INACTIVATION OF BACTERIAL AND VIRAL PATHOGENS

IN BERRY PRODUCTS BY HIGH PRESSURE AND PULSED LIGHT

by

Yaoxin Huang

A dissertation submitted to the Faculty of the University of Delaware in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Animal and Food Sciences

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ABSTRACT

Foodborne pathogens such as *Salmonella*, *Escherichia coli* O157:H7 and human norovirus are major causative agents of foodborne illnesses in the US and have been frequently associated with foodborne outbreaks of berry products in recent years. Since current industrial practices are not designed to reduce potential pathogen contamination, there has been a growing interest in the application of nonthermal processing technologies including high pressure processing (HPP) and pulsed light (PL) as commercial alternatives to traditional thermal processing methods in food industry. The objectives of the present study were 1) to evaluate the potential of using HHP alone or in combination with frozen storage to inactivate bacterial pathogens and 2) to evaluate and develop a feasible PL treatment strategy for decontamination of berries from both bacterial and viral pathogens.

In the first project, fresh strawberry puree was inoculated with high (~6 log CFU/g) and low (~3 log CFU/g) levels of *E. coli* O157:H7 or *Salmonella* and stored at -18 °C for 12 weeks to determine bacterial survival. Both pathogens were able to survive for at least 4 and 12 weeks in the case of low and high inocula, respectively. Pressure treatment at 450 MPa for 2 min at 21 °C was able to eliminate (< 1 CFU/5g) both pathogens in strawberry puree. Frozen storage at -18 °C after pressure treatment substantially enhanced the inactivation of both pathogens as only 250 − 300 MPa was needed to eliminate both pathogens. Natural yeasts and molds in strawberry puree were effectively reduced by pressure treatment at 300 MPa for 2 min at 21 °C. HPP did not cause any adverse impact on physical properties such as color, soluble solids content, pH and viscosity of strawberry puree.

In the second project, blueberries inoculated with *E. coli* O157:H7 or *Salmonella* were first treated with PL directly (dry PL) for 5 – 60 s. Dry PL treatments adversely changed the appearance of the blueberries and a maximum temperature of 64.8 °C was recorded on the blueberry surface. Therefore, a prototype water-assisted PL (WPL) treatment was developed, in which berries were immersed in agitated water during the PL treatment. This new strategy not only resolved two main challenges in the application of PL, including sample heating and shadowing effects, in fresh produce, but was more effective than chlorine washing in reducing bacterial pathogens on blueberries. After a 60-s WPL treatment, the populations of *E. coli* O157:H7 inoculated on calyx and skin of blueberries were reduced by 3.0 and > 5.8 log CFU/g, respectively and *Salmonella* was reduced by 3.6 and > 5.9 log CFU/g, respectively. No change in the visual appearance of blueberries was observed after WPL treatments and sample heating was substantially reduced.

In the third project, the effectiveness of the WPL treatment was assessed for the inactivation of *E. coli* O157:H7, *Salmonella* and murine norovirus (MNV), a human norovirus surrogate, on strawberries and raspberries. The combinations of WPL treatment with 1% hydrogen peroxide (H_2O_2) and 100 ppm sodium dodecyl sulfate (SDS) were also evaluated. Significantly higher ($P < 0.05$) reductions of *E. coli* O157:H7 were obtained using 60-s WPL treatment than washing with 10 ppm chlorine. Compared with washing with chlorine, SDS and H_2O_2, the combination of WPL treatment with 1% H_2O_2 for 60 s significantly reduced ($P < 0.05$) *E. coli* O157:H7 on strawberries and raspberries by 3.3- and 5.3-log units, respectively. For decontamination of MNV, a 60-s WPL treatment reduced the viral titers on

strawberries and raspberries by 1.8- and 3.6-log units, respectively. However, no more reduction was obtained using the combination of WPL and 1% H_2O_2.

In the final study, a small scaled-up WPL system was developed. The effects of organic load, water turbidity, berry type and PL energy output on the inactivation of *Salmonella* were thoroughly investigated. The combination of WPL and 1% H_2O_2 (WPL-H_2O_2), as an advanced oxidation process, was the most effective treatment since it reduced *Salmonella* on raspberries and blueberries by 4.0 and > 5.6 log CFU/g, respectively, in clear water. When high organic load and SiO_2, as a soil simulator, were added into wash water, the free chlorine level in chlorinated water decreased significantly ($P < 0.05$); however, no significant difference ($P > 0.05$) was observed for the decontamination efficacy of 1-min WPL-H_2O_2 treatment. No significant difference ($P > 0.05$) was observed between treatments at high and low PL fluences. Even in the presence of high organic load and water turbidity, no viable bacterial cells were recovered from the wash water, which showed that WPL-H_2O_2 could effectively prevent the risk of cross-contamination during treatment.

Taken together, our study showed that HPP is a promising alternative intervention method that can potentially eliminate bacterial pathogens in strawberry puree with minimal impact on the quality of puree. Water-assisted PL treatment, with minimal impact on the quality of berries, provides a new strategy to use PL as a practical chlorine alternative in the fresh produce industry.

Chapter 1

INTRODUCTION

It is estimated that 48 million Americans suffer from foodborne illnesses every year (Scallan et al., 2011). Foodborne pathogens such as *Salmonella, Escherichia coli* O157:H7 and human norovirus (HuNoV) are major causative agents and are frequently associated with foodborne outbreaks of fresh produce. During 1990 – 2005, HuNoV was ranked as the top causative agent for fresh produce outbreaks, responsible for 40% of all the outbreaks, followed by *Salmonella* spp. (18%), *Escherichia coli* (8%), *Clostridium* spp. (6%) and hepatitis A virus (4%) (Doyle and Erickson, 2008). The estimated annual economic losses due to HuNoV, *Salmonella* and *E. coli* O157:H7 illnesses were about 2.6 billion, 3.7 billion and 271 million dollars, respectively (USDA-ERS, 2014a).

