International Journal of Food Microbiology 210 (2015) 136–142

Contents lists available at ScienceDirect

International Journal of Food Microbiology

journal homepage: www.elsevier.com/locate/ijfoodmicro

Ultraviolet-C light inactivation of *Escherichia coli* O157:H7 and *Listeria monocytogenes* on organic fruit surfaces

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ARTICLE INFO

Article history:
Received 2 February 2015
Received in revised form 9 June 2015
Accepted 22 June 2015
Available online 24 June 2015

Keywords:
Contact angle
Hydrophobic
Surface roughness
Surface energy
Weibull model

ABSTRACT

This study investigated UV-C light inactivation of *Escherichia coli* O157:H7 and *Listeria monocytogenes* on the surface of organic apples, pears, strawberries, red raspberries and cantaloupes. Fruit surfaces spot inoculated with cocktail strains of *E. coli* O157:H7 and *L. monocytogenes* were exposed to UV-C doses up to 11.9 kJ/m^2 at 23 °C. Fruit surface roughness, contact angle, and surface energy were determined and correlated with UV-C inactivation kinetics.

Results demonstrate that bacterial pathogens on fruit surfaces respond differently to UV-C light exposure. *E. coli* O157:H7 on apple and pear surfaces was reduced by 2.9 and 2.1 log CFU/g, respectively when treated with UV-C light at 0.92 kJ/m^2 (60 s). For berries, the reduction of *E. coli* O157:H7 was lower with 2.0 (strawberry) and 1.1 log CFU/g (raspberry) achieved after UV-C treatment at 7.2 kJ/m^2 (8 min) and at 10.5 kJ/m^2 (12 min), respectively. Similarly, a higher reduction of *L. monocytogenes* was observed on apple (1.6 log CFU/g at 3.75 kJ/m^2) and pear (1.7 log CFU/g at 11.9 kJ/m^2) surfaces compared to cantaloupe and strawberry surfaces (both achieved 1.0 log CFU/g at 11.9 kJ/m^2). *L. monocytogenes* shows more resistance than *E. coli* O157:H7. Inactivation rates were higher for less hydrophobic fruits with smoother surfaces (apples and pears) as compared to fruits with rougher surfaces (cantaloupe, strawberry and raspberry). Findings indicate that UV-C light can effectively reduce *E. coli* O157:H7 and *L. monocytogenes* populations on fruit and berry surfaces. However, surface characteristics influence the efficacy of UV-C light.

1. Introduction

Aqueous sanitizers such as sodium hypochlorite, chlorine dioxide and peroxyacetic acid are commonly used to disinfect fresh produce, despite their limited efficacy in reducing human pathogens (Beuchat et al., 1998; Sapers, 2001; Wisniewsky et al., 2000). Current practices in fruit packing houses include multiple applications of aqueous sanitizers, as well as mechanical force such as brushing (Beuchat et al., 1998; Sapers, 2001; Wisniewsky et al., 2000). Some of these practices are effective in reducing the microbial load on fruit surfaces. However, this is limited to only a few products due to the soft, delicate surfaces of many fruits, particularly raspberries and strawberries. Recent outbreaks of food-borne pathogens, e.g. *Escherichia coli* O157:H7 and *Listeria monocytogenes* on fruits and berries have threatened the health and safety of consumers. Thus, there is a critical need to develop novel disinfection technologies that substitute for conventional washing and sanitizing techniques.

The UV-C light of wavelength of 254 nm is approved for microbial reduction in food and juice (US-FDA, 2011). Some juice processing plants in the U.S. now use UV pasteurization. However, UV-C light remains a new method for disinfecting fresh produce surfaces. Recent findings support the use of UV-C light application for surface disinfection (Bialka and Demirci, 2008; Collignon and Korsten, 2010; Syamaladevi et al., 2013; Syamaladevi et al., 2014; Syamaladevi et al., 2015; Yun et al., 2013). In fact, UV-C light may eliminate the need for mechanical scrubbing and help maintain the integrity and texture of berries during disinfection. It is also effective for treating other delicate produce surfaces. For example, Yaun et al. (2004) found that UV-C light is more effective against *E. coli* O157:H7 cells that are inoculated onto surfaces of leaf lettuce and apples than 20–320 ppm of chlorine. Reducing *E. coli* O157:H7 on apple surfaces (>2.9 log CFU/g) with UV-C treatment of <1.0 kJ/m^2 was as effective or more effective than treatment with pre-ozonated water (for 3 min) (Achen and Yousef, 2001), spray application of chlorinated water (200 ppm) (Beuchat et al., 1998), and ClO_2 gas treatment (1.1 mg l^{-1} for 10 min) (Du et al., 2003). Similarly, Kim and Hung (2012) found that UV-C light is more effective than electrolyzed water and ozone in inactivating *E. coli* O157:H7. Applying UV light at 20 mW/cm^2 was found to reduce the population of *E. coli* O157:H7 between 1.5 and 2.1 log°CFU/g on blueberry calyx and 3.1–5.5 log CFU/g on blueberry skin following 1–10 min treatments. On the other hand, ozone (4000 mg/l) and

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http://dx.doi.org/10.1016/j.ijfoodmicro.2015.06.018
0168-1605/

electrolyzed water treatment reduced *E. coli* O157:H7 by only 0.7-log CFU/g on calyx and 0.1 to 0.2 log CFU/g and 0.9 to 1.1 log CFU/g on blueberry skins, respectively (Kim and Hung, 2012).

