Inactivation of *Escherichia coli* Population on Fruit Surfaces Using Ultraviolet-C Light: Influence of Fruit Surface Characteristics

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Financial Support:

This research work was funded, in part, with a grant from Pear Bureau Northwest.

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Abstract

Ultraviolet-C (UV-C 254 nm) light is a possible alternative for chemical disinfection of fresh fruits. However, studies on the influence of surface characteristics on the kinetics of UV-C inactivation of microorganisms on fruits are limited. In this study, UV-C inactivation of generic *Escherichia coli* (ATCC 23716), a nonpathogenic surrogate strain for *E. coli* O157:H7, was inoculated onto the skin surface intact pear, pear with surface wounds and the skin surface of intact peach. Disc shaped (0.057 m diameter x 0.01 m height) fruit surface were exposed at room temperature to UV-C light ranging from 0 to 7.56±0.52 kJ/m^2 and microbial inactivation kinetics determined. Maximum reductions of 3.70±0.125 log CFU/g were achieved for *E. coli* on intact pear surfaces ($P < 0.05$), with lesser reduction on wounded pear (3.10±0.329 log CFU/g) and peach surfaces (2.91±0.284 log CFU/g) after 4 minutes UV-C exposure at 7.56 kJ/m^2 UV. The Weibull scale factor (α) values of UV-C inactivation for *E. coli* on an intact pear surface was 0.001±0.0007 min (0.235±0.001 kJ/m^2), wounded pear surface, 0.003±0.001 min (0.240±0.002 kJ/m^2) and peach surface, 0.001±0.0007 (0.235±0.001 kJ/m^2). The time required for a 90% reduction in *E. coli* cell numbers or the reliable life time (t_R) calculated with the Weibull model for intact pear surfaces (0.019±0.009 min, 0.268±0.017 kJ/m^2) was smaller than for wounded pear (0.062±0.013 min, 0.348±0.024 kJ/m^2) and peach surfaces (0.074±0.012, 0.371±0.022 kJ/m^2), suggesting that the wounds on pear surfaces and trichomes (100-1000 μm) on peach surfaces helped to shield and protect microorganisms from UV-C radiation. There was likely a more uniform distribution of bacterial cells onto pear surfaces due to its smaller surface roughness, spreading coefficient, and hydrophobic nature compared to peach. Fourier transform infrared (FT-IR) spectroscopy indicate that bacterial membrane damage (phospholipids, protein secondary structures and polysaccharides) and changes to DNA/RNA in *E. coli* resulted from UV-C treatment. UV-C can reduce *E. coli* populations on fresh fruit surfaces but the efficacy of

UV treatment is dependent upon the morphological and surface properties of the fruit and surface integrity.

Key words: Atomic force microscopy, FT-IR spectroscopy, Scanning Electron Microscopy, surface disinfection, surface morphology

Introduction

Chemical sanitizers such as hypochlorite solution can leave a chemical residue (Beuchat et al. 1998) on the fruit surface and may not be effective (Sapers, 2001). Alternatives to chemical treatment for surface sanitation of fresh fruits and vegetables have been studied (Novak et al. 2008; Bialka and Demirci, 2007) including ultraviolet light (200 and 280 nm (UV-C), more specifically, at 254 nm) which can be effective for microbial inactivation on fruits and vegetable surfaces (Table 1) (Bintsis et al., 2000; Gonzalez-Aguilar et al. 2001; Cia et al. 2007; Allende and Artes, 2003; Erkan et al. 2001). However, kinetic parameters of UV-C inactivation of microorganisms on different food surfaces are not reported and comparative data for different fruit surfaces subjected to the same treatment are not readily available. UV-C has been approved by FDA for the inactivation of microorganisms on food product surfaces and reduction of microorganisms in juice products (US-FDA, 2011).

The efficacy of surface disinfection by UV-C on fruit surfaces is influenced by several factors including: UV-C dose (J/m^2), UV-C dose rate (W/m^2), exposure time (s), surface characteristics, and initial bacterial inoculum level (Otto et al. 2011) and bacterial type. Since UV-C light has limited penetration depth, plant morphological characteristics such as roughness and presence of wounds on fruit surfaces impact microbial inactivation (Wong et al. 1998; Woodling and Moraru, 2005; Schenk et al. 2008); understanding these influences is needed if this technology is

to be commercialized. However, little information is available on the influence of fruit surface properties on the efficacy of UV-C for surface decontamination.

The established microbial inactivation mechanism by UV-C exposure is DNA dimer formation (Cutler and Zimmerman, 2011). Further, indirect photochemical effects such as free radical formation may also induce ultra-structural changes (Cutler and Zimmerman, 2011). Biochemical and morphological changes to bacteria from exposure to continuous UV-C in food matrices is not well understood. However recent experiments with Fourier transform infrared (FT-IR) spectroscopy examined ultra-structural and chemical changes in microorganisms exposed to continuous UV-C showing changes to microbial cell membrane composition, for example in a study of *Cronobacter sakazakii* in dry infant formula following treatment with UV radiation (Liu et al., 2012). Infrared spectral features reflect the biochemical compositions of cell wall and cell membranes and can elucidate both the nature and degree of microbial cell injury (Lu et al., 2011a-d).

The objectives of this study were to determine how surface properties of fruit affect UV-C inactivation of generic *E. coli* and investigate the type and degree of cell injury using morphological, physical and spectroscopic methods.

Materials and Methods

Treatment surfaces and target microorganism

Fresh D'Anjou pears and O'Henry peaches were purchased from a local retail store (Dissmore's IGA, Pullman, WA) during July to September 2011, and stored at 4 ^{o}C for less than 2 weeks before conducting the experiments.

The National Advisory Committee on the Microbiological Criteria for Foods recommends the use of surrogate microorganisms in place of pathogens in pilot plant studies involving food (Gurtler et al. 2010). Therefore, in the current work, a generic *Escherichia coli* (ATCC 23716) strain obtained from School of Food Science, Washington State University was selected as a surrogate microorganism due to safety concerns. Since the main purpose of this research was not to design/validate an inactivation process but to understand the influence of fruit surface morphology and physical characteristics on bacterial inactivation by UV-C, use of a surrogate was appropriate. This microbe has been used as a surrogate for *E. coli* O157:H7 in many thermal and non-thermal process studies (Yuk et al. 2009; Geveke and Brunkhorst, 2008; Jin et al. 2008) including UV-C inactivation studies on egg white (Geveke, 2008).

Inoculum preparation

The *E. coli* culture was stored in 30% (wt/wt) glycerol (20% water v/v) at -80 ^{o}C in tryptic soy broth (TSB, Hardy Diagnostics, Santa Maria, CA). Frozen cultures were activated by two successive passages, first inoculating 0.1 ml in 9 ml of TSB and incubated at 37 ^{o}C for 18 to 24 hours. Then one milliliter of the inoculum was added to 100ml of TSB and incubated at 37 ^{o}C for 18 to 24 hour. This stationary phase culture served as a stock culture for inoculation of fruit surfaces. The average initial inoculum level in all the experiments was $4.5\pm1.2\times10^{9}$ CFU/ml.

Fruit surface preparation

Fresh whole pears or peaches were washed with distilled water. The fruits were then air dried inside a biological safety cabinet for 0.5-1 h at room temperature to remove surface moisture. A sharpened, ethanol sterilized stainless steel cutting disc and knives were used to slice axial section of the pears and peaches into 0.057 m diameter and approximately 0.01 m thick discs (approx. 30 g) leaving the peel on. Each fruit disc was kept on the sterile Petri dishes with the

peel surface facing up. A sterile needle was used to wound surfaces of pear slices (one wound per fruit disc, in the equatorial zone; 2 mm diameter and 1 mm depth. The stock culture was mixed vigorously by hand 25 times in a 30 cm arc, then 0.5 ml of the *E. coli* ATCC 23716 culture was aseptically and uniformly inoculated onto the peel surfaces of pear and peach fruits and wounded region of pear slices. UV-C treatments were carried out approximately 10 minutes after inoculation.

Ultraviolet-C treatment

The ultraviolet -C treatment of the fruit discs were carried out inside a UVC Emitter™ Table-top System (Reyco Systems, Meridian ID) at a wavelength of 254 nm at room temperature. This equipment consists of a motorized roller conveyor (base) placed below an array of four 110 V 16-inch Steril-Aire™ UVC Emitters™ mounted in a stainless steel hood (0.45×0.30 m). The height of these arrays above the base was adjustable from 0.05 to 0.2 m height above the base. The UVC array was comprised of four Steril-Aire™ 16SE food-grade, shatter resistant, sleeved UVC Emitters™ mounted in bulk head fittings. For the current experiments, the UVC emitters were adjusted to 0.1 m above the fruit disks during irradiation treatments The UV power was measured using a UV radiometer (EIT UVICURE PLUS II, EIT, Inc., Sterling, VA, USA). Based upon the preliminary experiments, specific UV doses of 0.59±0.07, 1.14±0.08, 2.16±0.16, 4.00±0.33, 5.71±0.26, and 7.56±0.52 kJ/m^2 corresponding to 0.25, 0.5, 1, 2, 3, and 4 min were selected to treat the inoculated pear and peach discs at the center of the UV-C chamber. A digital timer was used to control the UV exposure times. The temperature of the chamber was 23 ^{o}C as monitored using a digital thermometer and no change in the temperature was observed during the time of UV-C exposure used in this study. Inoculated and non irradiated fruit discs were used as

control. Sample preparation and UV-C treatment was conducted inside a Class II laminar hood to avoid post irradiation contamination.

