

**INACTIVATION OF *ESCHERICHIA COLI* O157:H7, *SALMONELLA*  
*ENTERICA* AND MURINE NOROVIRUS ON BLUEBERRIES USING A  
NOVEL WATER-ASSISTED ULTRAVIOLET LIGHT PROCESS**

by

Chuhan Liu

A thesis submitted to the Faculty of the University of Delaware in partial  
fulfillment of the requirements for the degree of Master of Science in Food Science

Spring 2015

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## ABSTRACT

Fresh produce, such as blueberries, has been associated with foodborne illnesses. The most common causative agents include *Escherichia coli* O157:H7, *Salmonella* and norovirus. Chlorinated water has been widely used by the food industry to wash fresh produce to achieve some level of microbial decontamination. However, chlorine wash can lead to the formation of carcinogenic substances. The ability of ultraviolet (UV) light to inactivate bacteria and viruses is well established; however, its application on food commodities is limited because of their shadowing effect. In the present study, a novel set-up using water-assisted UV processing was developed to inactivate *Escherichia coli* O157:H7, *Salmonella* and murine norovirus (MNV-1) on fresh blueberries. The effect of different wash water qualities was also investigated for MNV-1 inactivation. Blueberry samples were exposed to UVC light alone (dry UV) or immersed in agitated water during UV treatment (wet UV). Wet UV treatment generally showed higher efficacies than dry UV treatment for both bacteria and virus inactivation. *E. coli* was most easily killed on skin-inoculated blueberries, followed by calyx-inoculated berries. Wet UV treatment of 10 min resulted in 5.2- and 3.9-log reductions of *E. coli* for skin- and calyx-inoculation methods, respectively. Dip-inoculated blueberries were the most difficult to decontaminate and 1.6-log reduction of *E. coli* was achieved after 10-min wet UV treatment. With a similar result, MNV-1 was more easily killed on skin-inoculated than on calyx-inoculated blueberries. Wet UV treatment of 5 min resulted in >4.36 and 3.04-log reductions of MNV on skin- and calyx-inoculated blueberries, respectively. Wet UV treatments

were comparable with a 10-ppm chlorine wash. Addition of 100-ppm sodium dodecyl sulfate (SDS), 0.5% levulinic acid or 10 ppm chlorine to washing solutions did not significantly enhance the wet UV treatment for bacteria inactivation. UV irradiation combined with 10 ppm chlorine wash was comparable to wet UV treatment alone for MNV-1 inactivation. Presence of 2% blueberry juice in wash water provided protection to MNV-1 from UV irradiation or chlorine wash treatment. Inactivation efficacy was comparable between UV+DI water wash and UV+DI water (5% blueberry crush) wash. Overall, this study shows that UV treatment could be used as an alternative to chlorine wash for blueberries and potentially for other fresh produce.

## Chapter 1

### INTRODUCTION

As one of the most popular fruits, blueberries have many benefits to human health, such as anticancer, antioxidant and anti-inflammatory activities (Roy, Lundy, & Kalicki, 2009). The consumption of blueberries in the U.S. has been on the rise due to the increasing recognition of their potential health benefits. The U.S. Department of Agriculture (USDA) reported that between 1994 and 2003, the consumption of fresh blueberries in the U.S. increased about 1.6 times (Roy et al., 2009). Fresh blueberries are harvested manually or mechanically and field packed into retail containers (Harris et al., 2003). Fresh blueberries destined for the fresh market are not washed following harvesting. Berries that are to be processed are usually washed with potable water or chlorinated water. Washed berries are sometimes mixed with up to 30% sucrose before freezing (Harris et al., 2003). Thus, blueberries can occasionally lead to food safety problems since they are mostly consumed raw or minimally processed. Blueberries can become contaminated at any point on the farm-to-table continuum, including irrigation, picking, and post-harvest processing (Rodas Bourquin, Salazar, Gomez, & Wise, 2009). Fresh berries and berry products have been implicated in several foodborne outbreaks (Calder et al., 2003; The U.S. Food and Drug Administration [FDA], 2001; Oregon Health Authority, 2011). In 2003, contaminated raw blueberries were reported to be the source of an outbreak of hepatitis A (Calder et al., 2003). In 2009, blueberries contaminated with *Salmonella* Muenchen resulted in a multistate outbreak that caused 14 cases of illnesses (Centers for Diseases Control and

Prevention [CDC], 2014). In 2010, an outbreak of six cases of *Salmonella* Newport infection in northwestern Minnesota was investigated and results identified blueberries as the cause (Miller , Rigdon, Robinson, Hedberg, &Smith, 2013). In 2011, an outbreak in Oregon was associated with fresh strawberries contaminated with *Escherichia coli* O157:H7, which caused at least 10 people to become sick and one death (Oregon Health Authority, 2011). Frozen strawberries and raspberries have also been frequently associated with HAV and human norovirus outbreaks (Cotterelle et al., 2005; Hutin et al., 1999; Korsager, Hede, Boggild, Bottiger, & Molbak, 2005; Mäde , Trübner, Neubert, Höhne, & Johne, 2013; Niu et al., 1992). Therefore, there is an urgent need to develop effective decontamination technologies for berries.

Nonthermal food processing technologies have been gaining more and more interest as alternatives to traditional thermal processing. Nonthermal processing technologies can be used to lower foodborne pathogen levels while maintaining nutritional and sensory characteristics (Butz & Tauscher, 2002). Some of the commonly seen nonthermal food processing technologies include high pressure processing (HPP), pulsed electric fields (PEF), ultraviolet light (UV), and irradiation. UV light has been applied in juice pasteurization and it has been shown that UV has little detrimental effect on phenolic compounds and anthocyanins (Pala & Toklucu, 2013). UV light treatment has been shown to be effective for inactivation of bacteria (Allende, McEvoy, Luo, Artes, & Wang, 2006; Kim & Hung, 2012), protozoan oocysts (Clancy , Hargy, Marshall, & Dyksen, 1998), fungi (Gunduz & Pazir, 2013) and viruses (Nuanualsuwan, Thongtha, Kamolsiripichaiporn, & Subharat, 2008).

Therefore, the objectives of the current research were to (i) evaluate the efficacy of UV light on decontaminating fresh blueberries inoculated with *E. coli*

O157:H7 and *Salmonella* with or without washing treatments, (ii) determine the efficacy of UV on the inactivation of murine norovirus (MNV) on fresh blueberries with or without washing treatments, (iii) investigate the efficacy of water-assisted UV treatment on the inactivation of MNV on blueberries on a large scale.

## REFERENCES

- Allende, A., McEvoy, J. L., Luo, Y., Artes, F., & Wang, C. Y. (2006). Effectiveness of two-sided UV-C treatments in inhibiting natural microflora and extending the shelf-life of minimally processed 'Red oak leaf' lettuce. *Food Microbiology*, 23(3), 241-249.
- Butz, P., & Tauscher, B. (2002). Emerging technologies: Chemical aspects. *Food Research International*, 35(2-3), 279-284.  
doi:[http://dx.doi.org/10.1016/S0963-9969\(01\)00197-1](http://dx.doi.org/10.1016/S0963-9969(01)00197-1)
- Calder, L., Simmons, G., Thornley, C., Taylor, P., Pritchard, K., Greening, G., & Bishop, J. (2003). An outbreak of hepatitis A associated with consumption of raw blueberries. *Epidemiology & Infection* 131, 745-751.
- Clancy, J. L., Hargy, T. M., Marshall, M. M., & Dyksen, J. E. (1998). UV light inactivation of *Cryptosporidium* oocysts. *Journal of the American Water Works Association*, 90, 92-102.
- Centers for Disease Control and Prevention (CDC). (2014). *Reports of Selected Salmonella Outbreaks Investigations*. Retrieved November 8, 2014, from <http://www.cdc.gov/salmonella/outbreaks.html>.
- Cotterelle, B., Drougard, C., Rolland, J., Becamel, M., Boudon, M., Pinede, S., Traore, O., Balay, K., Pothier, P., & Espie, E. (2005). Outbreak of norovirus infection associated with the consumption of frozen raspberries. France, March 2005. *Euro Surveill*, 10(17), 2690. Retrieved October 25, 2014, from <http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=2690>.
- Gunduz, G. T., & Pazir, F. (2013). Inactivation of *Penicillium digitatum* and *Penicillium italicum* under In Vitro and In Vivo Conditions by Using UV-C Light. *Journal of Food Protection*, 76(10), 1761-1766
- Harris, L.J., Farber, J.N., Beuchat, L.R., Parish, M.E., Suslow, T.V., Garrett, E.H., & Busta, F.F. (2003). Outbreaks Associated with Fresh Produce: Incidence, Growth, and Survival of Pathogens in Fresh and Fresh-Cut Produce. *Comprehensive Reviews in Food Science and Food Safety*, 2, 78-141.

- Hutin, Y. J., Pool, V., Cramer, E. H., Najnan, O. V., Weth, J., Williams, I. T., Goldstein, S. T., Gensheimer, K. F., Bell, B. P., Shapiro, C. N., Alter, M. J., & Margolis, H. S. (1999). A multistate, foodborne outbreak of hepatitis A. National Hepatitis A Investigation Team. *The New England Journal of Medicine*, *340*, 595-602.
- Kim, C., & Hung, Y. (2012). Inactivation of *E. coli* O157:H7 on blueberries by electrolyzed water, ultraviolet light, and ozone. *Journal of Food Science*, *77*(4), 206 - 211.
- Korsager, B., Hede, S., Boggild, H., Bottiger, B., & Molbak, K. (2005). Two outbreaks of norovirus infections associated with the consumption of imported frozen raspberries, Denmark, May-June 2005. *Euro Surveill*, *2005*. *10*(25), 2729. Retrieved October 25, 2014, from <http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=2729>
- Mäde, D., Trübner, K., Neubert, E., Höhne, M., & Johne, R. (2013). Detection and typing of norovirus from frozen strawberries involved in a large-scale gastroenteritis outbreak in Germany. *Food and environmental virology*, *5*, 162-168.
- Miller, B. D., Rigdon, C. E., Robinson, T. J., Hedberg, C., & Smith, K. E. (2013). Use of global trade item numbers in the investigation of a *Salmonella* Newport outbreak associated with blueberries in Minnesota, 2010. *Journal of Food Protection*, *76*, 762-769.
- Niu, M. T., Polish, L. B., Robertson, B. H., Khanna, B.K., Woodruff, B. A., Shapiro, C. N., Miller, M. A., Smith, J. D., Gedrose, J. K., & Alter, M. J. (1992). Multistate outbreak of hepatitis A associated with frozen strawberries. *Journal of Infectious Diseases*, *166*, 518-524.
- Nuanualsuwan, S., Thongtha, P., Kamolsiripichaiporn, S., & Subharat, S. (2008). UV inactivation and model of UV inactivation of foot-and-mouth disease viruses in suspension. *International Journal of Food Microbiology*, *127*, 1-2
- Oregon Health Authority. (2011). *Lab tests confirm source of E. coli O157 from deer droppings in strawberry fields in NW Oregon*. Retrieved October 25, 2014 from <http://www.oregon.gov>
- Pala, Ç. U., & Toklucu, A. K. (2013). Effects of UV-C light processing on some quality characteristics of grape juices. *Food and Bioprocess Technology*, *6*(3), 719-725.

- Roy J. H., Lundy, S., & Kalicki, B. (2009). Blueberries "their role in health". *Pennington Nutrition Series*, No. 2.
- Rodas, A. G., Bourquin, L., Salazar, C. G., Gomez, A.V., & Wise, J.C., 2009. *Good Agricultural Practices for Food Safety in Blueberry Production: Basic Principles*. Retrieved November 8, 2014, from [http://www.gaps.msue.msu.edu/blue\\_manual.pdf](http://www.gaps.msue.msu.edu/blue_manual.pdf).
- The U.S. Food and Drug Administration (FDA). (2001). *FDA survey of imported fresh produce FY 1999 field assignment*. Retrieved October 25, 2014, from <http://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/ProducePlantProducts/ucm118891.htm>.

## **Chapter 2**

### **LITERATURE REVIEW**

#### **2.1 Berry Products: Contamination Involved in Foodborne Diseases**

##### **2.1.1 Blueberry and Blueberry Products**

The United States is the world's largest producer of blueberries, accounting for over 50% of world output (USDA, 2013). A total of 564.4 million pounds of cultivated and wild blueberries were harvested in 2012. In the U.S., blueberries rank as the second most important commercial berry crop, with a total crop value of almost \$851 million in 2012. Michigan is the nation's leading producer of cultivated blueberries, harvesting 87 million pounds in 2012. Other top producers included Oregon, Georgia and New Jersey (Agricultural Marketing Resource Center [AgMRC], 2013).

As one of the most popular fruits, blueberries have many benefits to human health, such as anticancer, antioxidant and anti-inflammatory activities (Roy et al., 2009). Fresh blueberries are harvested manually or mechanically and then field packed into retail containers (Harris et al., 2003). Fresh blueberries destined for the fresh market are not washed following harvesting. Berries that are to be processed are usually washed with potable water or chlorinated water.

### **2.1.2 Foodborne Diseases and Outbreaks Associated with Berry Products**

Blueberries can occasionally lead to food safety problems since they are mostly consumed raw or minimally processed. Blueberries can become contaminated at any point on the farm-to-table continuum, including irrigation, picking, and post-harvest processes (Rodas et al., 2009).

Fresh berries and berry products have been implicated in several foodborne outbreaks (Calder et al., 2003; FDA, 2001; Oregon Health Authority, 2011). In 2003, contaminated raw blueberries resulted in an outbreak of hepatitis A (Calder et al., 2003). In 2010, an outbreak of six cases of *Salmonella* Newport infection in northwestern Minnesota was investigated and results identified blueberries as the cause (Miller et al., 2013). In 2011, an outbreak in Oregon was associated with fresh strawberries contaminated with *Escherichia coli* O157:H7, which caused at least 10 illnesses and one death (Oregon Health Authority, 2011). Frozen strawberries and raspberries have also been frequently associated with HAV and human norovirus outbreaks (Cotterelle et al., 2005; Hutin et al., 1999; Korsager et al., 2005; Mäde et al., 2013; Niu et al., 1992).

## **2.2 *Escherichia coli* O157:H7**

### **2.2.1 Characteristics**

*E. coli* is a Gram-negative, non-spore-forming facultative anaerobe in the family *Enterobacteriaceae*. *E. coli* normally lives in the intestines of animals and people. Most *E. coli* bacteria are harmless; however, some are pathogenic and can cause either diarrhea or illness outside of the intestinal tract.

Serotyping and serogrouping of *E. coli* is used for subdividing the species into serovars. Serotyping in *E. coli* involves serological identification of three surface

antigens: O (somatic lipopolysaccharide), H (flagellar), and K (capsular). Pathogenic *E. coli* are also categorized into pathotypes based on the presence of certain virulence factors and toxin production and their interaction pattern with mammalian cells or tissues. There are six pathotypes of pathogenic *E. coli*: (i) enterohemorrhagic *E. coli* (EHEC), (ii) enterotoxigenic *E. coli* (ETEC), (iii) enteropathogenic *E. coli* (EPEC), (iv) enteroaggregative *E. coli* (EAEC), (v) enteroinvasive *E. coli* (EIEC), and (vi) diffusely adherent *E. coli* (DAEC) (CDC, 2012b). The most commonly identified EHEC in North America is *E. coli* O157:H7 and it is also considered the prototypical serotype of EHEC.

*E. coli* O157:H7 are phenotypically distinct from other *E. coli* in that it generally does not ferment sorbitol and does not have  $\beta$ -glucuronidase activity (FDA, 2011). It has an optimum growth temperature of 37 °C, grows rapidly at 30 – 42 °C, doesn't grow at temperatures lower than 10 °C and does not grow or grow poorly at 44 °C or above (Bhunja, 2008). The bacteria are destroyed at 70 °C or higher. *E. coli* O157:H7 is relatively acid-tolerant and can grow at pH levels of 4.4 – 9.0. It can survive for extended periods in foods at pH levels of 3.5 – 5.5 (Riemann & Cliver, 2006).

#### **2.2.1.1 Illness**

*E. coli* O157:H7 bacteria live in the guts of ruminant animals, including goats, cattle, deer, sheep and elk. The major source for human illnesses is cattle. The infectious dose for *E. coli* O157:H7 is estimated to be 10 – 100 cells (FDA, 2011). The incubation period for *E. coli* is 1-10 days and the illness lasts for 5-10 days. Infection may lead to a wide variety of symptoms, including diarrhea (usually bloody), nausea, abdominal cramps, vomiting and chills. Fever is usually rare. It can also

potentially develop a rare life-threatening complication known as hemolytic uremic syndrome (HUS), which is the most common cause of sudden, acute kidney failure among children in the United States (US Department of Health and Human Services [USDHHS], 2014a). About 15% of children infected with *E. coli* O157:H7 develop HUS (Iowa Department of Public Health, 2014). EHEC infection occurs in all age groups; however, children and elderly people appear to be at increased risk for complications.

*E. coli* O157:H7 produces two different Shiga-like toxins (Stx1 and Stx2). These Shiga-like toxins are very similar, if not identical, to the toxins produced by *Shigella dysenteriae*. These toxins inhibit protein synthesis, leading to apoptosis and/or necrosis of receptor-bearing, susceptible, microvascular endothelial cells. In addition to Shiga-like toxins, *E. coli* O157:H7 has other virulence characteristics, such as attaching and effacing activity and hemolysin production (Pruimboom-Brees et al., 2000).

### **2.2.2 Transmission**

Transmission of *E. coli* O157:H7 can occur in many ways, including through drinking water contaminated with animal or human feces containing the bacteria; eating raw fruits and vegetables contaminated with feces of infected animals; drinking unpasteurized cider, apple juice or dairy products; eating undercooked contaminated ground beef, and person-to-person transmission (Iowa Department of Public Health, 2014).

### **2.2.3 Outbreaks**

*E. coli* O157:H7 has been the cause of multiple outbreaks associated with fresh produce. There was a case of multistate outbreak of *E. coli* O157:H7 infections linked to ready-to-eat salads in 2013, where 33 people were infected and two of them developed HUS (CDC, 2013a). *E. coli* O157:H7 contamination of organic spinach and spring mix blend in 2012 was reported from five states (CDC, 2012c). In 2011, an outbreak in Oregon was associated with fresh strawberries contaminated with *Escherichia coli* O157:H7, which caused at least 10 people to become sick one death (Oregon Health Authority, 2011).

## **2.3 Salmonella**

### **2.3.1 Characteristics**

*Salmonella* is a rod-shaped, Gram negative, non-spore forming facultative anaerobe that belongs to the family *Enterobacteriaceae*. There are two species in the genus of *Salmonella*, namely *enterica* and *bongori*. *Salmonella enterica* is subdivided into six subspecies: *enterica*, *arizonae*, *salamae*, *diarizonae*, *houtenae* and *indica*. The habitat for subspecies *enterica* is warm-blooded animals (Su & Chiu, 2007; Murray, Baron, Jorgensen, Landry & Pfaller, 2007; Porwollik et al., 2004). The usual habitat for the other subspecies is cold-blooded animals and the environment (Murray et al., 2007). *Salmonella enterica* subspecies *enterica* includes 2610 serotypes and the most well known ones are Typhi, Paratyphi, Enteritidis, and Typhimurium. The serotypes are characterized by three different surface antigens: oligosaccharide (O) antigen, flagellar (H) antigen, and polysaccharide (Vi) antigen (in Typhi and Paratyphi serotypes) (Bronze & Greenfield, 2005).

*Salmonella* grows at temperatures from 6 – 46 °C with an optimum growing temperature of 37 °C. The bacterium is easily killed by through cooking and pasteurization. The pH range of *Salmonella* growth ranges from 4.1 to 9.0. The optimum growth pH is 6.5 – 7.5. The bacteria grow at a water activity of 0.93 and above (Julie, 2014).

### **2.3.2 Illness**

The reservoir hosts of *Salmonella* are domestic and wild animals, including cattle, poultry, swine, flies and wild birds, as well as humans with a chronic carrier state (Ryan & Ray, 2004; Krauss et al., 2003; Richmond and McKinney, 1999; Greenberg, 1964). Humans are usually the final host (Krauss et al., 2003).

Every year, *Salmonella* is estimated to cause one million illnesses in the U.S. with 19,000 hospitalizations and 380 deaths (CDC, 2014). Infection with the bacteria is named salmonellosis. Symptoms of salmonellosis include diarrhea, abdominal cramps, vomiting and fever (CDC, 2014; USDHHS, 2014b). Most people develop these symptoms 12 to 72 hours after infection. The illness usually lasts for 4 to 7 days and most people recover without treatment; however, young children, the elderly and the immunocompromised are more likely to have severe infections and may need to be hospitalized. It's estimated that about 400 people die each year from acute salmonellosis (CDC, 2014). The infective dose can be as few as 15-20 cells, depending on age and health of host, and strain of *Salmonella* (Julie, 2014).