Berry fruits, including strawberries, raspberries and blueberries are popular fruit crops for their unique flavors, textures and health benefits. In the last decade, berry fruits have gained a significantly larger percentage in people's diet although the total amount of fruits that Americans are consuming is decreasing (USDA-ERS, 2014b). Unfortunately, berry fruits are susceptible to contamination with pathogenic bacteria and viruses since they are usually grown in open fields. Berries can be contaminated by pathogens in the field through soil, feces, irrigation water, insects, animals, or during harvesting by human contacts, harvesting equipment, and transport containers (Beuchat, 2002). A number of foodborne outbreaks due to the consumption of fresh and frozen berry products have been reported. In 2011, an outbreak of *E. coli*

O157:H7 in the state of Oregon was traced to contaminated fresh strawberries, which caused 15 cases of illnesses and two deaths (Laidler et al., 2013). In 2012, norovirus-contaminated frozen strawberries caused the largest foodborne outbreak in Germany, which affected about 11,000 people (Mäde et al., 2013). In spite of the facts, current production practices are not designed to reduce potential pathogen contamination. Therefore, in recent years, there has been a sustained effort to develop new nonthermal processing technologies such as high pressure processing (HPP) and pulsed light (PL) as commercial alternatives to traditional thermal processing methods.

The primary advantage of these nonthermal processing technologies is that they can inactivate pathogenic and spoilage microorganisms without the use of high temperatures (≥ 70 °C) that can dramatically alter the sensorial and nutritional qualities of fresh produce. HPP has been showed to have little detrimental effect on vitamins and other nutrient components with good retention of product flavor quality (Patterson, 2005) and can be used to inactivate undesirable spoilage enzymes in some cases (San Martin et al., 2002). Pulsed light (PL) technology has been shown to be highly effective for rapid inactivation of bacteria, fungi and viruses *in vitro* (Elmnasser et al., 2007; Oms-Oliu et al., 2008). PL has also been successfully used to decontaminate both solid and liquid foods, food contact materials and food packaging materials (Bialka and Demirci, 2008; McDonald et al., 2000; Sauer and Moraru, 2009).

This dissertation aims to evaluate the effectiveness of HPP and PL to inactivate *E. coli* O157:H7, *Salmonella* and HuNoV in fresh berries (strawberries, raspberries and blueberries) and strawberry puree. Efforts have been made to modify and optimize the treatment system and parameters to provide a feasible processing method for berry

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products. The impact of those treatments on the main quality parameters of berry products was evaluated. Therefore, results presented in this dissertation can provide new strategies using nonthermal processing technologies for the processing of berry products. The survival and inactivation of bacterial pathogens in strawberry puree using HPP was assessed (Chapter 3). The inactivation of bacterial pathogens on fresh blueberry surfaces was evaluated by PL treatment and a novel water-assisted PL (WPL) prototype system was developed (Chapter 4). The decontamination of strawberries and raspberries from both bacterial and viral pathogens was evaluated using the combination of PL with sodium dodecyl sulfate and hydrogen peroxide (Chapter 5). A small scaled up WPL system was then designed, fabricated, and tested for its effectiveness in reducing *Salmonella* on blueberries and raspberries. The effects of PL fluence, organic load, and water turbidity on the WPL treatments and the system's ability to prevent cross-contamination were thoroughly evaluated (Chapter 6).

REFERENCES

Beuchat, L.R., 2002. Ecological factors influencing survival and growth of human pathogens on raw fruits and vegetables. Microbes Infect. 4, 413–423. doi:10.1016/S1286-4579(02)01555-1

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. J. Food Sci. 73, M201–M207. doi:10.1111/j.1750-3841.2008.00743.x

Doyle, M.P., Erickson, M.C., 2008. Summer meeting 2007 - the problems with fresh produce: an overview. J. Appl. Microbiol. 105, 317–30. doi:10.1111/j.1365-2672.2008.03746.x

Elmnasser, N., Guillou, S., Leroi, F., Orange, N., Bakhrouf, a, Federighi, M., 2007. Pulsed-light system as a novel food decontamination technology: a review. Can. J. Microbiol. 53, 813–21. doi:10.1139/W07-042

Laidler, M.R., Tourdjman, M., Buser, G.L., Hostetler, T., Repp, K.K., Leman, R., Samadpour, M., Keene, W.E., 2013. *Escherichia coli* O157:H7 infections associated with consumption of locally grown strawberries contaminated by deer. Clin. Infect. Dis. 57, 1129–34. doi:10.1093/cid/cit468

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 Environ. Virol. 5, 162–168. doi:10.1007/s12560-013-9118-0

McDonald, K.F., Curry, R.D., Clevenger, T.E., Brazos, B.J., Unklesbay, K., Eisenstark, A., Baker, S., Golden, J., Morgan, R., 2000. The Development of photosensitized pulsed and continuous ultraviolet decontamination techniques for surfaces and solutions. IEEE Trans. Plasma Sci. 28, 89–96.

Oms-Oliu, G., Martín-Belloso, O., Soliva-Fortuny, R., 2008. Pulsed light treatments for food preservation. A review. Food Bioprocess Technol. 3, 13–23. doi:10.1007/s11947-008-0147-x

Patterson, M.F., 2005. Microbiology of pressure-treated foods. J. Appl. Microbiol. 98, 1400–9. doi:10.1111/j.1365-2672.2005.02564.x

San Martin, M., Barbosa-Canovas, G., Swanson, B., 2002. Food processing by high hydrostatic pressure. Crit. Rev. Food Sci. Nutr. 42, 627–645.

Sauer, A., Moraru, C.I., 2009. Inactivation of *Escherichia coli* ATCC 25922 and *Escherichia coli* O157: H7 in apple juice and apple cider, using pulsed light treatment. J. Food Prot. 72, 937–944.