Microbial cell enumeration

After the UV-C treatments, the fruit discs were aseptically transferred into separate sterilized stomacher bags containing 100 ml of sterile 0.1% peptone water (Becton, Dickinson and Co., Cockeysville, MD). The samples were blended (Stomacher® 400 CIRCULATOR, Seward Laboratory Systems Inc. Port Saint Lucie, FL, USA) for 3 minutes. A one ml portion of the supernatant were aseptically transferred into 9 ml 0.1% peptone water and serial dilutions prepared, with 0.1 ml sample spread plated on tryptic soy agar (TSA, Hardy Diagnostics, Santa Maria, CA) in triplicate. Agar plates were incubated for 24±2h at 37 ºC and colony forming units (CFU) counted. Each experiment was repeated at least three times. The initial level of colony forming units on pear and peach surfaces were approximately $2.05\pm1.07\times10^{8}$ CFU/g for fruit slices.

UV-C inactivation kinetics

The Weibull equation has a shape factor and hence it is more flexible in describing microbial inactivation kinetics (Cunha et al. 1998) and is used to model microbial, enzymatic and other degradation reactions in foods (Odriozola-Serrano et al. 2009; Cunha et al. 1998). The Weibull equation is

$$N = N_o \exp\left[-\left(\frac{t}{\alpha}\right)^{\gamma}\right] \quad (1)$$

Where N is the number of surviving bacteria after time t, N_o is the initial concentration of the microorganism, α is the scale factor and γ is the shape parameter determining the shape of the

curve. The value of $\gamma > 1$ yields a survival curve with a convex shape; while a value of $\gamma < 1$ yields a curve with a concave shape which indicates higher microbial resistance. When $\gamma = 1$, the Weibull model is equivalent to the 1st order model. The values of α and γ are determined by non-linear optimization. The reliable life time (t_R), estimated from Weibull parameters (α and γ), is the time required for 90% reduction in the number of target microorganism (Van Boekel, 2002) and is similar to decimal reduction time (D value). The value of t_R can be estimated from

$$t_R = \alpha \left(2.303\right)^{\frac{1}{\gamma}} \quad (2)$$

where α is the scale factor and γ is the shape parameter of the Weibull equation.

Characterization of fruit surfaces

Microscopy Techniques

Environmental Scanning Electron Microscopy (ESEM): The fresh pear and peach fruit samples were cut from their outside surfaces into 2-3 mm slices with a stainless steel razor and analyzed using an environmental scanning electron microscope (ESEM) (Quanta 200 ESEM, FEI Co. [Field Emission Instrument], Hillsboro, OR) with magnifications from 100 to 800. At least 2 to 3 slices from different parts of each fruit sample were taken for surface morphology analysis (N=3) and micrographs generated (Leica Microsystems Inc., Buffalo Grove, IL, USA).

Atomic Force Microscopy (AFM): To determine surface roughness, external fruit surfaces of 1 cm^2 were mounted to AFM sample disks (Hershko et al. 1998; Yang et al. 2005) and measurements taken using a Veeco Multimode Picoforce coupled with NanoScope IIIa controller, a 3× 3 μm^2 J-scanner and a silicon cantilever. The resonance frequency was 200–300 kHz and nominal spring constant was 40 N/m respectively with a scan rate of 1.5 Hz. The

integral and proportional gains were 0.3 and 0.5, respectively. At least 3 different locations on each fruit peel surface were imaged (N=2).

Contact angle and surface energy determination: Contact angle is a measure of surface hydrophobicity and was conducted using a sessile drop method using a face contact angle set-up equipped with a camera (VCA Optima, AST Products Inc., MA, USA) (Bernard et al., 2011). External skin sections from pear and peach of approximately 2 × 2 cm^2 and 1 mm thickness were cut with a sharp knife. Small drops (0.5–1.0 µl) of a polar liquid (double-distilled water) or a nonpolar liquid (diiodomethane (99% purity; Sigma-Aldrich) were deposited onto the fruit surfaces using a microliter syringe and a 0.5-mm diameter needle at room temperature (23 ^oC). Twenty data points were taken for each fruit sample (N=20). Side-view images were captured using a camera and the contact angles between the drops and the surfaces were calculated.

Surface energy calculation: The surface energy of the fruit peel surfaces was determined from contact angle measurements using Fowkes' equation (Ribeiro et al. 2007; Bernard et al., 2011):

$$W_a = \gamma_L(1+\cos\theta) = 2\left(\sqrt{\gamma_L^d \gamma_S^d}\right) + \sqrt{\gamma_L^p \gamma_S^p} \quad (3)$$

$$W_c = 2\gamma_L \quad (4)$$

$$W_s = W_a - W_c \quad (5)$$

W_a = Reversible work of adhesion (mN/m)

γ_L = Surface enrgy of the liquid (mN/m)

θ = contact angle between solid and liquid

γ_L^d = Dispersion component of the surface energy of the liquid (mN/m)

γ_S^d = Dispersion component of the surface energy of the solid (mN/m)

γ_L^p = Polar component of the surface energy of the liquid (mN/m)

γ_S^p = Polar component of the surface energy of the solid (mN/m)

W_c = Cohesion coefficient (mN/m)

W_s = Spreading coefficient (mN/m)

W_a is related to spreading or adhesion of the liquid on the solid surface while W_c is the cohesion of liquid molecules causing contraction (Ribeiro et al. 2007). The spreading coefficient (W_s), also known as wettability, is related to spreading of liquid on the solid surface (Ribeiro et al. 2007).

Microscopy for E. coli ATCC 23716

Environmental Scanning Electron Microscopy (ESEM): Following UV treatment, (0, 2.16 and 7.56 kJ/m^2 UV doses) the fruit discs were aseptically transferred into separate sterilized stomacher bags containing 100 ml of sterile 0.1% peptone water. The samples were blended for 3 minutes. Bacterial samples were fixed with glutaraldehyde and osmium tetroxide, then rinsed using 0.1 M phosphate buffer and dehydrated with ethanol/water in increasing concentrations of ethanol (30%, 70%, 95%, 100%). After dehydration, the bacteria samples were sputter coated with gold and examined (Machado et al. 2010) using an environmental scanning electron microscope (Quanta 200 ESEM, FEI Co [Field Emission Instruments], Hillsboro, OR).

FT-IR spectroscopy

After UV-C treatment (0 and 7.56 kJ/m^2 UV doses) and homogenization by stomacher, FT-IR spectra were taken on the supernatant (10 mL) . Spectral interference from food matrices is the challenge with this technique and filtration as previously described (Liu et al., 2012, Lu et al., 2011a) was used in the current study. Supernatant was filtered through a 10.0 µm pore size

polycarbonate membrane filter (K99CP04700; GE Water & Process Technologies, Trevose, PA) and then through a 0.2 μm pore size aluminum oxide membrane filter (25 mm diameter, Anodisc, Whatman Inc., Clifton, NJ) under vacuum to harvest bacterial cells. The anodisc membrane filter was removed from the Whatman vacuum filtration apparatus (Whatman Catalog number 1960-032) and air dried under laminar flow at room temperature for 30 min yielding a homogeneous film of bacterial cells (Lin et al., 2004).

A Nicolet 380 FT-IR spectrometer (Thermo Electron Inc., San Jose, California) was used to collect spectral features of recovered bacteria. The aluminum oxide membrane filter coated with a layer of bacterial cells was placed in direct contact with the diamond crystal cell of attenuated total reflectance (ATR) detector. FT-IR spectral features were recorded at the wavenumbers of 4002 to 399 cm^{-1} with a spectral resolution of 8 cm^{-1} and each spectrum was added together by 32 interferograms. Eight spectra were acquired for untreated and UV-C treated *E. coli* at different locations on the aluminum oxide membrane filter for a total of 24 spectra for each group of bacterial cells (N=3).

Spectroscopic based chemometric analyses

FT-IR spectra were automatic baseline corrected following a smooth of Gaussian function of 9.463 cm^{-1}. The processed spectra were read by Matlab (Math Works Inc., Natick, MA). The spectral reproducibility was determined by calculating D_{y1y2} according to the procedure of Liu et al. (2012). Second derivative transformations (with a gap value of 12 cm^{-1}) were conducted to magnify the visualization of minor differences among raw spectra (Lu et al., 2011c). Two different types of chemometric models were established to segregate untreated and UV-C treated samples based upon the spectral features between 1800 cm^{-1} to 900 cm^{-1} ("fingerprint" region).

Principal component analysis (PCA), an unsupervised chemometric method, was used to generate a two dimensional model for segregation of different samples into distinct clusters (Lu et al., 2010). Hierarchical cluster analysis is a supervised chemometric method using prior knowledge (*i.e.*, sample name) to create a dendrogram for category differentiation (Lu et al., 2011a-d).