A major obstacle toward the use of UV-C light for commercial disinfection of fruits is their complex surface characteristics, which influence the efficacy of UV-C treatment. Our previous research found that fruit surface characteristics influenced the UV-C inactivation kinetics of blue molds (Syamaladevi et al., 2013 and 2015). However, further research is needed to determine the influence of fruit surface morphology on UV-C inactivation of foodborne pathogens. This will inform the design of UV-C sanitization systems for fruits. Therefore, this study aimed to determine the influence of fruit surface properties on the UV-C inactivation rate of *E. coli* O157:H7 and *L. monocytogenes*. Findings can be used to inform development of new sanitization systems and protect consumer health and safety.

2. Materials and methods

2.1. Treatment surfaces and target microorganism

Fresh organic Fuji apples, D'Anjou pears, cantaloupes from Guatemala strawberries and raspberries (Driscoll's Associates, Inc.; Watsonville, CA), were purchased from a local retail outlet and stored at 7 °C for less than 24 h before the experiments. *E. coli* O157:H7 and *L. monocytogenes* isolates were selected to align with those used in other studies that examined bacterial response on fruit surfaces. *E. coli* O157:H7 strains ATCC 43890 (the human feces isolate known to produce the shiga-like toxin I), ATCC 43895 (the raw hamburger outbreak strain known to produce the shiga-like toxins I and II), and SEA 13B88 (the unpasteurized Odwalla apple juice outbreak isolate) and *L. monocytogenes* strains NRRL B-33006 (serotype 1/2b, isolated from garlic), NRRL B-33069 (serotype 1/2a, isolated from bovine milk) and NRRL B-33385 (serotype 4b, isolated from clinical isolate) were used in this study. Apple, pear and strawberry surfaces were inoculated with both bacteria, while raspberry surfaces were inoculated with *E. coli* O157:H7 and cantaloupe surfaces were inoculated with only *L. monocytogenes*.

2.2. Inoculum preparation

The bacterial cultures were stored in 30% (wt/wt) glycerol (20% water v/v) at −80 °C in a tryptic soy broth (TSB, Hardy Diagnostics, Santa Maria, CA). Frozen cultures were activated in three successive passes, first inoculating 100 μl in 9 ml of TSB (for *E. coli* O157:H7) and TSB + 0.6% yeast extract (for *L. monocytogenes*) and incubated at 37 °C for 18 to 24 h. On the second day, 1 ml of each bacterial culture was inoculated into 9 ml of respective broth and incubated at 37 °C for 18 to 24 h. On the third day, 1 ml of each bacterial culture was inoculated into 99 ml TSB + glucose (for *E. coli* O157:H7) and TSB + 0.6% yeast extract (for *L. monocytogenes*) and incubated at 37 °C for 18 to 24 h. On the fourth day, the bacterial cocktail was prepared, and the experiment was performed.

For the cocktail preparation, 100 ml of each of the three strains was mixed into an empty sterile 500 ml bottle through a sterilized glass funnel. The average initial inoculum level of *E. coli* O157:H7 in all experiments was 8.8 log CFU/ml, while this figure was 8.9 log CFU/ml for *L. monocytogenes*.

2.3. Fruit surface preparation and inoculation

Fresh whole apples, pears and cantaloupes were washed with deionized water and air-dried inside a biological safety cabinet for 1 h at room temperature to remove surface moisture. Although berries were unwashed, the stem portion of the strawberries was removed. A sharp stainless steel cutting disc and knives, both sterilized with ethanol, were used to slice the axial sections of apples, pears and cantaloupes to discs of 4 cm diameter and approximately 0.8 cm thickness (approx. 20 g), with the peel left on. Berries were bisected longitudinally. For apples, pears, cantaloupes and strawberries each fruit disc was kept on sterile petri dishes, with the uncut peel surface facing up. Three raspberry discs were positioned on each sterile petri dish, with the cut surface on the bottom and the epidermal surface facing up. The bacterial cocktail was agitated 25 times in a 30 cm arc to ensure thorough mixing. Next, 500 μl was spot inoculated onto the epidermal surfaces of each cantaloupe disc, 100 μl on each apple, pear and strawberry disc, and 50 μl on each raspberry disc. The inoculated fruit discs were dried for an hour inside a biological safety cabinet.

2.4. Ultraviolet-C light treatment

Prior to each experiment, two fruit discs were selected as the untreated, uninoculated controls. After inoculation, four fruit discs were selected as inoculated control samples. Two sample discs were randomly selected for each UV-C light treatments inside a UV-C Emitter™ Table-top System (Reyco Systems, Meridian ID) at a wavelength of 254 nm at 23 °C, as described by Syamaladevi et al. (2013). This equipment consists of an array of two 110 V 16-inch Steril-Aire™ UV-C Emitters™ mounted in a stainless steel hood (0.45 × 0.30 m). The height of the UV-C emitters was adjusted to 0.1 m above the fruit discs during irradiation treatments. A UV radiometer (EIT UVICURE

Table 1
Average logarithmic reduction levels of *Escherichia coli* O157:H7 on selected organic fruit surfaces (N = 3).