Scallan, E., Griffin, P.M., Angulo, F.J., Tauxe, R. V, Hoekstra, R.M., 2011. Foodborne illness acquired in the United States--unspecified agents. Emerg. Infect. Dis. 17, 16–22. doi:10.3201/eid1701.091101p2

USDA-ERS, 2014a. USDA Economic Research Service - Cost Estimates of Foodborne Illnesses. URL http://www.ers.usda.gov/data-products/cost-estimates-of-foodborne-illnesses.aspx#.VDW27r4mUfy (accessed 11.23.14).

USDA-ERS, 2014b. USDA Economic Research Service - Food Availability (Per Capita) Data System. URL http://www.ers.usda.gov/data-products/food-availability-%28per-capita%29-data-system.aspx (accessed 12.23.14).

Chapter 2

LITERATURE REVIEW

2.1 Characterization of Berry Crops

2.1.1 Strawberry

Strawberries belong to the family Rosaceae and the genus *Fragaria*. The strawberry fruit is widely appreciated for its characteristic aroma, sweetness, color, and texture. The cultivated strawberry (*Fragaria × ananassa*) is a hybrid between two octoploid wild species from the Americas, *F. chiloensis* and *F. virginiana*. Botanically, the fruit of strawberry is not a berry, but an aggregate accessory fruit, composed of fleshy red receptacle with achenes (fertilized ovules, often called 'seeds') arranged spirally on the outside of the receptacle (Strik, 2007). Strawberries, although widely grown, were considered to be a seasonal crop with a modest yield of small, soft berries best suited for production in temperate regions. However, 200 years of breeding has resulted in varieties adapted to environments ranging from areas with very cold winters, such as Norway and Finland, to subtropical, such as North Africa (Brennan et al., 2014).

2.1.2 Raspberry

Raspberries, also known as bramble fruits, belong to the genus *Rubus* and family Rosaceae. The raspberry is an aggregate fruit composed of bright red hairy drupelets which adhere to one another and contain seeds. Raspberry plants are biennial and grow on 3- to 6-ft erect stalks with many short thorns, which is why they are also called 'caneberries'. This fruit plant grows best in climates with cool summers and moderate winters. The raspberry has been traced back to 45 A.D., when it was called

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by its Greek name, Ida, after Mount Ida where the berry was gathered. The cultivation of red raspberries dates back to the 4th century A.D. (Deuel and Plotto, 2004). Today, most of the cultivated raspberries are derived from the wild red raspberries (*Rubus ideaus*) and black berries (*Rubus occidentalis*) (Sinha, 2006).

2.1.3 Blueberry

Blueberries are members of the Ericaceae family, within the genus *Vaccinium, subgenus Cyanococcus.* The blueberry fruit is a true berry, consisting of an ovary with up to 100 seeds. Ripe fruits are generally blue or purple in color with a surface wax layer called the 'bloom'. Blueberry is a perennial crop that can produce for more than 30 years (Girard and Sinha, 2006). Commercially, there are both wild and cultivated blueberry species. The highbush blueberry (*V. corymbosum* and *V. australe*) and rabbiteye blueberry (*V. ashei*) are cultivated while the lowbush blueberries (*V. angustifolium*) are only handled in a semi-domesticated way. Highbush blueberries are the types most extensively planted worldwide and can be further subdivided into northern, southern or intermediate types, depending on their winter hardiness (Brennan et al., 2014).

2.2 Berry and Berry Production

Reactive oxidant species (ROS) and free radicals are produced in an extensive range of physiological processes. When there is an imbalance between the insufficiency of antioxidant defense mechanism and intense production of ROS, it can lead to chronic disorders, including cancer, cardiovascular diseases, inflammation, diabetes, Parkinson´s and Alzheimer´s disease and other pathologies processes (Paredes-López et al., 2010). Berry fruits, such as strawberries, blueberries and

raspberries, are often referred to as natural functional foods since they are rich sources of antioxidant phenolics including flavonoids (anthocyanins, flavonols, flavones, flavanols, flavanones, and isoflavonoids), stilbenes, tannins, and phenolic acids (Seeram, 2008). Phenolic compounds have been reported to exhibit many biologically significant mechanisms of action, such as scavenging or detoxification of ROS, blocking ROS production, impacting cell cycle, suppression of tumors, modulation of signal transduction, apoptosis, detoxifying enzymes and metabolism. The World Health Organization (WHO) emphasizes the importance of antioxidant activity of phenolic components, especially from small colorful fruits, for prevention of the most important health problems such as cardiovascular diseases, diabetes, cancer, and obesity (WHO, 2002).

As a result, the production and consumption of berries in the United States has been steadily increasing in the past decades. From 2000 to 2010, the consumption of fresh blueberries experienced a five-fold increase and a four-fold increase was observed for the consumption of raspberries (USDA-ERS, 2014a). Responding to the greater consumption demands, from 1970 to 2009, the production of strawberries in the U.S. has increased from 496 million lbs. to 2801.3 million lbs. with total value approximately $2.1 billion dollars (USDA-ERS, 2014a). Currently, the United States is the largest producer of strawberries and blueberries in the world. In 2012, fresh market strawberry fruit accounted for 80 percent of total strawberry production in the U.S., valued at $2.2 billion while processing strawberries accounted for nearly 6 billion lbs., valued at nearly $200 million (Boriss et al., 2014). Interestingly, the overall consumption of fruits in the US has been decreasing, down from a high of

131.3 lbs. in 1999 to 107.6 lbs. of fresh and processed fruit per person in 2012 (USDA-ERS, 2014b).