Statistical analysis

The data for inactivation of *E. coli* by UV-C were analyzed for statistical significance using SAS 9.1 (SAS Institute, Inc., Cary, NC). A value of $P < 0.05$ was selected as statistically significant using the Two-Way ANOVA by Fisher's Least Significant Difference (LSD) method. UV-C treatment time and type of surface were the two factors considered for the Two-Way ANOVA analysis. Further, One-way ANOVA by Fisher's Least Significant Difference (LSD) method was also performed when no interaction between UV-C treatment time and type of surface was found *E. coli* inactivation rate. Further, we conducted contrast test to determine the statistical significance in log reductions between surfaces at each UV-C exposure time individually.

Results and Discussion

UV inactivation kinetics of E. coli on fruit surface

The average population of *E. coli* on fruit surfaces prior to UV-C treatment was $2.1\pm1.1\times10^8$ CFU/g. UV-C treatment significantly reduced the number of *E. coli* on intact pear skin, wounded pear skin and peach skin surfaces ($P < 0.05$) (Figure 1). No significant interaction between treatment surface and time was observed ($P \geq 0.05$). Cell numbers decreased significantly during the first 2 minutes of treatment by 3.59±0.096 log CFU/g for intact pear surfaces and 2.60±0.069 to 2.50±0.151 log CFU/g for *E. coli* cells on the wounded pear skin and intact peach skin. UV-C was relatively ineffective after 2 min and no significant difference in *E. coli* inactivation was

observed between 2 min (4.00±0.33 kJ/m^2) ($P \geq 0.05$) and 4 min (7.56±0.52 kJ/m^2) treatments for all three surfaces. The difference in log reductions in *E. coli* population between intact pear and peach surfaces was not significant ($P \geq 0.05$) after 15 and 30 seconds of UV exposure. However, at higher exposure times beyond 30 seconds, there was a significant difference in log reduction among three selected surfaces. UV-C was most effective on intact pear surface with a 3.70±0.125 log CFU/g reduction observed after 4 min treatment time. The log reduction of *E. coli* on pear surface was significantly higher that that of peach and wounded pear surface after 4 min ($P < 0.05$) reflecting the physical protection from UV light on bacterial cells within the damaged pear tissue due to poor UV-C penetration and the protective effect of the hair-like projections (trichomes) on the surface of the peach. Inactivation of *E. coli* was lower for wounded pear and peach surfaces compared to intact pear surfaces. No significant difference in *E. coli* inactivation by UV-C was found for wounded pear and peach surfaces ($P \geq 0.05$).

A number of earlier studies have been conducted to determine the effectiveness of UV irradiation on microbial control on food surfaces (Table 1). In general, UV can be effective and potentially more effective than chemical sanitizers. Inactivation of *E. coli* in foods appears to be predominantly from a nonthermal effect (Geveke et al. 2008). At equivalent UV-C intensities, the log reduction in the population of *E. coli* in egg white increased from 1.63 to 2.48 log CFU/g when the temperature increased from 30 to 50 ^{o}C (Geveke et al. 2008). However, the effect of temperature was negligible as only 0.13 log CFU/g reduction in the *E. coli* population was observed at 50 ^{o}C without UV energy (Geveke et al. 2008). Yaun et al. (2004) used UV light to reduce the population of *Salmonella* spp. and *E. coli* O157:H7 on leaf lettuce, tomato and apples surfaces and found that UV-C was more effective against these foodborne pathogens than 20-320 ppm chlorine (Yaun et al. 2004). Schenk et al. (2008) reported inactivation (2.6 to 3.4 log CFU/g

reduction) in the populations of *Listeria innocua*, *Listeria monocytogenes*, *E. coli* and *Zygosaccharomyces bailii* on pear slices without peel with lower reduction ranging from 1.8 to 2.5 log CFU/g on pear slices with the peel attached. In studies with other food products, UV has been found to provide greater inactivation of microbes on the surface rather than in the underlying tissue. UV light at 20 mW/cm^2 reduced the population of *E. coli* O157:H7 between 1.53-2.14 log CFU/g on blueberry calyx and 3.11-5.53 log CFU/g on blueberry skin following 1-10 min treatments (Kim and Hung, 2012) and was more effective than electrolyzed water and ozone inactivating *E. coli* O157:H7 (Kim and Hung, 2012) (Table 1). Manzocco et al. (2011) reported reductions in *Enterobacteriaceae* between 1.65-2.14 log CFU/g on fresh cut melon cubes exposed to UV-C light. However, a direct comparison between the inactivation rates of similar/different microorganisms on different/similar surfaces respectively may not be possible, as the survival of microorganisms depends upon several other factors such as type of strain, initial inoculums level, surface characteristics, and growth conditions (Guerrero-Beltran and Barbosa-Canovas, 2004).

The *E. coli* inactivation kinetics by UV-C treatment on intact pear, wounded pear and peach surfaces (Figure 1) fitted a non-linear Weibull model (R^2 = 0.99). The α (0.003±0.001 min, 0.240±0.002 kJ/m^2) values and reliable life time (t_R = 0.062±0.013 min, 0.348±0.024 kJ/m^2) of UV-C inactivation kinetics of *E. coli* on the wounded pear surface were significantly greater than ($P < 0.05$) those of intact pear surface (α = 0.001±0.0007 min, 0.235±0.001 kJ/m^2), t_R = 0.019±0.009 min (0.269±0.017 kJ/m^2) (Table 2). Further, no significant difference in the values of α (0.004±0.0004 min, 0.241±0.0008 kJ/m^2) and reliable life time (t_R = 0.074±0.012 min, 0.371±0.022 kJ/m^2) for UV-C inactivation kinetics of *E. coli* were observed between peach and wounded pear surfaces ($P \geq 0.05$). Further, the α values and reliable life time (t_R) of UV-C

inactivation kinetics of *E. coli* on peach surface were significantly greater than that of intact pear surface ($P < 0.05$). This may indicate that the population of *E. coli* may have differing susceptibility to UV-C exposure over time (Van Boekel, 2002) but more likely indicates that the penetration of UV radiation may not be sufficient to target cells entrained within the interstitial spaces of plant tissue. UV effectiveness is matrix dependent. Chun et al. (2009) reported Weibull scale factor (α) values for the UV-inactivation of *L. monocytogenes*, *S. enterica* Typhimurium, and *C. jejuni* on agar plates were 0.78 J/m^2, 0.82 J/m^2, 0.78 J/m^2, respectively where the reliable life time (t_R) values were 2.48 J/m^2, 2.39 J/m^2 and 2.18 J/m^2, respectively.

Surface characteristics of fruits

The surface characteristics of fruits may influence the effectiveness of UV-C inactivation of *E. coli*. Environmental scanning electron microscopy (ESEM) shows surface characteristics of intact pear (Figure 2A), wounded pear (Figure 2B) and peach (Figure 2C). The greater survival of *E. coli* on wounded pear and peach surfaces could be attributed to the shielding of microbes by the wounds on pear surfaces and the trichomes on peach surfaces (Figure 3). Trichomes are approximately 100 - 1000 μm, being 10 - 100 times larger than the *E. coli* cells.

Food surface properties such as hydrophobicity, electric charge and roughness may influence the adhesion and distribution of bacterial cells on food surface (Araujo et al. 2010). Contact angle is related to the hydrophobicity of the surface and spreadability of liquid on the surface. The contact angles (θ) for water on intact pear and peach surfaces were 96.8±7.7 and 138.7±4.7^o, and for diiodomethane were 38.7±5.0 and 56.1±9.1^o respectively (Figure 4 and Table 3). Velasquez et al. (2011) reported contact angles for selected test liquids on 16 fruit surfaces where the determined contact angle for water on pear surface was 89.7^o. In general, surfaces with water contact angle value > 65 are considered to be hydrophobic where $\theta < 65$ are considered to be

hydrophilic (Vogler, 1998). Also, contact angle $\theta = 0$ indicate complete wetting, $0 < \theta < 90$ indicates surface spreading of the liquid and $\theta > 90$ indicates a surface upon which the liquid forms a bead (Woodling and Moraru 2005). Contact angle measurements indicated that pear and peach surfaces were hydrophobic in nature, however, pear surfaces were less hydrophobic than peach surfaces; hence a broader spatial distribution of bacterial cells could be achieved (Choi et al. 2002). Furthermore, the peach surface and trichomes possess a cuticular covering, containing high concentrations of the hydrophobic component cutan (Fernandez et al. 2011). The higher effectiveness of UV-C inactivation of *E. coli* cells on pear surfaces could also be attributed to the lower hydrophobicity of pear surfaces compared to peach. It is likely that there was greater adherence of bacteria along with a more uniform distribution of cells onto pear surfaces compared to peach surfaces. The greater effectiveness of the UV treatment for smooth pear surface may have been due to the lack of protective features such as trichomes, since based on physical properties alone, pear surface properties were more amenable to surface attachment of *E coli* cells.

Bacterial adhesion and surface distribution are dependent upon hydrophobicity. *E. coli* K12 is moderately hydrophilic group with $\theta < 65^{o}$ (Mitik-Dineva et al. 2009; Burks et al. 2003). Thermodynamically, hydrophilic cells tend to adhere onto hydrophilic substrates (Bos et al. 1999) and hydrophobic interactions play an important role in the adhesion of hydrophilic cells to hydrophobic substrates (Ong et al. 1999). Ong et al. (1999) reported that *E. coli* D21 adhered more strongly to hydrophobic OTS-treated glass than hydrophilic glass. In the current study, hydrophilic *E. coli* cells may be adhered less strongly onto hydrophobic intact peach surfaces and this reduced attachment may have counteracted some of the protective effect of trichomes.