### **2.3.3 Transmission**

Foods associated with *Salmonella* include contaminated poultry, raw meat, eggs, unpasteurized milk or juice, cheese and contaminated raw fruits and vegetables

(USDHHS, 2014b). Humans can get infected when consuming contaminated foods and water, through contact with infected feces and animals, animal feed or humans. Flies can infect foods and thus also pose a risk for transmission to humans (Public Health Agency of Canada, 2011).

### **2.3.4 Outbreaks**

*Salmonella* infection is one of the most common types of food poisoning. In 2009, blueberries contaminated with *Salmonella* Muenchen resulted in a multistate outbreak that caused 14 cases of illnesses (CDC, 2012). In 2010, an outbreak of six cases of *Salmonella* Newport infection in northwestern Minnesota was investigated and results identified blueberries as the cause (Miller et al., 2013). Other foods have also been implicated in recent *Salmonella* infections, including chicken, cucumbers, ground beef, peanut butter, cantaloupe, and alfalfa sprouts (CDC, 2012).

## **2.4 Norovirus**

### **2.4.1 Characteristics**

Norovirus was first discovered in 1968 and was named after an outbreak in Norwalk, Ohio, USA. The outbreak involved acute infectious non-bacterial gastroenteritis in an elementary school (Kapikian et al., 1972). Norovirus is a non-enveloped, single stranded RNA virus. The RNA genome of the virus consists of about 7700 nucleotides. The virus is 26 - 34 nm in diameter. It's small, round, with an amorphous surface and ragged outer edge (McIver, 2005). It has icosahedral capsid symmetry.

Noroviruses belong to the family *Caliciviridae*. Noroviruses are currently classified into five gene-groups designated GI, GII, GIII, GIV and GV. Genogroup I

includes Norwalk virus and GV includes murine norovirus (MNV-1). GI, GII and GIV groups infect humans while Genogroups III and V are detected in cattle, mice and pigs. GI and GII are more commonly known to infect humans and to cause acute gastroenteritis (Ando, Noel & Fankhauser, 2000).

Noroviruses are highly resistant to disinfection techniques (Hutson, Atmar & Estes, 2004). They are also heat and low-pH resistant. In a study by Dolin et al. (1972), noroviruses maintained infectivity after 30 min at 60 °C, and for 3 h after exposure to pH 2.7 at room temperature.

Straub et al. (2007) demonstrated that human noroviruses could infect and replicate in a 3-dimensional (3-D), organoid model of human small intestinal epithelium. Before this discovery, human norovirus infectivity assays could only be carried out on human volunteers, which are rare and costly. ELISA and reverse-transcription PCR (RT-PCR) are the most frequently used techniques for the detection and diagnosis of norovirus (Carter, 2005). Surrogates are also used in research and studies to simulate response of human norovirus to environmental conditions and sanitizing treatments. Murine norovirus (MNV-1) is commonly used as a surrogate for human norovirus. Studies have shown that MNV-1 is more environmentally stable (Bae & Schwab, 2007) and persistent over a wider range of pH values (Hirneisen & Kniel, 2013).

#### **2.4.2 Illness**

Noroviruses are the most common cause of acute gastroenteritis in the United States and cause 19 - 21 million illnesses and contribute to 56,000 - 71,000 hospitalizations and 570 - 800 deaths each year (CDC, 2013b). Human noroviruses cause about 58% (5.5 million cases) of foodborne illnesses in the United States each

year (Hirneisen & Kniel, 2012). The low infectious dose of norovirus infection (estimated median infectious dose of 18 viruses) (Moe, 2009) and the high attack rate of 90-100% (Lees, 2000) make it extremely hard to prevent and to control norovirus infections.

Noroviruses cause acute onset of diarrhea, nausea, abdominal cramps and vomiting. Diarrhea is more common in adults while a greater proportion of children experience vomiting. Other symptoms may occur as well, such as headache, fever, and malaise (Parashar, Dow & Fankhauser, 1998). Norovirus gastroenteritis has an incubation period of 24 – 48 hours, but can extend to 12 – 50 hours. Symptoms may last for 12 – 72 hours (Rockx et al., 2002).

The illness is usually self-limiting without any serious long-term sequela; however, more severe clinical disease could be seen in the elderly and those who are immunocompromised (Estes, Prasad, & Atmar, 2006).

### **2.4.3 Transmission**

Transmission of noroviruses occurs through a variety of routes, but is primarily a result of person-to-person contact, ingestion of contaminated foods or water, contact with contaminated surfaces, and transmission via aerosolized vomit (Fankhauser, Noel, Monroe, Ando, & Glass, 1998). It is possible for norovirus to spread through aerosolized vomit by landing on surfaces or being inhaled or swallowed by a person. There is no evidence showing that people can get infected by inhaling the virus (CDC, 2013b).

People usually begin shedding noroviruses once they have symptoms; however, it is also possible for an infected individual to shed norovirus before

symptoms appear. Virus shedding may continue for 2 weeks or more after an infected person recovers (CDC, 2013b).

#### **2.4.4 Outbreaks**

Noroviruses are the leading cause of reported outbreaks of gastroenteritis. Norovirus outbreaks occur throughout the year with over 80% of the outbreaks occurring from November to April (CDC, 2013c). Norovirus outbreaks have been associated with foods such as fruits, leafy vegetables, shellfish, and deli meat. In 2012, 14 people got sick from eating contaminated grape salad and norovirus genogroup II was confirmed as the cause. In another case of GII outbreak in 2012, 24 people got sick from eating asparagus. Lettuce was identified as contaminated source in an outbreak in Oregon in 2011 and norovirus genogroup II was the cause of the outbreak (CDC, 2012a).

#### **2.5 Interventions to Control Foodborne Pathogens in Fresh Produce**

Numerous studies have been done on foodborne pathogen inactivation in or on fresh produce. Some of the commonly used intervention methods include washing with/without disinfectants, irradiation, refrigeration/frozen storage, high pressure processing (HPP) and exposure to gaseous chemicals (Lukasik et al., 2003; Bidawid, Farber, & Sattar, 2000; Yu et al., 1995; Bialka & Demirci, 2008; Butot, Putallaz, & Sánchez, 2008; Knudsen, Yamamoto, & Harris, 2001; Jordan, Pascual, Bracey, & Mackey, 2001; Han, Selby, Schultze, Nelson, & Linton, 2004).

Various studies have been done to evaluate the efficacy of washing treatments on fresh produce decontamination. Lukasik et al. (2003) studied the efficacy of physical and disinfectant washes on the inactivation of poliovirus 1, bacteriophages,

*Salmonella*, and *E. coli* O157:H7 on strawberries. They found that gentle agitation of contaminated strawberries in water for 2 min led to microbial population reductions of 41-79% (water temperature of 22°C) and 62-90% (43°C), while significant reductions (> 98%) of bacteria and viruses were achieved with sodium hypochlorite (50-330 ppm of free chlorine). They also found that solutions containing vinegar (10%) and table salt (2% NaCl) reduced bacteria by about 90% and that vinegar wash reduced virus population by about 95%.

Irradiation is approved for use in the United States for several food commodities (FDA, 2013a). Bidawid et al. (2000) studied the inactivation of HAV by gamma irradiation and the data indicated that gamma irradiation doses between 2.7 and 3.0 kGy were required to obtain  $\geq 90\%$  reduction in HAV populations on lettuce and strawberries. Another study (Yu et al., 1995) found that electron beam irradiation with doses of 1 and 2 kGy could extend shelf life of strawberries by 2 and 4 days, respectively. However, the intensity of red color decreased as the irradiation dose increased from 0 to 2 kGy. Pulsed light (PL) is an emerging non-thermal technology for rapid inactivation of microorganisms on food surfaces, equipment and food packaging materials that involves the use of short duration pulses of intense broad spectrum rich in UVC light (100-280 nm). Pulsed light technology was adopted by the food industry in 1996 when it was approved for the use in production, processing and handling of foods by FDA (FDA, 2013). Bialka and Demirci (2008) PL treated strawberries and raspberries at varying doses and times. They found that on raspberries, maximum reductions of *Salmonella* and *E. coli* O157:H7 were 3.4 and 3.9 log CFU/g at 59.2 and 72 J/cm<sup>2</sup>. On strawberries, maximum reductions were 2.8 and 2.1 log CFU/g at 34.2 and 25.7 J/cm<sup>2</sup>, respectively.

The effects of refrigeration and frozen storage on survival of foodborne pathogens have been studied. Butot et al. (2008) reported that frozen storage for 3 months had limited effects on hepatitis A virus and rotavirus survival in blueberries, raspberries and strawberries. Knudsen et al. (2001) investigated the effect of refrigeration and frozen storage on the survival of *E. coli* O157:H7 and *Salmonella* on fresh and frozen strawberries. They concluded that both pathogens were capable of survival but not growth on the surface of fresh strawberries when they were stored at 5°C for up to 7 days and that they can survive in frozen strawberries for over one month.

High pressure processing (HPP) involves applying 80k-130k pounds per square inch of pressure to a sample and applying this extreme pressure for 2-5 min to kill most vegetative microorganisms (USDA, 2006). HPP has become a commercial pasteurization process for a variety of products on the market, including juices and fruit smoothies, jams, guacamole, tomato-based salsas, ready-to-eat meat and seafood such as oysters (Grove et al., 2006). Jordan et al. (2001) reported that pressure treatment of 500 MPa for 5 min at 20 °C achieved an immediate 5-log reduction of *E. coli* O157:H7 in apple juice (pH 3.5) and tomato juice (pH 4.1). Kingsley et al. (2005) pressure treated strawberry puree and sliced green onions contaminated with HAV and found that pressure treatment of 375 MPa for 5 min reduced HAV in strawberry puree and sliced green onions by 4.32 and 4.75 log PFU/g, respectively.

Gaseous decontamination methods have shown to be effective for decontamination of some small fruits. In a study by Han et al. (2004), strawberries treated with 4 mg/L gaseous chlorine dioxide (ClO<sub>2</sub>) for 30 min and continuous treatment with 3 mg/L ClO<sub>2</sub> for 10 min achieved more than 5 log reductions of *E. coli*

O157:H7 and *Listeria monocytogenes*. Sy et al. (2005) reported that gaseous chlorine dioxide treatment of 8 mg/L significantly reduced *Salmonella* on blueberries, strawberries and raspberries by 2.4-3.7, 3.8-4.4, and 1.5 log CFU/g, respectively.

## **2.6 Ultraviolet (UV) Light Processing**

Ultraviolet light (UV light) is non-ionizing electromagnetic radiation with a wavelength range (100 – 400 nm) shorter than visible light (USDHHS, 2014c). UV light can cause damage to organisms ranging from bacteria to humans.

UV radiation is divided into different regions based on its wavelength. Shortwave UV light (UV-C) has wavelength range from 200 to 280 nm (Cutler & Zimmerman, 2011) and has been shown to be able to inactivate a wide range of microorganisms (Hijnen, Beerendonk, & Medema, 2006,). UVC can be produced by mercury-vapor lamps where the mercury is vaporized in low-pressure plasma. At wavelengths of 200-280 nm, UV is able to penetrate cellular membranes and alter the DNA and RNA of the microorganisms. UV energy at the wavelength of 254 nm readily affects the double-bond stability between adjacent carbons in DNA and RNA (Cutler & Zimmerman, 2011). UV light also produces 6-4 pyrimidine-pyrimidone and other photoproducts at lower ratios (Chandrasekhar & Houten, 2000; Harm, 1980).

Microorganisms that contain genomic RNA, such as RNA viruses, also go through morphological changes after UV light exposure (Katagiri, Hinuma, & Ishida, 1967; Miller & Plagemann, 1973; De Sena & Jarvis, 1981). De Sena and Jarvis (1981) conducted research on the effect of UV irradiation on type I poliovirus and found that UV exposure resulted in permeability of the capsid to RNase. Miller and Plagemann (1973) UV irradiated purified mengovirus and found altered proteins and a structural change in the capsid.

UV dose is the energy necessary to kill a microorganism. It can be measured in micro Joule per square centimeter:  $\text{Dose (mJ/cm}^2\text{)} = \text{Intensity (mW/cm}^2\text{)} \times \text{Exposure time (s)}$  (Thurston-Enriquez, Haas, Jacangelo, Riley, & Gerba, 2003). Microorganism inactivation is described as the reduction of the microorganism after being exposed to a harmful mechanism. Usually there is a linear relationship between the log of inactivation ( $\log_{10}[N/N_0]$ ) and the UV dose, where  $N_0$  stands for the initial concentration of microorganism and  $N$  is the final concentration of the microorganism after UV light exposure (Thurston-Enriquez et al., 2003; Mamane-Gravetz & Linden, 2005; Hijnen et al., 2006); however, there are two major deviations from the first-order kinetic of inactivation. One is described as shoulder effect, where no inactivation is observed at low doses, followed by linear inactivation (Hiatt, 1964; Sommer, Haider, Cabaj, Pribil, & Lhotsky, 1998; Mamane-Gravetz & Linden, 2005). The other deviation is called tailing, where no further increase in inactivation is seen at high doses (Hiatt, 1964; Hijnen et al., 2006).

As a nonthermal technology, UV has less detrimental effects on nutrients and can better retain the fresh-like characteristics and flavors of foods compared with thermal processing (Falguera et al., 2011; Pala & Toklucu, 2013). UV treatment reduced populations of *E. coli* and *Listeria innocua* by more than 99% in apple cider without changing the liquid's flavor (USDA, 2006). In addition, equipment setup is simple and relatively low in cost. UV light is also a clean technology that leaves no residual activity even at high doses (Yaun et al., 2003). However, the application of UV as a decontamination treatment for food surfaces has not been successful due to the shallow penetration depth of UV (Shama, 1999). In distilled water, the loss of radiation intensity is up to 30% at 40 cm from the surface (Snowball & Hornsey,

1988). The success of using UV for liquid treatment relies on thin layers of liquid and consistent turbulent flow (Bintsis, Litopoulou-Tzanetaki, & Robinson, 2000).

### **2.6.1 Effects of UV Irradiation on Microorganisms in Food Products**

UV light has been approved by the FDA as a treatment for controlling surface microorganisms on food products (FDA, 2013b). Studies have been done to evaluate the efficacy of UV light on microorganism reduction.

#### **2.6.1.1 Drinking Water Disinfection**

UV irradiation has been shown to be effective against all waterborne pathogens (Hijnen et al., 2006). The use of UV irradiation for water disinfection became the primary process after the discovery that it is highly efficient in the inactivation of *Cryptosporidium parvum* oocysts (Clancy et al., 1998) and *Giardia*, two of the main water pathogens. Substantial inactivation of (oo)cysts of both protozoa has been shown at UV fluences lower than 20 mJ/cm<sup>2</sup> by low pressure and medium pressure lamps (Hijnen et al., 2006).

#### **2.6.1.2 Application to Fresh Produce Disinfection**

Fresh produce such as salad or fruits are mostly consumed raw or minimally processed and thus can lead to food safety issues. Various studies have shown ultraviolet light to be effective against pathogenic microorganisms on fresh produce such as lettuce and berry products (Allende et al., 2006; Kim & Hung, 2012).

Allende et al. (2006) studied the application of UV light on minimally processed “Red Oak Leaf” lettuce and found that UV doses as low as 3 mJ/cm<sup>2</sup> inhibited growth of most of the bacteria associated with fresh produce, including *Enterobacter*, *Escherichia*, and *Pseudomonas*. They also found that with a UV-C dose

of 85 J/m<sup>2</sup>, a complete growth inhibition could be reached for *Salmonella* Typhimurium, *Yersinia aldovae*, *Leuconostoc carnosum* and *Erwinia carotovora*. Kim and Hung (2012) found that UV light treatment at 20 mW/cm<sup>2</sup> for 10 minutes could achieve 2.14 and greater than 4.05 log reductions of *Escherichia coli* O157:H7 on the calyx and skin of blueberries, respectively. According to Fino & Kniel (2008), significant virus reductions could be seen on lettuce, green onions and strawberries with a UV dose of 240 mJ/ cm<sup>2</sup>. Low to moderate levels of UV-C radiation could also be used for sanitizing minimally processed spinach leaves as an alternative to chlorine and to preserve their quality (Artés-Hernández, Escalona, Robles, Martínez-Hernández, & Artés, 2009).

#### **2.6.1.3 Effect of UV on Meat Products**

Several researchers have demonstrated the effectiveness of UV irradiation to reduce pathogenic microorganisms on surface of chicken, seafood and red meat. Wong et al. (1998) evaluated the effect of ultraviolet light on the reduction of *E. coli* and *Salmonella* Senftenberg on pork skin and muscle. They demonstrated the effectiveness of UV light to reduce the two pathogens on pork meat surfaces. The study also showed that *E. coli* was more resistant to UV treatment than *Salmonella* Senftenberg in all test conditions.

In a study conducted by Wallner-Pendleton et al. (1994), UV treatment reduced the population of *Salmonella* Typhimurium by 61% in broiler chicken halves without affecting its color. Several other studies have demonstrated the effectiveness of UV treatment against *Listeria monocytogenes*, *Salmonella* Typhimurium and *Campylobacter jejuni* on chicken breast (Chun, Kim, Lee, Yu, & Song, 2010; Wallner-Pendleton et al., 1994). It has also been shown that UV-C irradiation could be

used for pathogen inactivation in ready-to-eat sliced ham (Chun, Kim, Chung, Won, & Song, 2009).

UV deputation procedures are mandatory in the shellfish industry to reduce pathogen population before human consumption (Sunnotel et al., 2007). UV deputation can reduce fecal coliforms and *Salmonella* spp. in shellfish. Sunnotel et al. (2007) studied the application of UV on the inactivation of *Cryptosporidium* spp., which have significant resistance to environmental stress. The study found that standard UV treatment resulted in a 13-fold reduction of recovered, viable *Cryptosporidium parvum* oocysts from spiked Pacific oysters. However, the low number of viable oocysts surviving after the UV deputation process still poses a public health risk if the oysters are consumed raw (Sunnotel et al., 2007). Thus, an improved deputation procedure and an increased periodic monitoring program are needed to minimize the risk of cryptosporidiosis.

#### **2.6.1.4 Liquid Pasteurization**

UV light has been applied for liquid food pasteurization. Burton (1951) conducted a study where milk was pumped at high speed through transparent tubes with a diameter of 1 cm, which were UV irradiated. Eighty percent of the UV radiation reached the milk and destroyed about 99% of the bacteria in the milk. Matak et al. (2005) inoculated fresh goat milk with *Listeria monocytogenes* with an initial population of  $10^7$  CFU/ml. A greater than 5-log reduction was achieved when the milk received a UV dose of about  $15.8 \text{ mJ/cm}^2$ . However, in a later study, it was found that severe sensory and chemical changes occurred in goat's milk that was UV irradiated for 18 seconds with a dose of  $15.8 \text{ mJ/cm}^2$  (Matak et al., 2007).

UV light has also been applied in juice pasteurization. Franz et al. (2009) found UV-C treatment to be able to reduce *E. coli* and *Lactobacillus brevis* to undetectable levels in commercial naturally cloudy apple juice from an initial concentration of  $10^6$  CFU/ml and  $10^4$  CFU/ml. Keyser et al. (2008) used UV light to reduce microbial loads in different fruit juices and nectars. The maximum reduction for yeast was 0.53, 2.51 and 2.42 log in grape, cranberry and grape juices, respectively, with a flow rate of 1.02 L/min for 30 min of treatment.

Various studies have shown UV irradiation to be efficient in microorganism inactivation in liquid egg. It is an alternative treatment to thermal processing and high hydrostatic pressure but may have negative impacts on product properties due to protein denaturation (Unluturk, Handan, Tari, & Atilgan, 2008). Unluturk et al. (2008) treated liquid egg products contaminated with a non-pathogenic *Escherichia coli* strain (ATCC 8739) for 0, 5, 10 and 20 min by using a collimated beam apparatus and found that for liquid egg white, a >2-log reduction was achieved when the fluid depth was 0.153 cm with a UV intensity of  $1.314 \text{ mW/cm}^2$ . They also observed 0.675 and 0.316 log reductions in liquid egg yolk and liquid whole egg at the same conditions, respectively. Their results indicated that UVC irradiation could be combined with mild heat treatment for the pasteurization of liquid egg products.

