BIOAG PROJECT FINAL REPORT

TITLE: Sustainable sanitation technique for postharvest quality and safety of organic fruits

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KEY WORDS: *E. coli O157:H7*, Food quality, Food safety, *Penicillium expansum*, Ultraviolet light

ABSTRACT

The project investigated the application of ultraviolet-C light for sanitizing organic fruit surfaces. We evaluated the efficacy of ultraviolet-C light for inactivating pathogenic bacteria *E. coli O157:H7* and spoilage mold *Penicillium expansum* on selected organic fruit surfaces. Maximum reductions of 2.09±0.08 and 2.94±0.18 log CFU/g were achieved for *E. coli* on pear and apple surface, respectively after UV-C exposure at an average 0.91 kJ/m^2 UV dose. While only 0.98±0.06 and 1.95±0.33 log CFU/g were achieved for red raspberry and strawberry surface, respectively after 7.2 kJ/m^2 UV dose. For *Penicillium expansum*, the maximum reduction of 2.1±0.10 and 1.8±0.3 log CFU/g was achieved on cherry and apple surface, respectively after 1.3 kJ/m^2 UV dose while 2.8±0.1 and 2.6±0.3 log CFU/g was achieved on red raspberry and strawberry, respectively after 3.3 kJ/m^2 UV dose. Higher UV-C doses will require reducing microbial populations on red raspberry and strawberry surfaces. The higher surface roughness of raspberry and strawberry helped to shield microorganisms from UV-C light. The changes in color and textural parameters, and ^{o}Brix of fruits were insignificant after UV-C doses at the level of 1.3-2.0 kJ/m^2.

PROJECT DESCRIPTION

The postharvest life of small fruits such as cherries, red raspberries, strawberries and blueberries is limited by fungal growth caused by several pathogens including *Penicillium expansum* and *B. cinerea*, resulting in great economic losses. In fact, the lack of effective postharvest control of mold is the most important factor limiting the sale of small fruit in distant markets (Ellis et al., 2008). Although hot water treatments, biological control, and chemical applications have shown to reduce postharvest rots of fruits, each has limitations that can affect commercial applicability. Due to the delicate nature of small fruits, hot water treatment is not a viable option to control fruit rot. Biological methods have not lived up to their early promise, and very few biofungicides are commercially available. Chemically resistant fungi and bacteria have developed due to the intensive use of chemical applications, and the chemical solutions commonly used for to sanitize fruits are regulated for organic produce. In search of viable alternatives the organic industry has shifted its focus to physical methods of postharvest fruit protection. Small fruits such as berries and cherries are generally considered safe from pathogenic bacteria because of their high acid content. However, fresh fruits have occasionally been implicated in food-borne illnesses. Bacterial pathogens such *Salmonella* and *Listeria monocytogenes* have been isolated in fresh and frozen berries (Bower et al., 2003). Recent outbreaks of *E. coli O157:H7*, *Salmonella* and *Listeria monocytogenes* in fresh fruits have challenged the belief that pathogenic bacteria cannot grow in high-acid foods. The U.S. Food and Drug Administration (FDA) Food Safety Modernization Act now requires growers and packers of all fresh produce to adapt preventive strategies for microbial controls to minimize the risk of human pathogens.

The project is designed to obtain mechanistic understanding of bacteria and mold inactivation on diverse fruit surfaces using UV-C light technology. Specific objectives of the proposed research are:

(1) to determine the ultraviolet-C light inactivation kinetics of generic *E. coli* O157:H7and and *Penicillium expansum* on organic fruit surfaces; and (2) to assess the physical and chemical quality of fruit after UV treatments.