The surface energy values of the intact pear and peach surfaces were determined using equations 3, 4, and 5. Surface free energy components for test liquids are presented in Table 4. Since surface energy of the solid (γ_s) values were less than 100 mN/m, pear and peach surfaces are low energy surfaces with surface interactions with liquids being through apolar weak dispersive forces such as van der Waals forces (Zisman, 1964; Velazquez et al. 2011). Surface free energy of more hydrophobic peach surfaces (36.6±6.4 mN/m) was less than that of pear surfaces (40.6±2.9 mN/m) (Table 3). Velazquez et al. (2011) reported surface energy values of 16 fruits including a pear, between 37 and 44 mN/m. Pear surfaces exhibited greater work of adhesion (W_a = 64.3±9.7 mN/m) and spreading coefficient/wettability (W_s = -81.5±9.5mN/m) in comparison to the peach surfaces (where W_a = 18.3±3.9 mN/m; W_s = 127.5±3.9 mN/m), and this would support finding for better wetting, spreading and distribution of *E. coli* on pear surfaces based upon surface angle measurements. Hydrophobic/hydrophilic interactions between substrates and bacterial surfaces play a major role in the adhesion/attachment of bacterial cells. Ong et al. (1999) reported that adhesion of more hydrophilic or high surface energy *E. coli* D21 cells was better on high energy substrate such as glass and mica. However, a clear understanding on the effect of surface adhesion of bacteria cells and UV-C inactivation is yet to be achieved, it can be presumed from these physical properties results that washing with water would be ineffective in removing Gram-negative surface microflora because surface energy and surface features would encourage surface adhesion of bacteria to fruit surfaces.

The root mean square surface roughness (R_q) was 2136±7 nm and the average surface roughness (R_a) values (1859±12 nm) for the intact pear surface. Surface roughness analysis by AFM could not be conducted on peach surfaces due to their higher surface roughness and the presence of trichomes. The reported R_a values of uncoated onion skin and shaved peach surface

were 78 nm and 6.5 nm respectively while the R_q value of shaved peach surface was 8.1 nm (Hershko et al. 1998; Yang et al. 2005). Greater surface roughness may result in increased surface adhesion of microorganisms due to increased surface area and potential shielding of bacteria which may result in protecting microbes from shear forces associated with washing steps (Scheuerman et al. 1998).

Inactivation mechanism:

Environmental Scanning Electron Microscopy

ESEM analysis revealed few readily discernible structural changes to *E. coli* following 1 min and 4 min UV-C treatments (Figure 5). Others have observed no structural disruption or surface irregularities from pulsed UV treated *Bacillus subtillis* and *Aspergillus niger* using scanning electron microscopy (Levy et al. 2012).

Fourier Transform Infrared (FT-IR) Spectroscopy:

Spectroscopic methods such as infrared spectroscopy, can determine the degree and chemical nature of bacterial injury caused by various antimicrobial treatments such as UV as show here and in recent work (Liu et al. 2012); sonication (Lin et al., 2004), cold and freezing (Lu et al., 2011a), sanitizer treatments (Al-Qadiri et al., 2008a), heat (Al-Qadiri et al., 2008b) and exposure to bioactive compounds derived from vegetables (Lu et al., 2011a; Lu et al., 2011b). Second derivative transformations and other chemometric models (*i.e.*, PCA, HCA and partial least squares regression, PLSR) are employed to either magnify minor biochemical compositional variations from raw spectral features or to segregate samples based upon treatment levels. Microbial cell injury detected spectroscopically has been verified by studies of bacterial survival,

leakage of cellular contents, and ultrastructural changes resulting from physical and chemical treatments (Lu et al. 2011 b, c, d).

FT-IR spectral features of intact pear surface following inoculation and after 4 min treatment with UV-C radiation at 7.56 kJ/m^2 UV dose are shown in Figure 6. Because of irregularities in food surfaces, to ensure reliable measurements, spectral reproducibility was determined (D_{y1y2} values ranged from 13.56 ± 2.15 to 17.89 ± 3.94), showing good reproducibility for each treatment. The intragroup variation of spectral features was significantly ($P < 0.05$) smaller than the intergroup variation of spectral features. Thus, spectral subtraction between groups was feasible (Liu et al., 2012). Spectral subtraction (Figure 6B – Figure 6A and Figure 6C – Figure 6A) was separately employed to remove spectral interference from the fruit surface allowing for examination of only the spectral features from the bacteria.

Second derivative transformations were performed to more easily examine the chemical compositional variations between *E. coli* cells before and after 4 min treatment of UV-C radiation at 7.56 kJ/m^2 (Figure 7). The band at 1018 cm^{-1} is related to υ(CO), υ(CC), δ(OCH), and ring structure of polysaccharides and/or pectin (Movasaghi et al., 2008). The band at 1105 cm^{-1} is assigned to carbohydrates (Lu et al., 2011a). The band shift from 1240 cm^{-1} to 1224 cm^{-1} indicates DNA variations in bacterial cells before and after UV-C treatment. The band at 1224 cm^{-1} is assigned to asymmetric stretching of phosphate groups of phosphodiester linkages in DNA and RNA (Naumann, 2001) while the band at 1240 cm^{-1} is assigned to PO_2 asymmetric vibrations of nucleic acids (Naumann, 2001). The band at 1444 cm^{-1} is due to δ(CH_2) of lipids and/or fatty acids (Lu et al., 2011a-d). The bands at 1545 cm^{-1} and 1647 cm^{-1} are assigned to amide II and amide I (Lu et al., 2011a-d), respectively, both of which are secondary protein structures. Collectively, the variations of phospholipids, protein secondary structures and

polysaccharides are related to the bacterial cell membrane damage by UV-C treatment. Further, the DNA/RNA structural variations can be observed from second derivative transformed FT-IR spectra and have been validated in earlier studies that show that UV-C radiation distorts the DNA helix, which blocks microbial replication and subsequently causes *E. coli* death (Cutler and Zimmerman, 2011).

Unsaturated organic compounds, which are the building structures of DNA and RNA and are important for cell maintenance including, pyramidines, purines and flavin are susceptible to UV-C radiation (Cutler and Zimmerman, 2011). Absorption of UV-C by these unsaturated organic compounds resulted in hydration of the nucleic acid base or base dimerization, *i.e.*, DNA dimers (thymine and cytosine) and RNA dimers (uracil and cytosine) (Jagger, 1967; Cutler and Zimmerman, 2011). The most common photoproducts of nucleic acids by exposure to UV-C are cyclobutyl pyrimidine dimers (Guerrero-Beltran and Barbosa-Canovas, 2004). Cutler and Zimmerman (2011) reported that sugars and phosphates of nucleic acids do not absorb radiation above 210 nm, however, these FT-IR results show variation in phosphate and polysaccharides in the nucleic acids resulting in cell membrane damage.

Two types of chemometric models, namely PCA and HCA, were established and validated for segregation of untreated *E. coli* samples from UV-C treated *E. coli* samples (Figure 8). The wavenumber regions between 1800 and 900 cm^{-1} were selected for model analysis. The tight clusters (Figure 8A) demonstrated significant differences ($P < 0.05$) between untreated and UV-C treated samples. In addition, the interclass distances based upon Mahalanobis distance measurement ranged from 10.29 to 13.42. Clusters with interclass distance values higher than 3 are believed to be significantly different from each other (Lu et al., 2011a-d). The composite dendrogram derived from hierarchical cluster analysis was well established and sorted on the

basis of different groups (Figure 8B). Taken together, both types of segregation chemometric models show that cell injury occurred since untreated and UV-C treated bacterial samples could be clearly differentiated.

For scaling up of the UV-C process to use in the industrial level, it is important to identify the efficacy of UV-C light for the inactivation of pathogenic bacteria and other microorganisms on whole fruits including pear and this research is progressing. Further, it is important to understand the energy required for surface disinfection of fruits by UV-C.

Conclusions

Physical and morphological characteristic of fruit surface have a great impact on the inactivation kinetics of *E. coli* by UV-C. UV-C treatment can reduce *E. coli* 23716 on discs of surfaces of intact pear >3 log CFU/g following a 0 to 4 minute treatment at 7.56 kJ/m^2. The presence of wounds on pear surfaces and trichomes on peach surfaces shielded the *E. coli* against UV-C, resulting in its reduced effectiveness. UV-C inactivation kinetics of *E. coli* fitted Weibull equation. Further, the surface roughness of peach, and the relatively lower hydrophobicity of pear explain in part the lower effectiveness of UV-C treatment for peach relative to pear surfaces. Bacterial cell membranes (phospholipids, protein secondary structures and polysaccharides) were damaged by UV-C radiation treatment and DNA/RNA structural variations were observed by FT-IR suggesting that these were the major causes of *E. coli* injury and inactivation. The results of this study indicate that the surface characteristics influence the efficacy of UV-C to achieve specific levels of reduction in *E. coli* population, which is an important consideration for the design of UV-C systems for sanitization of fruit surfaces.

Acknowledgements

This research work was funded, in part, with a grant from Pear Bureau Northwest. Technical assistance from Mr. Pavlo Rudenko and Dr. Elena Ten, School of Mechanical and Materials Engineering, Dr. Valerie Lynch-Holm, Franceschi Microscopy and Imaging Center, and Mr. Ofero Caparino, Biological Systems Engineering, WSU are greatly acknowledged.