In the brewing industry, companies have adopted UV irradiation for water treatment to ensure a high quality, pure final product (Brewing, 2013). Lu et al. (2010), used a thin film apparatus with quartz optical fibers for UV light delivery to inactivate microorganisms in beer. The apparatus reduced *E. coli* and *L. brevis* in beer from 6 log CFU/ml to below 10 CFU/ml and from 4 log CFU/ml to non-detectable level at UV

doses of 16.1 and 9.7 mJ/cm<sup>2</sup>, respectively. However, the reduction of *S. cerevisiae* was not significant.

## **2.6.2 Effects of UV Irradiation on Food Components and Properties**

### **2.6.2.1 Impact on Fruit Juices**

Pala and Toklucu (2013) conducted a study in which white and red grape juices were UVC light treated with doses of 12.6 and 25.2 J/ml, respectively. They tested the effects of UV treatment on antioxidant activity, phenolics and total anthocyanins of grape juices. Their results showed that while the microbial loads of grape juices were completely inactivated after UV treatment with a dose of 25.2 J/ml, the antioxidant activity and phenolics were maintained after UV treatment. The loss in monomeric anthocyanins in red grape juice was 8.7% after the treatment. Meanwhile, thermal treatment at 85 °C for 15 min led to a 11.8% loss of anthocyanin in red grape juice, which was a significant difference compared to the UVC treatment.

Feng et al. (2013) evaluated the effects of UVC treatment on microbial inactivation and physico-chemical properties of watermelon juice using Teflon<sup>®</sup>-coil for 37 days of storage at 5°C after UVC treatment. They found that UVC treatments with doses of 2.7 and 37.5 J/ml inactivated all (2.6 log CFU/ml) coliforms, and UV treatment with a dose of 37.5 J/ml reduced yeast/mold and total aerobes by 0.99 and 1.47 log CFU/ml, respectively. Under these test conditions, UVC treatment did not result in significant changes in color, pH, °Brix, lycopene, or phenolics. However, the UVC treated juices had a higher a\* (redness) and lower b\* (yellowness) color than untreated juice.

The effects of ultraviolet treatment on apple cider and apple juice were also examined by various studies. Fan and Geveke (2007) investigated the possible formation of furan by ultraviolet C treatment in apple cider. Their results showed that UVC induced furan formation from fructose in the cider. However, with a UVC dose that could inactivate 5 log of *E. coli* K12, only very low concentrations of furan were induced (<1 ppb). Falguera et al. (2011) studied the effect of UV irradiation on apple juices from different varieties. They observed a loss of vitamin C between 4% and 6% in apple juices from the varieties Starking, Golden, and Fuji, after UV irradiation for 120 min. For the variety King David, vitamin C loss was 70% after the same processing conditions, due to its lack of pigmentation. Ibarz et al. (2005) observed a color change in UV irradiated apple juice where the luminosity increased and the a\* and b\* values decreased significantly, indicating that the compounds that give brown color were destroyed in the process.

#### **2.6.2.2 Impact on Food Toxins**

Mycotoxins pose serious problems for consumer safety. These toxins are not affected by conventional heat treatments. Various studies have been done to seek alternative treatment to eliminate or to reduce its content in foods. It has been found that UV radiation could destroy aflatoxin to some degree. Leeson et al. (1995) found that it was possible to destroy aflatoxin in peanuts; however, limited decomposition was observed for citrinin and ochratoxin A when they were treated with UV light (Neely & West, 1972).

UV irradiation has been used for aflatoxin degradation in milk (Yousef & Marth, 1986). Yousef and Marth UV treated raw whole milk containing 1 ppb aflatoxin M<sub>1</sub> at 5°C, 25°C and 65°C and observed toxin degradation at all temperatures.

However, the amount of toxin decreased nonlinearly when treatment temperature increased. The study also found that the presence of 0.002% benzoyl peroxide did not change the extent of aflatoxin M<sub>1</sub> degradation by UV irradiation, whereas the presence of 0.05% hydrogen peroxide increased the extent of toxin degradation by 28.4% when raw whole milk was UV irradiated for 20 min at 25°C.

Murata et al. (2008) evaluated the effects of mild and strong UV irradiation (254 nm UV-C) on two feeds contaminated with the mycotoxins, deoxynivalenol and zearalenone, with an initial toxin concentration of 30 mg/kg. When exposed to mild irradiation (0.1 mW/cm<sup>2</sup>), both of the toxins were reduced as radiation time increased and became undetectable at 60 min. Strong UV irradiation (24 mW/cm<sup>2</sup>) reduced the toxins more rapidly in the same time-dependent manner.

## REFERENCES

- Allende, A., McEvoy, J. L., Luo, Y., Artes, F., & Wang, C. Y. (2006). Effectiveness of two-sided UV-C treatments in inhibiting natural microflora and extending the shelf-life of minimally processed 'Red oak leaf' lettuce. *Food Microbiology*, 23(3), 241-249. doi:10.1016/j.fm.2005.04.009
- Ando, T., Noel, J., and Fankhauser, R. (2000). Genetic classification of "Norwalk-like viruses". *Journal of Infectious Diseases*, 181: 336-348.
- Artés-Hernández, F., Escalona, V. H., Robles, P. A., Martínez-Hernández, G. B., & Artés, F. (2009). Effect of UV-C radiation on quality of minimally processed spinach leaves. *Journal of the Science of Food and Agriculture*, 89(3), 414-421.
- Bae, J., & Schwab, K. J. (2007). Evaluation of Murine Norovirus, Feline Calicivirus, Poliovirus, and MS2 as Surrogates for Human Norovirus in a Model of Viral Persistence in Surface Water and Groundwater. *Applied and Environmental Microbiology*, 74 (2): 477-484.
- Bhunia, A. K. (2008). *Foodborne microbial pathogens: Mechanisms and pathogenesis*. New York: Springer.
- Bidawid, S., Farber, J. M., & Sattar, S. A. (2000). Inactivation of hepatitis A virus (HAV) in fruits and vegetables by gamma irradiation. *International Journal of Food Microbiology*, 57, 91-97.
- Bialka, K. L., & Demirci, A. (2008). Efficacy of Pulsed UV-Light for the Decontamination of *Escherichia coli* O157:H7 and *Salmonella* spp. on Raspberries and Strawberries. *Journal of Food Science*, 73, 5.
- Bintsis, T., Litopoulou-Tzanetaki, E., & Robinson, R.K. (2000). Existing and potential applications of ultraviolet light at the food industry - a critical review. *Journal of the Science of Food and Agriculture*, 80, 637-645.
- Brewing: UV disinfection keeps beer contaminant-free. (2013). *Filtration + Separation*, 50(2), 45.
- Bronze, M. S., & Greenfield, R. A. (Ed.). (2005). *Biodefense Principles and Pathogens horizon bioscience*.

- Burton, H. (1951). Ultra-violet irradiation of milk. *Dairy Science Abstracts*, 13, 229-244
- Butot, S., Putallaz, T., & Sánchez, G. (2008). Effects of sanitation, freezing and frozen storage on enteric viruses in berries and herbs. *International Journal of Food Microbiology*, 126, 1-2.
- Calder, L., Simmons, G., Thornley, C., Taylor, P., Pritchard, K., Greening, G., & Bishop, J. (2003). An outbreak of hepatitis A associated with consumption of raw blueberries. *Epidemiology & Infection*, 131, 745-751.
- Carter, M.J. (2005). Enterically infecting viruses: pathogenicity, transmission and significance for food and waterborne infection. *Journal of Applied Microbiology*, 98, 1354- 1380.
- Centers for Disease Control and Prevention (CDC). (2014). *Salmonella*. Retrieved November 12, 2014, from <http://www.cdc.gov/salmonella/>
- Centers for Disease Control and Prevention (CDC). (2013a). *Multistate outbreak of shiga toxin-producing Escherichia coli O157:H7 infections linked to ready-to-eat salads (final update)*. Retrieved November 10, 2014, from <http://www.cdc.gov/ecoli/2013/O157H7-11-13/index.html>
- Centers for Disease Control and Prevention (CDC). (2013b). *Norovirus*. Retrieved November 12, 2014, from <http://www.cdc.gov/norovirus/index.html>
- Centers for Disease Control and Prevention (CDC). (2013c). *Surveillance for Norovirus Outbreaks*. Retrieved November 12, 2014, from <http://www.cdc.gov/features/dsnorovirus/>
- Centers for Disease Control and Prevention (CDC). (2012a). *Foodborne Outbreak Online Database (FOOD)*. Retrieved November 10, 2014, from <http://wwwn.cdc.gov/foodborneoutbreaks/Default.aspx>
- Centers for Disease Control and Prevention (CDC). (2012b). *General Information Escherichia coli (E. coli)*. Retrieved November 8, 2014, from <http://www.cdc.gov/ecoli/general/index.html>.
- Centers for Disease Control and Prevention (CDC). (2012c). *Multistate outbreak of shiga toxin-producing Escherichia coli O157:H7 infections linked to organic spinach and spring mix blend*. Retrieved November 10, 2014, from <http://www.cdc.gov/ecoli/2012/O157H7-11-12/index.html>

- Centers for Disease Control and Prevention (CDC). (2012d). *Drinking water – water treatment*. Retrieved November 14, 2014, from [http://www.cdc.gov/healthywater/drinking/public/water\\_treatment.html](http://www.cdc.gov/healthywater/drinking/public/water_treatment.html)
- Chandrasekhar, D., & Houten, B. (2000). *In vivo* formation and repair of cyclobutane pyrimidine dimers and 6-4 photoproducts measured at the gene and nucleotide level in *Escherichia coli*. *Mutation Research*, 450, 19-40.
- Chun, H., Kim, J., Chung, K., Won, M., & Song, K. B. (2009). Inactivation kinetics of *Listeria monocytogenes*, *Salmonella enterica* serovar Typhimurium, and *Campylobacter jejuni* in ready-to-eat sliced ham using UV-C irradiation. *Meat Science*, 83(4), 599-603.
- Chun, H. H., Kim, J. Y., Lee, B. D., Yu, D. J., & Song, K. B. (2010). Effect of UV-C irradiation on the inactivation of inoculated pathogens and quality of chicken breasts during storage. *Food Control*, 21(3), 276-280.
- City of Boulder Colorado. (2012). *City using new ultraviolet light technology to safely treat Boulder's wastewater before sending it downstream*. Retrieved August 14, 2014, from <https://bouldercolorado.gov/newsroom/dec-7-2012-city-using-new-ultraviolet-light-technology-to-safely-treat-boulders-wastewater-before-sending-it-downstream>.
- Clancy, J. L., Hargy, T. M., Marshall, M. M., & Dyksen, J. E. (1998). UV light inactivation of *Cryptosporidium oocysts*. *Journal of the American Water Works Association*, 90, 92-102
- Clancy, S. (2008). DNA damage & repair: mechanisms for maintaining DNA integrity. *Nature Education* 1(1), 103.
- Cotterelle, B., Drougard, C., Rolland, J., Becamel, M., Boudon, M., Pinede, S., Traore, O., Balay, K., Pothier, P., & Espie, E. (2005). Outbreak of norovirus infection associated with the consumption of frozen raspberries. France, March 2005. *Euro Surveill*, 10(17), 2690. Retrieved October 25, 2014, from <http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=2690>.
- Cutler, T. D., & Zimmerman, J. J. (2011). Ultraviolet irradiation and the mechanisms underlying its inactivation of infectious agents. *Animal Health Research Reviews*, 12(1), 15-23.
- De Sena, J., & Jarvis, D. (1981). Modification of the poliovirus capsid by ultraviolet light. *Canadian Journal of Microbiology*, 27, 1185-1193.

- Dolin, R., Blacklow, N. R., DuPont, H., Buscho, R. F., Wyatt, R. G., Kasel, J. A., Hornick, R., & Chanock, R. M. (1972). Biological properties of Norwalk agent of acute infectious nonbacterial gastroenteritis. *Proceedings of the Society for Experimental Biology and Medicine*, 140(2): 578-583.
- Estes, M., Prasad, B., & Atmar, R. (2006). Noroviruses everywhere: has something changed? *Current Opinion in Infectious Diseases*, 19, 467-474.
- Falguera, V., Pagán, J., Garza, S., Garvín, A., & Ibarz, A. (2011). Ultraviolet processing of liquid food: A review Part 2: Effects on microorganisms and on food components and properties. *Food Research International*, 44, 1580-1588.
- Fan X, & Geveke, D. J. (2007). Furan formation in sugar solution and apple cider upon ultraviolet treatment. *Journal of Agricultural and Food Chemistry*, 55(19), 7816-21.
- Fankhauser, R. L., Noel, J. S., Monroe, S. S., Ando, T., & Glass, R. I. (1998). Molecular epidemiology of "Norwalk-like viruses" in outbreaks of gastroenteritis in the United States. *Journal of Infectious Diseases*, 178(6), 1571-1578.
- Feng, M., Ghafoor, K., Seo, B., Yang, K., & Park, J. (2013). Effects of ultraviolet-C treatment in teflon®-coil on microbial populations and physico-chemical characteristics of watermelon juice. *Innovative Food Science & Emerging Technologies*, 19(0), 133-139.  
doi:<http://dx.doi.org.proxy.nss.udel.edu/10.1016/j.ifset.2013.05.005>
- Fino, V. R., & Kniel, K. E. (2008). UV light inactivation of hepatitis A virus, Aichi virus, and feline calicivirus on strawberries, green onions, and lettuce. *Journal of Food Protection*, 71(5), 908-13.
- Charles F., Specht, I., Cho, G., Graef, V., & Stahl, M. R. (2009). UV-C-inactivation of microorganisms in naturally cloudy apple juice using novel inactivation equipment based on Dean vortex technology. *Food Control*, 20(12), 1103-1107.
- Geveke, D. J. (2005). UV Inactivation of Bacteria in Apple Cider. *Journal of Food Protection*, 68, 1739-42
- Greenberg, B. (1964). Experimental Transmission of Salmonella Typhimurium by Houseflies to Man. *American Journal of Hygiene*, 80, 149 – 156.

- Grove, S. F., Lee, A., Lewis, T., Stewart, C. M., Chen, H., & Hoover, D. G. (2006). Inactivation of foodborne viruses of significance by high pressure and other processes. *Journal of Food Protection*, 69(4), 957-68.
- Han, Y., Selby, T. L., Schultze, K. K., Nelson, P. E., & Linton, R. H. (2004). Decontamination of strawberries using batch and continuous chlorine dioxide gas treatments. *Journal of Food Protection*, 67(11), 2450-5.
- Harm, W. (1980). *Biological effects of ultraviolet radiation*. New York, NY: Cambridge University Press.
- Harris, L.J., Farber, J.N., Beuchat, L.R., Parish, M.E., Suslow, T.V., Garrett, E.H., & Busta, F.F. (2003). Outbreaks Associated with Fresh Produce: Incidence, Growth, and Survival of Pathogens in Fresh and Fresh-Cut Produce. *Comprehensive Reviews in Food Science and Food Safety*, 2, 78-141.
- Hiatt, C. (1964). Kinetics of the inactivation of viruses. *Bacteriology Reviews*, 28(2), 150-163.
- Hijnen, W. A. M., Beerendonk, E. F., & Medema, G. J., (2006). Inactivation credit of UV radiation for viruses, bacteria and protozoan (oo)cysts in water: A review. *Water Research*, 40, 3-22.
- Hirneisen, K. A., Kniel, K. E. (2013). Comparing human norovirus surrogates: murine norovirus and Tulane virus. *Journal of Food Protection*, 76(1), 139-43.
- Hirneisen, K. A., Kniel, K. E. (2012). Comparison of ELISA attachment and infectivity assays for murine norovirus. *Journal of Virological Methods*, 186, 1-2, 14-20.
- Hutin, Y. J., Pool, V., Cramer, E. H., Najnan, O. V., Weth, J., Williams, I. T., Goldstein, S. T., Gensheimer, K. F., Bell, B. P., Shapiro, C. N., Alter, M. J., & Margolis, H. S. (1999). A multistate, foodborne outbreak of hepatitis. A National Hepatitis A Investigation Team. *The New England Journal of Medicine*, 340, 595-602.
- Hutson, A. M., Atmar, R. L., & Estes, M.K. (2004). Norovirus disease: changing epidemiology and host susceptibility factors. *Trends in Microbiology*, 12(6): 279-287.
- Ibarz, A., Pagán, J., Panadés, R., & Garza, S. (2005). Photochemical destruction of color compounds in fruit juices. *Journal of Food Engineering*, 69(2), 155-160.

- Iowa Department of Public Health. (2014). *E. coli (shiga-toxin)*. Retrieved November 8, 2014, from <http://www.idph.state.ia.us/Cade/DiseaseIndex.aspx?disease=E.%20coli%20%2528shiga-toxin%2529>
- Jordan, S., Pascual, C., Bracey, E., & Mackey, B. (2001). Inactivation and injury of pressure-resistant strains of *Escherichia coli* O157 and *Listeria monocytogenes* in fruit juices. *Journal of applied microbiology*, *91*, 463-469.
- Julie, A. A. 2014. *Salmonella*. Retrieved November 12, 2014, from <http://food.unl.edu/safety/salmonella>
- Kapikian, A.Z., Wyatt, R.G., Dolin, R., Thornhill, T.S., Kalica, A.R., & Chanock, R.M. (1972). Visualization by immune electron microscopy of a 27-nm particle associated with acute infectious nonbacterial gastroenteritis. *Journal of Virology*, *10*, 1075-1081.
- Katagiri, S., Hinuma, Y., & Ishida, N. (1967). Biophysical properties of poliovirus particles irradiated with ultraviolet light. *Virology*, *32*, 337-343.
- Keyser, M., Müller, I. A., Cilliers, F. P., Nel, W., & Gouws, P. A. (2008). Ultraviolet radiation as a non-thermal treatment for the inactivation of microorganisms in fruit juice. *Innovative Food Science and Emerging Technologies*, *9*(3), 348-354.
- Kim, C., & Hung, Y. (2012). Inactivation of *E. coli* O157:H7 on blueberries by electrolyzed water, ultraviolet light, and ozone. *Journal of Food Science*, *77*(4), 206-211.
- Kingsley, D. H., Guan, D., & Hoover, D. G. (2005). Pressure inactivation of hepatitis A virus in strawberry puree and sliced green onions. *Journal of Food Protection*, *68*(8), 1748-51.
- Korsager, B., Hede, S., Boggild, H., Bottiger, B., & Molbak, K. (2005). Two outbreaks of norovirus infections associated with the consumption of imported frozen raspberries, Denmark, May-June 2005. *Euro Surveill*, *2005*, *10*(25), 2729. Retrieved October 25, 2014, from <http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=2729>
- Knudsen, D. M., Yamamoto, S. A., & Harris, L. J. (2001). Survival of *Salmonella* spp. and *Escherichia coli* O157:H7 on fresh and frozen strawberries. *Journal of Food Protection*, *64*(10), 1483-8.

- Krauss, H., Weber, A., Appel, M., Enders, B., Isenberg, H. D., Schiefer, H. G., Slenczka, W., von Graevenitz, A., & Zahner, H. (Eds.). (2003). *Zoonoses Infectious Diseases Transmissible from Animals to Humans* (3rd ed.). Washington: ASM press.
- Lee, J., Zoh, K., & Ko, G. (2008). Inactivation and UV disinfection of murine norovirus with TiO<sub>2</sub> under various environmental conditions. *Applied and Environmental Microbiology*, 74(7), 2111-7.
- Lees, D. (2000). Viruses and bivalve shellfish, *International Journal of Food Microbiology*, 59, 1-2, 25, 81-116.
- Leeson, S., Diaz G., Gonzalo J., & Summers, J. D. (1995). *Poultry metabolic disorders and mycotoxins*. Guelph: University books.
- Lu, G., Li, C., Liu, P., Cui, H., Yao, Y., & Zhang, Q. (2010). UV inactivation of microorganisms in beer by a novel thin-film apparatus. *Food Control*, 21(10), 1312-1317.
- Lukasik, J., Bradley, M. L., Scott, T. M., Dea, M., Koo, A., Hsu, W. Y., Bartz, J. A., ... & Farrah, S. R. (2003). Reduction of poliovirus 1, bacteriophages, *Salmonella montevideo*, and *Escherichia coli* O157:H7 on strawberries by physical and disinfectant washes. *Journal of Food Protection*, 66(2), 188-93.
- Mäde, D., Trübner, K., Neubert, E., Höhne, M., & Johne, R. (2013). Detection and typing of norovirus from frozen strawberries involved in a large-scale gastroenteritis outbreak in Germany. *Food and environmental virology*, 5, 162-168.
- Mamane-Gravetz, H. & Linden, K. (2005). Relationship between physiochemical properties, aggregation and UV inactivation of isolated environmental spores in water. *Journal of Applied Microbiology*, 98, 351-363.
- Matak, K. E., Churey, J. J., Worobo R. W., Sumner S. S., Hovingh, E., Hackney, C. R., & Pierson M. D. (2005). Efficacy of UV light for the reduction of *Listeria monocytogenes* in goat's milk. *Journal of Food Protection*, 68(10), 2212-6.
- Matak, K. E., Sumner, S. S., Duncan, S. E., Hovingh, E., Worobo, R. W., Hackney, C. R., & Pierson, M. D. (2007). Effects of ultraviolet irradiation on chemical and sensory properties of goat milk. *Journal of Dairy Science*, 90(7), 3178-3186.