Berry crops are usually grown and sold via three marketing channels that include U-pick or direct on-farm sales, fresh sales via local or more distant stores, and processing fresh berries into frozen fruit, puree, dried fruit or juice, which are sold directly to consumers or food manufacturers (Kniel and Shearer, 2009). In general, fresh berries destined for the fresh market are hand-picked and directly packed into the final container, usually clear plastic clamshells (Strik, 2007). Producing berry fruit for fresh market sales is very labor intensive as it requires experienced pickers to harvest, sort and pack the fruits. Mechanical harvesting can be less expensive than employing manual labor. However, it is only possible for fruits like blueberries destined for the industrial processing market since machine harvest can easily bruise or damage the delicate berry crops. It was reported that the firmness of blueberries harvested by machines was decreased by 36% as compared to those harvested by hand (Nunez-Barrios et al., 2005). Bruising and injury on the berry surface can substantially impact the shelf life of fresh berries or even disqualify the berries for fresh market. Postharvest handling and storage of berries destined for the fresh market is a race with time, as these small fruits are highly perishable commodities. Unlike other fresh produce, berries destined for the fresh market are not washed following harvesting and prior to shipment to consumers in order to prolong their shelf life. Rapid cooling is required to remove field heat from the berries, which not only slows down the berries' respiration rate and growth of spoilage microorganisms but also decreases the enzyme activity which leads to softening (Bower, 2007). Packaging is also important for retaining the integrity and quality of berry crops. Clamshell containers are now widely

used to provide a rigid packaging to provide mechanic protection of these high-value fresh berries from potential bruising caused by vibrations during storage and transportation (Bower, 2007). Cushioned packaging material may also be used during harvest to provide additional protection (Brown et al., 1996).

It has become possible to have year-round berries and berry products available to consumers due to developments in breeding, storage, transportation, and processing. Processed berries can be frozen or used in other products including juice, jelly, jam, fruit fillings, ice cream, various dessert, etc. Processed berry products include frozen purees and puree concentrates; frozen whole or sliced berries; juice concentrate; dehydrated berries; jams, preserves, or condiments; fruit fillings and syrups; beverages and wines, etc. (Deuel and Plotto, 2004). Berries that are to be processed are typically washed with potable water or water containing antimicrobials (e.g., chlorine). Some of the berries may be chopped. Washed berries are often mixed with up to 30% sucrose before freezing as sugar pack frozen berries (Harris et al., 2003). Berry puree is processed from fresh berries or frozen sort-outs that do not meet standards for fresh fruit, straight pack, or individually quick frozen (IQF) berries. Berries free of rot or mold will be selected. Berry puree is produced by screening chopped berries in a pulper or finisher to remove extraneous material (leaves, caps). Sometimes, sucrose is added to the puree prior to concentration to achieve higher soluble solids in the finished product. (Deuel and Plotto, 2004). To extend shelf life and inactivate pathogens, a thermal treatment (88 °C for 1.5 – 2 min) is usually employed. However, consumers are increasingly demanding minimally-processed foods in order to maintain nutritional and flavor aspects of the berry fruits. Therefore, the pasteurization processes of berry puree are sometimes mitigated and a mild heating step of either 30 s

at 65 °C or 15 s 75 °C is often used nowadays (Baert et al., 2008). Unfortunately, those limited thermal pasteurization process are sometimes not sufficient to eliminate foodborne pathogens.

2.3   Sources of Contamination

From a food-safety point of view, berries can become contaminated at any point in the production chain with viral or bacterial pathogens. The contamination can occur either pre- or post-harvest. Pre-harvest sources include soil, feces, irrigation water, reconstituted fungicides and insecticides, dust, insects, inadequately composted manure, wild or domestic animals and human handling (Beuchat, 2002). Human handling can contribute to postharvest contamination along with harvesting equipment, transport containers, insects, dust, rinse water, ice, transport vehicles and processing equipment (Beuchat and Ryu, 1997; Beuchat, 2002).

2.3.1   Soil and manure

The use of animal manure as fertilizer has been practiced for millennia around the world despite the fact that animal manure is a well-recognized potential source of pathogens that can directly or indirectly lead to foodborne illnesses through consumption of contaminated water or foods (Millner, 2009). It has been reported that *E. coli* O157:H7 and *Salmonella* may survive in soil for 7 – 25 weeks depending on the soil type, moisture level, temperature and source of contamination (Erickson et al., 2010; Lang and Smith, 2007; Zhang et al., 2009). Mootian et al. (2009) showed that about 30% of the lettuce samples initially irrigated with contaminated water or grown in contaminated soil (including manure-amended soil) for 15 days were positive for *E. coli* O157:H7. A field experiments was carried out in Nottinghamshire, UK to

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determine the survival of several bacterial pathogens in livestock manures during storage and following land application (Nicholson et al., 2005). All these pathogens survived for less than one month in solid manure heaps where temperatures were greater than 55 °C during the storage. Following manure spreading to land, *E. coli* O157, *Salmonella* and *Campylobacter* generally survived in the soil for up to one month depending on soil type whereas *Listeria* commonly survived for more than one month. Viruses have also been shown to persist in biosolids and manures and survival was dependent upon manure and virus type (Wei et al., 2010a, 2010b, 2009). In order to decrease the risk of "manure-borne" pathogens, the USDA restricts application of non-composted manure on soil to more than 120 days prior to the harvest of a product whose edible portion has direct contact with the soil surface or soil particles (USDA, 2002).

2.3.2 Irrigation water

The quality of irrigation water and type of irrigation system influence the microbial safety of fresh produce (Brackett, 1999; Olaimat and Holley, 2012). A study designed to track *E. coli* O157:H7 in a major produce production region of California suggests that the pathogen, when found in water, is generally close to a point source (Cooley et al., 2007). The incidence of *E coli* O157:H7 increased significantly when there was an increased flow rate in the rivers caused by heavy rain. The authors also pointed out that in periods of high water flow, often associated with flooding, the pathogen may be transported over 30 km. Nicholson et al. (2005) reported that *E. coli* O157, *Salmonella* and *Campylobacter* survived in stored slurries and dirty water for up to three months, with *Listeria* surviving for up to six months. In another study, Okafo et al. (2003) reported that unregulated release of untreated sewage into rivers

and streams can result in water contamination , which can place any crops irrigated with the water at risk of being contaminated. Recently, Brassard et al. (2012) evaluated the presence of pathogenic human and zoonotic viruses on irrigated, field-grown strawberry plants in Quebec, Canada. Strawberries were shown to be positive for Norovirus genogroup I, rotavirus, and swine hepatitis E virus genogroup 3, and irrigation water was suspected as the contamination origin.