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LIST OF FIGURES

Figure 6 FT-IR spectral features of pear fruit surface without *E. coli* inoculation (A), with *E. coli* inoculation (B), and with *E. coli* inoculation and 4 min treatment of UV radiation (C). In this figure, various spectra were shown in each panel and indicate good reproducibility for spectral features.

Figure 7 Second derivative transformations of FT-IR spectral features of inoculated pear fruit surface, untreated (black) and UV treated for 4 min (blue).

Figure 8 The variations of *E. coli* on peach surfaces untreated (control) and treated by UV radiation for 4 mins using principal component analysis (A) and hierachical cluster analysis (B).

Table 1. Previous studies on surface disinfection of foods by UV-C

Food Surface	Microorganism	Treatment conditions	*D* value or Weibull model parameters	Reference
Pork skin Pork muscle	*Escherichia coli* GM 1829 *S. senfetenberg*	Distance: 10-30 cm 20, 50, 80, 100, 500, 1000 $\mu W/cm^2$ 0-1920 s	Log linear model When exposed to 100 $\mu W/cm^2$, *D* value for *E. coli* on a). Tryptic soy agar: 242 s b). Pork muscle: 1282 s c). Pork skin: 1370 s *D* value for *S.* Senftenberg on a). Tryptic soy agar: 15 s b). Pork muscle: 1163 s c). Pork skin: 595 s When exposed to 1000 $\mu W/cm^2$, *D* value for *E. coli* on a). Tryptic soy agar: 177 s b). Pork muscle: 1205 s c). Pork skin: 592s *D* value for *S.* Senftenberg on a). Tryptic soy agar: 21s b). Pork muscle: 1064 s c). Pork skin: 490 s	Wong et al. (1998)
Single lamp annular UV reactor	*Yersinia pseudotuberculosis* *Escherichia coli* K12	Flow rates: 3, 6, 12.5, and 36 ml/s	For *E. coli* K12*: k* from Series event model = 0.675 cm^2/mJ *k* from first order model = 0.557 cm^2/mJ For *Yersinia pseudotuberculosis: k* from Series event model = 0.984 cm^2/mJ *k* from first order model = 0.325 cm^2/mJ	Koutchma et al. (2007)
ready-to-eat sliced ham	*Listeria monocytogenes* *Salmonella enterica Serovar Typhimurium*, *Campylobacter Jejuni*	UV doses used: 1000, 2000, 4000, 6000, and 8000 J/m^2	Weibull parameters: *L. monocytogenes*: $\alpha = 0.78$, $\beta = 0.72$ and $d_R = 2.48$ *S.* Typhimurium: $\alpha = 0.82$, $\beta = 0.78$ and $d_R = 2.39$ *C. jejuni*: $\alpha = 0.78$, $\beta = 0.82$ $d_R = 2.18$	Chun et al. (2009)
Plastic surface (Petri dishes)	*Bacillus subtilis*	Distance: 142 cm 0.14 mW/cm^2	99.9% reduction in *B. subtilis* population	McDonald et al. (2000)

		30-960 s		
Apple disc	*Escherichia coli* ATCC 11229 *Listeria innocua* ATCC 33090 *Saccharomyces cerevisae* KE 162	10 min (5.6 kJ/m^2) 15 min (8.4 kJ/m^2) 20 min (14.1 kJ/m^2)	Reduction in microbial population varied between 1 to 1.9 log CFU/g	Gomez et al. (2010)
Leaf lettuce Tomato Apple	*Salmonella* spp. *Escherichia coli* O157:H7	1.5 to 24 mW/cm^2	Leaf lettuce: 2.65 and 2.79 log CFU/g maximum reduction in *Salmonella* spp. and *E.coli* O157:H7 respectively Tomato: 2.19 log CFU/g maximum reduction in *Salmonella* spp. Apple: 3.3 log CFU/g maximum reduction in *E.coli* O157:H7	Yaun et al. (2004)
Plates containing tryptic soy agar and nalidixic acid	*Salmonella* *Escherichia coli* O157:H7	1.5 to 30 mW/cm^2 5 to 75 s	5 log CFU/g reduction in Salmonella population was achieved for a UV dose of >14.5 mW/cm^2 5 log CFU/g reduction in E. coli O157:H7 population was achieved for a UV dose of >8.4 mW/cm^2	Yaun et al. (2003)
Fat free franks Bratwurst Drumsticks Shell eggs Chicken breast Pork chop Roma tomato Jalapeno pepper	*Salmonella* spp. *Staphylococcus aureus* *Listeria monocytogenes*	Distance: 20 cm 0.5 to 4 J/cm^2	Fat free franks: *Salmonella* spp.: 1.56 to 2.19 log CFU/g reduction (CFU/g) *Staphylococcus aureus*: 1.27 to 1.97 log CFU/g reduction (CFU/g) *Listeria monocytogenes*: 1.5 to 2.14 log CFU/g reduction (CFU/g) Bratwurst: *Salmonella* spp.: 1.14 to 1.51 log CFU/g reduction (CFU/g) *Staphylococcus aureus*: 1.1 to 1.38 log CFU/g reduction (CFU/g) *Listeria monocytogenes*: 1.42 to 1.78 log CFU/g reduction (CFU/g) Drumsticks: *Salmonella* spp.: 0.39 to 0.45 log CFU/g reduction (CFU/g) *Staphylococcus aureus*: 0.42 to 0.42 log CFU/g reduction (CFU/g) *Listeria monocytogenes*: 0.48 to 0.63 log CFU/g reduction (CFU/g) Shell eggs:	Sommers et al. (2010)

			Salmonella spp.: 0.43 to 0.98 log CFU/g reduction (CFU/g) *Staphylococcus aureus*: 0.12 to 0.81 log CFU/g reduction (CFU/g) *Listeria monocytogenes*: 0.28 to 1.16 log CFU/g reduction (CFU/g) Chicken breast: *Salmonella* spp.: 0.33 to 0.32 log CFU/g reduction (CFU/g) *Staphylococcus aureus*: 0.33 to 0.44 log CFU/g reduction (CFU/g) *Listeria monocytogenes*: 0.25 to 0.37 log CFU/g reduction (CFU/g) Pork chop: *Salmonella* spp.: 0.43 to 0.53 log CFU/g reduction (CFU/g) *Staphylococcus aureus*: 0.50 to 0.49 log CFU/g reduction (CFU/g) *Listeria monocytogenes*: 0.61 to 0.65 log CFU/g reduction (CFU/g) Roma tomato: *Salmonella* spp.: 3.08 to 3.82 log CFU/g reduction (CFU/g) *Staphylococcus aureus*: 3.13 to 3.62 log CFU/g reduction (CFU/g) *Listeria monocytogenes*: 2.59 to 3.60 log CFU/g reduction (CFU/g) Jalapeno pepper: *Salmonella* spp.: 3.02 to 3.79 log CFU/g reduction (CFU/g) *Staphylococcus aureus*: 3.09 to 3.33 log CFU/g reduction (CFU/g) *Listeria monocytogenes*: 3.11 to 3.72 log CFU/g reduction (CFU/g)	
Fresh cut pear with and without peel	*Listeria innocua* ATCC 33090 *Listeria monocytogenes* ATCC 19114D *Escherichia coli* ATCC 11229	Distance: 10 cm 0 to 87 kJ/cm^2 0 to 20 min	Reduction in the population of the selected bacteria varied from between 2.6 and 3.4 log CFU/g for pear slices without peel Reduction in the population of the selected bacteria varied from between 1.8 and 2.5 log CFU/g for pear slices with peel	Schenk et al. (2008)

	Zygosaccharomyces bailii NRRL 7256			
Fresh cut melon	*Enterobacteriaceae*	0 to 12 kJ/m^2	2 log CFU/g reduction in the bacteria	Manzocco et al. (2011)
Blueberry	*E. coli* O157:H7	20 mW/cm^2 Distance: 0.9 cm 1, 5 and 10 min	Reduction in the population of *E. coli* O157:H7 varied from between 1.53-2.14 log CFU/g on calyx and 3.11-5.53 log CFU/g on skin	Kim and Hung (2012)

Table 2. Average and standard deviation values of Weibull model parameters for *E. coli* inactivation on selected fruit surfaces UV-C

Fruit surface	α min (kJ/m^2)	γ	t_R min (kJ/m^2)	R^2
Pear surface	0.001±0.0007 (0.235±0.001)	0.25±0.03	0.019±0.009 (0.268±0.017)	0.99
Wounded pear surface	0.003±0.001 (0.240±0.002)	0.28±0.03	0.062±0.013 (0.348±0.024)	0.99
Peach surface	0.004±0.0004 (0.241±0.0008)	0.28±0.01	0.074±0.012 (0.371±0.022)	0.99

The values in bracket are α and t_R in kJ/m^2

Table 3. Average and standard deviation values of surface energy parameters of selected fruits (N= 20)