OUTPUTS

Work Completed:

We investigated UV-C inactivation of pathogenic bacteria *E. coli* O157:H7 (Table 1) and blue mold i.e. *Penicillium expansum* on selected organic fruit surfaces (Tables 2). The results indicated that it is possible to reduce up to 2 log CFU/g *E. coli* pear and apple surfaces using 1 kJ/m2 UV does, with lesser reduction on red raspberry (0.0.53±0.06) and strawberry (0.93±0.04) surfaces after UV-C exposure at 1.0 kJ/m^2 UV dose. Similarly, for *Penicillium expansum*, higher reduction was achieved on cherry and apple surfaces compare to strawberry and cherry surfaces. The trichomes/irregular surface morphology of raspberry and strawberry (Table 3) protected microorganisms from UV-C light (Figures 1 and 2). UV-C can reduce *E. coli* and *Penicillium expansum* 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. Both hydrophobic nature of surface and spreading coefficient affect the distribution of microbial cells when applied in the fruit surface which in turn affected the efficacy of UV-C treatment on different fruit surfaces (Table 4). The effect of UV-C treatment on color, texture and soluble solids of fresh fruits was not significant (Tables 5-7).

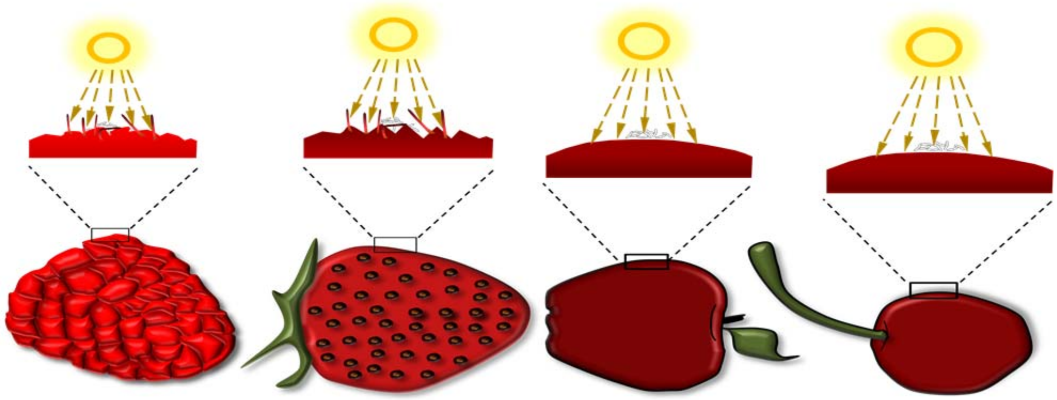

Figure 1 Schematic diagram presenting possible "shielding" of microorganisms from UV-C light by trichomes/irregular surface morphology of raspberries and strawberries compared to relatively smoother surfaces of apple and cherry

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

Time (s)	Pear		Apple		Raspberry		Strawberry	
	UV intensity (kJ/m^2)	Log reduction	UV intensity (kJ/m^2)	Log reduction	UV intensity (kJ/m^2)	Log reduction	UV intensity (kJ/m^2)	Log reduction
0	0	0^{a}	0	0^{a}	0	0^{a}	0	0^{a}
10	0.15	1.20 ± 0.21^{ghij}	0.14	2.06 ± 0.35^{mn}				
20	0.31	1.28 ± 0.24^{hij}	0.30	2.20 ± 0.43^{mn}				
30	0.48	1.50 ± 0.24^{ijk}	0.46	2.38 ± 0.44^{no}	0.49	0.38 ± 0.08^{b}	0.5	0.51 ± 0.19^{bc}
40	0.65	1.52 ± 0.23^{jk}	0.61	2.54 ± 0.32^{op}				
50	0.81	1.91 ± 0.24^{lm}	0.75	2.71 ± 0.25^{pq}				
60	0.98	2.09 ± 0.08^{mn}	0.86	2.94 ± 0.18^{q}	0.95	0.53 ± 0.06^{bc}	1.0	0.93 ± 0.04^{efg}
120					1.9	0.59 ± 0.13^{bcd}	2.0	1.18 ± 0.05^{ghi}
240					3.7	0.69 ± 0.09^{cde}	3.9	1.24 ± 0.11^{ghij}
360					5.3	0.85 ± 0.09^{def}	5.6	1.63 ± 0.40^{kl}
480					7.2	0.98 ± 0.06^{fgh}	7.1	1.95 ± 0.33^{lm}
600					8.9	1.08 ± 0.11^{fgh}		
720					10.9	1.09 ± 0.01^{fgh}		