- Miller, B. D., Rigdon, C. E., Robinson, T. J., Hedberg, C., & Smith, K. E. (2013). Use of global trade item numbers in the investigation of a *Salmonella* Newport outbreak associated with blueberries in Minnesota, 2010. *Journal of Food Protection*, 76, 762-769.
- Miller, R. & Plagemann, P. (1973). Effect of ultraviolet light on Mengovirus: formation of uracil dimers, instability and degradation of capsid, and covalent linkage of protein to viral RNA. *Journal of Virology*, 13(3), 729-739.
- Moe, C. L. (2009). Preventing Norovirus Transmission: How Should We Handle Food Handlers. *Clinical Infectious Diseases*, 48(1), 38-40.
- Murray, P.R., Baron, E. J., Jorgensen, J. H., Landry, M. L., & Pfaller, M. A. (Eds.). (2007). *Manual of Clinical Microbiology* (9<sup>th</sup> ed.). Washington: ASM Press.
- Murata, H., Mitsumatsu, M., & Shimada, N. (2008). Reduction of feed-contaminating mycotoxins by ultraviolet irradiation: An in vitro study. *Food Additives & Contaminants: Part A*, 25(9), 1107-1110.
- McIver, C. K. (2005). *A compendium of laboratory diagnostic methods for common and unusual pathogens – an Australian perspective*. Melbourne: Australian Society for Microbiology.
- Neely, W. C., & West, A. D. (1972). Spectroanalytical parameters of fungal metabolites. 3. Ochratoxin A. *Journal - Association of Official Analytical Chemists*, 55, 1305 - 1309.
- Niu, M. T., Polish, L. B., Robertson, B. H., Khanna, B.K., Woodruff, B. A., Shapiro, C. N., Miller, M. A., Smith, J. D., Gedrose, J. K., & Alter, M. J. (1992). Multistate outbreak of hepatitis A associated with frozen strawberries. *Journal of Infectious Diseases*, 166, 518-524.
- Oregon Health Authority. (2011). Lab tests confirm source of *E. coli* O157 from deer droppings in strawberry fields in NW Oregon. Retrieved October 25, 2014 from <http://www.oregon.gov>
- Pala, C. U., & Toklucu, A. K. (2013). Effects of UV-C processing on some quality characteristics of grape juices. *Food and Bioprocess Technology*, 6(3), 719-725.
- Parashar, U. D., Dow L., & Fankhauser, R. L., Humphrey, C. D., Miller, J., Ando, T., ... Glass, R. I. (1998). An outbreak of viral gastroenteritis associated with consumption of sandwiches: implications for the control of transmission by food handlers. *Epidemiology and Infection*, 121(3), 615-621.

- Porwollik, S., Boyd, E. F., Choy, C., Cheng, P., Florea, L., Proctor, E., & McClelland, M. (2004). Characterization of *Salmonella enterica* subspecies I genovars by use of microarrays. *Journal of Bacteriology*, 186(17), 5883-5898.
- Pruimboom-Brees, I. M., Morgan, T. W., Ackermann, M. R., Nystrom, E. D., Samuel, J. E., Cornick, N. A., & Moon, H. W. (2000). Cattle lack vascular receptors for *Escherichia coli* O157:H7 Shiga toxins. *Proceedings of the National Academy of Sciences of the United States of America*, 97 (19), 10325-9.
- Public Health Agency of Canada. (2011). *Salmonella enterica spp. pathogenic safety data sheet – infectious substances*. Retrieved November 12, 2014, from <http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/salmonella-ent-eng.php>
- Riemann, H., & Cliver, D. O. (2006). *Foodborne infections and intoxications* (3rd ed.). Amsterdam, Netherlands ; Burlington, MA: Elsevier Academic Press.
- Richmond, J. Y., & McKinney, R. W. (Eds.). (1999). *Biosafety in Microbiological and Biomedical Laboratories* (4<sup>th</sup> ed.). Washington: CDC-NIH.
- Rockx, B., De Wit, M., Vennema, H., Vinjé, J., De Bruin, E., Van Duynhoven, Y., & Koopmans, M. (2002). Natural history of human calicivirus infection: a prospective cohort study. *Clinical Infectious Diseases*, 35(3), 246-53.
- Roy J. H., Lundy, S., & Kalicki, B. (2009). Blueberries "their role in health". *Pennington Nutrition Series*, No. 2.
- Rodas, A. G., Bourquin, L., Salazar, C. G., Gomez, A.V., & Wise, J.C., 2009. Good Agricultural Practices for Food Safety in Blueberry Production: Basic Principles. Retrieved November 8, 2014, from [http://www.gaps.msue.msu.edu/blue\\_manual.pdf](http://www.gaps.msue.msu.edu/blue_manual.pdf).
- Ryan, K. J., & Ray, C. G. (Eds.). (2004.). *Sherris Medical Microbiology: An Introduction to Infectious Disease*. (Fourth Edition. ed.). New York.: McGraw-Hill.
- Snowball, M. R., & Hornsey, I. S. (1988). Purification of water supplies using ultra-violet Light. In R. K. Robinson (Ed.). *Development in food microbiology*. London: Elsevier Applied Science.
- Shama, G. (1999). *Ultraviolet light*. In: R.K. Robinson, C. Batt and P. Patel (eds), *Encyclopedia of Food Microbiology-3*. London: Academic Press, 2208-2214.

- Sommer, R., Haider, T., Cabaj, A., Pribil, W., & Lhotsky M. (1998). Time fluencereciprocity in UV disinfection of water. *Water Science and Technology*, 38(12), 145-150.
- Straub, T. M., Höner zu Bentrup, K., Orosz-Coghlan, P. O., Dohnalkova, A., Mayer, B. K., Bartholomew, R. A., Valdez, C. O., Brunkner-Lea, C. J., Gerba, C. P., Abbaszadegan, C., & Nickerson, C. A. (2007). In vitro cell culture infectivity assay for human noroviruses. *Emerging Infectious Diseases*, 13(3), 396-403.
- Su, L. H., & Chiu, C. H. (2007). Salmonella: clinical importance and evolution of nomenclature. *Chang Gung Medical Journal*, 30 (3), 210-219.
- Sunnotel O., Snelling W. J., McDonough, N., Browne, L., Moore, J. E, Dooley, J. S., & Lowery C. J. (2007). Effectiveness of standard UV deputation at inactivating *Cryptosporidium parvum* recovered from spiked pacific oysters (crassostrea gigas). *Applied and Environmental Microbiology*, 73(16), 5083-7.
- Sy, K. V., McWatters, K. H., & Beuchat, L. R. (2005). Efficacy of gaseous chlorine dioxide as a sanitizer for killing Salmonella, yeasts, and molds on blueberries, strawberries, and raspberries. *Journal of Food Protection*, 68(6), 1165-75.
- The U.S. Food and Drug Administration (FDA). (2013a). *Pulsed light for the treatment of food. 21 CFR 179.41*, Retrieved November 14, 2014, from <http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=179.41>
- The U.S. Food and Drug Administration (FDA). (2013b). *Ionizing radiation for the treatment of food. 21CFR179.26*. Retrieved November 14, 2014, from <http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfCFR/CFRSearch.cfm?fr=179.26>
- The U.S. Food and Drug Administration (FDA). (2011). *Bacteriological Analytical Manual Chapter 4A Diarrheagenic Escherichia coli*. Retrieved November 8, 2014, from <http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm070080.htm>
- The U.S. Food and Drug Administration (FDA). (2001). *FDA survey of imported fresh produce FY 1999 field assignment*. Retrieved October 25, 2014, from <http://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/ProducePlantProducts/ucm118891.htm>.

- Thurston-Enriquez, J. A., Haas, C. N., Jacangelo, J., Riley, K., & Gerba, C. P. (2003). Inactivation of feline calicivirus and adenovirus type 40 by UV radiation. *Applied and Environmental Microbiology*, 69, 577-582.
- Unluturk S., Handan B. A., Tari C., & Atilgan M. R. (2008). Use of UV-C radiation as a non-thermal process for liquid egg products (LEP). *Journal of Food Engineering*, 85(4), 561-568.
- U.S. Department of Health and Human Services. (2014a). *Hemolytic Uremic Syndrome in Children*. Retrieved November 8, 2014, from [http://kidney.niddk.nih.gov/kudiseases/pubs/childkidneydiseases/hemolytic\\_uremic\\_syndrome/#what](http://kidney.niddk.nih.gov/kudiseases/pubs/childkidneydiseases/hemolytic_uremic_syndrome/#what).
- U.S. Department of Health and Human Services. (2014b). *Salmonella*. Retrieved November 12, 2014, from <http://www.foodsafety.gov/poisoning/causes/bacteriaviruses/salmonella/>
- U.S. Department of Health and Human Services. (2014c). *Scientific Review of Ultraviolet (UV) Radiation, Broad Spectrum and UVA, UVB, and UVC*. Retrieved December 10, 2014, from <http://ntp.niehs.nih.gov/pubhealth/roc/listings/u/uv/summary/index.html>
- U.S. Department of Agriculture (USDA). (2013). *U.S. Blueberry Industry*. Retrieved November 10, 2014, from <http://usda.mannlib.cornell.edu/MannUsda/viewDocumentInfo.do?documentID=1765>
- U.S. Department of Agriculture (USDA). (2006). *Nonthermal Food Processing Heats Up*. Retrieved November 14, 2014, from <http://www.ars.usda.gov/is/pr/2006/061018.htm>
- Wallner-Pendleton, E. A., Sumner, S. S., Froning, G. W., & Stetson, L.E. (1994). The use of ultraviolet radiation to reduce *Salmonella* and psychrotrophic bacterial contamination on poultry carcasses. *Poultry Science*, 73(8), 1327.
- Wong, E., Linton, R. H., & Gerrard, D.E. (1998). Reduction of *Escherichia coli* and *Salmonella* Senftenberg on pork skin and pork muscle using ultraviolet light. *Food Microbiology*, 15(4), 415-423.
- Yaun, B. R., Marcy, J. E., Eifert, J. D., & Sumner, S. S. (2003). Response of *Salmonella* and *Escherichia coli* O157:H7 to UV energy. *Journal of Food Protection*, 66, 1071-1073.

- Yousef, A. E., & Marth, E. H. (1986). Use of ultraviolet energy to degrade aflatoxin M1 in raw or heated milk with and without added peroxide. *Journal of Dairy Science*, 69(9), 2243-2247. [http://dx.doi.org/10.3168/jds.S0022-0302\(86\)80663-4](http://dx.doi.org/10.3168/jds.S0022-0302(86)80663-4)
- Yu, L., Reitmeier, C. A., Gleason, M. L., Nonnecke, G. R., Olson, D. G., & Gladon, R. J. (1995). Quality of Electron Beam Irradiated Strawberries. *Journal of Food Science*, 60(5), 1084-1087.

### Chapter 3

## INACTIVATION OF *ESCHERICHIA COLI* O157:H7 AND *SALMONELLA* ON BLUEBERRIES USING A NOVEL WATER-ASSISTED ULTRAVIOLET LIGHT PROCESS

### ABSTRACT

Ultraviolet light (UV) has antimicrobial effects, but the shadowing effect has limited its application. In this study, a novel setup using water-assisted UV processing was developed to inactivate *Escherichia coli* O157:H7 and *Salmonella* on blueberries. Blueberries were dip- or spot-inoculated with *E. coli* or *Salmonella*. Blueberries inoculated with *E. coli* were treated for 2–10 min with UV directly (dry UV) or immersed in agitated water during UV treatment (wet UV). *E. coli* was most easily killed on skin-inoculated blueberries with a 5.2-log reduction after 10-min wet UV treatment. Dip- inoculated blueberries were the most difficult to be decontaminated with a 1.6-log reduction after 10-min wet UV treatment. Wet UV treatment generally showed higher efficacies than dry UV treatment, achieving an average of 1.4 log more reduction for spot-inoculated blueberries. For dip-inoculated blueberries, chlorine washing and UV treatments were less effective, achieving < 2 log reductions of *E. coli*. Thus, the efficacy of combinations of wet UV with sodium dodecyl sulfate (SDS), levulinic acid or chlorine was evaluated. Inoculated blueberries were UV-treated while being immersed in agitated water containing 100 ppm SDS, 0.5% levulinic acid or 10 ppm chlorine. The three chemicals did not significantly enhance the wet UV treatment.

Findings of this study suggest that UV treatment could be used as an alternative to chlorine washing for blueberries and potentially for other fresh produce.

### **3.1 Introduction**

As one of the most popular fruits, blueberries have many benefits to human health, such as anticancer, antioxidant and anti-inflammatory activities (Roy, Lundy, & Kalicki, 2009). The consumption of blueberries in the U.S. has been on the rise due to the increasing recognition of the potential health benefits of blueberries. The U.S. Department of Agriculture (USDA) reported that between 1994 and 2003, the consumption of fresh blueberries in the U.S. increased about 1.6 times (Roy et al., 2009). However, blueberries can occasionally lead to food safety problems since they are mostly consumed raw or minimally processed. Blueberries can become contaminated at any point on the farm-to-table continuum, including irrigation, picking, and post-harvest processes (Rodas, Bourquin, Salazar, Gomez, & Wise, 2009). Fresh berries and berry products have been implicated in several foodborne outbreaks (Calder et al., 2003; FDA, 2001; Oregon Health Authority, 2011). In 2003, contaminated raw blueberries were reported to be the source of an outbreak of hepatitis A (Calder et al., 2003). In 2009, blueberries contaminated with *Salmonella* Muenchen resulted in a multistate outbreak that caused 14 cases of illnesses (Centers for Diseases Control and Prevention, 2012a). In 2010, an outbreak of six cases of *Salmonella* Newport infection in northwestern Minnesota was investigated and results identified blueberries as the cause (Miller, Rigdon, Robinson, Hedberg, & Smith, 2013). In 2011, an outbreak in Oregon was associated with fresh strawberries contaminated with *Escherichia coli* O157:H7, which caused at least 10 illnesses and one death (Oregon Health Authority, 2011). Frozen strawberries and raspberries have

also been frequently associated with HAV and human norovirus outbreaks (Cotterelle et al., 2005; Hutin et al., 1999; Korsager, Hede, Boggild, Bottiger, & Molbak, 2005; Mäde, Trübner, Neubert, Höhne, & Johne, 2013; Niu et al., 1992). Therefore, there is an urgent need to develop effective decontamination technologies for berries.

Shortwave ultraviolet light (UVC, simplified as UV in this study) has been shown to be able to inactivate a wide range of microorganisms (Hijnen, Beerendonk, & Medema, 2006). At wavelengths of 200-280 nm, UV is mutagenic to microorganisms including bacteria and viruses (Cutler & Zimmerman, 2011). UV light has been approved by the FDA as a treatment for controlling surface microorganisms on food products (FDA, 2013). Studies have been done to evaluate the efficacy of UV light on microorganism reduction. On agar surfaces, UV light reduced *E. coli* O157:H7 and *Salmonella* by 5 log (Yaun, Marcy, Eifert, & Sumner, 2003). Various studies have shown UV light treatment to be effective on bacterial reduction on food surfaces such as blueberries (Kim & Hung, 2012) and lettuce (Allende, McEvoy, Luo, Artes, & Wang, 2006). Kim and Hung (2012) found that UV light treatment at 20 mW/cm<sup>2</sup> for 10 min achieved 2.1 and > 4.1 log reductions of *E. coli* O157:H7 on the calyx and skin of blueberries, respectively. UV has also been successfully used to treat liquids such as wastewater, drinking water, and apple ciders (City of Boulder Colorado, 2012; CDC, 2012b; Geveke, 2005).

As a nonthermal disinfection treatment, UV has less detrimental effects on nutrients and can better retain the fresh-like characteristics and flavors of foods compared with thermal processing (Falguera, Pagán, Garza, Garvín, & Ibarz, 2011; Pala & Toklucu, 2013). In addition, equipment setup is simple and relatively low in cost. However, the application of UV as a decontamination treatment for food surfaces

has not been successful due to two important limitations. One is that microorganisms on a food surface must directly face a UV lamp to be inactivated due to the shallow penetration depth of UV (Shama, 1999). In addition, items to be treated might be exposed to different doses of UV light. To overcome these two limitations, we developed a water-assisted UV system in which blueberry samples were immersed in agitated water during UV treatment. With this new system, the blueberry samples could randomly move and rotate in the agitated water, thus allowing all the blueberry surfaces to be exposed to UV light and receive more uniform UV exposure since the samples were moving around randomly during the UV treatment.

The objectives of this study were to evaluate the efficacy of UV light on the inactivation of *E. coli* O157:H7 and *Salmonella* on blueberries and to investigate whether chemicals could be used to enhance the inactivation of both pathogens using this UV system.

## **3.2 Materials and Methods**

### **3.2.1 Bacterial Strains and Inoculum Preparation**

Five strains of *E. coli* O157:H7 and four strains of *Salmonella enterica* of different serotypes, as shown in Table 3.1, were used in this study. All the strains were nalidixic acid-resistant. *E. coli* O157:H7 strains were kindly provided by Dr. Joerger and Dr. Kniel at the University of Delaware and *Salmonella* strains by Dr. Gurtler at the U.S. Department of Agriculture. Individual strains were grown in the presence of 50 µg/mL nalidixic acid (Fisher Scientific, Hampton, NH, USA) and maintained at 4 °C on tryptic soy agar (Difco Laboratories, Sparks, MD, USA) supplemented with 0.6% yeast extract and 50 µg/mL nalidixic acid (Difco) (TSAYE-N) as described previously

by Huang and Chen (2011). Individual cultures were grown in tryptic soy broth (Difco) supplemented with 0.6% yeast extract and 50 µg/mL of nalidixic acid (TSBYE-N) overnight at 35 °C and transferred into 10 mL of fresh TSBYE-N at 35 °C for 24 h. Five mL of each individual culture was mixed to form a five-strain cocktail of *E. coli* O157:H7 or a four-strain cocktail of *Salmonella*. The cocktails were centrifuged at 2450 × g for 10 min (Centra CL2, Centrifuge, Thermo Scientific, Waltham, MA, USA) and the pellet was resuspended in sterile 0.1% peptone water (Difco). The final inoculums contained about 10<sup>9</sup> CFU/ml.

Table 3.1: Bacterial Strain Information

Species	Serotype	Strain	Origin
<i>Escherichia coli</i>	O157:H7	Cider	Cider outbreak
<i>Escherichia coli</i>	O157:H7	250	Sprout outbreak
<i>Escherichia coli</i>	O157:H7	251	Lettuce outbreak
<i>Escherichia coli</i>	O157:H7	J58	Lettuce isolate
<i>Escherichia coli</i>	O157:H7	H1730	Lettuce outbreak
<i>Salmonella enterica</i>	Stanley	HO588	Sprout outbreak
<i>Salmonella enterica</i>	St. Paul	02-517-1	Cantaloupe outbreak
<i>Salmonella enterica</i>	Newport	H1275	Sprout outbreak
<i>Salmonella enterica</i>	Montevideo	G4639	Tomato outbreak

### **3.2.2 Inoculation of Blueberries**

Fresh blueberries were purchased from local grocery stores the day before each experiment and stored at 4 °C until use. The blueberries were UV-treated for 10 min in a biosafety hood (NuAire Lab Equipment, Plymouth, MN, USA) at room temperature (22 °C) to reduce the background microflora. For spot-inoculation, 75 µL of *E. coli* O157:H7 cocktail was deposited on either the skin or the calyx tissue of blueberries in small droplets to simulate two contamination conditions. For dip-inoculation, blueberries were dipped in the cocktail of *E. coli* O157:H7 or *Salmonella* for 1 min and allowed to dry in the biological safety hood for 2 h at room temperature (~22°C).

### **3.2.3 UV Light Treatment**

UV treatments were conducted using a Reyco UVC Emitter Table Top Test System (Medirian, ID, USA). The test system is an enclosed chamber that contains mercury lamps emitting UV light at 254 nm. UV intensity was measured by placing the sensor of a UV radiometer (UVP, Upland, CA, USA) right above the surface of blueberry samples. Intensity was measured before each treatment. During UV treatments the chamber was fully closed. Inoculated blueberry samples were placed in the center of the UV chamber.