Fruit surface	Contact angle (θ)		$\gamma_s \times 10^3$ (mN/m)	$\gamma_s^d \times 10^3$ (mN/m)	$\gamma_s^p \times 10^3$ (mN/m)	$W_a \times 10^3$ (mN/m)	$W_s \times 10^3$ (mN/m)
	Water	Diiodomethane					
Pear	96.8±7.7	38.7±5.0	40.6±2.9	40.2±2.5	0.490±0.9	64.3±9.7	-81.5±9.5
Peach	138.7±4.7	56.1±9.1	36.6±6.4	30.8±5.2	5.79±1.6	18.3±3.9	127.5±3.9

where γ_S^d = Dispersion component of the surface energy of the solid (mN/m), γ_S^p = Polar component of the surface energy of the solid (mN/m), W_a = Reversible work of adhesion (mN/m), W_c = Cohesion coefficient (mN/m), W_s = Spreading coefficient (mN/m)

Table 4. Surface free energy components for test liquids used in this work

Liquid	$\gamma_L \times 10^3$ (mN/m)	$\gamma_L^d \times 10^3$ (mN/m)	$\gamma_L^p \times 10^3$ (mN/m)
Water	72.9	21.9	51
Diiodomethane	50.8	50.8	0

where γ_L = Surface enrgy of the liquid (mN/m), γ_L^d = Dispersion component of the surface energy of the liquid (mN/m), and γ_L^p = Polar component of the surface energy of the liquid (mN/m)

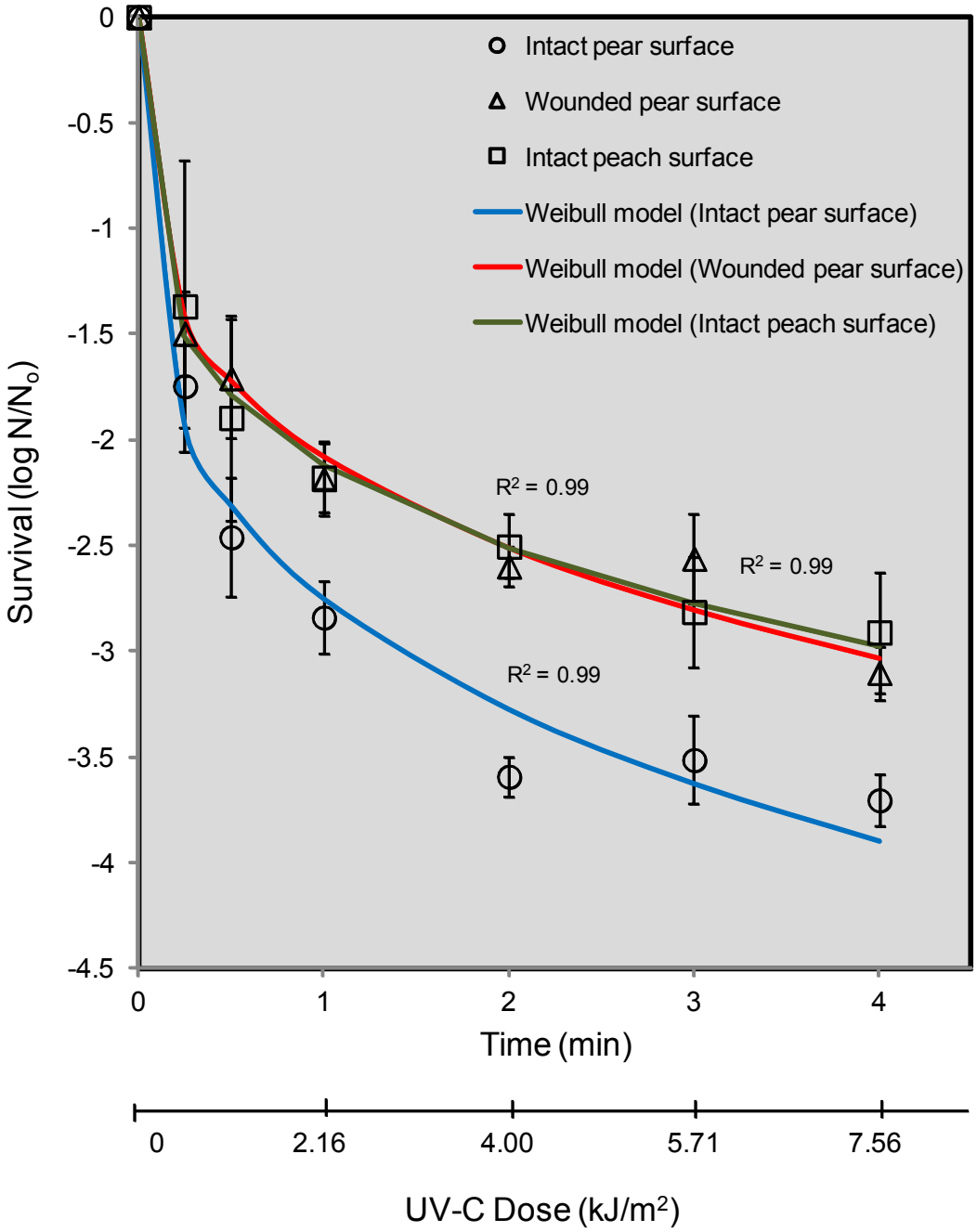

Intact pear surface
Wounded pear surface
Intact peach surface
Weibull model (Intact pear surface)
Weibull model (Wounded pear surface)
Weibull model (Intact peach surface)
R² = 0.99
R² = 0.99
R² = 0.99
Survival (log N/N₀)
0
-0.5
-1
-1.5
-2
-2.5
-3
-3.5
-4
-4.5
0
1
2
3
4
Time (min)
0
2.16
4.00
5.71
7.56
UV-C Dose (kJ/m²)

Figure 1

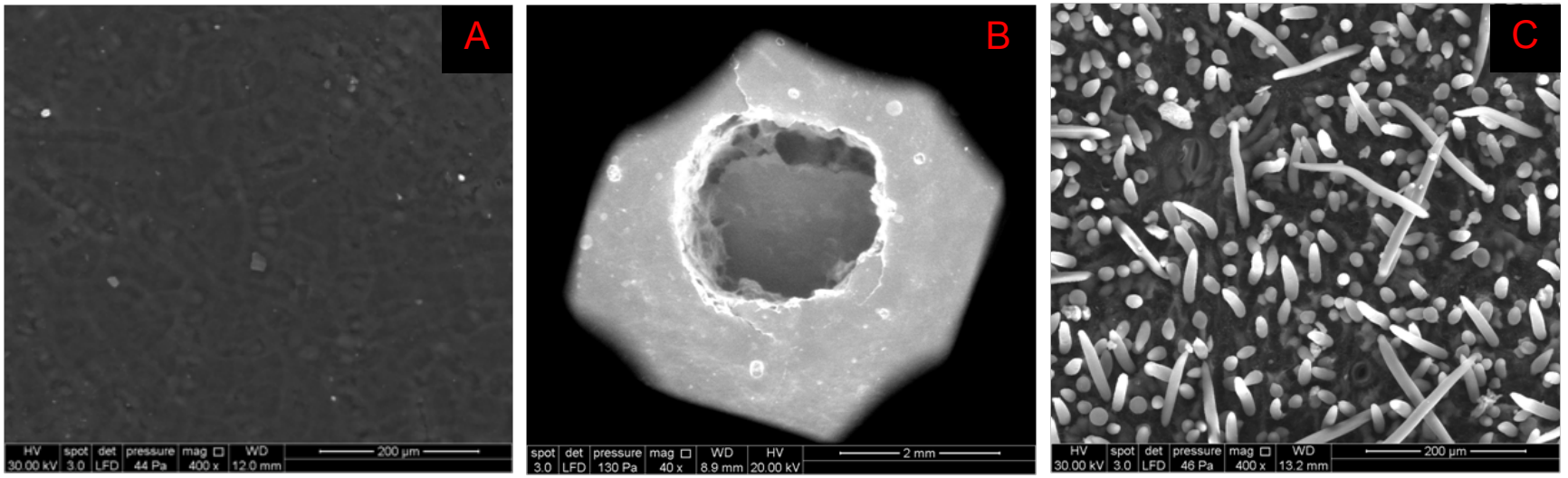

A
HV 30.00 kV
spot 3.0
det LFD
pressure 44 Pa
mag 400 x
WD 12.0 mm
200 µm
B
spot 3.0
det LFD
pressure 130 Pa
mag 40 x
WD 8.9 mm
HV 20.00 kV
2 mm
C
HV 30.00 kV
spot 3.0
det LFD
pressure 46 Pa
mag 400 x
WD 13.2 mm
200 µm