Time (s)	Average UV-C dose (kJ/m^2)	*Escherichia coli* O157:H7 (log N/N_o)			
		Apple	Pear	Raspberry	Strawberry
0	0	0^{A}	0^{A}	0^{A}	0^{A}
10	0.15	-2.1 ± 0.4^{MN}	-1.2 ± 0.2^{GHIJ}		
20	0.31	-2.2 ± 0.4^{MN}	-1.3 ± 0.2^{HIJ}		
30	0.49	-2.4 ± 0.4^{NO}	-1.5 ± 0.2^{IJK}	-0.4 ± 0.1^{B}	-0.5 ± 0.2^{BC}
40	0.63	-2.5 ± 0.3^{OP}	-1.5 ± 0.2^{JK}		
50	0.78	-2.7 ± 0.3^{PQ}	-1.9 ± 0.2^{LM}		
60	0.92	-2.9 ± 0.2^{Q}	-2.1 ± 0.1^{MN}	-0.5 ± 0.1^{BC}	-0.9 ± 0.0^{EFG}
120	1.89			-0.6 ± 0.1^{BCD}	-1.2 ± 0.1^{GHI}
240	3.66			-0.7 ± 0.1^{CDE}	-1.2 ± 0.1^{GHIJ}
360	5.21			-0.9 ± 0.1^{DEF}	-1.6 ± 0.4^{KL}
480	7.17			-1.0 ± 0.1^{FGH}	-2.0 ± 0.3^{LM}
600	8.56			-1.1 ± 0.1^{FGH}	
720	10.5			-1.1 ± 0.0^{FGH}	

The average population of *E. coli* O157:H7 before UV-C treatment on apple, pear, strawberry and raspberry surfaces was 6.7 ± 0.1, 6.3 ± 0.2, 6.7 ± 0.1 and 6.0 ± 0.1 log CFU/g, respectively. Different superscripts in rows and columns represent statistically significant differences between log reduction values in number of *E. coli* O157:H7 cells obtained at selected UV doses (UV treatment times) ($p < 0.05$).

Table 2
Average logarithmic reduction levels of *Listeria monocytogenes* on selected organic fruit surfaces (N = 3).

Time (s)	Average UV-C dose (kJ/m^2)	*Listeria monocytogenes* reduction (log N/N_o)			
		Apple	Pear	Strawberry	Cantaloupe
0	0	0^{A}	0^{A}	0^{A}	0^{A}
10	0.17	-0.7 ± 0.2^{HI}	-0.7 ± 0.0^{HIJ}	-0.6 ± 0.1^{DE}	-0.3 ± 0.1^{B}
20	0.37	-0.8 ± 0.2^{IJ}	-0.9 ± 0.3^{JKL}	-0.6 ± 0.1^{DEF}	-0.3 ± 0.1^{B}
30	0.56	-0.9 ± 0.2^{JK}	-0.9 ± 0.2^{KLM}	-0.6 ± 0.1^{EFGH}	-0.4 ± 0.1^{B}
40	0.66	-0.8 ± 0.1^{JK}			
50	0.79	-1.0 ± 0.1^{LM}			
60	1.10	-1.0 ± 0.1^{LM}	-0.9 ± 0.2^{JKLM}	-0.6 ± 0.1^{DEFG}	-0.4 ± 0.1^{BC}
90	1.27	-1.1 ± 0.1^{MN}			
120	2.02	-1.1 ± 0.2^{MNO}	-1.0 ± 0.3^{LMNO}	-0.6 ± 0.1^{DEFGH}	-0.5 ± 0.1^{CD}
180	2.33	-1.2 ± 0.1^{NOP}			
240	3.65	-1.4 ± 0.1^{Q}	-1.2 ± 0.2^{OPQ}	-0.7 ± 0.1^{FGHI}	-0.7 ± 0.1^{EFGH}
300	3.75	-1.6 ± 0.1^{S}			
360	5.30		-1.3 ± 0.3^{PQR}	-0.7 ± 0.1^{GHI}	-0.7 ± 0.1^{EFGH}
480	6.89		-1.3 ± 0.3^{QR}	-0.9 ± 0.1^{JK}	-0.7 ± 0.0^{FGHI}
600	8.82		-1.5 ± 0.3^{RS}	-0.9 ± 0.1^{JK}	-0.8 ± 0.1^{JK}
720	10.3		-1.6 ± 0.2^{S}	-0.9 ± 0.1^{JK}	-0.9 ± 0.1^{JK}
840	11.9		-1.7 ± 0.1^{S}	-1.0 ± 0.0^{KL}	-1.0 ± 0.1^{KL}

The average population of *L. monocytogenes* before UV-C treatment on apple, pear, strawberry and cantaloupe surfaces was 5.5 ± 0.1, 5.6 ± 0.1, 5.0 ± 0.5 and 6.3 ± 0.1 log CFU/g, respectively. Different superscripts in rows and columns represent statistically significant differences between *Listeria monocytogenes* log reduction values in number of cells obtained at selected UV doses (UV treatment times) ($p < 0.05$).

PLUS II, EIT, Inc., Sterling, VA, USA) was used to measure the UVC doses following each treatment by placing the radiometer exactly at the same location for equivalent treatment times.

Preliminary experiments were performed to establish UVC treatment doses, inoculation method and amount of inoculum required for each type of fruits. The first experiment was performed with apple discs using *E. coli* O157:H7. Twenty apple discs were used for identifying suitable inoculation method: 10 discs were examined with spot inoculation and 10 with spread inoculation. Spot inoculation resulted in uniform microbial populations among the samples tested. Similarly, for each type of fruits preliminary experiment was performed to establish suitable inoculum volume. During preliminary studies for establishing suitable UVC treatment doses, apple discs were samples every 30 s interval. The 30 s interval was too long resulted in more than 2.0 log reduction of E. coli O157:H7 within the first 30 s. Thus 10 s interval was selected for apples. The treatment doses established for apples and pears were not effective for berries. A very low reduction was observed for the equivalent treatment doses. Thus, treatment times/doses were subsequently increased for berries. UV-C doses ranged from 0.14 to 11.87 kJ/m^2, corresponding to treatment times of 10–840 s. Average UV-C intensity and treatment times used for each experiment are shown in Tables 1 and 2. A digital timer was used to control UV exposure times. The temperature of the chamber was 23 °C, as monitored with a digital thermometer. No temperature change was observed during UV-C exposure. Inoculated and non-irradiated fruit discs were used as controls.