2.3.3 Wild Animals

Fresh berries are utilized as an important food source by a variety of wild animals such as birds, deer, rodents, bears and insects. As the majority of agricultural production takes place in rural areas that is also habitat to a variety of wild animals, it is impossible to keep wild animals out of farmland. The role of wild animals as a direct source of produce contamination has been recently demonstrated. In the *E. coli* O157:H7 outbreak associated with contaminated baby spinach in 2006, compelling evidence showed that feral swine may have contributed to the contamination (Cooley et al., 2007; Jay et al., 2007). In addition, feral swine were also identified as carriers of *E. coli* O157:H7 in a survey of wildlife in Sweden (Wahlström et al., 2003). Black-tailed deer were investigated as potential sources of *E. coli* O157 following two produce-related outbreaks in the United States. In 2011, fresh strawberries from a farm in Oregon were linked with an *E. coli* O157:H7 outbreak, which caused at least 15 people to become sick and two deaths. Deer droppings in strawberry field were confirmed as the source of *E. coli* O157:H7 (Laidler et al., 2013). In 1996, an *E. coli* O157:H7 outbreak was epidemiologically linked to a particular brand of unpasteurized apple juice. A small sampling of deer droppings in an apple orchard

revealed the presence of *E. coli* O157:H7, but the strain was not genetically related to the human outbreak strain (Cody et al., 1999).

2.3.4 Harvesting and processing

Harvesting and processing influence the quality and microbiological safety of berries. These activities include human and mechanical contact, immersion in water, and cutting or slicing. These operations not only create surfaces to which enteric pathogens can more easily attach, but can also enhance bacterial growth by releasing large amounts of juice which can be readily used by microbes (Brackett, 1999; Doyle and Erickson, 2008; Davis et al., 1988). Infected workers are thought to be the primary source of viruses that cause foodborne illnesses, since pathogens such as norovirus and hepatitis A have exclusively human reservoirs (Berger et al., 2010; Warriner et al., 2009). Extensive human handling during harvesting and processing of frozen berries may explain the greater association of outbreaks with frozen berries (Harris et al., 2003). It was reported that manual removal of strawberry stems by workers in the field during picking might be a potential mechanism for strawberry contamination (Niu et al., 1992). In norovirus infections, vomitus can be highly infectious and lead to rapid spread of aerosols containing HuNoV. The persons who vomit may not perceive themselves as ill, and thus not exclude themselves from working in the kitchen (Lynch et al., 2009). León-Félix et al. (2010) evaluated the impact of harvesting and packinghouse operation on the contamination of green bell pepper with norovirus, fecal coliforms and *E. coli* in northwest Mexico. Norovirus and *E. coli* were absent from pickers' hands at the beginning of labor activities but detected 3 h later. Norovirus was detected on 45 and 30% of green bell peppers from the fields and packing houses, respectively. Fecal coliforms were detected in 85 and 15% of field

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and packed GBP, respectively, while *E. coli* was absent. The report showed that handling of fresh produce by workers during harvesting and packing operations had negative impacts on fresh produce safety. A review of food- and water-borne outbreak events due to norovirus between 2000 and 2007 revealed that the food handler was responsible for the outbreak in 42.5% of the cases, contaminating sandwiches and catered meals, followed by water (27.5%), bivalve shellfish (17.5%) and raspberries (10%) (Baert et al., 2009).

2.4   Incidence of Foodborne Illness Associated with Berry Consumption

Foodborne illness is a significant health and economic burden worldwide. It is estimated by the Centers for Disease Control and Prevention that about 48 million people (1 in 6 Americans) get sick, 128,000 are hospitalized, and 3,000 die each year from foodborne diseases (CDC, 2010). The total economic burden from health losses due to foodborne illnesses in the United States was estimated to be about $77.7 billion annually (Scharff, 2012). Leafy vegetables and fruits/nuts are the 2nd and 4th leading food vehicles for foodborne illnesses, responsible for 13% and 11% of all the foodborne outbreaks in the US (Gould et al., 2013).

While the production and consumption of berries and berry products have been steadily increasing, outbreaks of foodborne illnesses related to the consumption of berry products have been reported frequently in recent years. Frozen raspberries have been reported to have caused multiple large-scale HuNoV outbreaks in 2005 and 2009 (Friedman et al., 2005; Sarvikivi et al., 2012). In 2012, the largest foodborne outbreak in Germany was associated with norovirus-contaminated frozen strawberries, which affected more than 11,000 people (Mäde et al., 2013). Multistate foodborne outbreak of hepatitis A virus (HAV) was linked to frozen strawberries in in 1990 and 1997

(Hutin et al., 1999; Niu et al., 1992). In the 1997 HAV outbreak, a total of 213 cases of HAV were reported from 23 schools in Michigan and 29 cases from 13 schools in Maine (Hutin et al., 1999). Contaminated raw blueberries were reported to be the source of an outbreak of hepatitis A in 2003 (Calder et al., 2003). Fresh raspberries and blackberries imported from Guatemala were linked to several large outbreaks of *Cyclospora cayetanensis* (CDC, 1997; Dawson, 2005; Herwaldt and Beach, 1999; Herwaldt and Ackers, 1997; Katz, 1999). In 2011, fresh strawberries from a farm in Oregon were linked with an *E. coli* O157:H7 outbreak, which caused at least 15 people to become and two deaths. Deer droppings in the strawberry field were confirmed as the source of *E. coli* O157:H7 (Laidler et al., 2013). Since 1990, consumption of contaminated berries and berry products has been responsible for at least 41 outbreaks of illnesses in the United States, resulting in over 3800 reported cases of foodborne illness (Table 2.1).

