fruit surface structures, as there were many trichomes and stomata capable of

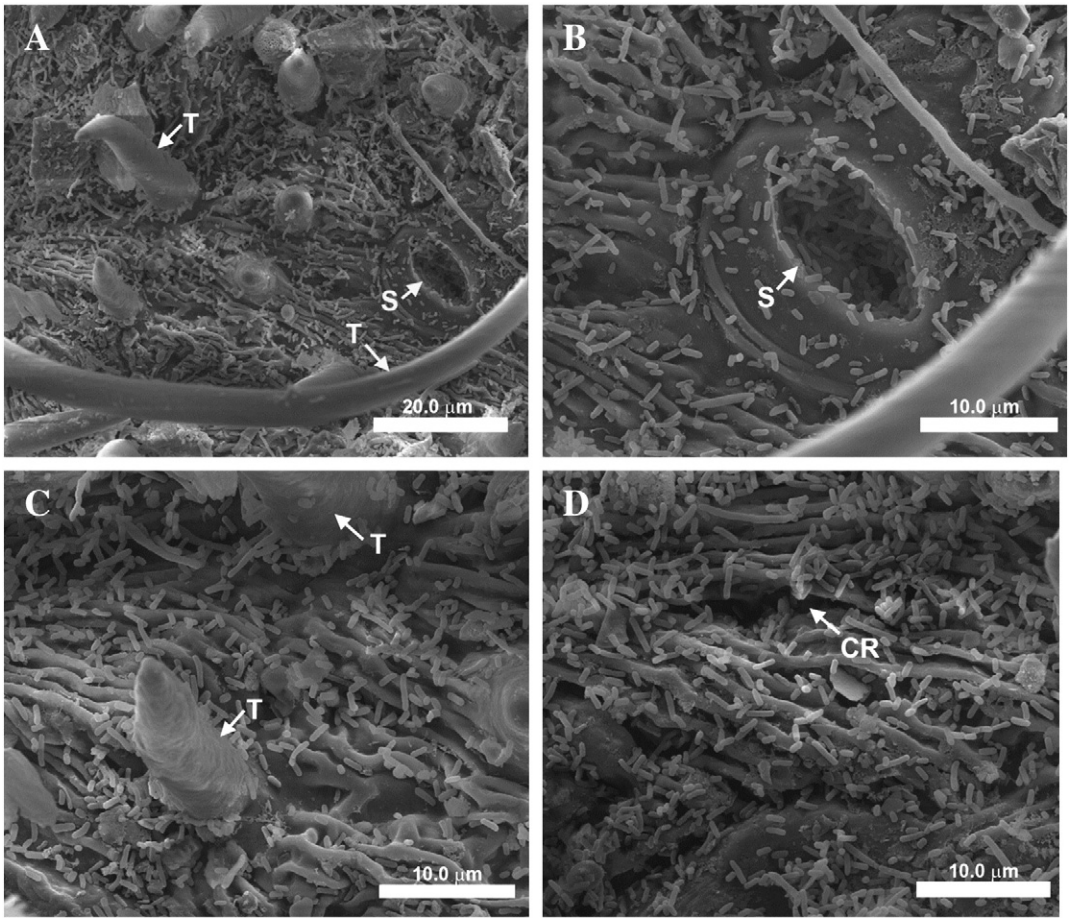

Fig. 3. Scanning electron microscope images of apricot surface with inoculated *E. coli* O157:H7. T:trichome, S:stomata, CR:cracks.

eliciting this phenomenon (Fig. 3) on the surface of apricot fruit. Another explanation is that there may be heterogeneity among the bacterial population, where a proportion of bacterial cells is more resistant to UV-C than other portions of the same bacteria.