### **3.2.4 Effect of Water on UV Light Inactivation of *E. coli* O157:H7 on Blueberries**

UV treatments of 2-10 min were conducted using the Reyco UVC Emitter Table Top Test System (Medirian, ID, USA). Inoculated blueberries were either treated by UV directly (dry UV treatment) or immersed in agitated water during the UV treatment (wet UV treatment). For dry UV treatment, three blueberries were placed on a petri-dish centered in the UV chamber and directly illuminated by UV.

The inoculation sites of the spot-inoculated berries were directly facing the UV lamps during the treatment. The dip-inoculated samples were flipped over once in the middle of each treatment to allow even UV exposure on both sides of the blueberries. For wet UV treatment, three blueberries were immersed in 150 mL agitated deionized (DI) water in a 250-mL glass beaker containing a 2.5-cm stir bar during the UV treatment. The depth of the water was 5 cm. An ultra-thin magnetic stirrer (Lab Disc, Fisher Scientific) was placed under the beaker to agitate the water in the beaker so that random rotation and movement of blueberries could be achieved. For comparison, blueberries were also washed with 150 mL agitated water for 10 min or with 10 ppm chlorinated water for 1 min in the beaker with the stir bar. Chlorinated water was prepared by adding commercial bleach (Clorox, Oakland, CA, USA) into DI water to obtain 10 ppm of free chlorine and then adjusted to pH 6.9 using hydrochloric acid. The free chlorine level was determined by free chlorine micro check test strips (HF Scientific, Ft. Myer, FL). All the water and solutions used in the wet UV treatment were at 4 °C. After treatments, the blueberry and wash water samples were immediately subjected to microbial analyses as described in Section 3.2.6.

### **3.2.5 Effect of Chemicals on Wet UV light Inactivation of *E. coli* O157:H7 and *Salmonella* Dip-inoculated on Blueberries**

Blueberries were dip-inoculated with *E. coli* O157:H7 or *Salmonella* and dried as described in Section 3.2.2. The blueberries were washed in 100 ppm of sodium dodecyl sulfate (SDS) (Amresco, Solon, OH, USA), chlorinated water (10 ppm free chlorine), or 0.5% levulinic acid (Sigma-Aldrich, St. Louis, MO, USA) for 10 min with or without UV exposure using the same experimental setup described in Section 3.2.4. All washing solutions were kept and used at 4 °C. After treatments, the

blueberry samples were immediately subjected to microbial analyses as described in Section 3.2.6.

### **3.2.6 Microbiological Analysis**

Wash water (1 ml) was withdrawn immediately after treatments and surface plated on TSAYE-N followed by incubation at 35 °C for 48 h. For chlorinated water treatment, 1 mL wash water was mixed with an equal volume of 0.1% sterile sodium thiosulfate (Sigma-Aldrich, USA) to neutralize the residual chlorine before plating. The detection limit was 1 CFU/ml of wash water. For blueberry sample, each sample consisting of three blueberries (~5 g) was aseptically transferred into a sterile filter bag (Whirl-Pak, Nasco, USA) containing 45 ml D/E (Dey/Engley) neutralizing broth (Difco). The mixture (pH 6.77) was pummeled in a stomacher (Seward 400, Seward, London, U.K.) for 2 min at 260 rpm. The homogenate was serially diluted in sterile 0.1% peptone water and surface-plated on TSAYE-N followed by incubation at 35 °C for 48 h. Colonies of *E. coli* O157:H7 or *Salmonella* formed on the plates were counted. The detection limit of plating was 1 log CFU/g of blueberries.

### **3.2.7 Statistical Analysis**

At least three independent trials were conducted for each experiment. Colony counts were converted to log CFU/g. Means and standard deviations were calculated. Statistical analyses were conducted using JMP (SAS Cary, NC, USA). Two-tail t-test and Tukey's one-way multiple comparisons were used to determine significant differences between treatments at the 95% confidence level ( $P < 0.05$ ).

### 3.3 Results

#### 3.3.1 Effect of Water on UV Light Inactivation of *E. coli* O157:H7 on Blueberries

The effect of presence of water on UV inactivation of *E. coli* O157:H7 on spot- and dip-inoculated blueberries was evaluated (Table 3.2). The UV intensity received by blueberry samples was 7.9 mW/cm<sup>2</sup> and 4.6 mW/cm<sup>2</sup> during dry and wet UV treatments, respectively. The initial populations (log CFU/g) of *E. coli* O157:H7 were  $7.1 \pm 0.1$ ,  $7.2 \pm 0.3$ , and  $6.2 \pm 0.5$  for skin, calyx and dip inoculated blueberries, respectively. For spot inoculation onto skin and calyx, the inactivation of *E. coli* O157:H7 was increased by both dry and wet UV treatments as the UV treatment time increased. For example, the dry UV treatment for 2, 5 and 10 min reduced *E. coli* O157:H7 spot-inoculated on the blueberry skin by 2.0, 3.7, and 4.0 log, respectively. In general, the wet UV treatment was more effective in inactivating *E. coli* O157:H7 spot-inoculated onto blueberries than the dry UV treatment. Except for one treatment (5-min UV treatment for the skin spot inoculation), all the other wet UV treatments were significantly more effective than the corresponding dry UV treatments for the skin and calyx-inoculation ( $P < 0.05$ ). The average differences in the log reductions between the wet and dry UV treatments were 0.9 log and 1.9 log for the skin and calyx inoculation, respectively. For skin spot inoculation, the 2-min, 5-min and 10-min wet UV treatments were comparable to the 1-min chlorine wash (not significant,  $P > 0.05$ ). For calyx spot inoculation, three wet UV treatments had 1.2-1.8 log more reductions of *E. coli* O157:H7 than the 1-min chlorine wash and the differences were significantly different ( $P < 0.05$ ).

For dip inoculated samples, extending the UV treatment time did not significantly enhance the killing effect. No significant differences in log reduction

between the dry and wet treatments were observed ( $P > 0.05$ ). In addition, the killing effect of dry and wet UV treatments was similar to the 1-min chlorine and 10-min water wash treatments. Generally speaking, *E. coli* O157:H7 dip inoculated onto blueberries was the most difficult to be inactivated and *E. coli* O157:H7 spot inoculated onto blueberry skin was the easiest to be killed. For example, the 10-min wet UV treatment reduced the populations of *E. coli* O157:H7 by 5.2, 3.9, and 1.6 log for skin spot inoculation, calyx spot inoculation, and dip inoculation, respectively.

To evaluate the effect of wet UV treatments and chlorine and water washing on wash water quality, water samples were immediately taken for microbiological analysis after treatments were finished. Results are shown in Table 3.2. When chlorine was used in the wash water, all the water samples were negative for *E. coli* O157:H7 regardless of the inoculation methods. No survival of *E. coli* O157:H7 was found for the water samples from the skin and dip inoculation methods. Low *E. coli* counts were found in the water samples from the 2-min and 5-min UV treatments. Extending the UV treatment time to 10 min completely inactivate the pathogen in the water sample. Very high bacterial counts were observed in all the water samples from the 10-min water wash.

Table 3.2: Comparison of Dry UV and Wet UV Treatments of Blueberries Inoculated with *E. coli* O157:H7 on Blueberries. Data represent mean bacterial reductions (log CFU/g) of at least three replicates  $\pm$  one standard deviation.

Treatment	Log reduction of <i>E. coli</i> O157:H7 on berries (log CFU/g)						<i>E. coli</i> O157:H7 survival in wash water (log CFU/mL)		
	Skin-Spot Inoculation		Calyx-Spot Inoculation		Dip Inoculation		Skin	Calyx	Dip
	Dry	Wet	Dry	Wet	Dry	Wet			
2-min UV	2.0 $\pm$ 0.1 <sup>abA</sup>	3.4 $\pm$ 0.6 <sup>bA</sup>	0.8 $\pm$ 0.6 <sup>aA</sup>	3.3 $\pm$ 0.1 <sup>bAB</sup>	1.3 $\pm$ 0.4 <sup>aA</sup>	1.6 $\pm$ 0.3 <sup>aa</sup>	UD**	1.2 $\pm$ 1.1	UD
5-min UV	3.7 $\pm$ 0.2 <sup>ab</sup>	3.9 $\pm$ 1.0 <sup>aA</sup>	1.7 $\pm$ 0.5 <sup>abAB</sup>	3.5 $\pm$ 0.5 <sup>bA</sup>	1.0 $\pm$ 0.6 <sup>aA</sup>	1.1 $\pm$ 0.1 <sup>aA</sup>	ND***	<0.1 $\pm$ 0.2 <sup>*****</sup>	ND
10-min UV	4.0 $\pm$ 0.1 <sup>ab</sup>	5.2 $\pm$ 0.5 <sup>bb</sup>	2.5 $\pm$ 0.5 <sup>ab</sup>	3.9 $\pm$ 0.8 <sup>bA</sup>	1.6 $\pm$ 0.3 <sup>aa</sup>	1.6 $\pm$ 0.4 <sup>aa</sup>	ND	UD	ND
1-min chlorine wash	N/A*	4.1 $\pm$ 0.5 <sup>AB</sup>	N/A	2.1 $\pm$ 0.6 <sup>C</sup>	N/A	0.9 $\pm$ 0.6 <sup>A</sup>	UD	UD	UD
10-min water wash	N/A	3.2 $\pm$ 0.3 <sup>A</sup>	N/A	2.2 $\pm$ 0.4 <sup>BC</sup>	N/A	1.0 $\pm$ 0.5 <sup>A</sup>	5.2 $\pm$ 0.1	5.1 $\pm$ 0.1	3.5 $\pm$ 0.2

Initial bacteria populations (log CFU/g) were 7.1  $\pm$  0.1, 7.2  $\pm$  0.3, and 6.2  $\pm$  0.5 for skin, calyx, and dip inoculated blueberries, respectively. Within the same category of “Skin”, “Calyx” and “Dip Inoculation”, data in the same row with different lowercase letters are significantly different ( $P < 0.05$ ) (two-tail t-test). Data in the same column with different uppercase letters are significantly different ( $P < 0.05$ ).

\*: Not applicable; \*\*: Un-detectable by plating (detection limit: 1 CFU/mL); \*\*\*: Not done; \*\*\*\*\*: Two out of the three replicates had zero plate counts (negative samples). The plating detection limit was 1 CFU/ml and this value was used for the two negative samples to calculate the mean and standard deviation.

### 3.3.2 Effect of Chemicals on Wet UV Light Inactivation of *E. coli* O157:H7 and *Salmonella* Dip-inoculated on Blueberries

In order to improve the decontamination efficacy for the dip-inoculated blueberries, combinations of UV and washing with SDS, levulinic acid, and chlorine were tested. The measured UV intensity was 7.9 mW/cm<sup>2</sup> for all UV treatments. Initial bacteria populations were  $6.1 \pm 0.6$  and  $5.9 \pm 0.3$  log CFU/g for *E. coli* O157:H7 and *Salmonella*, respectively. The results of the UV-chemical treatment inactivation of *E. coli* O157:H7 and *Salmonella* are shown in Figure 3.1. *Salmonella* showed resistance to most of the UV and chemical treatments comparable to that seen with *E. coli* O157:H7 except for two treatments, UV+10 ppm chlorine and 0.5% levulinic acid. *Salmonella* was significantly more resistant to the treatment of UV+10 ppm chlorine, but significantly more sensitive to the treatment of 0.5% levulinic acid than *E. coli* O157:H7. In general, the least effective treatments for inactivation of both pathogens were 100 ppm SDS, 0.5% levulinic acid, and UV+0.5% levulinic acid. The wet UV treatment alone (UV+water in the figure) was as effective as or more effective in inactivating both pathogens than the other treatments. Adding 10 ppm chlorine, 100 ppm SDS or 0.5% levulinic acid+100 ppm SDS to the wash water used in the UV treatment did not significantly enhanced the efficacy of the wet UV treatment alone. On the contrary, adding 0.5% levulinic acid to the wet UV system (UV+0.5% levulinic acid) reduced its efficacy in the inactivation of *Salmonella*. The treatment of UV+0.5% levulinic acid only achieved a 0.4-log reduction.

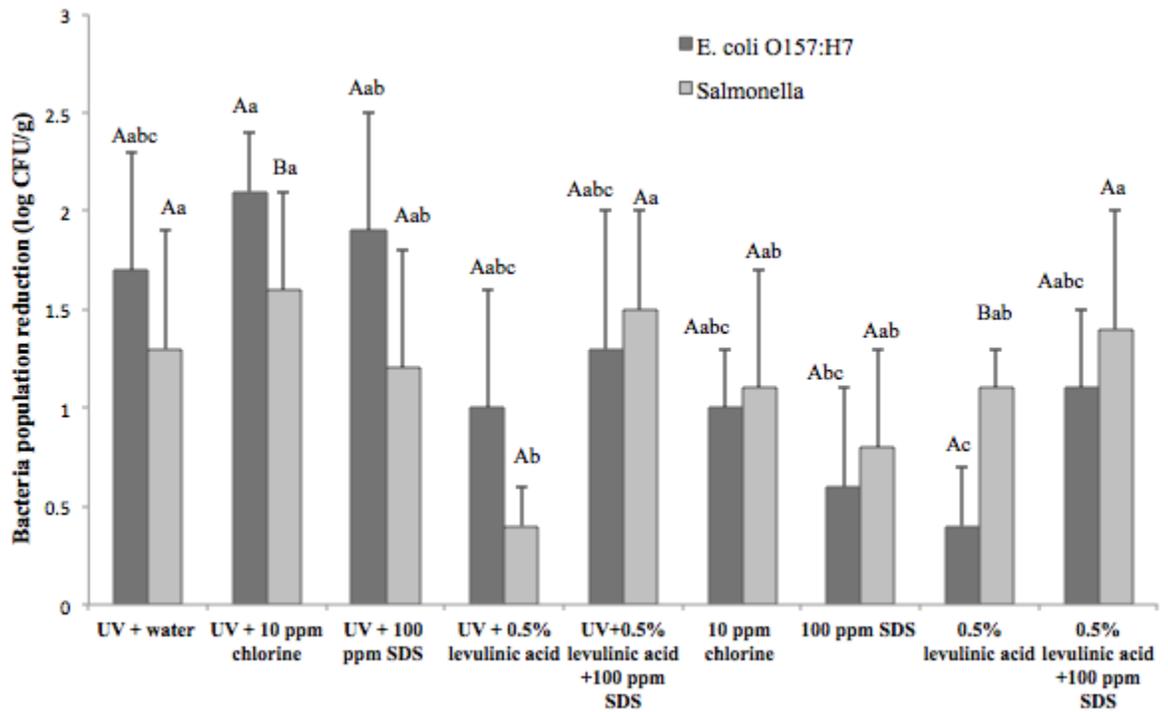


Figure 3.1: Effect of Chemicals on Wet UV Light Inactivation of *E. coli* O157:H7 and *Salmonella* Dip-inoculated on Blueberries. Blueberries dip-inoculated with *E. coli* O157:H7 and *Salmonella* were washed with water or chemicals for 10 min during UV treatment. Data represent mean bacterial reductions (log CFU/g) of at least three replicates  $\pm$  one standard deviation. Initial bacteria populations were  $6.1 \pm 0.6$  and  $5.9 \pm 0.3$  log CFU/g for *E. coli* O157:H7 and *Salmonella*, respectively. Error bars represent one standard deviation. For the same pathogen (*E. coli* O157:H7 or *Salmonella*), bars with different lowercase letters are significantly different ( $P < 0.05$ ). Within the same treatment, bars with different uppercase letters are significantly different ( $P < 0.05$ ).

### 3.4 Discussion

Two inoculation methods, spot and dip inoculation, were used in this study. The spot inoculation was used to simulate contamination caused by unhygienic contact with workers' hands, soil, or equipment during harvest, packing or processing, while the dip inoculation was to simulate contamination during washing. Generally the skin-inoculated blueberries showed the highest bacterial reduction followed by the calyx-inoculated blueberries, while the dip-inoculated ones had the lowest microbial population reduction. This is probably due to the different surface structures of blueberry's skin, calyx, and depressed ring (at the top of the fruit where the stem was attached) and the method of inoculation. It is likely that *E. coli* O157:H7 attached better to the rougher surfaces of the depressed ring and calyx than to the smooth skin. In addition, the rougher surface structures probably allow more shielding/shadowing of microorganisms inside surface irregularities or crevices during treatments. The dip inoculation method also provided a much larger surface for *E. coli* O157:H7 to attach to than the spot inoculation, which made it more difficult to wash off the bacterial cells during the wet UV treatment and chlorine wash. It is known that UV has a very limited penetration depth in nontransparent solid and is only capable of targeting superficial microorganisms on food surfaces. Therefore, bacterial cells hiding in the sub-surface of the depressed ring and calyx can be protected from UV. Similar findings were also reported by other researchers. Kim and Hung (2012) observed a higher survival of *E. coli* O157:H7 on the calyx-inoculated blueberries than the skin-inoculated ones after UV treatment.

For skin and calyx inoculated blueberries, longer UV treatment generally achieved higher microbial reductions for both dry and wet UV treatments (Table 3.2). UV dose is the product of UV intensity and time of exposure, thus longer treatment

time could provide more damage to bacterial cells (Environmental Protection Agency [EPA], 2006). This is in agreement with results reported by other studies. Kim and Hung (2012) observed decreased surviving populations of *E. coli* O157:H7 on blueberries with increased UV treatment time. Geveke (2008) found that in liquid egg white, log reductions of *E. coli* K12 and UV treatment times followed first-order kinetics with a correlation coefficient of 0.94 (Geveke, 2008). However, for the dip-inoculated blueberries, treatment time hardly had any effect on bacterial inactivation. It is likely that most of the bacterial cells on the dip-inoculated blueberries were hiding in the sub-surface of the blueberry skin, the irregularities or crevices of calyx and depressed ring, making them inaccessible to UV. It is also possible that these cells were able to attach tightly to the blueberry surface, which made it difficult to remove them by washing. Therefore, extending the UV treatment time did not increase inactivation.

Wet UV treatment was more efficient than dry UV treatment for spot-inoculated blueberries. However, the UV intensity received by the blueberries during the wet UV treatment was lower than that received by the blueberries during the dry UV treatment. In addition, during dry UV treatment, the inoculation site was facing the UV lamps all the time and was the only surface to receive all the UV energy; however, for wet UV treatment, the surface being illuminated with UV was always changing as the berries were randomly moving and rotating in the agitated water. Therefore, the inoculation sites on the blueberries received much less UV energy in the wet treatment than in the dry UV treatment. We would have expected that the dry UV treatment was more effective in inactivating pathogens than the wet UV treatment based on the UV energy the blueberries received. On the contrary, the wet UV

treatment was generally more effective in inactivating *E. coli* O157:H7 spot-inoculated onto blueberries than the dry UV treatment. The agitated water in the wet UV treatment probably helped remove the bacterial cells from the blueberry surface into water, making the bacteria more susceptible to UV light as it has a better penetration ability in clear water than in organic matter (Guerrero-Beltran & Barbosa-Canovas, 2004). For the dip-inoculated blueberries, however, wet UV did not differ from dry UV treatment.

Since *E. coli* O157:H7 dip-inoculated on blueberries was the most difficult to be inactivated, we investigated whether surfactant (SDS), organic acid (levulinic acid), and sanitizer (chlorine) could be used to enhance the efficacy of the wet UV treatment. SDS, an anionic surfactant, is generally recognized as safe (GRAS) by the FDA (21 CFR 172.822). SDS itself did not have any antimicrobial activity. Washing with SDS alone achieved only < 1 log reduction in both pathogens which was similar to that achieved by water washing. It was hoped that SDS could enhance the wet UV treatment by helping release bacterial cells tightly bound on the berry surface into water where they could be easily inactivated by the UV light. However, we did not observe such an enhanced inactivation effect. Levulinic acid (21 CFR, 172.515), an organic acid, also has GRAS status. Levulinic acid alone had very limited effect on *E. coli* O157:H7 (0.4 log reduction). Its effectiveness against *Salmonella* was comparable to that of UV and other chemical washing treatments. However, when levulinic acid was combined with UV (the treatment of UV+levulinic acid), it significantly reduced the effectiveness of the wet UV treatment against *Salmonella* (0.4 log reduction). The reason for this is not fully clear, but it is possible that levulinic acid was degraded by UV. The combination of SDS and levulinic acid has been shown as a highly effective

sanitizer. It was reported that > 5 log-reductions of *E. coli* O157:H7 and *Salmonella* were achieved on lettuce, poultry skin, and alfalfa seeds (T. Zhao, P. Zhao, & Doyle, 2009; Zhao et al., 2010). However, in our study, washing with SDS and levulinic acid only reduced *E. coli* O157:H7 and *Salmonella* on dip-inoculated blueberries by < 1.5-log CFU/g. It is possible that blueberries could better harbor pathogens than lettuce or alfalfa seeds due to their irregularities and crevices at the calyx and the depressed ring. The difference could also be due to the different inoculation protocols used in the two studies. UV treatment did not further enhance the effectiveness of the combined treatment of levulinic acid+SDS. Although UV treatment coupled with chlorine washing achieved the most log reductions of both *E. coli* O157:H7 and *Salmonella* on dip-inoculated blueberries, these log reductions were not significantly different from those obtained by the treatment of UV+water.