2.5. Microbial cell enumeration

After UV-C treatment, fruit or berry discs were placed immediately into a sterile stomacher bag (BA 604I CPG Standard Bag, Seward Limited, UK). Then 100 ml of neutralizing Dey-Engley broth was poured over each disc (DE, Hardy Diagnostics, Santa Maria, CA.) Samples were blended (Stomacher® 400 CIRCULATOR, Seward Laboratory Systems Inc. Port Saint Lucie, FL, USA) for 1 min. The supernatant after stomaching was serial diluted in 9 ml of 0.1% peptone water. Appropriate dilutions were spread-plated in duplicate on Sorbitol MacConkey Agar (SMAC, Hardy Diagnostics, Santa Maria, CA), supplemented with Cefixime–Tellurite Supplement (CT, Invitrogen Dynal AS, Oslo, Norway) for *E. coli* O157:H7 and Modified Oxford Agar (MOX, Hardy Diagnostics, Santa Maria, CA) for *L. monocytogenes*. Cells were enumerated after incubation at 37 °C for 24 ± 2 h. The extended incubation of the counting plates up to 48 h did not yield higher counts. Each experiment was repeated at least three times. The initial level of colony forming units on apple, pear, strawberry and raspberry surfaces ranged from 6.1 to 6.7 log CFU/g.

2.6. UV-C inactivation of bacteria

We used the Weibull equation to describe the UV-C inactivation kinetics of selected bacteria. The equation is

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

where N is the number of surviving bacteria after UV-C energy E (kJ/m^2), N_o is the initial number of bacteria, α is the scale factor and β is the shape parameter determining the shape of the curve (Peleg and Cole, 1998). Values of α and γ were determined by non-linear optimization with the Statistica® version 5 computer program. Weibull parameters were used to determine the UV-C energy (E_{90}) required for

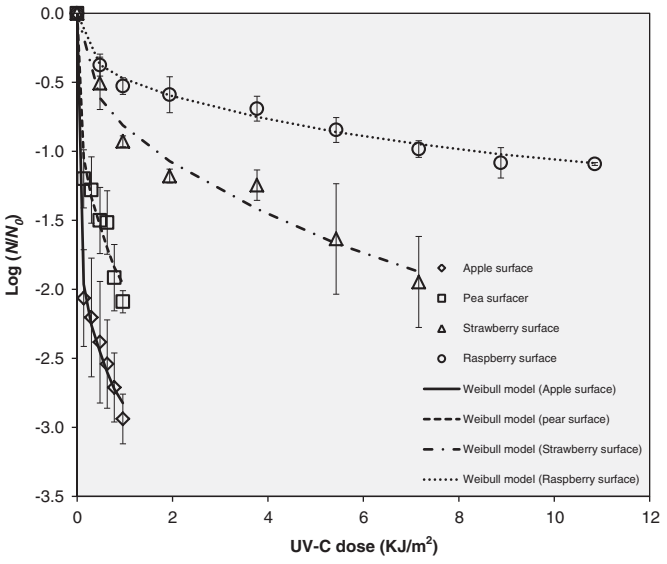

Fig. 1. Kinetics of *E. coli* O157:H7 inactivation by UV-C (no is the initial population of *E. coli* O157:H7 and N is the population after UV-C treatment time t with a specific UV intensity).

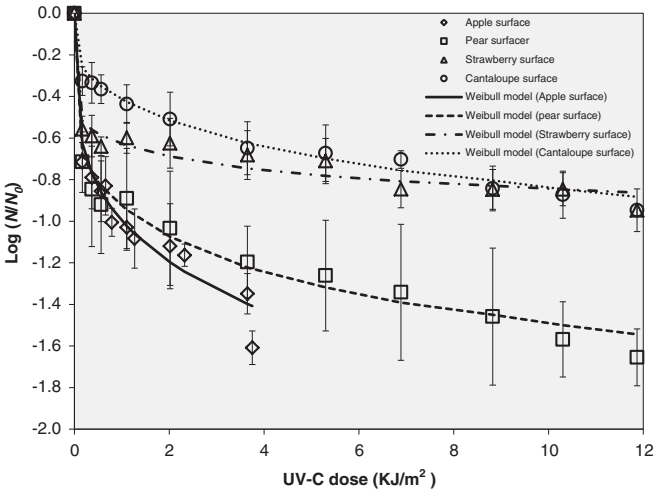

Fig. 2. Kinetics of *E. coli* O157:H7 inactivation by UV-C (No is the initial population of *E. coli* O157:H7 and N is the population after UV-C treatment time t with a specific UV intensity).

a 90% reduction in target microorganisms (van Boekel, 2002). The value of E_{90} can be determined as:

$$E_{90} = \alpha(2.303)^{\frac{1}{\beta}}. \quad (2)$$

2.7. Roughness of fruit surfaces

The surface roughness of fruits was determined as described by Syamaladevi et al. (2015). Briefly, a 10 × 10 mm section of apples, pears and cantaloupes was removed, and the flesh was cut back to ~2–3 mm from the skin. The sample was placed on the Tencor P15 stylus profilometer stage. A scan of 1 mm × 1 mm was made using 12 parallel scans on a 92 μm spacing at a rate of 200 μm/s. For berry surfaces, scans were made over the peak of the drupelet using a 500 μm × 500 μm scan area with 12 scans at a 46 μm spacing at a rate of 100 μm/s.