Weibull modeling indicated that the t_R value (UV-C dose required to achieve the first log reduction) and the β value (shape parameter of the reduction–dose curve) (Bialka et al., 2008) were not significant ($P > 0.05$) different between pathogenic bacteria and attenuated bacteria (data not shown). Therefore, it appears that the attenuated bacteria were similar as the corresponding pathogenic bacteria, suggesting that Shiga toxin-negative bacteria and the mixture of attenuated *S.* Typhimurium may be used as surrogates for corresponding pathogenic *E. coli* O157:H7 and *Salmonella* spp. Food processing establishments should ensure that the introduction of non-pathogenic cultures does not create unsanitary conditions in the facility or cause the food to become adulterated. In the present study, Shiga toxin-negative *E. coli* O157:H7 and attenuated *S.* Typhimurium/LT2 were used. Even though detection of the presence of Shiga toxin-negative *E. coli* O157:H7 would not automatically be considered as adulterated, to avoid possible confusion between Shiga toxin-positive and negative *E. coli* O157:H7, the use of generic *E. coli* may be tested. It should also be emphasized that the LT2 strain is recently re-classified by ATCC as a BSL-2 bacterium, as a result, the bacterium should not be used in a commercial processing facility.

3.4. Changes in E. coli O157:H7 and Salmonella spp. on apricots during storage at two different temperatures

Populations of *E. coli* O157:H7 inoculated on apricots were reduced by 0.8–1.2 log CFU/fruit, as measured on the day of the UV-C treatments (Fig. 4). Populations on all apricots decreased during storage at 2 °C. Samples treated with 74 mJ/cm^2 of UV-C had reductions similar to the non-treated controls, while *E. coli* populations on apricots treated with 442 mJ/cm^2 (60-s) UV-C decreased much more rapidly following UV-C treatments. After 21 days of storage, *E. coli* populations on fruit treated with 442 mJ/cm^2 UV-C were 3.5 log CFU/fruit lower than those on the non-treated control.

During 8 days of storage at 20 °C, the population of *E. coli* O157:H7 on non-treated apricots, as well as those treated with 74 mJ/cm^2 (10 s) UV-C, experienced similar bacterial reductions (Fig. 5); nevertheless, at the end of 8 days of storage at 20 °C, more than 6 log CFU/fruit remained on the non-treated fruit. Populations of *E. coli* O157:H7 on the apricot treated with 442 mJ/cm^2 UV-C decreased rapidly during storage, especially between 4 and 8 days. After 8 days of storage, *E. coli* O157:H7 populations on the fruit, treated with 442 mJ/cm^2 UV-C, were 3.3 and 4.2 log CFU/fruit lower than the non-treated fruit, when enumerated on TSA and SMAC, respectively.

Salmonella populations on all apricots decreased during storage at 2 °C (Fig. 6); however, populations on fruit treated with the two doses of UV-C decreased much more rapidly during post-UV storage. Approximately 1 log CFU of *Salmonella* per fruit was inactivated on non-treated fruit during 21 days of storage, while reductions on fruit treated with 74 and 442 mJ/cm^2 were 2–3 log and >4.5 log CFU/fruit, respectively. On the day of UV treatment, UV-C treatments at 74 and 442 mJ/cm^2 reduced *Salmonella* spp. by 0.7 and 1.0 log CFU/fruit, respectively, when assessed on TSA, and by1.4 and 1.5 log CFU/fruit, respectively, on XLT-4 medium. However, after 21 days of storage at 2 °C, populations on fruit treated with 74 and 442 mJ/cm^2 UV-C were 2.4–3.1 and >4 log CFU/fruit, respectively, lower than those from non-treated control.