Blueberries are typically sold as fresh berries, or processed into frozen berries, puree and other products. To reduce microbial load and enhance food safety, the food industry generally uses 10 ppm free chlorine to wash blueberries intended for further processing such as freezing (for frozen berries) and thermal pasteurization (for puree). Chlorine is also widely used to wash other types of fresh produce such as tomatoes, sprouting seeds, lettuce and spinach. However, the use of chlorine-based chemical sanitizers can generate hazardous fumes that have raised public health concerns (Beuchat, 1997). Chlorine residues remaining on the food products could also potentially harm human health due to corrosive acid formation in the presence of water (FAO & WHO, 2008; New York State Department of Health, 2004). Residual chlorine in wastewater is also toxic to many kinds of aquatic life and can form carcinogenic trihalomethanes upon reaction with organic materials in the water; thus,

wastewater is required to be dechlorinated prior to discharge into aquatic waters (EPA, 2000). Due to the various drawbacks of using chlorine as a fresh produce sanitizer, alternative decontamination methods have been proposed and studied. In the current study, the wet UV treatments were generally more effective or as effective as chlorine washing, indicating water-assisted UV treatment can be an alternative to chlorine washing for the fresh produce industry. In comparison to chlorine wash, the major benefits of using UV is that it leaves no chemical residue on the produce and does not create chemical disposal issues. The wet UV system developed in this study would also be acceptable for organic producers.

### **3.5 Conclusion**

For spot inoculation onto skin and calyx, the inactivation of *E. coli* O157:H7 was increased by both dry and wet UV treatments as the UV treatment time increased. The wet UV treatment was generally more effective in inactivating *E. coli* spot-inoculated onto blueberries than the dry UV treatment. The wet UV treatments were generally more effective or as effective as the chlorine washing. SDS, levulinic acid and chlorine did not significantly enhance the efficacy of the wet UV treatment in inactivating *E. coli* O157:H7 and *Salmonella* dip-inoculated on blueberries. Findings of this study suggest that the wet UV treatment could be used as an alternative to chlorine washing for blueberries and potentially for other fresh produce. In an industry setting, the wet UV system can be easily set up since UV lamps could be placed on top of a washing tank in a closed chamber and no additional processing step is needed.

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## REFERENCES

- Akbas, M. Y., & Ölmez, H. (2007). Inactivation of *Escherichia coli* and *Listeria monocytogenes* on iceberg lettuce by dip wash treatments with organic acids. *Letters in Applied Microbiology*, *44*, 619-624.
- Allende, A., McEvoy, J. L., Luo, Y., Artes, F., & Wang, C. Y. (2006). Effectiveness of two sided UV-C treatments in inhibiting natural microflora and extending the shelf-life of minimally processed 'Red oak leaf' lettuce. *Food Microbiology*, *23*, 241-249.
- Bachmann, R. (1975). Sterilization by intense ultraviolet radiation. *The Brown Boveri Review*, *62*, 206–209.
- Beuchat, L. R. (1997). Comparison of chemical treatments to kill *Salmonella* on alfalfa seeds destined for sprout production. *International Journal of Food Microbiology*, *34*, 329-333.
- Boulder Colorado. (2012). *City using new ultraviolet light technology to safely treat Boulder's wastewater before sending it downstream*. Retrieved October 25, 2014, from <https://bouldercolorado.gov/newsroom/dec-7-2012-city-using-new-ultraviolet-light-technology-to-safely-treat-boulders-wastewater-before-sending-it-downstream>.
- Calder, L., Simmons, G., Thornley, C., Taylor, P., Pritchard, K., Greening, G., & Bishop, J. (2003). An outbreak of hepatitis A associated with consumption of raw blueberries. *Epidemiology & Infection*, *131*, 745-751.
- Centers for Disease Control and Prevention. (2012a). *Foodborne Outbreak Online Database (FOOD)*. Retrieved November 10, 2014, from <http://wwwn.cdc.gov/foodborneoutbreaks/Default.aspx>
- Centers for Disease Control and Prevention. (2012b). *Water treatment*. Retrieved October 25, 2014, from [http://www.cdc.gov/healthywater/drinking/public/water\\_treatment.html](http://www.cdc.gov/healthywater/drinking/public/water_treatment.html).
- Clancy, S. (2008). DNA damage & repair: mechanisms for maintaining DNA integrity. *Nature Education*, *1*(1), 103.

- Cotterelle, B., Drougard, C., Rolland, J., Becamel, M., Boudon, M., Pinede, S., Traore, O., Balay, K., Pothier, P., & Espie, E. (2005). Outbreak of norovirus infection associated with the consumption of frozen raspberries. France, March 2005. *Euro Surveill*, 10(17), 2690. Retrieved October 25, 2014, from <http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=2690>.
- Cutler T. D., & Zimmerman, J.J. (2011). Ultraviolet irradiation and the mechanisms underlying its inactivation of infectious agents. *Animal Health Research Reviews*, 12(1), 15-23.
- Dickson, J. S. (1992). Acetic acid action on beef tissue surfaces contaminated with *Salmonella* Typhimurium. *Journal of Food Science*, 57, 297-301.
- Doyle, M. P., & Erickson, M. C. (2008). Summer meeting 2007—the problems with fresh produce: an overview. *Journal of Applied Microbiology*, 105, 317-330.
- Du, J., Han, Y., & Linton, R. H. (2002). Inactivation by chlorine dioxide gas (ClO<sub>2</sub>) of *Listeria monocytogenes* spotted onto different apple surfaces. *Food Microbiology*, 19, 481-490.
- Environmental Protection Agency (EPA). (2006). *Ultraviolet disinfection guidance manual for the final long term 2 enhanced surface water treatment rule*. Retrieved October 25, 2014, from [http://www.epa.gov/safewater/disinfection/lt2/pdfs/guide\\_lt2\\_uvguidance.pdf](http://www.epa.gov/safewater/disinfection/lt2/pdfs/guide_lt2_uvguidance.pdf)
- Environmental Protection Agency (EPA). (2000). *Wastewater technology fact sheet: dechlorination*. Retrieved October 25, 2014, from [http://water.epa.gov/scitech/wastetech/upload/2002\\_06\\_28\\_mtb\\_dechlorination.pdf](http://water.epa.gov/scitech/wastetech/upload/2002_06_28_mtb_dechlorination.pdf).
- Environmental Protection Agency (EPA). (1993). *R.E.D. Facts lauryl sulfate salts*. Retrieved October 25, 2014, from <http://www.epa.gov/oppsrrd1/REDs/factsheets/4061fact.pdf>.
- Escudero, M. E., Velázquez, L., Favier, G., & de Guzmán, A. M., 2003. Effectiveness of chlorine, organic acids and UV treatments in reducing *Escherichia coli* O157:H7 and *Yersinia enterocolitica* on apples. *Central European Journal of Public Health*, 11, 68-72.
- Falguera, V., Pagán, J., Garza, S., Garvín, A., & Ibarz, A. (2011). Ultraviolet processing of liquid food: A review Part 2: Effects on microorganisms and on food components and properties. *Food Research International*, 44, 1580-1588.

- Food and Agriculture Organization of the United Nations, & World Health Organization. (2008). *Benefits and risks of the use of chlorine-containing disinfectants in food production and food processing. (Joint FAO/WHO Expert Meeting)*. Geneva, Switzerland: WHO Document Production Services.
- Food and Drug Administration (FDA). (2013). *Irradiation in the production, processing and handling of food. Code of Federal Regulations Title 21, 179.39*. Retrieved October 25, 2014, from <http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfCFR/CFRSearch.cfm?fr=179.39>
- Food and Drug Administration (FDA). (2001). *FDA survey of imported fresh produce FY 1999 field assignment*. Retrieved October 25, 2014, from <http://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/ProducePlantProducts/ucm118891.htm>.
- Food and Drug Administration (FDA). (1998). *Guide to minimize microbial food safety hazards for fresh fruits and vegetables*. Retrieved October 25, 2014, from <http://www.fda.gov/downloads/Food/GuidanceComplianceRegulatoryInformation/GuidanceDocuments/ProduceandPlanProducts/UCM169112.pdf>.
- Franz, C. M. A. P., Specht, I., Cho, G., Graef, V., & Stahl, M. R. (2009). UV-C-inactivation of microorganisms in naturally cloudy apple juice using novel inactivation equipment based on dean vortex technology. *Food Control*, 20, 1103-1107.
- Geveke, D. J. (2008). UV inactivation of *E. coli* in liquid egg white. *Food and Bioprocess Technology*, 1, 201.
- Geveke, D. J. (2005). UV Inactivation of Bacteria in Apple Cider. *Journal of Food Protection*, 68, 1739-42.
- Guerrero-Beltran, J. A., & Barbosa-Canovas, G. V. (2004). Advantages and limitations on processing foods by UV light. *Food Science and Technology International*, 10, 137-147.
- Hijnen, W. A. M., Beerendonk, E. F., & Medema, G. J. (2006). Inactivation credit of UV radiation for viruses, bacteria and protozoan (oo)cysts in water: A review. *Water Research*, 40, 3-22.
- Huang, Y., & Chen, H. (2011). Effect of organic acids, hydrogen peroxide and mild heat on inactivation of *Escherichia coli* O157:H7 on baby spinach. *Food Control*, 22, 1178-1183.

- Hutin, Y. J., Pool, V., Cramer, E. H., Najnan, O. V., Weth, J., Williams, I. T., Goldstein, S. T., Gensheimer, K. F., Bell, B. P., Shapiro, C. N., Alter, M. J., & Margolis, H. S. (1999). A multistate, foodborne outbreak of hepatitis A. National Hepatitis A Investigation Team. *The New England Journal of Medicine*, *340*, 595-602.
- Izat, A. L., Colberg, M., Adams, M. H., Reiber, M. A., & Waldroup, P. W. (1989). Production and processing studies to reduce the incidence of *Salmonellae* on commercial broilers. *Journal of Food Protection*, *52*, 670-673.
- Kim, C., & Hung, Y. (2012). Inactivation of *E. coli* O157:H7 on blueberries by electrolyzed water, ultraviolet light, and ozone. *Journal of Food Science*, *77*, 206-211.
- Korsager, B., Hede, S., Boggild, H., Bottiger, B., & Molbak, K. (2005). Two outbreaks of norovirus infections associated with the consumption of imported frozen raspberries, Denmark, May-June 2005. *Euro Surveill.* *2005*, *10*(25), 2729. Retrieved October 25, 2014, from <http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=2729>
- Mäde, D., Trübner, K., Neubert, E., Höhne, M., & Johne, R. (2013). Detection and typing of norovirus from frozen strawberries involved in a large-scale gastroenteritis outbreak in Germany. *Food and Environmental Virology*, *5*, 162-168.
- Miller, B. D., Rigdon, C. E., Robinson, T. J., Hedberg, C., & Smith, K. E. (2013). Use of global trade item numbers in the investigation of a *Salmonella* Newport outbreak associated with blueberries in Minnesota, 2010. *Journal of Food Protection*, *76*, 762-769.
- New York State Department of Health. (2004). *The facts about chlorine*. Retrieved October 25, 2014, from [http://www.health.ny.gov/environmental/emergency/chemical\\_terrorism/chlorine\\_general.htm](http://www.health.ny.gov/environmental/emergency/chemical_terrorism/chlorine_general.htm).
- Niu, M. T., Polish, L. B., Robertson, B. H., Khanna, B.K., Woodruff, B. A., Shapiro, C. N., Miller, M. A., Smith, J. D., Gedrose, J. K., & Alter, M. J. (1992). Multistate outbreak of hepatitis A associated with frozen strawberries. *Journal of Infectious Diseases*, *166*, 518-524.
- Oregon Health Authority. (2011). *Lab tests confirm source of E. coli O157 from deer droppings in strawberry fields in NW Oregon*. Retrieved October 25, 2014 from <http://www.oregon.gov>

- Pala, C. U., & Toklucu, A. K. (2013). Effects of UV-C processing on some quality characteristics of grape juices. *Food and Bioprocess Technology*, 6(3), 719-725.
- Rodas, A. G., Bourquin, L., Salazar, C. G., Gomez, A.V., & Wise, J.C. (2009). *Good Agricultural Practices for Food Safety in Blueberry Production: Basic Principles*. Retrieved October 25, 2014, from [http://www.gaps.msue.msu.edu/blue\\_manual.pdf](http://www.gaps.msue.msu.edu/blue_manual.pdf).
- Roy J. H., Lundy, S., & Kalicki, B. (2009). Bluberries "their role in health". *Pennington Nutrition Series*, No. 2.
- Shama, G. (1999). *Ultraviolet light*. In: R.K. Robinson, C. Batt and P. Patel (eds), *Encyclopedia of Food Microbiology-3*. London: Academic Press, 2208-2214.
- Sy, K. V., McWatters, K. H., & Beuchat, L. R. (2005). Efficacy of gaseous chlorine dioxide as a sanitizer for killing *Salmonella*, yeasts, and molds on blueberries, strawberries, and raspberries. *Journal of Food Protection*, 68(6), 1165-75.
- Yaun, B. R., Marcy, J. E., Eifert, J. D., & Sumner, S. S. (2003). Response of *Salmonella* and *Escherichia coli* O157:H7 to UV energy. *Journal of Food Protection*, 66, 1071-1073.
- Zhao, T., Doyle, M. P., & Zhao, P. (2009). Inactivation of *Salmonella* and *Escherichia coli* O157:H7 on lettuce and poultry skin by combinations of levulinic acid and sodium dodecyl sulfate. *Journal of Food Protection*, 72, 928-936.
- Zhao, T., Zhao, P., & Doyle, M. P. (2010). Inactivation of *Escherichia coli* O157:H7 and *Salmonella* Typhimurium DT 104 on alfalfa seeds by levulinic acid and sodium dodecyl sulfate. *Journal of Food Protection*, 73, 2010-2017.

**Chapter 4**

**APPLICATION OF WATER-ASSISTED ULTRAVIOLET LIGHT  
PROCESSING ON THE INACTIVATION OF MURINE NOROVIRUS ON  
BLUEBERRIES**

**ABSTRACT**

Ultraviolet light (UV) has antimicrobial effects. However, shadowing effects have limited its application. In this study, a novel set-up using water-assisted UV processing was developed to inactivate murine norovirus (MNV-1) on fresh blueberries for both small and large-scale experimental setups. Blueberries were skin-inoculated with MNV-1 and treated for 1-5 min with UV directly (dry UV) or immersed in agitated water during UV treatment (water-assisted UV). The effect of presence of 2% (v/v) blueberry juice or 5% blueberry crush (w/w) in wash water was also evaluated. Results showed that water-assisted UV treatment generally showed higher efficacies than dry UV treatment. With a UV fluence of 3 J/cm<sup>2</sup>, MNV reductions of >4.36- and 3.04-log were achieved by wet and dry UV treatments, respectively. The average reduction difference between dry and water-assisted UV treatments was greater than 1.3 log. Water-assisted UV showed similar inactivating efficacy as 10-ppm chlorine wash, achieving over 4-log reduction of MNV-1 after 2-min treatment. MNV-1 was more easily killed on skin-inoculated blueberries compared with calyx-inoculated berries. When double deionized water was used for wet UV treatment (UV fluence of 1.2 J/cm<sup>2</sup>), MNV reductions of >3.2- and 1.81-log

were achieved for skin- and calyx-inoculated berries, respectively. Presence of 2% blueberry juice in wash water provided protection to MNV-1 from UV irradiation or 10-ppm chlorine wash treatment. To improve the inactivation efficacy, the effect of combining water-assisted UV treatment with chlorine wash was evaluated. Inoculated blueberries were UV-treated while being immersed in agitated water containing 10 ppm free chlorine. The UV+chlorine treatment had better or similar inactivation efficiency as water-assisted UV and chlorine wash alone. Findings of this study suggest that UV treatment could be used as an alternative to chlorine wash for blueberries and potentially for other fresh produce.

#### **4.1 Introduction**

Blueberry is a high-value fruit that has many benefits to human health, such as antioxidant and anti-inflammatory activities (Roy, Lundy, & Kalicki, 2009). Fresh blueberries are harvested manually or mechanically and then field-packed into retail containers (Harris et al., 2003). Fresh berries destined for the fresh market are not washed following harvesting. Berries to be processed are usually washed with potable water or chlorinated water. Since the berries are consumed raw or minimally processed, they could lead to food safety problems. In 2003, contaminated raw blueberries led to an outbreak of hepatitis A (Calder et al., 2003). In 2009, blueberries contaminated with *Salmonella* Muenchen resulted in a multistate outbreak, which caused 14 cases of illnesses (Centers for Diseases Control and Prevention, 2012a). In a massive human norovirus (HuNoV) gastroenteritis outbreak in 2012, frozen strawberries were identified as the cause and the outbreak that affected about 11,000 people (Mäde, Trübner, Neubert, Höhne, & Johne, 2013). Indeed, norovirus is the leading cause of acute gastroenteritis in the United States, which causes 19 - 21

million illnesses and contributes to 56,000 - 71,000 hospitalizations and 570 - 800 deaths each year (CDC, 2013).

Chlorine has been widely used for fresh produce decontamination. It is low in cost, has minimal impact on the quality of the food product and has been shown to be effective in killing pathogens in suspensions (Gonzalez, Luo, Ruiz-Cruz, & McEvoy, 2004; Gil, Selma, Lopez-Galvez, & Allende, 2009). It is critical that a relatively constant level of free chlorine be maintained in washing solutions to ensure its efficacy against microbial contamination. Chlorine can react rapidly with organic matter in the washing solution and form by-products like trihalomethanes, halo ketones and chloropicrin (Gil et al., 2009). To maintain a constant free chlorine level, it is necessary to replenish the chlorine during washing process, thus leading to the accumulation of toxic chlorine by-products and generation of harmful chlorine off-gas (Suslow, 2011). Therefore, there has been a sustained effort to find chlorine alternatives.

Shortwave ultraviolet light (UVC, simplified as UV in this study) has been shown to be able to inactivate a wide range of microorganisms (Hijnen, Beerendonk, & Medema, 2006). UV light has been approved by the FDA as a treatment for controlling surface microorganisms on food products (FDA, 2013). Various studies have shown UV light treatment to be effective on bacterial and viral reduction on food surfaces such as blueberries, strawberries, lettuce and onions (Kim & Hung, 2012; Allende, McEvoy, Luo, Artes, & Wang, 2006; Fino & Kniel, 2008). According to Fino & Kniel (2008), significant virus reductions could be seen on lettuce, green onions and strawberries with a UV dose of 240 mJ/cm<sup>2</sup>. UV has also been successfully

used to treat liquids such as wastewater, drinking water, and apple ciders (City of Boulder Colorado, 2012; CDC, 2012b; Geveke, 2005).

UV has fewer detrimental effects on nutrients and can better retain the fresh-like characteristics and flavors of foods compared with thermal processing (Falguera, Pagán, Garza, Garvín, & Ibarz, 2011; Pala & Toklucu, 2013). In addition, the equipment setup is simple and relatively low in cost. However, the application of UV as a decontamination treatment for food surfaces is limited. The microorganisms on a food surface must directly face a UV lamp to be inactivated due to the shallow penetration depth of UV (Shama, 1999). Samples positioned in different parts of the UV chamber might also be exposed to different doses of UV light. To overcome these two limitations, a water-assisted UV system was developed where blueberry samples were immersed in agitated water during UV treatment. The blueberry samples could randomly move and rotate in the agitated water, thus allowing all blueberry surfaces to be exposed to UV light and receive more uniform UV exposure.

Since HuNoVs cannot be propagated in cell cultures (Duizer, 2004), surrogate viruses that share similar molecular and/or pathological features with HuNoVs are used. Murine norovirus (MNV-1) is commonly used as a surrogate for human norovirus. Studies have shown that MNV-1 is environmental stable (Bae & Schwab, 2007) and persistent over a wide range of pH values (Hirneisen & Kniel, 2013). MNV was also found to be more resistant to UV irradiation than feline calicivirus (Park, Linden, & Sobsey, 2011), which is a commonly used surrogate for HuNoV.

The overall goal of this study was to evaluate the efficacy of water-assisted UV irradiation on the inactivation of murine norovirus on blueberries. First, the effect of water-assisted UV irradiation in comparison to UV irradiation alone (dry UV) was

evaluated. Second, since blueberries might be damaged during the washing process and thus lead to berry exudates being released into the washing solution, the efficiency of water-assisted UV on MNV-1 inactivation was assessed when wash water contained blueberry juice or crush.