Different superscripts in rows and columns represent statistical 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 *P. expansum* on selected organic fruit surfaces (N = 3)

Time (s)	Cherry		Apple		Raspberry		Strawberry	
	UV intensity (kJ/m^2)	Log reduction	UV intensity (kJ/m^2)	Log reduction	UV intensity (kJ/m^2)	Log reduction	UV intensity (kJ/m^2)	Log reduction
0	0	0	0	0	0	0	0	0
10	0.14	0.20±0.05[a]	0.13	0.24±0.15[a]	-	-	0.14	0.79±0.23[bcd]
20	0.30	0.58±0.17[abc]	0.243	0.44±0.26[ab]	-	-	0.30	0.99±0.30[cde]
30	0.46	0.98±0.08[cde]	-	-	0.28	0.54±0.28[abc]	0.46	1.3±0.2[ef]
40	-	-	0.47	0.81±0.26[bcd]	-	-	-	-
60	0.86	1.8±0.11[gh]	0.667	1.3±0.3[def]	0.59	1.3±0.11[def]	0.86	2.0±0.3[ghi]
90	1.4	2.1±0.10[hij]	0.95	1.6±0.2[fg]	-	-	-	-
120	2.1	2.4±0.15[ijk]	1.2	1.8±0.3[gh]	1.2	1.5±0.6[fg]	2.1	2.5±0.4[jk]
180	-	-	-	-	1.7	1.8±0.3[gh]	3.3	2.6±0.3[jk]
240	-	-	-	-	2.2	1.7±0.5[fgh]	-	-
360	-	-	-	-	3.3	2.8±0.1[k]	-	-

Different superscripts in rows and colums represent statistical significant differences between log reduction values in number of *P. expansum* cells obtained at selected UV doses (UV treatment times) ($p < 0.05$)

Table 3. Root mean square surface roughness (R_q) and average surface roughness (R_a) values of selected fruits

Fruit	S_q (µm)	S_a (µm)
Strawberry	296	287
Apple	30.3	25.4
Raspberry	78.6	62.4

Table 4. Average and standard deviation values of surface energy parameters of organic 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					
Strawberry	76.3±9.2	35.6±11	46.4±6.1	41.3±5.2	5.07±3.6	90.2±12	-55.6±11
Apple	81.8±12.5	42.5±8.4	42.9±6.4	38.7±4.3	4.31±3.2	84.6±15	-61.2±15
Raspberry	91.0±10.0[a]	77.3±8.0	24.4±5.9	19.0±4.2	5.4±4.3	71.7±13	-74.2±12
Cherry	84.1±5.4[b]	65.2±6.9	31.3±3.3	25.6±3.9	5.7±2.6	80.4±6.8	-65.5±6.7

where γ_S = surface energy (mN/m), 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_s = Spreading coefficient (mN/m)