2.8. Contact angle and surface energy

Contact angle measurements were conducted with a sessile drop method (face contact angle: VCA Optima, AST Products Inc., MA, USA) (Bernard et al., 2011). Using a microliter syringe and a 0.5-mm diameter needle, a small volume (0.5–1.0 μl) of a polar liquid (double-distilled water) or a nonpolar liquid (diiodomethane (99% purity; Sigma-Aldrich) were dropped onto the fruit surfaces (approximately 2 × 2 cm^2 and 1 mm thickness) at room temperature (23 °C). Surface energy values of the selected fruit surfaces were calculated from contact angle measurements using Fowkes' equation (Ribeiro et al., 2007; Bernard et al., 2011; Syamaladevi et al., 2013).

2.9. Statistical analysis

Experiments were repeated at least three times. The effect of UV-C doses on fruit and berry surfaces was analyzed for statistical significance with the mixed-model procedure of SAS ver 9.1 (SAS Institute, Inc., Cary, NC, USA).

3. Results and discussion

3.1. UV-C inactivation kinetics of E. coli O157:H7 and L. monocytogenes on fruit surfaces

The average population of *E. coli* O157:H7 before UV-C treatment on apple, pear, strawberry and raspberry surfaces was in the range of 6.0 to 6.7 log CFU/g. Upon exposure to UV-C light, a significant effect ($p < 0.0001$) was observed between treatments for each type of fruit (Fig. 1, Table 1). A significant reduction ($p < 0.05$) was achieved for an average dose of 0.14 kJ/m^2 UV-C energy (with 10 s treatment time) in both apples (2.1 ± 0.4 log CFU/g reduction) and pears (1.2 ± 0.2 log CFU/g reduction). Cell numbers decreased significantly after every 20 s UV-C treatments in apples. There was a reduction of 2.9 ± 0.2 log CFU/g after treatment, with an average UV-C dose of 0.92 kJ/m^2 (one minute treatment). For pears, microbial levels remained similar for average UV-C treatment doses between 0.15 and 0.63 kJ/m^2 (10–40 s treatment time). Cell numbers were further reduced after an average UV-C energy dose of 0.92 kJ/m^2 (one minute treatment), with a total reduction of 2.1 ± 0.1 log CFU/g on pear surfaces.

E coli O157:H7 was found to be more UV-C resistant on visibly rougher pear surfaces. Therefore, pears required a 0.92 kJ/m^2 UV-C dose, while apples required only a 0.15 kJ/m^2 UV-C dose to reduce cell numbers to similar levels. For both strawberries and raspberries, *E. coli* O157:H7 levels decreased significantly ($p < 0.05$) after UV-C treatment, with an average dose of 0.49 kJ/m^2 (30 s treatment). After UV-C treatment with an average dose of 0.92 kJ/m^2 (one minute treatment), *E. coli* O157:H7 levels were significantly ($p < 0.05$) reduced on strawberry surfaces, and remained constant until a four minute treatment was achieved. For raspberries, significant reduction was achieved after 2 and 6 minute treatments, with no further reduction even up to a 12 min treatment time. A total reduction of 1.0 ± 0.1 log CFU/g was achieved in raspberries after 8 min of treatment (average dose 7.17 kJ/m^2). This result was similar to that achieved with 1 min of treatment for strawberries (average dose 0.99 kJ/m^2). Inactivation by UV was much less effective on berry surfaces than apple or pear surfaces.

In our study, the average population of *L. monocytogenes* before UV-C treatment on apple, pear, strawberry and cantaloupe surfaces was in the range of 5.1 to 6.3 log CFU/g. Significant reductions ($p < 0.05$) were observed in *L. monocytogenes* populations, with higher reductions in apple (1.6 log at 3.75 kJ/m^2) and pear (1.7 log at 11.9 kJ/m^2) surfaces compared to cantaloupe and strawberry surfaces (1.0 log at 11.87 kJ/m^2) (Fig. 2, Table 2). Reductions achieved during the first 5 min of treatment on apple surfaces were similar to those achieved after 14 min of treatment on pear surfaces (1.6 log CFU/g). The bacteria were more resistant to UV-C treatment on strawberry and cantaloupe surfaces, with less than a log reduction (1.0 log CFU/g) after 14 min of

Table 3
Weibull model parameters for UV-C inactivation of *E. coli* O157:H7 and *Listeria monocytogenes* on different fruits.

Fruit surface	*E. coli* O157:H7			*Listeria monocytogenes*		
	α, kJ/m^2 (s)	β	E_{90}, kJ/m^2 (s)	α, kJ/m^2 (s)	β	E_{90}, kJ/m^2 (s)
Apple	0.001 (0.054)	0.275	0.017 (1.12)	0.034 (2.30)	0.255	0.895 (60.5)
Pear	0.010 (0.676)	0.331	0.124 (8.40)	0.020 (1.55)	0.205	1.35 (90.9)
Cantaloupe				0.991 (67.0)	0.296	16.6 (1121.4)
Raspberry	0.756 (51.1)	0.34	8.60 (581.4)			
Strawberry	0.245 (16.6)	0.432	1.69 (114.2)	0.046 (3.11)	0.123	40.6 (2741.4)

Where α is the scale factor, β is the shape parameter determining the shape of the curve and E_{90} is the UV-C energy required for a 90% reduction in target microorganisms.