Figure 2

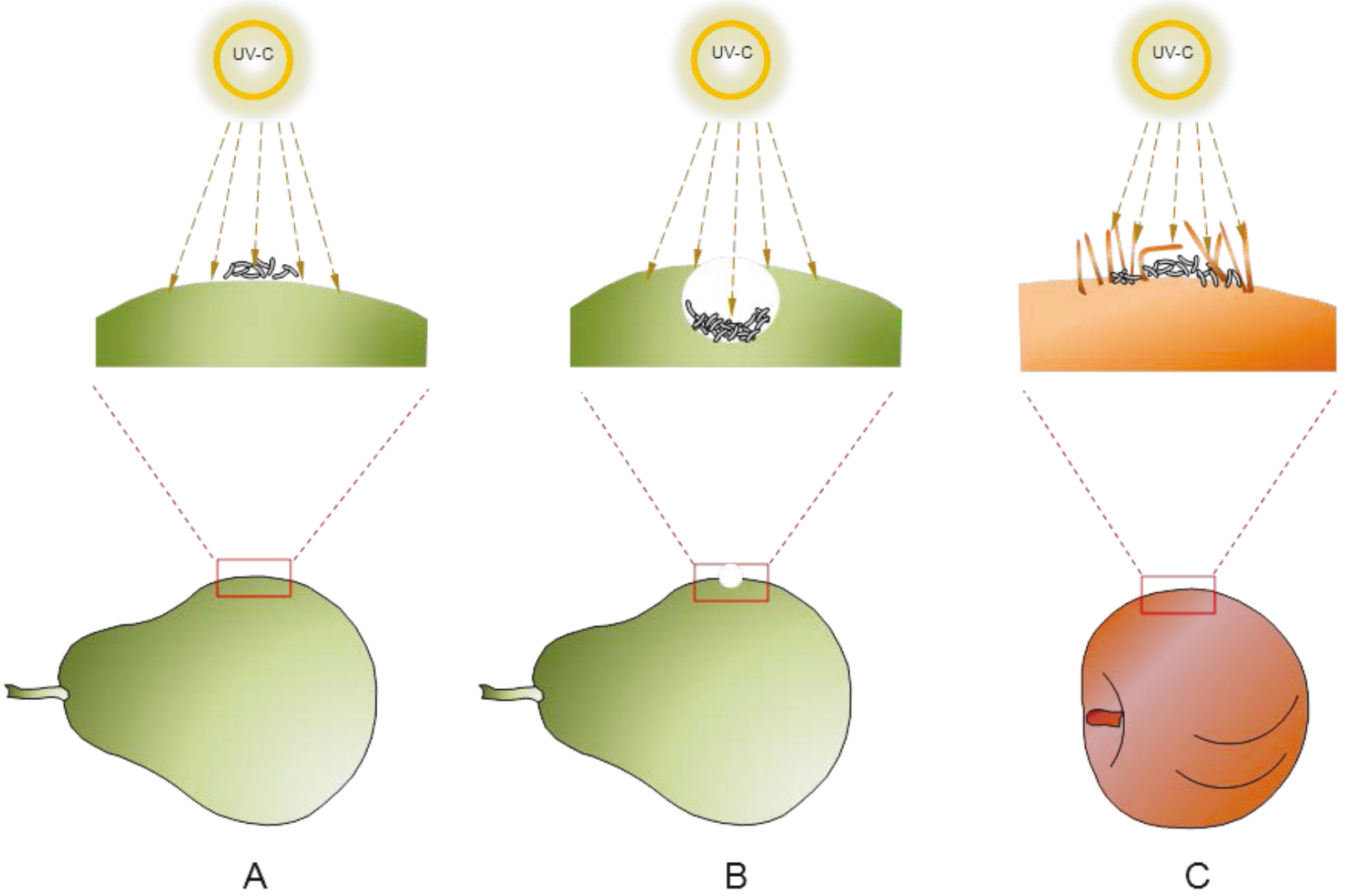


Figure 3

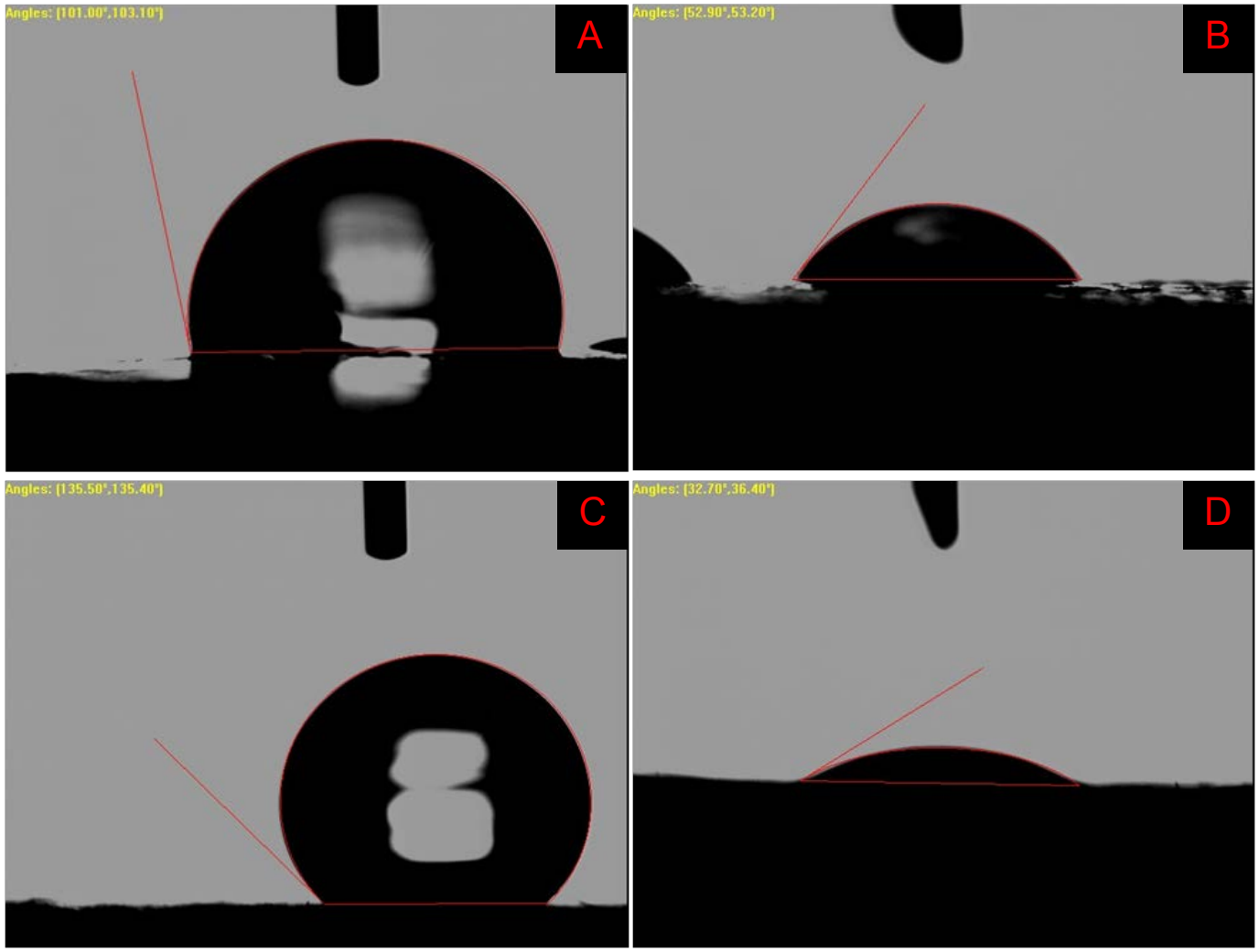

Angles: (101.00°,103.10°)
A
Angles: (52.90°,53.20°)
B
Angles: (135.50°,135.40°)
C
Angles: (32.70°,36.40°)
D

Figure 4

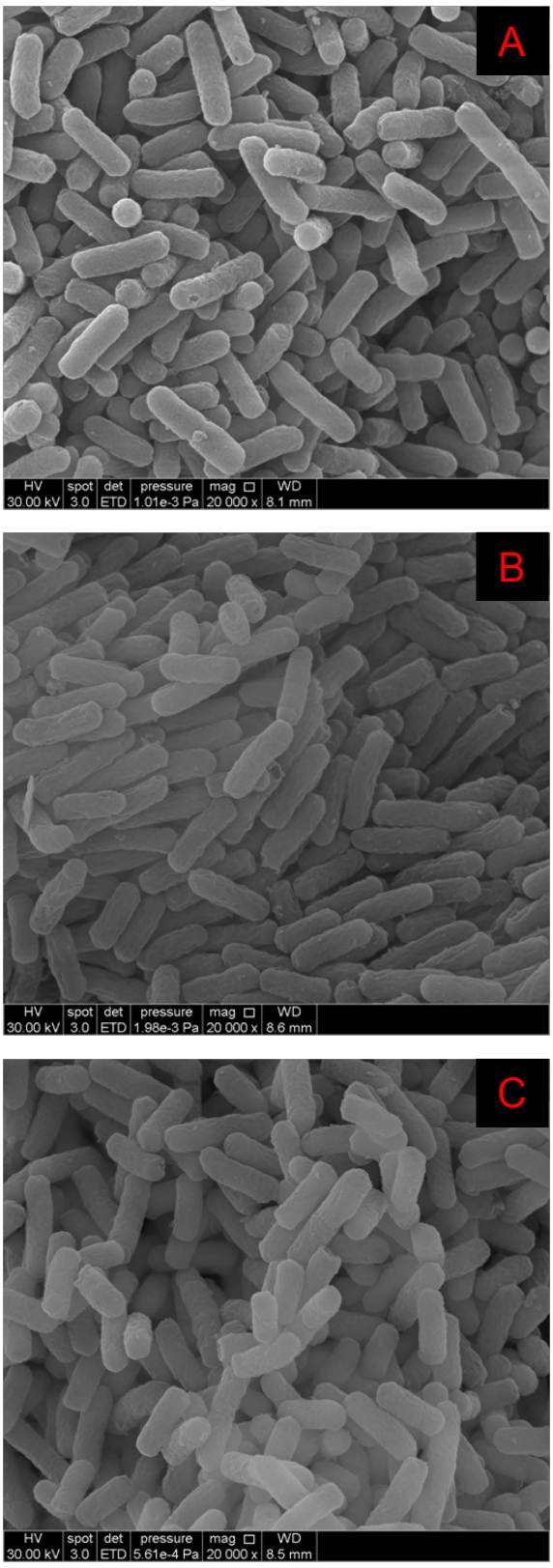

A
HV spot det pressure mag WD
30.00 kV 3.0 ETD 1.01e-3 Pa 20 000 x 8.1 mm
B
HV spot det pressure mag WD
30.00 kV 3.0 ETD 1.98e-3 Pa 20 000 x 8.6 mm
C
HV spot det pressure mag WD
30.00 kV 3.0 ETD 5.61e-4 Pa 20 000 x 8.5 mm