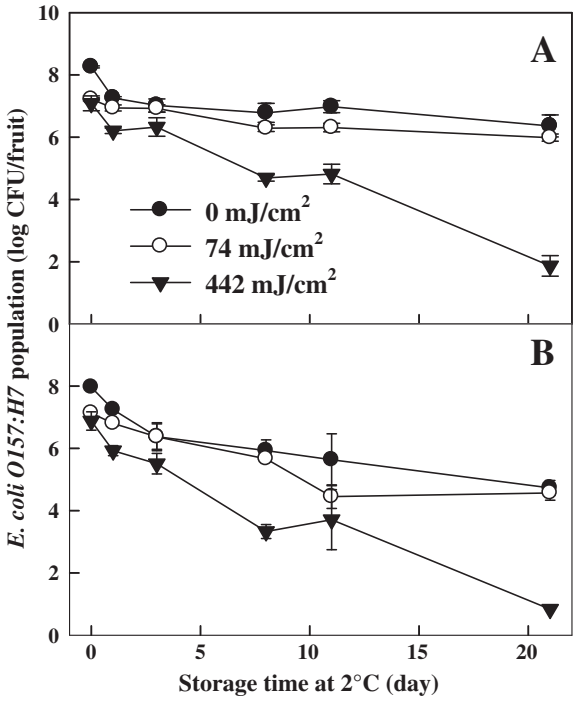

Fig. 4. Changes in populations of *E. coli* O157:H7 on apricots during storage at 2 °C. Apricots were inoculated with a cocktail of five strains of *E. coli* O157:H7, followed by UV treatments at doses of 0, 74 mJ/cm^2 and 442 mJ/cm^2. The population of survival *E. coli* on apricots was recovered and enumerated using (A) TSA with 100 μg/mL rifampicin, and (B) SMAC during storage. Vertical bars represent standard deviations. Detection limit was 1.2 log CFU/fruit.

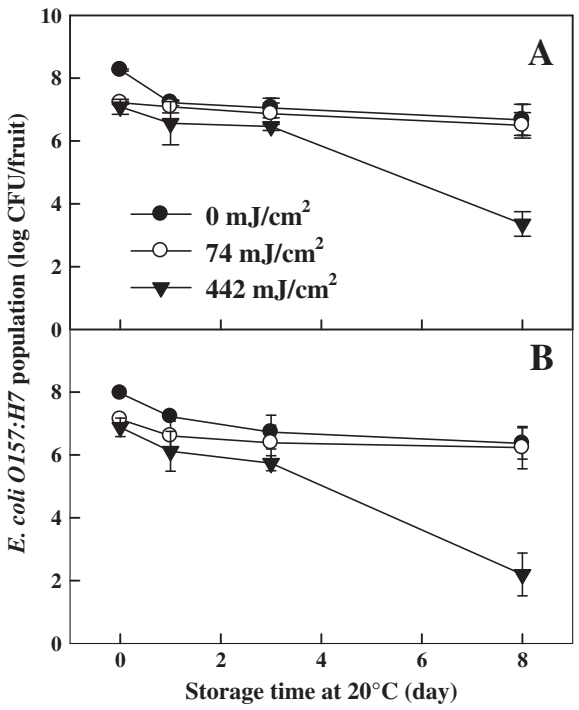

Fig. 5. Changes in populations of *E. coli* O157:H7 on apricots during storage at 20 °C. Apricots were inoculated with a cocktail of five strains of *E. coli* O157:H7 followed by UV treatments at doses of 0, 74 mJ/cm^2 and 442 mJ/cm^2. *E. coli* on apricots was recovered and enumerated using (A) TSA with 100 μg/mL rifampicin, and (B) SMAC. Vertical bars represent standard deviations. Detection limit was 1.2 log CFU/fruit.

When apricot fruit was stored at 20 °C, *Salmonella* populations on apricots also decreased; however, the reduction of *Salmonella* populations on fruit treated with 74 mJ/cm^2 was the lowest during storage (Fig. 7). Even though UV-C treatment at 74 mJ/cm^2 reduced *Salmonella* populations by 0.7 and 1.4 log CFU/fruit when enumerated on TSA and XLT-4, respectively, in comparison with non-treated controls on the day of treatment, there was no significant difference between non-treated controls and fruit treated with 74 mJ/cm^2 after 8 days at 20 °C. On the other hand, *Salmonella* populations on fruit treated with 442 mJ/cm^2 UV-C decreased rapidly during storage. Following 8 days at 20 °C, populations of *Salmonella* on fruit treated with 442 mJ/cm^2 were 2 and 4 log CFU/fruit lower than those from the non-treated fruit enumerated on TSA and XLT-4 media, respectively.