Table 4
Root mean square surface roughness (R_q) and average surface roughness (R_a) values of selected fruits.

Fruit	R_q (μm)	R_a (μm)
Apple	30.3	25.4
Pear	40.2	32.8
Cantaloupe	55.8	47.7
Raspberry	78.6	62.4
Strawberry	296	287

treatment. For all fruit surfaces, reduction achieved within the first minute of treatment (1.10 ± 0.16 kJ/m^2) was significantly ($p < 0.05$) higher than during the remaining 2 to 12 min of treatment.

Our results show that *L. monocytogenes* was more resistant to UV-C treatment than *E. coli* O157:H7 on fruit surfaces. Reduction of *E. coli* O157:H7 achieved within 10 s UV-C treatment on apple surfaces was higher than that of *L. monocytogenes* after 5 min treatment. Comparable results were observed on pear and strawberry surfaces, with *E. coli* O157:H7 reduction of 2.1 and 0.9 log CFU/g, respectively, within 60 s treatment. However, *L. monocytogenes* reductions on similar surfaces after 14 min treatment were only 1.7 and 1.0 log CFU/g, respectively. Similar observations have been reported on the resistance of *L. monocytogenes* toward UV-C treatment (Gabriel and Nakano, 2009).

In a study conducted by suspending bacterial pathogens on phosphate buffer saline (PBS) and apple juice, *L. monocytogenes* had significantly higher resistance to UV treatment than *Salmonella* and *E. coli* O157:H7 (Gabriel and Nakano, 2009). Guerrero-Beltrán and Barbosa-Cánovas (2005) reported greater resistance of *Listeria innocua* (ATCC 51742) to UV radiation than *E. coli* (ATCC11775) suspended in apple juice. Factors such as presence of organic food material and biofilm formation can affect how well UV-C treatment reduces *L. monocytogenes* (Bernbom et al., 2011). Furthermore, *L. monocytogenes* can better attach to and colonize fruit surfaces than *E. coli* O157:H7 cells (Collignon and Korsten, 2010). The effectiveness of UV-C light treatment against *L. monocytogenes* strains may also be affected by the pre-stress conditions of the strains (McKinney et al., 2009).

In our study, the survival curves for both *E. coli* O157:H7 and *L. monocytogenes* were non-linear. Weibull model for inactivation kinetics was found to be appropriate. The β values, which determine the shape of the curve for both fruits and berries, were in the range of 0.12–0.43 (Table 3). The survival curve had concave shape, indicating that surviving microbes were more resistant to inactivation or could quickly adapt to applied stress (Mafart et al., 2002). Studies indicate that the stress response of *E. coli* O157:H7 and *L. monocytogenes* to sublethal, environmental stresses such as starvation, acid stress, or temperature changes provides cross-protection to a variety of physical and chemical stresses (Leenanon and Drake, 2001; McKinney et al., 2009). The bacterial strains used in this study were isolated from different sources (*E. coli* O157:H7: human feces, outbreak strains from raw hamburger and Odwalla apple juice; *L. monocytogenes*: garlic, bovine milk and human feces). This may have affected their stress response mechanisms. Similar trends were observed by Bialka et al. (2008), who applied the Weibull model on the inactivation curves of *E. coli* O157:H7 and *Salmonella* enterica for raspberries and strawberries exposed to ozone or pulsed UV-light.

3.2. Influence of surface properties on UV-C inactivation of bacteria

Several factors related to fruit surfaces may influence the inactivation rate of microorganisms by UV-C. These include surface roughness, surface hydrophobicity and the presence of trichomes (Birmpa et al., 2013; Syamaladevi et al., 2013; Yaun et al., 2004; Yun et al., 2013). These properties also influence the attachment of bacterial pathogens onto fruit surfaces (Collignon and Korsten, 2010). This hinders the removal of pathogens from fruit surfaces during a variety of treatments (Liao and Sapers, 2000; Wang et al., 2008) and alters the susceptibility of microbes to decontamination treatment (Burnett et al., 2000). In general, higher UV-C doses are required to reduce microorganisms on fruit surfaces that are rougher, have lower spreading coefficients, or that contain trichomes (Syamaladevi et al., 2014; Syamaladevi et al., 2015).

We found that the surface roughness values of the strawberries were higher than that of apples and pears by roughly one order of magnitude. This can be attributed to the presence of indentations and seeds. There may be protection of microbial cells from UV-C light on opaque seeds and dimpled strawberry surface (Table 4 and Fig. 3). We observed similar roughness values for cantaloupe and raspberry surfaces (Table 4 and Fig. 3).

The contact angle (θ), which is related to the hydrophobicity of the surface (Velazquez et al., 2011), reflects microbial adherence (Syamaladevi et al., 2013). Hydrophobic surfaces have a contact angle value of $\theta > 65$ for water, while angles of $\theta < 65$ are considered to be