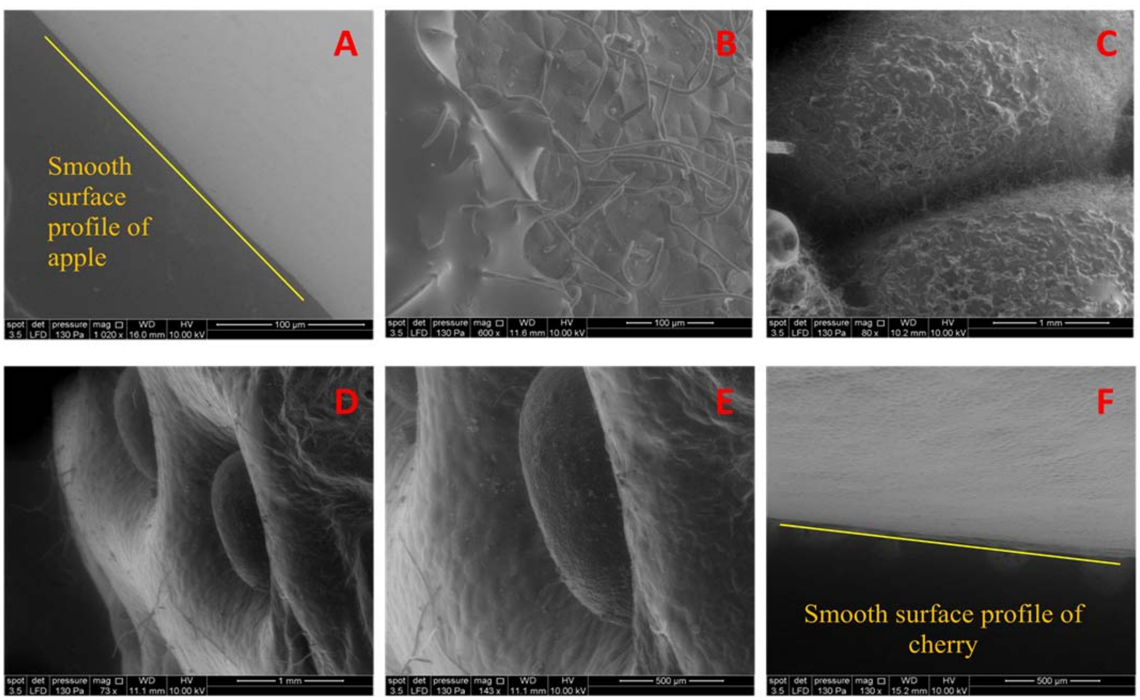

Figure 2 Surface profile of (A) apple (B, C) raspberry (D, E) strawberry (F) cherry observed though an environmental scanning electron microscopy

Table 5. °Brix of untreated and UV-C treated organic fruits (N = 3)

Fruit		°Brix
Raspberry	Untreated	10.1±0.2
	UV-C treated	10.3±0.1
Strawberry	Untreated	8.1±0.1
	UV-C treated	9.5±0.3
Apple	Untreated	14.1±0.0
	UV-C treated	14.2±0.2
Cherry	Untreated	18.7±0.2
	UV-C treated	17.9±0.9

Table 6. Color parameters of untreated and UV-C treated organic fruits (N = 10)

Fruit		L^*	*a*	*b*
Raspberry	Untreated	26.9±1.9	19.2±2.9	4.7±1.1
	UV-C treated	26.6±1.7	21.8±2.8	5.4±1.4
Strawberry	Untreated	35.1±2.7	34.2±4.8	17.1±4.3
	UV-C treated	38.0±3.1	35.9±2.8	20.7±4.8
Apple	Untreated	53.2±5.6	20.5±9.2	25.2±4.5
	UV-C treated	50.9±6.9	22.0±8.8	24.4±4.9
Cherry	Untreated	31.7±2.2	21.5±6.1	6.9±2.5
	UV-C treated	33.9±2.8	27.3±5.5	10.0±3.3

Table 7. Textual parameters of untreated and UV-C treated organic fruits (N=5)

Fruit		Hardness (N)	Adhesiveness (N.s)	Springiness	Cohesive-ness	Chewi-ness
Raspberry	Untreated	5.0±1.5	-0.03±0.02	0.32±0.05	0.20±0.03	0.32±0.11
	UV-C treated	4.2±0.7	-0.06±0.03	0.36±0.02	0.20±0.02	0.84±0.09
Strawberry	Untreated	36.6±7.5	-0.27±0.21	0.26±0.04	0.15±0.02	1.4±0.5
	UV-C treated	36.0±9.1	-0.33±0.15	0.27±0.04	0.14±0.01	1.4±0.4

Fruit		Maximum force required to puncture/compress the fruit tissue (N)
Apple	Untreated	66.0±4.3
	UV-C treated	63.5±5.2
Cherry	Untreated	3.9±0.3
	UV-C treated	4.7±1.4

- Publications, Handouts, Other Text & Web Products:

Conference Presentations:

Adhikari, A., Syamaladevi, R., Lupien, S., Dugan, F., Killinger, K., and Sablani, S. S. (2012) Ultraviolet-C Light Inactivation of *Penicillium Expansum* and *Escherichia Coli* on Organic Fruit Surfaces, CSANR Celebration Symposium, Pullman, WA, on December 06.