Our results indicate that *E. coli* and *Salmonella* survived well on non-treated apricots during storage either at 2 °C or 20 °C. More than 5 log CFU/fruit of *E. coli* and *Salmonella* spp. remained on the fruit after 8 days of storage at 20 °C or 21 days at 2 °C. Following UV-C treatment, particularly at a dose of 442 mJ/cm^2, bacteria on apricots decreased more rapidly than those on non-treated fruit during post-UV storage at either 2 °C or 20 °C. Escalona et al. (2010) found that UV-C radiation at doses of 2.4–24 mJ/cm^2 reduced *S. enterica* loads during the first 4 days of a 14-day storage study at 5 °C, after which, the bacterium increased significantly on radiated leaves when compared to the control. The same authors (Escalona et al., 2010) also found that UV treatment (2.4–24 kJ/m^2) reduced *Listeria monocytogenes* populations by 2 logs on cut spinach. However, after 14 days of storage at 4 °C, the control reached 4.2–4.7 log CFU/g, while UV-C-treated leaves increased to 3.6–4.5 log CFU/g, indicating that the pathogen grew faster on UV-treated leaves than on the control. The dose used in our present study was much higher than those used by Escalona et al. (2010). Our results demonstrated that a lower dose of UV (i.e., 74 mJ/cm^2) increased reductions of bacterial populations only for *Salmonella* spp. stored at 2 °C. Higher doses of UV-C (i.e., 442 mJ/cm^2) increased lethality of UV-C during storage, regardless of storage temperature. Sommers et al. (2009) found that *L. monocytogenes* levels on UV-C-treated (1 J/cm^2) frankfurters that contained potassium lactate and sodium diacetate decreased much more rapidly than those on non-UVC-treated frankfurter samples during 8 weeks of refrigerated storage.

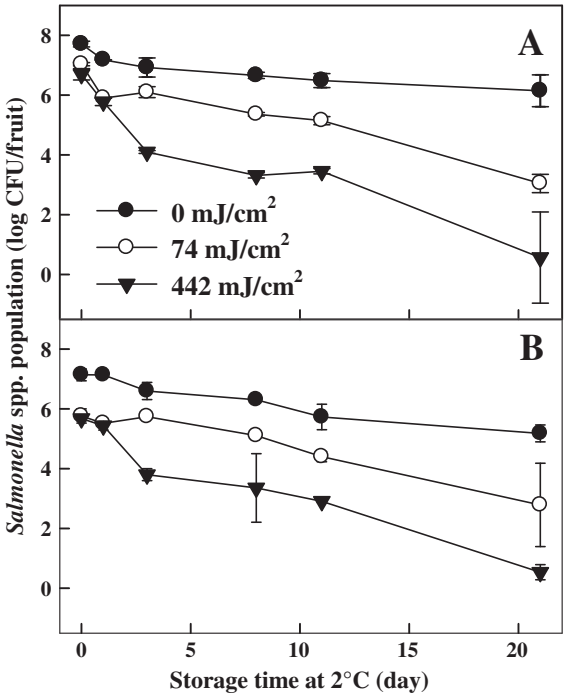

Fig. 6. Changes in populations of *Salmonella* spp. on apricots during storage at 2 °C. Apricots were inoculated with a cocktail of four strains of *Salmonella* spp. followed by UV treatments at doses of 0, 74 mJ/cm^2 and 442 mJ/cm^2. *Salmonella* spp. on apricots was recovered and enumerated using (A) TSA with 100 μg/mL nalidixic acid, and (B) XLT-4 during storage. Vertical bars represent standard deviations. Detection limit was 1.2 log CFU/fruit.