Figure 5

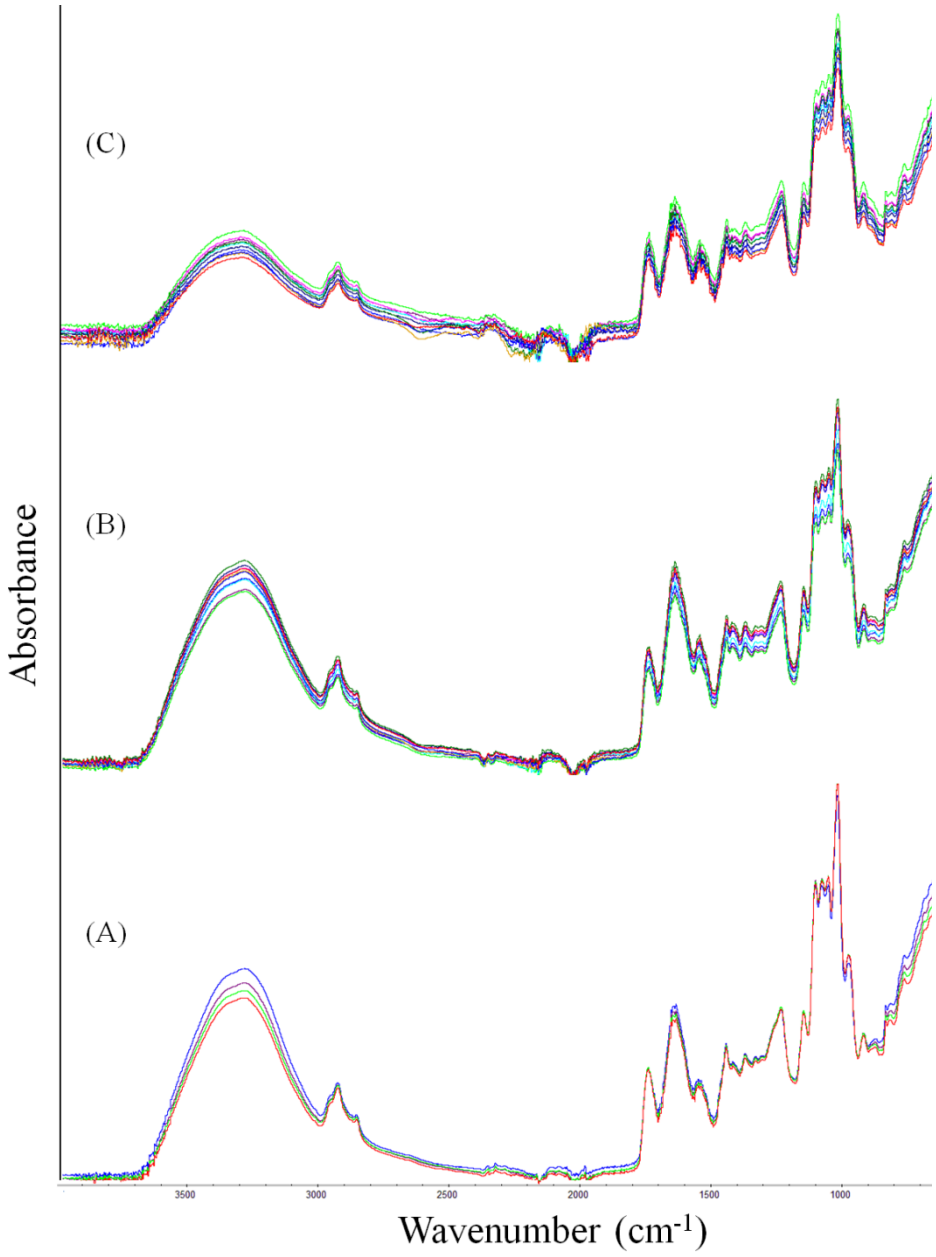

(C)
(B)
(A)
Absorbance
3500
3000
2500
2000
1500
1000
Wavenumber (cm-1)

Figure 6

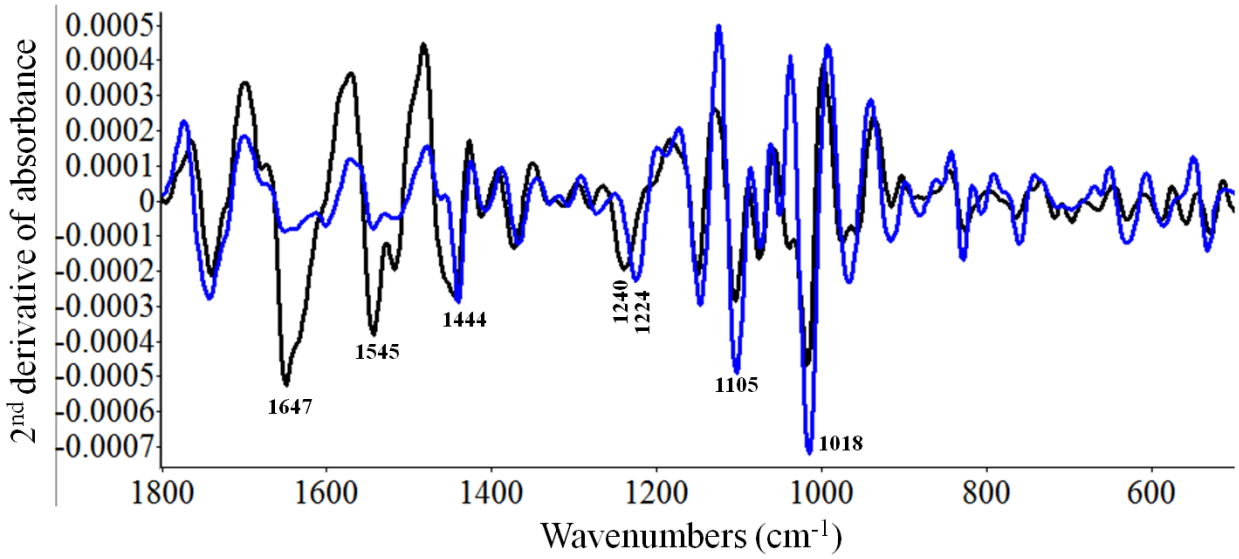
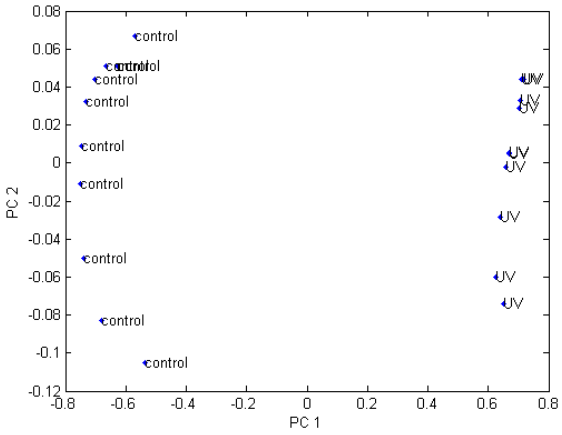

0.0005
0.0004
0.0003
0.0002
0.0001
0
-0.0001
-0.0002
-0.0003
-0.0004
-0.0005
-0.0006
-0.0007
2nd derivative of absorbance
1647
1545
1444
1240
1224
1105
1018
1800
1600
1400
1200
1000
800
600
Wavenumbers (cm-1)

Figure 7

(A)


0.08
0.06
0.04
0.02
0
-0.02
-0.04
-0.06
-0.08
-0.1
-0.12
PC 2
-0.8
-0.6
-0.4
-0.2
0
0.2
0.4
0.6
0.8
PC 1
control
UV

(B)

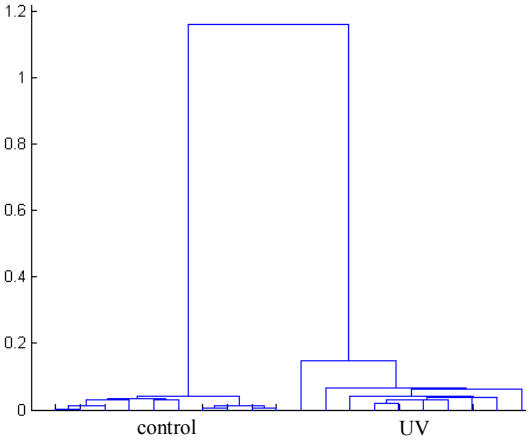

1.2
1
0.8
0.6
0.4
0.2
0
control
UV

Figure 8