In a recent survey, samples of leafy greens, soft red fruits and other types of fresh produce (tomatoes, cucumber and fruit salads) were tested in Belgium, Canada, and France (Baert et al., 2011). Norovirus was detected by real-time reverse transcription-polymerase chain reaction (RT-PCR) in 34.5% (N = 29) and 6.7% (N = 150) of the soft red fruits samples tested in Belgium and France, respectively and 28.2% (N = 641), 33.3% (N = 6) and 50% (N = 6) of leafy greens tested in Canada, Belgium and France, respectively (Baert et al., 2011). In 1999, FDA conducted a 1000-sample survey focused on high-volume imported fresh produce including broccoli, cantaloupe, celery, cilantro, culantro, loose-leaf lettuce, parsley, scallions (green onions), strawberries and tomatoes. During the survey, 143 strawberry samples from five countries (Argentina, Belgium, Canada, Mexico, and New Zealand) were

analyzed for *Salmonella* and *E. coli* O157:H7. Only 1 out of 143 imported strawberries samples was positive for *Salmonella* (FDA, 2001). Results from these very limited number of surveys and studies showed that both bacterial and viral contamination were detected in the market. An increased awareness that berries play a role in the transmission of both bacterial and viral pathogens led to these epidemiological surveys which in turn enhanced our understanding of the relationship between contaminated berries and foodborne illness (Butot et al., 2007).

Table 2.1 List of berry-related outbreaks in the U.S. from the period of 1990-2011

Year	Food	Pathogen	Cases	States
1990	Strawberries	Hepatitis A	51	Multi-state
1993	Melon; Strawberries	*C. jejuni*	48	Minnesota
1995	Raspberries	*C. cayetanensis*	32	New York
1995	Raspberries	*C. cayetanensis*	38	Florida
1996	Raspberries	*C. cayetanensis*	1465	Multi-state
1996	Strawberries	*Cyclospora* spp.	10	Texas
1996	Raspberries and others	*C. cayetanensis*	8	New York
1996	Berry dessert	*Cyclospora* spp.	16	Texas
1997	Raspberries	*C. cayetanensis*	1012	Multi-state
1997	Strawberries	Hepatitis A	256	Multi-state
1998	Strawberries	Hepatitis A	29	Texas
1998	Strawberries and others	Norovirus	41	Iowa
1999	Berries	*C. cayetanensis*	94	Florida
1999	Strawberries and others	Norovirus	63	Minnesota
1999	Strawberries and others	*Shigella sonnei*	3	California
2000	Raspberries; Blackberries	*C. cayetanensis*	19	Georgia
2000	Raspberries	*C. cayetanensis*	54	Pennsylvania
2000	Strawberries	Hepatitis A	8	Massachusetts
2000	Strawberries and others	Norovirus	100	California
2001	Strawberries and others	Viral	32	Colorado
2001	Strawberries and others	Norovirus	42	California
2002	Strawberries and others	Norovirus	15	Minnesota
2002	Raspberries	*C. cayetanensis*	26	Vermont
2003	Strawberries	*Salmonella* spp.	13	California
2004	Strawberries and others	Norovirus	62	Colorado

Table 2.1 Continued

Year	Food	Pathogen	Cases	States
2005	Strawberries	Norovirus	40	Georgia
2005	Strawberries and others	Norovirus	20	Washington
2006	Strawberries; Blueberries	*E. coli* O26	5	Massachusetts
2007	Raspberries and others	Norovirus	19	Illinois
2007	Strawberries	Norovirus	10	Georgia
2007	Strawberries and others	Hepatitis A	3	Florida
2007	Fresh berries in ice cream	Norovirus	17	California
2008	Berries	*C. cayetanensis*	3	Tennessee
2008	Mixed berries	*C. cayetanensis*	59	California
2009	Blackberries; Raspberries	*C. cayetanensis*	8	Connecticut
2009	Blueberries	*S.* Muenchen	14	Multistate
2010	Blueberries	*S.* Newport	6	Minnesota
2010	Strawberries and others	Norovirus	55	Ohio
2011	Strawberries	*E.coli* O157:H7	15	Oregon
2012	Strawberries and others	*C. jejuni*	15	Ohio
2012	Cranberry sauce	Norovirus	28	New Jersey

Source: Adapted from CDC-foodborne outbreak online database & Outbreak Alert!

database from Cspinet.org

2.5 Organisms of Major Concern

2.5.1 *Escherichia coli* O157:H7

2.5.1.1 General characteristics

E. coli O157:H7 is a Gram-negative, non-spore-forming facultative anaerobe in the family of *Enterobacteriaceae*. As opposed to other commensal strains, *E. coli* O157:H7 generally does not ferment sorbitol and does not have β-glucuronidase activity, which is a very useful marker for bacterial identification. It grows rapidly at $30 – 42°C$ and does not grow at temperatures below $10 °C$ or above $44°C$ with an optimum temperature of $37 °C$ (Bhunia, 2008). The organism is destroyed by thorough cooking of foods when all parts reach a temperature of $70 °C$ or higher, however, it can survive for weeks at $4 °C$ or even $-20°C$. Although there is some variability among strains, this pathogen is relatively acid-tolerant and can grow at pH levels ranging from $4.4 – 9.0$, a_w of > 0.95 and NaCl levels of $< 8.5\%$. In foods at pH levels of $3.5 – 5.5$, it can survive for extended periods (Fratamico and Smith, 2006).

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), K (capsular) and H (flagellar). Orskov and Orskov (1992) estimated that 173 O antigens, 80 K antigens and 56 H antigens existed. Based on the presence of certain virulence factors and their interaction pattern with mammalian cells or tissues and toxin production, pathogenic *E. coli* are also classified into six virotypes: (1) enterotoxigenic *E. coli* (ETEC), (2) enteropathogenic *E. coli* (EPEC), (3) enterohemorrhagic *E. coli* (EHEC), (4) enteroinvasive *E. coli* (EIEC), (5) enteroaggregative *E. coli* (EAEC), and (6) diffusely adhering *E. coli*

(DAEC) (Bhunia, 2008). *E. coli* O157: H7 is the first documented and most well studied EHEC strain, which is also considered the prototypical serotype of EHEC.