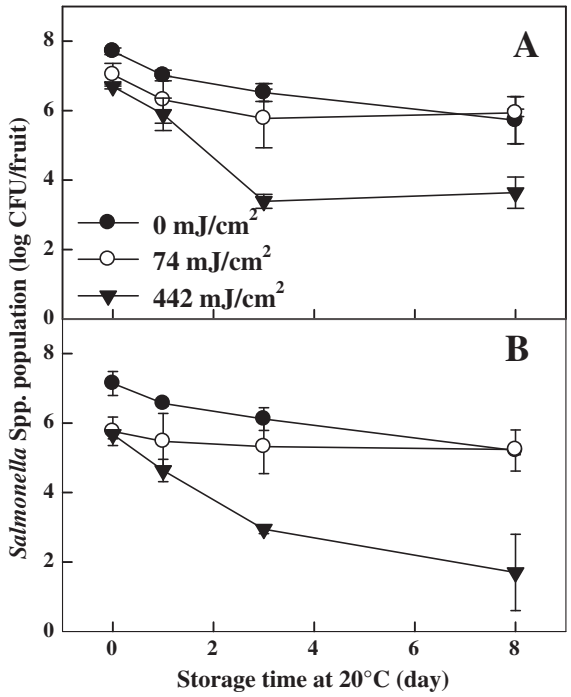

Fig. 7. Changes in population of *Salmonella* spp. on apricots during storage at 20 °C. Apricots were inoculated with a cocktail of four strains of *Salmonella* spp. followed by UV treatments at doses of 0, 74 mJ/cm^2 and 442 mJ/cm^2. *Salmonella* spp. on apricots was recovered and enumerated using (A) TSA with 100 μg/mL nalidixic acid, and (B) XLT-4 during storage. Vertical bars represent standard deviations. Detection limit was 1.2 log CFU/fruit.

Even though higher UV-C (442 mJ/cm^2) produced only marginal reductions of *E. coli* and *Salmonella*, when compared with the lower UV-C treatment (74 mJ/cm^2), differences in bacterial population between treatments were magnified during storage. The rapid decreases in population of *E. coli* O157:H7 and *Salmonella* on UV-C-treated fruit during post-UV-C storage may be attributed to the hormesis phenomenon, i.e. UV-C elicits a range of biochemical responses in fresh produce ranging from production of phytoalexin compounds to delays in ripening (Shama, 2007; Stevens et al., 1998). Studies are needed to explore the mechanism. Our results also reveal that reductions in the numbers of *E. coli* and *Salmonella* enumerated on selective media (XLT-4 and SMAC) were slightly lower than those recovered on non-selective media, indicating that some bacteria were injured by UV-C. However, the UV-C-injured cells were less than 10% of the total bacterial population, indicating that the injury could not explain the rapid decrease in bacterial populations that occurred on fruit treated with 442 mJ/cm^2 during post-UV-C storage. Therefore, either UV-C-induced chemical changes, occurring on the surface of apricots, inhibited the survival of bacteria during post-UV storage, or the subtle injury caused by UV-C was not detectable using selective media. Perhaps a study could be conducted using stainless steel or some other non-biotic materials to investigate whether similar reductions occurred as on apricots during the post-UV period.

Under the U.S. Food Safety Modernization Act, all food processors, including producers of fresh produce, must identify potential hazards and employ control measures to minimize bio-hazards. Our results suggest that UV-C could achieve ca. 1–2 log reductions of *E. coli* O157:H7 and *Salmonella* spp. within few seconds of treatments, which was similar or more effective than chlorine wash (Beuchat et al., 1998; Zhuang et al., 1995). In addition, bacterial populations, following UV-C treatment, decreased rapidly during storage. Our results suggest that UV-C may be used in combination with other control practices such as GAPs and GMPs, to enhance the microbial safety of the soft fruit.

Overall, our results suggest that UV-C at a dose of 74 mJ/cm^2 could achieve 0.9–1.5 log reductions of *Salmonella spp.* and *E. coli* O157:H7 on apricots. Higher doses of UV-C achieved limited additional reductions. However, a higher dose (442 mJ/cm^2) of UV-C resulted in a rapid decline in the populations of the two human pathogens on apricots during post-UV storage at 2 or 20 °C.

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