## **4.2 Materials and Methods**

### **4.2.1 Virus and Cell Lines**

MNV-1 and murine macrophage cell line RAW 264.7 were kindly provided by Dr. Jianrong Li at the Ohio State University. RAW 264.7 cells were cultured in Dulbecco's modified Eagle medium (DMEM) (Gibco, Life Technologies Corporation, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (FBS) (Gibco) and kept at 37 °C under 5% CO<sub>2</sub> atmosphere. To prepare MNV-1 stock, confluent RAW 264.7 cells were infected with MNV-1 at a multiplicity of infection (MOI) of 1. After 1 hour of incubation at 37 °C under 5% CO<sub>2</sub> atmosphere, 25 ml of DMEM (supplemented with 2% FBS) was added. MNV-1 was harvested 2 days after post-inoculation by three freezing-thawing cycles and subsequent centrifugation. The virus was stored at -80 °C until use.

### **4.2.2 Inoculation of Blueberries**

Fresh blueberries were purchased from local grocery stores the day before each experiment and stored at 4 °C until use. The blueberries were UV-treated for 10 min in a biosafety hood (NuAire Lab Equipment, Plymouth, MN, USA) at room temperature (22 °C) to reduce background microflora. MNV-1 of 50 µL was deposited on either the skin or the calyx tissue of blueberries in small droplets to simulate two contamination conditions. For small-scale experiments, three blueberries (~5 g) were

inoculated with MNV-1. For large-scale experiments, 10 out of 30 berries (~50 g) were inoculated with the virus. The inoculated blueberries were allowed to dry in the biological safety hood (NuAire Lab Equipment) for 2 h at room temperature (22 °C).

#### **4.2.3 UV Light Treatment**

The UV treatments were conducted using a Reyco UVC Emitter Table Top Test System (Medirion, ID, USA). The test system is an enclosed chamber, which contains mercury lamps that emit UV light at 254 nm. UV intensity was measured by placing the sensor of a UV radiometer (UVP, Upland, CA, USA) right above the surface of blueberry samples. Intensity was measured before each treatment. During UV treatments the chamber was fully closed. Inoculated blueberry samples were placed in the center of the UV chamber.

#### **4.2.4 Effect of Presence of Water during UV Irradiation on Inactivation of MNV-1 on Blueberries**

Skin-inoculated blueberries were either treated with UV directly (dry UV treatment) or immersed in agitated water during the UV treatment (water-assisted UV treatment) for 1-5 min. UV intensity for all treatments was 10 mW/cm<sup>2</sup>. For dry UV treatment, three skin-inoculated blueberries were placed on a petridish centered in the UV chamber and directly illuminated by UV. The inoculation sites of the spot-inoculated berries were directly facing the UV lamps during the treatment. For water-assisted UV treatment, three blueberries were immersed in 300 mL of agitated deionized (DI) water in a 500-mL quartz beaker containing a 2.5-cm stir bar during the UV treatment. The depth of the water was 6.5 cm. An ultra-thin magnetic stirrer (Lab Disc, Fisher Scientific) was placed under the beaker to agitate the water in the beaker to create turbulent flow so that random rotation and movement of blueberries could be

achieved. For comparison, blueberries were also washed with 300 mL of agitated water for 5 min or 10 ppm chlorinated water for 1-5 min in the beaker with the stirring bar. Chlorinated water was prepared by adding commercial bleach (Clorox, Oakland, CA, USA) into DI water to obtain 10 ppm of free chlorine and then adjusted to pH 6.5 using 10% citric acid. The free chlorine level was determined with a portable free chlorine meter (Hanna Instruments Inc., USA). All the water and solutions used in the water-assisted UV treatment were at room temperature (22 °C). After treatments, surviving virus in blueberry samples was extracted and quantified by a viral plaque assay as described in Sections 4.2.7 and 4.2.8. MNV-1 was also quantified in the spent wash water.

#### **4.2.5 Effect of Blueberry Juice on the Efficiency of Small-scale Water-assisted UV Inactivation of MNV-1 on Blueberries**

Three skin-inoculated blueberries were treated with water-assisted UV, chlorine wash, or a combined treatment of water-assisted UV and chlorine wash for 2 min. Blueberry juice (Knudsen & Sons Inc, USA) was added to the wash water right before each treatment to achieve a final concentration of 2% (v/v) juice. Turbidity of the wash water was tested using a portable turbidity meter (Hanna Instruments Inc, USA) and chemical oxygen demand (COD) value was tested using a COD colorimeter (YSI Inc, Yellow Springs, OH, USA). For chlorine treatments, chlorinated water was prepared by adding commercial bleach (Clorox) into DI water to obtain 50 ppm of free chlorine, then blueberry juice was added and the pH was adjusted to 6.5 using 1 M NaOH. The chlorinated water had a final free chlorine concentration of 10 ppm after 2% of berry juice was added. UV intensity for all treatments was 10 mW/cm<sup>2</sup>. After treatments, surviving virus in blueberry samples was extracted and quantified by a

viral plaque assay as described in Sections 4.2.7 and 4.2.8. MNV-1 was also quantified in the spent wash water.

#### **4.2.6 Effect of Blueberry Juice and Blueberry Crush on the Efficiency of Large-scale Water-assisted UV Inactivation of MNV-1 on Blueberries**

Ten out of thirty blueberries (~50 g) were skin- or calyx-inoculated with MNV-1 and dried as described in Section 4.2.2. The blueberries were treated with water-assisted UV, chlorine wash, or combined treatment of water-assisted UV and chlorine wash for 2 min. The wash water was modified by addition of 2.5 g manually crushed un-inoculated blueberries (representing 5% of the total blueberry weight) or blueberry juice to make a final juice concentration of 2% (v/v). For chlorine treatments with 5% crushed berries added, chlorinated water was prepared by adding commercial bleach (Clorox) into DI water to obtain 10 ppm of free chlorine (pH ~ 9). Addition of 5% of crushed berries did not change the free chlorine level in the wash water, but decreased the pH to approximately 7. For chlorine treatments with 2% blueberry juice, chlorinated water was prepared by adding commercial bleach (Clorox) into DI water to obtain 50 ppm of free chlorine, then blueberry juice was added and the pH was adjusted to 6.5 using 1 M NaOH. The chlorinated water had a final free chlorine concentration of 10 ppm after 2% of berry juice was added. For water-assisted UV treatments, 30 blueberries (~50 g) were immersed in 1000 mL of agitated washing solution in a 1.9-L shallow glass tray (20 cm long x 20 cm wide x 7 cm deep baking dish) (Pyrex, USA) containing a 6.5-cm stir bar during the UV treatment (UV intensity of 10 mW/cm<sup>2</sup>). The depth of the water was 4 cm. A stirring plate (Model No. 115007S, Fisher Scientific, USA) was placed under the glass tray to agitate the water in the tray to create turbulent flow so that random rotation and movement of

blueberries could be achieved. The water-assisted UV treatment setup is shown in Figure 4.1. For comparison, blueberries were also washed with 1000 mL of agitated chlorinated water for 2 min in the tray with the stir bar. After treatments, surviving virus in blueberry samples was extracted and quantified by a viral plaque assay as described in Sections 4.2.7 and 4.2.8. Spent wash water was also analyzed for MNV-1.



Figure 4.1: Large-scale Water-assisted UV Treatment Setup

#### **4.2.7 Extraction of MNV-1 from Blueberries and Sampling of Wash Water**

MNV-1 was extracted from blueberries using the method described by Kingsley et al. (2002) with significant modifications. Individual small-scale blueberry samples (~5 g) were transferred into sterile stomacher filter bags (Whirl-Pak, Nasco, USA) and individual large-scale blueberry samples (~ 50 g) were transferred into large sterile stomacher filter bags (Thermo Fisher Scientific Inc., USA). Two volumes of vegetable buffer (100 mM Tris [Thermo Fisher Scientific Inc.], 50 mM glycine

[Promega Corporation], 3% [m/v] beef extract [Becton Dickson Company], 50 mM MgCl<sub>2</sub> [Thermo Fisher Scientific Inc.], pH 9.5) were added to the bags and the samples were homogenized with a stomacher (Seward 400, Seward, London, U.K.) at 260 rpm for 1 min. The filtrate was taken and centrifuged at  $2,500 \times g$  for 10 min at 4 °C (Sorvall, Thermo Scientific, USA). The supernatants were used for subsequent plaque assays. To determine the extraction rate of MNV-1 from blueberries, blueberries were inoculated with 10-fold serial dilutions of the virus and dried as described above. The virus in the samples were extracted and quantified. To determine the quality of the spent wash water, 9 ml of the wash water was taken immediately after treatments without chlorine and mixed with 1 ml of PBS (10 $\times$ , pH 7.2) (Kerafast, Inc., Boston, MA, USA) and the mixture was used for viral plaque assays. For chlorinated water treatment, wash water was mixed with an equal volume of 0.1% sterile sodium thiosulfate (Sigma-Aldrich, USA) to neutralize the residual chlorine (Kemp & Schneider, 2000) and 9 ml of the mixture was added to 1 ml of PBS (10 $\times$ , pH 7.2) (Kerafast, Inc.) before plaque assays. The detection limit was 1 PFU/ml of wash water.

#### **4.2.8 Viral Plaque Assay**

MNV-1 was quantified using the procedure of Li et al. (2013) with modifications. Raw 264.7 cells were seeded into 6-well tissue culture plates (Thermo Fisher Scientific Inc.) at a density of about  $2 \times 10^6$  cells per well. After 24 h of incubation, cell monolayers were infected with 400  $\mu$ L of a 10-fold dilution series of the virus and plates were incubated for 1 h at 37 °C in a 5% CO<sub>2</sub> atmosphere with gentle manual agitation every 10-15 min. After incubation, the samples were removed and the cells were overlaid with 2.5 mL of Eagle minimum essential medium

(DMEM) supplemented with 5% (v/v) FBS, 1% (w/v) sodium bicarbonate, 10 mM HEPES (pH 7.7), 100 µg/ml streptomycin, 100 U/ml penicillin, 0.25 µg/ml amphotericin B, 2 mM L-glutamine (Life Technologies Corporation) and 0.5% (w/v) low-melting agarose (SeaPlaque, Lonza Group Ltd.). Plates were incubated at 37 °C in 5% CO<sub>2</sub> for 48 h, fixed in 3.7% formaldehyde for one hour and plaques were visualized by staining with 0.05% (w/v in 10% ethanol) crystal violet.

#### **4.2.9 Statistical Analysis**

Three independent trials were conducted for each experiment. Virus counts were converted to log PFU/sample and expressed as mean ± standard deviation. For treatments where the detection limit was reached, the detection limit viral count was used for calculating mean and standard deviation. Statistical analyses were conducted using SPSS (IBM, USA). One-way analysis of variance (ANOVA) and Tukey's multiple comparisons were used to determine significant differences between treatments at the 95% confidence level ( $P < 0.05$ ).

### **4.3 Results**

#### **4.3.1 MNV-1 Recovery Rate by the Vegetable Buffer Homogenization Method**

Recovery rates of MNV-1 using the vegetable buffer homogenization method are shown in Figure 4.2. The recovery rate was lower when the population of MNV-1 on blueberries was smaller. When the viral population on blueberries was 6.58 log PFU/sample, the recovery rate was 57%. When the viral population on the blueberry sample decreased to 3.58 log PFU/sample, only 4.9% of the MNV-1 could be recovered. However, there was an almost linear relationship ( $R^2 = 0.99$ ) between log (actual virus population) and log (recovered virus population). The equation for

calculation of actual MNV-1 population on blueberries was  $\log(\text{actual virus population}) = 0.6864 \times \log(\text{recovered population}) + 2.2039$ . This equation was applied for all viral survival calculations on blueberries.

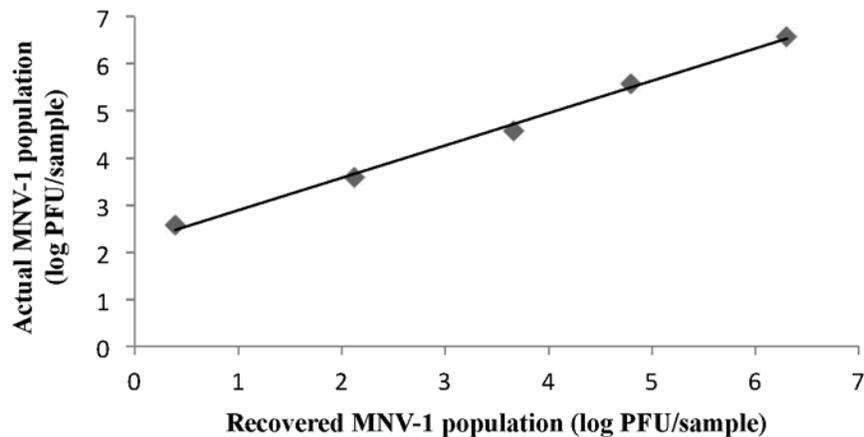


Figure 4.2: Recovery Rate of MNV-1 by Vegetable Buffer Homogenization Method

#### 4.3.2 Effect of Presence of Water During UV Irradiation on Inactivation of MNV-1 on Blueberries

The measured UV intensity was  $10 \text{ mW/cm}^2$  for both dry and water-assisted UV treatments. The effect of the presence of water on UV inactivation of MNV-1 on skin-inoculated blueberries was evaluated (Table 4.1). The initial viral population (log PFU/sample) was  $7.00 \pm 0.28$ . For dry UV treatment, although insignificant ( $P > 0.05$ ), the inactivation of MNV-1 increased as the UV treatment time increased. For water-assisted UV and 10-ppm chlorine treatment, MNV-1 was reduced to undetectable levels after a 2-min treatment. The 2-min and 5-min water-assisted UV treatments were significantly more effective than the 1-min water-assisted UV

treatment ( $P < 0.05$ ). Water wash alone for 5 min was not effective, achieving only a 1.73-log reduction of MNV-1. In general, the water-assisted UV treatment was more effective in inactivating MNV-1 skin-inoculated onto blueberries than the dry UV treatment. For both 2-min and 5-min treatments, the water-assisted UV treatments were significantly more effective than the corresponding dry UV treatments ( $P < 0.05$ ). The average difference in the log reductions between the water-assisted and dry UV treatments was greater than 1.3. Water-assisted UV treatments were comparable to 10-ppm chlorine wash for all treatment times. Wash water samples taken from the 1-min water-assisted UV treatments and 1-min chlorine treatments were tested for MNV-1 and no virus was found (below the detection limit of 1 PFU/ml).

Table 4.1: Comparison of Dry UV and Water-assisted UV Treatments of Blueberries Skin-inoculated With MNV-1

	Dry UV	Water-assisted UV	10 ppm chlorine	Water
1 min	2.43±0.32 <sup>aA</sup>	3.23±0.61 <sup>aA</sup>	3.44±0.18 <sup>aA</sup>	ND <sup>**</sup>
2 min	2.48±0.56 <sup>aA</sup>	>4.32±0.00 <sup>bB</sup> (3/3) <sup>*</sup>	>4.55±0.00 <sup>cB</sup> (3/3) <sup>*</sup>	ND <sup>**</sup>
5 min	3.04±0.23 <sup>aA</sup>	>4.36±0.06 <sup>bB</sup> (2/3) <sup>*</sup>	>4.31±0.00 <sup>bC</sup> (3/3) <sup>*</sup>	1.73±0.06 <sup>c</sup>

Data represent mean viral reduction (log PFU/sample) of three replicates ± one standard deviation. Initial viral population (log PFU/sample) was  $7.00 \pm 0.28$ . Data in the same row with different lowercase letters are significantly different ( $P < 0.05$ ). Data in the same column with different uppercase letters are significantly different ( $P < 0.05$ ). The detection limit was 2.75 log PFU/sample.

\*Content in parenthesis represent number of replicates below detection limit/total number of replicates. For replicates below detection limit, the detection limit value of 2.75 log PFU/sample was used for calculation.

\*\*ND: Not done.

### **4.3.3 Effect of Blueberry Juice on the Efficiency of Small-scale Water-assisted UV Inactivation of MNV-1 on Blueberries**

Blueberry juice was added to washing solutions to evaluate its impact on UV inactivation of MNV-1. Based on results obtained from Section 4.3.2, 2-min treatments were used in this part of the study. The initial viral population (log PFU/sample) was  $6.76 \pm 0.29$ . For 2-min water-assisted UV treatment, a viral reduction (log PFU/sample) of  $2.88 \pm 0.47$  was achieved. Chlorine (10 ppm) wash for 2 min resulted in a log reduction of  $2.05 \pm 0.02$ , which is significantly lower than that obtained by 2-min water-assisted UV treatment ( $P < 0.05$ ). In order to investigate if combining UV irradiation and chlorine wash could improve the decontamination efficacy, blueberries were washed with 10-ppm chlorine during UV treatment. Results showed that 2-min UV+Chlorine treatment achieved  $\geq 3.51$ -log reductions of MNV-1 (the detection limit was 2.75 log PFU/sample). When the actual data of 3.51 log was used for statistical analysis, the UV+Chlorine treatment was not significantly different from water-assisted UV treatment alone ( $P > 0.05$ ). All water samples were negative of MNV-1 ( $< 1$  PFU/ml).

#### 4.3.4 Effect of Blueberry Juice and Blueberry Crush on the Efficiency of Large-scale Water-assisted UV Inactivation of MNV-1 on Blueberries



Figure 4.3: Washing Solution in Quartz Beaker. Left to right: DI water, DI water with 2% (v/v) blueberry juice, DI water with 5% [w/w] crush.

Table 4.2: Water Quality Comparison

Wash water	Turbidity (NTU)	COD (mg/L)
DI water plus 2% juice	20.3±0.6	2150 ± 46
DI water plus 5% crush*	5.9±0.5*	97±43*

\*Blueberry debris was not suspended homogeneously in wash water. Water sample was taken randomly from the washing solution.

The effect of presence of blueberry juice and berry crush was investigated. Before experiments, wash water quality was tested. Figure 4.3 shows the appearances of the different conditions of wash water. When 2% blueberry juice was added to washing solutions, the color of the wash water turned to dark red. When 5% blueberry crush was added, the blueberry debris floated in the washing solution. Water quality

parameters are provided in Table 4.2. The COD value in DI water containing 2% blueberry juice was about 20 times the value in DI water containing 5% crush. MNV-1 inactivation results are shown in Table 4.3. Initial viral populations (log PFU/sample) for skin and calyx-inoculated blueberries were  $6.79 \pm 0.23$  and  $6.71 \pm 0.18$ , respectively. In general, MNV-1 was more easily inactivated on skin-inoculated blueberries than calyx-inoculated blueberries. For skin-inoculation, MNV-1 inactivation was most difficult when wash water contained 2% blueberry juice. The inactivation results were comparable between plain wash water and wash water containing 5% crushed berries; however, for calyx-inoculation, the differences in log reductions of MNV-1 among three different conditions of wash water were not significant ( $P > 0.05$ ). In addition, there was no significant difference in log reductions of MNV-1 among three treatments ( $P > 0.05$ ). Within the same inoculation method (skin or calyx) and water condition (DI water, 2% juice, or 5% crush), 2-min water-assisted UV treatment was comparable with 10 ppm chlorinated water wash or the combined treatment of UV+10 ppm chlorine wash except for one instance where skin-inoculated blueberries were treated in wash water containing 2% blueberry juice. In that instance, the combined treatment was significantly more effective than the single water-assisted UV and chlorine wash treatments. Viral counts in wash water were below the detection limit ( $< 1$  PFU/ml) for all the treatments except for the water-assisted UV treatments of skin and calyx-inoculated blueberries in wash water containing 2% juice. The viral counts in the wash water for those two treatments were  $< 3.33 \pm 2.52$  and  $< 1.33 \pm 0.58$  PFU/ml for skin and calyx-inoculated berries, respectively. One out of three replicates for skin-inoculated wash water sample was negative of MNV-1 ( $< 1$  PFU/ml) and two out of three replicates for calyx-inoculated

wash water sample reached detection limit ( $< 1$  PFU/ml). For replicates where virus was undetectable, the detection limit value (1 PFU/ml) was used for calculation.