2.5.1.2 Pathogenesis

E. coli O157:H7 is a relatively new pathogen. It was first described in 1977 by Konowalchuk et al. (1977) who found that certain diarrheagenic *E. coli* strains produced a cytotoxin that can kill Vero cells, thus they named it verotoxin (VT). In 1982, *E. coli* O157:H7 was recognized for the first time as a human pathogen when two outbreaks in Oregon and Michigan were associated with eating undercooked hamburgers from a fast food restaurant chain (Riley et al., 1983). This outbreak was characterized by a distinctive hemorrhagic colitis, and a rare isolate of O157:H7 serotype of *E. coli* was implicated as the agent of disease. During the past 30 years, an increasing number of *E. coli* O157:H7 outbreaks have gained a worldwide niche as a formidable public-health concern. In 1994, *E. coli* O157:H7 became a nationally notifiable infection and by 2000, reporting was mandatory in 48 states (Rangel et al., 2005). The US Department of Agriculture (USDA) has established a 'zero tolerance' policy for *E. coli* O157:H7 on ground beef. However, this policy has not resolved the problem, as an estimated 63,154 illnesses due to *E. coli* O157:H7 infection occur in the United States every year, leading to an estimated 2,138 hospitalizations and 20 deaths annually (Scallan et al., 2011).

E. coli O 157:H7 can produce two different Shiga-like toxins, namely Stx1 and Stx2. These Shiga-like toxins are very similar, if not identical, to the toxins produced by *Shigella dysenteriae.* Therefore, these *E. coli* strains are also called Shiga toxin-producing *E. coli* (STEC). Due to their toxic effects on Vero (African green monkey kidney) cells, *E. coli* O157:H7 is also known as verotoxin-producing *E. coli* (VTEC).

VTEC and STEC referred to the same types of bacteria: *E. coli* that produce one or more Shiga-like toxins. A strain of STEC may produce Stx1 or Stx2, or both of these toxins. The structure of the Stx toxins produced by *E. coli* O157:H7 consist of a 32 kDa protein A subunit that is covalently bound to five b subunits of 7 kDa (Joseph et al., 2002). The B subunit is responsible for toxin attachment to the glycolipid globotriaosylceramide (Gb3), the receptor for Shiga toxin, which is abundant in the cortex of the human kidney (Park et al., 2001). The protein A subunit is an *N*-glycosidase that inhibits protein synthesis. After entering the cytosol, the protein A subunit attacks the ribosome by specifically depurinating the adenine in the 28S rRNA (Sandvig et al., 1992).

In addition to Shiga-like toxins, *E. coli* O157:H7 can produce other virulence factors that may increase the severity of human illnesses. The pO157 is a 90-kb plasmid that is carried by almost all *E. coli* O157:H7 strains (Park et al., 2001). The plasmid encodes a couple of putative virulence factors such as enterohemolysins and a serine protease. The bacterial genes involved in the attaching and effacing (A/E) lesions are located on a 35-kb segment of chromosomal DNA termed the locus of enterocyte effacement (LEE). Among the multiple virulence factors encoded in the LEE, the *eae* gene encoding intimin and the *tir* gene encoding a translocated receptor for intimin are of great importance. The *eae* gene encodes a 94-97 kDa outer membrane protein called intimin, which allows *E. coli* O157:H7 to attach to, and subsequently efface intestinal epithelial cells (Joseph et al., 2002). Tir, a 90-kDa protein, is encoded by the tir gene in the LEE pathogenicity island and is injected into host cells by bacteria via a type III secretion system. Tir consists of at least 3 domains,

one of which function as a receptor for intimin, and another is involved in actin alignment and eventual pedestal formation.

2.5.2 *Salmonella*

2.5.2.1 General characteristics

Salmonella is a Gram-negative, facultative anaerobic, rod-shaped, non-sporeforming, motile bacterium in the family of Enterobacteriaceae. In the late 1800s, Dr. Theobald Smith, a researcher under Dr. Daniel E. Salmon in the USDA, was the first American to identify *Salmonella* as a separate strain or genus. However, the new bacterium was named after Salmon since Salmon's name was listed first as administrator on the research paper (FDA, 2007). Most *Salmonella* serotypes grow at a temperature range of 5 to 47 °C with an optimum temperature of 35 to 37 °C but some can grow at temperature as low as 2 to 4 °C or as high as 54 °C (Gray and Fedorka-Cray, 2002). *Salmonella* are sensitive to heat and often killed at temperature of 70 °C or above. Although they do not require sodium chloride for growth, *Salmonella* can grow in the presence of 0.4 to 4% of the salt. *Salmonella* grow in a pH range of 4 to 9 with the optimum between 6.5 and 7.5. They require high water activity between 0.99 and 0.94 for growth, but can also survive in dried foods with a_w < 0.2 for an extended periods of time (Pui et al., 2011).

Based on their somatic (O), flagellar (H), and capsular (Vi) antigenic patterns, *Salmonella* with 2463 different serotypes are now placed under 2 species: *Salmonella enterica* (2443 serotypes) and *Salmonella bongori* (20 serotypes) (Bhunia, 2008). *Salmonella enterica* is further divided into six subspecies, which are designated by roman numerals. *Salmonella enterica* subspecies I is mainly isolated from warm-

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blooded animals and accounts for more than 99% of clinical isolates whereas remaining subspecies and *S. bongori* are mainly isolated from cold-blooded animals and account for less than 1% of clinical isolates.