Syamaladevi, R. M., Adhikari, A., Lupien, S. L., Sablani, S. S., Dugan, F., Rasco, B., and Killinger, K. (2013) Ultraviolet-C Light Inactivation of *Penicillium Expansum* and *Escherichia Coli* O157:H7 on Organic Fruit Surfaces, Institute of Food Technologists Annual Meeting, Chicago, IL, July 13-16.

Peer-reviewed publications:

Syamaladevi, A., Lupien, S. L., Sablani, S. S., Dugan, F., Bhunia, K., and Killinger, K., (2013). Understanding the Influence of Surface Properties on Ultraviolet-C Light inactivation Kinetics of *Penicillium expansum* on Organic Fruits (In preparation)

Adhikari, A., Syamaladevi, R.M., Killinger, K., and Sablani, S. S., (2013). Fruit surface morphology influence on Ultraviolet-C Light inactivation Kinetics of *E. coli: O157 H7* (In preparation)

- Outreach & Education Activities:

The potential uses of UV-C technology for fruit sanitization and the results of our study were discussed with growers at the Good Agricultural Practices (GAPs) and Hazard Analysis and Critical Control Points (HACCP) workshops. The GAPs workshops were organized at Puyallup (February 08 and March 02, 2013) and Bellingham, WA, and HACCP workshop was conducted at Ellensburg (March 11-13, 2013). These workshops were attended by more than 60 participants.

- **IMPACTS:**

Short-Term: Both pathogenic bacteria and spoilage mold can be reduced up to 2-3 log CFU/g using UV-C light. The fruit surface morphology and properties affected efficacy of UV-C light for inactivation of *E. coli* and *Penicillium Expansum*. *E. coli* was found to be more UV-C resistant than *P. Expansum*.

Intermediate-Term: The information acquired can be used to design UV-C doses for sanitization of organic fruit surface thus increasing the microbial safety and extending the postharvest shelf-life of organic produce.

Long-Term: The improved microbial safety and extended postharvest life of organic produce will provide economic incentives to Washington organic agriculture industry. The research findings and new methods developed by this project may also lead to more environmentally-sound food handling methods and improved consumer health and safety throughout the nation.

ADDITIONAL FUNDING APPLIED FOR / SECURED:

The preliminary results have helped us to prepare following proposal which is under review process:

Ultraviolet Light based Hybrid Technologies to Control Foodborne Pathogens on Fresh Produce, USDA AFRI Food Safety Program, $424,907, 2013-2016 (Sablani, Rasco, Killinger and Syamaladevi)

GRADUATE STUDENTS FUNDED:

This project has supported two doctoral students: (1) Achyut Adhikari, School of Food Science, and (2) Kanishka Bhunia, Biological Systems Engineering. In addition, time slip research assistants, Ms. Katie Reeds, Horticulture and Roopesh Syamaladevi were supported by this project.

RECOMMENDATIONS FOR FUTURE RESEARCH:

Our research demonstrated that fruit surface morphology and surface integrity have significant influence on efficacy of UV-C treatment. In general, UV-C alone is effective in reducing microorganism population up to 1-3 log CFU/g depending up on types of microorganism and fruit surface characteristics. A combination of UV-C with other technologies such as electrolyzed water rinse and natural antimicrobial sprays needs to be investigated to achieve a 4-6 log CFU/g reduction of microorganisms on fresh produce as recommended by USDA.