Table 4.3: MNV-1 Inactivation by UV and/or Chlorine Wash With/out the Presence of 2% Blueberry Juice and 5% Blueberry Crush

Treatment	Log reduction of MNV-1 on blueberries (log PFU/sample)							
	Skin-inoculation				Calyx-inoculation			
	DI water	2% juice	5% crush		DI water	2% juice	5% juice	5% crush
Water-assisted UV 2 min	>3.20±0.00 <sup>A</sup> (3/3) <sup>*</sup>	1.63±0.22 <sup>A**</sup>	>3.00±0.07 <sup>A</sup> (2/3) <sup>*</sup>		1.81±0.58 <sup>A</sup>	1.25±0.18 <sup>A**</sup>		1.90±0.51 <sup>A</sup>
10 ppm chlorine 2 min	>3.20±0.00 <sup>A</sup> (3/3) <sup>*</sup>	1.84±0.29 <sup>A</sup>	>2.93±0.11 <sup>A</sup> (2/3) <sup>*</sup>		1.42±0.20 <sup>A</sup>	1.36±0.15 <sup>A</sup>		1.51±0.03 <sup>A</sup>
Water-assisted UV+10 ppm chlorine 2 min	ND <sup>***</sup>	2.88±0.22 <sup>B</sup>	>3.00±0.07 <sup>A</sup> (3/3) <sup>*</sup>		1.94±0.24 <sup>A</sup>	1.44±0.32 <sup>A</sup>		1.88±0.16 <sup>A</sup>

Data represent mean viral reductions (log PFU/sample) of three replicates ± one standard deviation. Initial virus populations (log PFU/sample) were 6.79 ± 0.23 and 6.71 ± 0.18 for skin and calyx inoculated blueberries, respectively. Data in the same column with different uppercase letters are significantly different (P < 0.05). The detection limit was 3.64 log PFU/sample.

\* Content in parenthesis represent number of replicates below detection limit/total number of replicates. For replicates below detection limit, the detection limit value of 3.64 log PFU/sample was used for calculation.

\*\* Detectable viral survival in wash water.

\*\*\*ND: Not done.

#### 4.4 Discussion

Results from this study demonstrated that water-assisted UV treatment was more efficient than dry UV treatment for skin-inoculated blueberries. During dry UV treatment, the inoculation site was constantly facing the UV lamps and was the only surface to receive UV energy; however, for water-assisted UV treatment, the surface being illuminated with UV was always changing, as the berries were randomly moving and rotating in the agitated water. Therefore, the inoculation sites on the blueberries received much less UV energy in the water-assisted treatment than in the dry UV treatment; nevertheless, the water-assisted UV treatment was generally more effective in inactivating MNV-1 skin-inoculated onto blueberries than the dry UV treatment. The agitated water in the water-assisted UV treatment probably helped remove the virus particles from the blueberry surface into water, making the virus more susceptible to UV light as it has a better penetration ability in clear water than in organic matter (Guerrero-Beltran & Barbosa-Canovas, 2004). No MNV-1 was detected in wash water using UV, but water wash alone resulted in high level of MNV-1 in spent wash water (data not shown). This suggested that the virus was indeed removed from the berry surface into the wash water and was killed by UV.

Inactivation results also showed that longer UV treatment generally achieved more viral reduction for both dry and water-assisted UV treatments (Table 4.1). For water-assisted UV treatments, 2-min water-assisted UV was significantly more effective than 1-min water-assisted UV. UV dose is the product of UV intensity and time of exposure, thus longer treatment time can cause more damage to virus particles. This finding is in agreement with results reported by other studies. Kim and Hung (2012) observed decreased surviving populations of *E. coli* O157:H7 on blueberries

with increased UV treatment times. Geveke (2008) found that in liquid egg white, log reduction of *E. coli* K12 and UV treatment time followed first-order kinetics where microbial reduction increased as treatment time increased.

In commercial fresh produce operations, double or triple sanitizer washes are sometimes used for reduction of potential microbial contamination and to prevent cross-contamination (Nou et al., 2011). Organic matters could be released into the washing solution from wounded tissues of fresh produce such as fresh-cut lettuce (Luo, 2007). The Same issue could exist with wash water for blueberry sanitation. Thus, the effect of blueberry juice in wash water was investigated. We used 2% blueberry juice in washing solutions as a worst case scenario for washing blueberries. Results showed that for small-scale experiments (~5 g/sample), MNV-1 reduction on skin-inoculated blueberries was about 2 log lower for all treatments when 2% blueberry juice existed in washing solution, compared with when pure DI water was used. This outcome was expected as UV has a low penetration depth in the presence of organic matter (Guerrero-Beltran & Barbosa-Canovas, 2004). The COD value of wash water containing 2% juice was 2150 mg/L, which was even higher than that of the spent plant lettuce wash water (1858 mg/L) used by Nou et al. (2011). Blueberries sold freshly in grocery stores grow on bushes that grow to about 6 feet tall and are mainly picked by hand (U.S. Highbush Blueberry Council, 2014). In contrast, lettuce is grown on the ground, thus is easier to be associated with soil and other organic matter from the ground. It is reasonable to assume that spent plant blueberry wash water is cleaner than that of lettuce wash water.

The UV intensity measured at the bottom of the quartz beaker was lower than 1 mW/cm<sup>2</sup> when washing water containing 2% blueberry juice was used. For chlorine

treatments, free chlorine was continuously reacting with organic matter in the water. The final chlorine concentration after the 2-min wash was about 6 ppm for chlorine wash alone. Thus a considerable amount of chlorine was depleted during washing treatment. Results also showed that the 2-min water-assisted UV treatment was significantly ( $P < 0.05$ ) more effective than 10-ppm chlorine wash. Combining UV irradiation and chlorine wash achieved 0.63 log more MNV-1 reduction than water-assisted UV treatment alone, but the difference was not significant.

Water-assisted UV treatment was effective on MNV-1 inactivation on skin-inoculated blueberries when DI water or DI water (2% juice) was used, thus we further investigated its efficiency on larger scale situations. Blueberries were skin- or calyx-inoculated and added to 2% blueberry juice or water containing 5% blueberry crush. Generally, skin-inoculated blueberries had higher viral reduction than the calyx-inoculated ones (Table 4.3). This result is probably due to the different surface structures of blueberry skin and calyx and the method of inoculation. It is likely that MNV-1 attached better to the rougher surfaces of the calyx than to the smooth skin. Moreover, the rougher surface structures probably allowed more shadowing of the virus inside the surface irregularities or crevices during treatments. It is known that UV has a very limited penetration depth in opaque commodities and is only capable of targeting superficial microorganisms on food surfaces. Therefore, viral particles hiding in the sub-surface of the calyx can be protected from UV. Similar findings were also reported by other researchers. Kim and Hung (2012) observed a higher survival of *E. coli* O157:H7 on calyx-inoculated blueberries than skin-inoculated ones after UV treatment. Du et al. (2002) found that chlorine dioxide gas was more effective at inactivating *Listeria monocytogenes* attached to the skin of apples than those attached

to the calyx or stem cavity. Raspberries and strawberries exhibited similar phenomena (Sy, McWatters, & Beuchat, 2005). Results also indicated that MNV-1 was most easily inactivated in DI water and most difficult when 2% juice was present. The presence of 2% blueberry juice served as an extreme situation for wash water quality and MNV-1 was detectable in spent wash water after water-assisted UV treatment alone when 2% juice was added. UV inactivation with washing solution containing 5% blueberry crush had similar inactivation efficiency as that with pure DI water. The UV intensity measured at the bottom of the quartz beaker was around 2 mW/cm<sup>2</sup> when 5% crush was added. It is possible that only the debris floating near the inoculated blueberries had an impact on the inactivation efficiency, which was minimal. The overall COD value of the 5% crush wash water was assumed to be low because adding 5% crush did not change the free chlorine content. Thus, it was expected that chlorine wash alone would have similar inactivation efficacy in water with 5% crush and in pure DI water. The results also demonstrated that water-assisted UV treatment had similar inactivating efficiency as chlorine wash for all treatments. To improve the inactivation efficiency, UV irradiation was combined with 10-ppm chlorine wash. No UV+Chlorine treatment was done for skin-inoculated blueberries when DI water was used, since water-assisted UV treatment and chlorine wash alone were both effective. For all other treatments, UV+Chlorine had better or similar inactivation efficiency as water-assisted UV or chlorine wash alone. Moreover, no virus was detectable when UV irradiation was combined with chlorine wash.

Based on results obtained above, MNV-1 skin-inoculated onto blueberries was generally more easily killed in small-scale experiments compared with large scale ones. It is possible that the non-inoculated blueberries provided shadowing for viral

particles and protected them from UV light during treatment. For chlorine wash alone, the difference was smaller between small and large-scale experiments.

#### **4.5 Conclusion**

Water-assisted UV treatment was generally more effective in inactivating MNV-1 skin-inoculated onto blueberries than the dry UV treatment. The water-assisted UV treatments were more effective or as effective as the 10-ppm chlorine washing. MNV-1 skin-inoculated onto blueberries was easier to be inactivated than that calyx-inoculated onto the berries. Presence of 2% blueberry juice in wash water provided protection for MNV-1 from both water-assisted UV and chlorine wash treatments. Overall, the water-assisted UV treatments were generally more effective or as effective as the chlorine washing, indicating water-assisted UV treatment can be an alternative to chlorine washing for blueberries and potentially for other fresh produce. In comparison to chlorine wash, the major benefits of using UV is that it leaves no chemical residue on the produce and does not create chemical disposal issues. The water-assisted UV system developed in this study would be also very beneficial for organic producers. In an industry setting, the water-assisted UV system can be easily setup since UV lamps could be placed on top of a washing tank in a closed chamber. Unlike chlorine washing, the water-assisted UV treatment does not require additional rinse step to remove chlorine residual.

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## REFERENCES

- Allende, A., McEvoy, J. L., Luo, Y., Artes, F., & Wang, C. Y. (2006). Effectiveness of two-sided UV-C treatments in inhibiting natural microflora and extending the shelf-life of minimally processed 'Red oak leaf' lettuce. *Food Microbiology*, 23(3), 241-249.
- Bae, J., & Schwab, K. J. (2007). Evaluation of Murine Norovirus, Feline Calicivirus, Poliovirus, and MS2 as Surrogates for Human Norovirus in a Model of Viral Persistence in Surface Water and Groundwater. *Applied and Environmental Microbiology*, 74 (2): 477-484.
- Beuchat, L. R. (1997). Comparison of chemical treatments to kill *Salmonella* on alfalfa seeds destined for sprout production. *International Journal of Food Microbiology*, 34, 329-333
- Calder, L., Simmons, G., Thornley, C., Taylor, P., Pritchard, K., Greening, G., & Bishop, J. (2003). An outbreak of hepatitis A associated with consumption of raw blueberries. *Epidemiology & Infection* 131, 745-751.
- Centers for Disease Control and Prevention (CDC). (2012a). *Foodborne Outbreak Online Database (FOOD)*. Retrieved November 10, 2014, from <http://wwwn.cdc.gov/foodborneoutbreaks/Default.aspx>
- Centers for Disease Control and Prevention. (2012b). *Water treatment*. Retrieved October 25, 2014, from [http://www.cdc.gov/healthywater/drinking/public/water\\_treatment.html](http://www.cdc.gov/healthywater/drinking/public/water_treatment.html).
- Centers for Disease Control and Prevention (CDC). (2013). *Norovirus*. Retrieved November 12, 2014, from <http://www.cdc.gov/norovirus/index.html>
- City of Boulder Colorado. (2012). *City using new ultraviolet light technology to safely treat Boulder's wastewater before sending it downstream*. Retrieved August 14, 2014, from <https://bouldercolorado.gov/newsroom/dec-7-2012-city-using-new-ultraviolet-light-technology-to-safely-treat-boulders-wastewater-before-sending-it-downstream>.
- Clancy, S. (2008). DNA damage & repair: mechanisms for maintaining DNA integrity. *Nature Education* 1(1), 103.

- Duizer, E., Schwab, K. J., Neill, F. H., Atmar, R. L., Koopmans, M. P., & Estes, M. K. (2004). Laboratory efforts to cultivate noroviruses. *The Journal of General Virology*, 85, 79-87.
- Du, J., Han, Y., & Linton, R. H. (2002). Inactivation by chlorine dioxide gas (ClO<sub>2</sub>) of *Listeria monocytogenes* spotted onto different apple surfaces. *Food Microbiology*, 19, 481-490.
- Environmental Protection Agency (EPA). (2000). *Wastewater technology fact sheet: dechlorination*. Retrieved October 25, 2014, from [http://water.epa.gov/scitech/wastetech/upload/2002\\_06\\_28\\_mtb\\_dechlorination.pdf](http://water.epa.gov/scitech/wastetech/upload/2002_06_28_mtb_dechlorination.pdf).
- Falguera, V., Pagán, J., Garza, S., Garvín, A., & Ibarz, A. (2011). Ultraviolet processing of liquid food: A review Part 2: Effects on microorganisms and on food components and properties. *Food Research International*, 44, 1580-1588.
- Fino, V. R., & Kniel, K. E. (2008). UV light inactivation of hepatitis A virus, Aichi virus, and feline calicivirus on strawberries, green onions, and lettuce. *Journal of Food Protection*, 71(5), 908-13.
- Food and Agriculture Organization of the United Nations, & World Health Organization. (2008). *Benefits and risks of the use of chlorine-containing disinfectants in food production and food processing. (Joint FAO/WHO Expert Meeting)*. Geneva, Switzerland: WHO Document Production Services.
- Geveke, D. J. (2005). UV Inactivation of Bacteria in Apple Cider. *Journal of Food Protection*, 68, 1739-42.
- Geveke, D. J. (2008). UV inactivation of *E. coli* in liquid egg white. *Food and Bioprocess Technology*, 1, 201.
- Gil, M. I., Selma, M. V., Lopez-Galvez, F., & Allende, A. (2009). Fresh-cut product sanitation and wash water disinfection: problems and solutions. *International Journal of Food Microbiology*, 134(1-2), 37-45.
- Gonzalez, R. J., Luo, Y., Ruiz-Cruz, S., & McEvoy, J. L. (2004). Efficacy of sanitizers to inactivate *Escherichia coli* O157:H7 on fresh-cut carrot shreds under simulated process water conditions. *Journal of Food Protection*, 67(11), 2375-80.
- Guerrero-Beltran, J. A., & Barbosa-Canovas, G. V. (2004). Advantages and limitations on processing foods by UV light. *Food Science and Technology International*, 10, 137-147.

- Hirneisen, K. A., Kniel, K. E. (2013). Comparing human norovirus surrogates: murine norovirus and Tulane virus. *Journal of Food Protection*, 76(1), 139-43.
- Harris, L.J., Farber, J.N., Beuchat, L.R., Parish, M.E., Suslow, T.V., Garrett, E.H., & Busta, F.F. (2003). Outbreaks Associated with Fresh Produce: Incidence, Growth, and Survival of Pathogens in Fresh and Fresh-Cut Produce. *Comprehensive Reviews in Food Science and Food Safety*, 2, 78-141.
- Hijnen, W. A. M., Beerendonk, E. F., & Medema, G. J., (2006). Inactivation credit of UV radiation for viruses, bacteria and protozoan (oo)cysts in water: A review. *Water Research*, 40, 3-22.
- Kingsley, D. H., Hoover, D. G., Papafragkou, E., Richards, G. P. (2002). Inactivation of hepatitis A virus and a calicivirus by high hydrostatic pressure. *Journal of Food Protection*, 65, 1605-1609.
- Kemp, G. K., Schneider, K. R. (2000). Validation of Thiosulfate for Neutralization of Acidified Sodium Chlorite in Microbiological Testing. *Poultry Science*, 79(12), 1857-60.
- Kim, C., & Hung, Y. (2012). Inactivation of *E. coli* O157:H7 on blueberries by electrolyzed water, ultraviolet light, and ozone. *Journal of Food Science*, 77(4), 206 - 211.
- Li, X., Ye, M., Neetoo, H., Golovan, S., Chen, H. (2013). Pressure inactivation of Tulane virus, a candidate surrogate for human norovirus and its potential application in food industry. *International Journal of Food Microbiology*, 162, 37-42.
- Luo, Y. (2007). Wash operation affect water quality and packaged fresh-cut romaine lettuce quality and microbial growth. *Horticultural Science*, 42,1413–19.
- Mäde, D., Trübner, K., Neubert, E., Höhne, M., & Johne, R. (2013). Detection and typing of norovirus from frozen strawberries involved in a large-scale gastroenteritis outbreak in Germany. *Food and environmental virology*, 5, 162-168.
- Miller, B. D., Rigdon, C. E., Robinson, T. J., Hedberg, C., & Smith, K. E. (2013). Use of global trade item numbers in the investigation of a *Salmonella* Newport outbreak associated with blueberries in Minnesota, 2010. *Journal of Food Protection*, 76, 762-769.

- Nou, X., Luo, Y., Hollar, L., Yang, Y., Feng, H., Millner, P., & Shelton, D. (2011). Chlorine stabilizer T0128 enhances efficacy of chlorine against cross-contamination by *E. coli* O157:H7 and *Salmonella* in fresh-cut lettuce processing. *Journal of Food Science*, 76(3), 218-24.
- New York State Department of Health. (2004). *The facts about chlorine*. Retrieved October 25, 2014, from [http://www.health.ny.gov/environmental/emergency/chemical\\_terrorism/chlorine\\_general.htm](http://www.health.ny.gov/environmental/emergency/chemical_terrorism/chlorine_general.htm).
- Oregon Health Authority. (2011). *Lab tests confirm source of E. coli O157 from deer droppings in strawberry fields in NW Oregon*. Retrieved October 25, 2014 from <http://www.oregon.gov>
- Pala, Ç. U., & Toklucu, A. K. (2013). Effects of UV-C light processing on some quality characteristics of grape juices. *Food and Bioprocess Technology*, 6(3), 719-725.
- Park, G. W., Linden, K. G., Sobsey, M. D. (2011). Inactivation of murine norovirus, feline calicivirus and echovirus 12 as surrogates for human norovirus (NoV) and coliphage (F+) MS2 by ultraviolet light (254 nm) and the effect of cell association on UV inactivation. *Letters in Applied Microbiology*, 52, 162-167.
- Rodas, A. G., Bourquin, L., Salazar, C. G., Gomez, A.V., & Wise, J.C. (2009). *Good Agricultural Practices for Food Safety in Blueberry Production: Basic Principles*. Retrieved October 25, 2014, from [http://www.gaps.msue.msu.edu/blue\\_manual.pdf](http://www.gaps.msue.msu.edu/blue_manual.pdf)
- Roy J. H., Lundy, S., & Kalicki, B. (2009). Blueberries "their role in health". *Pennington Nutrition Series*, No. 2.
- Shama, G. (1999). *Ultraviolet light*. In: R.K. Robinson, C. Batt and P. Patel (eds), *Encyclopedia of Food Microbiology-3*. London: Academic Press, 2208-2214.
- Suslow, T. V. (2001). *Water disinfection: a practical approach to calculating does values for preharvest and postharvest applications*. Retrieved December 11, 2014, from [anrcatalog.ucdavis.edu/pdf/7256.pdf](http://anrcatalog.ucdavis.edu/pdf/7256.pdf).
- Sy, K. V., McWatters, K. H., & Beuchat, L. R. (2005). Efficacy of gaseous chlorine dioxide as a sanitizer for killing *Salmonella*, yeasts, and molds on blueberries, strawberries, and raspberries. *Journal of Food Protection*, 68, 6, 1165-75.

The U.S. Food and Drug Administration (FDA). (2001). *FDA survey of imported fresh produce FY 1999 field assignment*. Retrieved October 25, 2014, from <http://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/ProducePlantProducts/ucm118891.htm>

The U.S. Food and Drug Administration (FDA). (2013). *Irradiation in the production, processing and handling of food. Code of Federal Regulations Title 21, 179.39*. Retrieved October 25, 2014 , from <http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfCFR/CFRSearch.cfm?fr=179.39>

U.S. Highbush Blueberry Council. (2014). *How Blueberries Grow*. Retrieved December 23, 2014, from <http://www.blueberrycouncil.org/growing-blueberries/how-blueberries-grow/>

## Chapter 5

### FUTURE RESEARCH

A novel set-up using water-assisted UV processing was developed and tested in this study. It demonstrated comparable effectiveness in inactivating *Escherichia coli* O157:H7, *Salmonella* spp., and murine norovirus on fresh blueberries as chlorine wash without noticeable damage to their appearance. However, no sensory test has been done on the treated blueberries. Thus, before the treatments can be scaled up, a sensory evaluation would be necessary with regard to the color, texture, flavor, and aroma of the treated samples. A shelf life study of the treated samples would also need to be done. For MNV-1 inactivation, dip-inoculation could be added as an addition of the inoculation method to simulate contamination during washing. Wash water quality plays a very important role in the inactivating efficiency of UV irradiation and chlorine wash. Thus, from a practical standpoint, it would be useful to know the quality of factory spent wash water for blueberry processing. The time that blueberries require for transportation, distribution and finally reaches our lab is unknown, which could result in discrepancy regarding the freshness of the samples. Thus it would be ideal to have freshly harvested blueberries for this project.