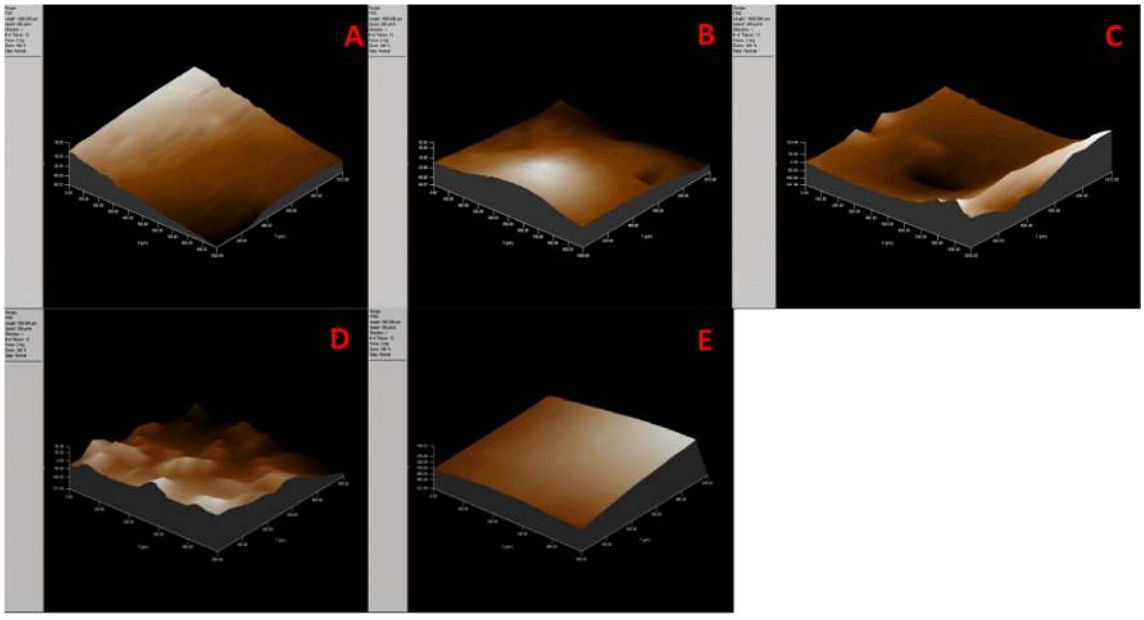

Fig. 3. Surface morphology of (A) apple (B) pear (C) cantaloupe (D) strawberry and (E) raspberry observed through a stylus profilometer.

Table 5
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)	$W_a \times 10^3$ (mN/m)	$W_s \times 10^3$ (mN/m)
	Water	Diiodomethane			
Apple	81.8 ± 12.5^{B}	42.5 ± 8.4^{C}	42.9 ± 6.4^{AB}	84.6 ± 15^{A}	-61.2 ± 15^{A}
Pear	96.8 ± 7.7^{A}	38.7 ± 5.0^{CD}	40.6 ± 2.9^{B}	64.3 ± 9.7^{B}	-81.5 ± 9.5^{B}
Cantaloupe	76.3 ± 12.8^{B}	63.7 ± 11.3^{B}	36.4 ± 2.0^{C}	89.7 ± 15^{A}	-56.1 ± 14.6^{A}
Raspberry	91.0 ± 10.0^{A}	77.3 ± 8.0^{A}	24.4 ± 5.9^{D}	71.7 ± 13^{B}	-74.2 ± 12^{B}
Strawberry	76.3 ± 9.2^{B}	35.6 ± 11^{D}	46.4 ± 6.1^{A}	90.2 ± 12^{A}	-55.6 ± 11^{A}

Where γ_S = surface energy of the solid (mN/m), W_a = reversible work of adhesion (mN/m), W_s = spreading coefficient (mN/m). Different superscripts represent statistical differences between surface energy parameters column-wise ($p < 0.05$).

hydrophilic, and $\theta = 0$ indicates a completely wettable surface. Surface spreading of a liquid occurs with a contact angle of $0 < \theta < 90$, and a liquid will bead at $\theta > 90$ (Vogler, 1998; Woodling and Moraru, 2005). Velasquez et al. (2011) reported contact angles for water on apple (Fuji) (91.6°), pear (89.7°) and strawberry (74.8°) surfaces. Our results were similar; raspberry had a water contact angle similar to apple and pear (91.0 ± 10.0°). Contact angles in diiodomethane were lower for apples (42.5 ± 8.4°), pears (38.7 ± 5.0°), and strawberries (35.6 ± 11.0°). However, the contact angles of cantaloupe (63.7 ± 11.3°) and raspberries (77.3 ± 8.0°) had the highest value and the greatest hydrophobicity (Table 5). Therefore, the hydrophobic characteristics of raspberries and cantaloupes may have contributed to the low inactivation rate.

Our study found that the surface roughness (R_q) and spreading coefficients (W_s) of fruits affect the UV-C inactivation kinetics and E_{90} of bacterial pathogens (Table 3 and Fig. 4). Results show that the bacterial pathogen inactivation rate was higher on apple and pear fruit surfaces. Both had a higher and more rapid reduction in microbial levels at a lower UV-C dose than berry and cantaloupe surfaces. The higher inactivation rates for *E. coli* O157:H7 and *L. monocytogenes* by UV-C on apple and pear surfaces can be attributed to their lower surface roughness and higher spreading coefficient values. The high surface roughness and low spreading coefficient of raspberries may be attributed to its higher E_{90} for *E. coli* O157:H7 and *L. monocytogenes* inactivation. Microorganisms can hide under the drupelets of raspberries, preventing direct exposure to UV-C light.

The higher E_{90} on raspberry (*E. coli* O157:H7) and cantaloupe (*L. monocytogenes*) may be due to the large number of trichomes and drupelets on raspberry surfaces (Syamaladevi et al., 2015) and the dense netting on cantaloupe surfaces (Annous et al., 2005). We found that UV-C light was effective only on exposed surfaces and rough surfaces shadow microbial cells, impairing the germicidal effect of UV-C light (Manzocco et al., 2011; Syamaladevi et al., 2013). Higher E_{90} values on fruit surfaces other than apples and pears may also be attributed to the inability of UV-C to penetrate into the interstices of the drupelets. This applies to the rough surfaces of raspberries, cantaloupes and strawberries. The less than 1 mm penetration depth for UV-C can affect the viability of microbial cells as reported on apples (Manzocco et al., 2011). This agrees with results from Syamaladevi et al. (2015) who observed that higher UV-C doses were required for *Penicillin expansum* on rough fruit surfaces such as strawberries and raspberries compared to apples and cherries.

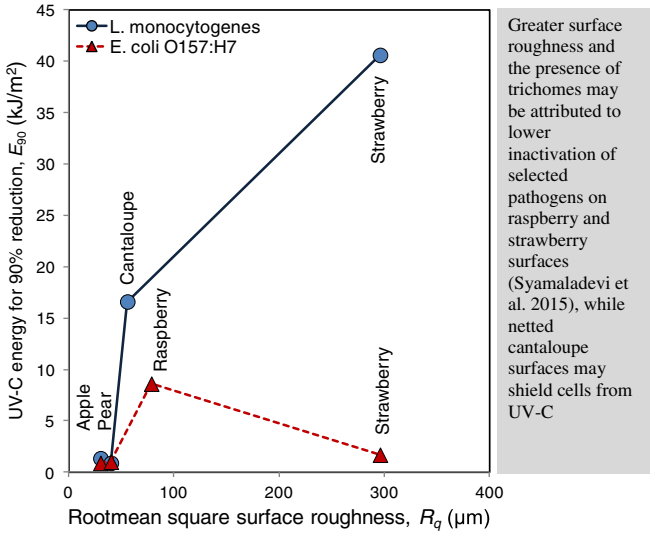

Fig. 4. Influence of root mean square surface roughness (R_q) on UV-C energy required for 90% reduction in bacterial population (E_{90}).

Limited studies performed UV-C inactivation of *E. coli* O157:H7 on food surfaces focused on mushrooms, fruit, berries, eggs whites, fish, and green leafy vegetables (Bialka et al., 2008; Birmpa et al., 2013; Guan et al., 2012; Kim and Hung, 2012; Unluturk et al., 2007; Yaun et al., 2004). UV-C was found to be more effective at reducing *E. coli* O157:H7 populations on apple surfaces (3.3 log at a UV-C dose of 9 mW/cm^2) than on lettuce (2.2 log reduction) (Yaun et al., 2004). Greater survival of generic *E. coli* was observed on the rougher peach surfaces compared to pear surfaces (Syamaladevi et al., 2013), indicating that the microbial inactivation is dependent upon the surface morphological characteristics of the fruit with similar results confirmed in this study with fruits with rougher surfaces.

The smaller UV-C inactivation rate and higher E_{90} for *E. coli* O157:H7 and *L. monocytogenes* on strawberries compared to apple and pear surfaces may be attributed to the higher roughness of strawberries (Figs. 3 and 4). Furthermore, the rough surface of strawberries contains hundreds of tiny seeds that could shield microbes from UV-C light. For strawberries, even with highest R_q, the largest W_s responsible for better spreading of the bacterial pathogens may have contributed to the lower t_R for *E. coli* O157:H7. This supports results by Birmpa et al. (2013), who observed a log reduction of only up to 1.4 log CFU/g in *E. coli*, *L. innocua*, *Salmonella* Enteritidis and *Staphylococcus aureus* on the surface of strawberries at UV-C light intensities of 72 kJ/m^2. A similar result was observed by Syamaladevi et al. (2015) for the plant pathogen *P. expansum*. Limited *E. coli* O157:H7 reduction (1.1 log CFU/g) was also reported on rough surfaces (mushroom caps) with a UV-C dose of up to 3.15 kJ/m^2 (Guan et al., 2012). A wound or roughened fruit surface shields and partially protects microorganisms during UV-C exposure (Syamaladevi et al., 2013). However, we observed an interesting pattern with *L. monocytogenes*, with the largest E_{90} on strawberry surfaces. This may be due to the higher resistance of *Listeria* spp. to UV-C treatment (Gabriel and Nakano, 2009; Guerrero-Beltrán and Barbosa-Cánovas, 2005).

4. Conclusions

In this study, we found that UV-C light effectively reduces *E. coli* O157:H7 (up to 2.9 log CFU/g at less than 1 kJ/m^2) and *L. monocytogenes* (up to 1.6 log CFU/g at 3.75 kJ/m^2) on fruit with smooth surfaces, such as apples. Inactivation rates were higher for fruits with smoother surfaces, such as apples and pears, and substantially lower for fruits with rougher surfaces (cantaloupe), dimples or seeds (strawberry), or drupelets (raspberry) that are impenetrable to UV-C

light. It was not possible to achieve a 2.0 log reduction of *E. coli* O157:H7 and *L. monocytogenes* on raspberry and cantaloupe surfaces with UV-C light of 11.9 kJ/m^2. Future studies on UV-C treatment, coupled with a chemically-based technology to inactivate cells that are hard to reach with UV-C light, may aid applications for disinfecting berries and fruits with rough surfaces. This study advances research on new ways to sanitize fruit and protect consumer health and safety. A potential use is on the packing line of fruits which may satisfy the preventive control requirements set forth by the proposed U.S. Food and Drug Administration Food Safety Modernization Act.

Acknowledgments

This research was partially funded with a Biological and Organic Agriculture (BIOAg) program grant from the Center for Sustaining Agriculture and Natural Resources at Washington State University. The authors wish to thank Dr. Michael Khbeis and Mr. Paul Schilling, Microfabrication Facility (MFF), University of Washington for conducting surface roughness measurements of fruits.

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