2.5.2.2 Pathogenesis

Salmonella is the leading bacterial causative agent for foodborne illnesses in the US. It is estimated that *Salmonella* causes approximately one million cases of foodborne illnesses and is the leading cause of hospitalizations (35%) and deaths (28%) due to foodborne illnesses in the US (Scallan et al., 2011). The estimated annual cost of *Salmonella* infection including factors such as medical costs and lost productivity is $4.4 billion USD (Scharff, 2012).

Salmonella are present in the intestinal tract of birds, reptiles, turtles, insects, farm animals and humans. *Salmonella* infections are usually acquired by the fecal-oral route through consumption of contaminated food such as eggs, poultry, milk, and meat. In recent years, however, fruits and vegetable have been increasingly reported in *Salmonella*-related foodborne outbreaks (Doyle and Erickson, 2008). In general, the infectious dose of *Salmonella* spp. was thought to be in excess of 10,000 cells but experiences from outbreaks have shown that under certain circumstances only a few (< 10 cells) bacterial cells have been sufficient to cause disease (Molbak et al., 2006). In human disease, the clinical pattern of salmonellosis can be divided into four disease patterns namely enteric fever, gastroenteritis, bacteremia and other complications of nontyphoidal salmonellosis as well as chronic carrier state (Pui et al., 2011). *Salmonella enterica* serovar Typhi is the most invasive type and can cause typhoid fever, a systemic disease in humans. *S. enterica* serovar Paratyphi causes typhoid-like infection in humans. *S. enterica* serotype Typhimurium and *S. enterica* serotype

Enteritidis cause self-limiting gastroenteritis or enterocolitis and are the most common serovars responsible for salmonellosis in the US (Bhunia, 2008). *Salmonella* gastroenteritis usually begins 24 – 48 h after ingestion of contaminated food or water with fever, nausea, and vomiting, followed by abdominal cramps and diarrhea. The diarrhea will usually persist as the predominant symptom for 1 – 4 days and usually resolves spontaneously within 7 days. Fever is present in about half of those manifesting clinical illness (Gray and Fedorka-Cray, 2002). The mortality rate for salmonellosis is 4.1%. About 1 – 5% of recovering patients may serve as a chronic carriers and shed *Salmonella* for 3 – 12 months. Systemic forms of the disease are more often seen in young children or immunocompromised persons.

The establishment of *Salmonella* infection depends on its ability to survive the gastric acid of the stomach and the ability of the pathogen to colonize and invade intestinal cells. The Peyer's patches of the distal ileum were identified as the primary invasion sites of *Salmonella*. M cells are specialized epithelial cells that are located above the follicles on the luminal surface of the Peyer's patch and function as antigen presenting cells by transporting the bacteria to lymphocytes and macrophages within the dome area (Gray and Fedorka-Cray, 2002). *Salmonella* virulence gene clusters are located in 12 pathogenicity islands (SPI) and near the tRNA genes. SPI-1 and SPI-2 are two DNA segments containing virulence genes involved in the intestinal phase of infection while the remaining SPIs are required for causing systemic infection, intracellular survival, fimbrial expression, antibiotic resistance and Mg^{2+} and iron uptake (Bhunia, 2008). SPI-1 is a 43-kb segment that contains 31 genes responsible for invasion of nonphagocytic cells and components of the Type III secretion system. SPI-2 is a 40-kb segment encoding 32 genes. The gene products are essential for

causing systemic infection and mediate bacterial replication within host macrophages (Bhunia, 2008).

2.5.3 Human noroviruses

2.5.3.1 General characteristics

Norovirus (formerly called 'Norwalk-like virus') was first discovered in Norwalk, Ohio in 1968 after an outbreak of acute gastroenteritis in an elementary school. Immune electron microscopy analysis of human stool samples identified a large number of small-round structured viral particles ranging from 27 – 38 nm in diameter and the virus was named after the location of the original isolation (Kapikian et al., 1972). The name of this virus was changed to norovirus after being identified in numerous outbreaks with similar symptoms occurring on cruise ships and in many other settings. In 2002, the name norovirus (*Norovirus* for the genus) was approved by the International Committee on Taxonomy of Viruses.

Noroviruses are a group of non-enveloped, icosahedral viruses with a plus-sense, single-stranded RNA genome of 7.5 – 7.7 kb that contains three open reading frames (ORFs) (Jiang et al., 1993). ORF1 encodes the nonstructural polyprotein which is cleaved by viral 3C-like protease into probably 6 proteins, including the viral Vpg, protease, and RNA-dependent RNA polymerase (Belliot et al., 2003; Sosnovtsev et al., 2006). ORF2 and ORF3 encode the major (VP1) and minor (VP2) capsid proteins, respectively (Zheng et al., 2006). The VP1 protein forms two domains: P (protruding, P1 and P2) and S (shell). The S domain of VP1 forms the inner shell of the capsid, while the P domain protrudes from the capsid surface and contributes to binding the histo-blood group antigen (HBGA) receptor (Cao et al., 2007) and antigenicity

(Donaldson et al., 2008). It is believed that the capsid protein not only provides shell structure for the virus but also contains cellular receptor binding site(s) and viral phenotype or serotype determinants. The function of VP2 associates with up-regulation of VP1 expression in *cis* and stabilization of VP1 in the virus structure (Bertolotti-Ciarlet et al., 2003).

HuNoVs belong to the genus *Norovirus* in the family *Caliciviridae*. Other genera within *Caliciviridae* are *Lagovirus* that infects rabbits and hares, *Vesivirus*, infecting multiple animal species including cats and sea lions, and *Sapovirus* that infects humans and a fifth genus called *Nebovirus* (Green et al., 2000; Mattison, 2011). HuNoVs have not been cultivated and thus direct serotyping using neutralizing antibodies is not possible, but RT-PCR and genomic sequencing has demonstrated that noroviruses are genetically and antigenically diverse (Zheng et al., 2006). A nomenclature scheme was proposed to classify noroviruses into at least 29 genetic clusters within five genogroups (GI, GII, GIII, GIV and GV) based on phylogenetic analysis of the capsid protein, VP1 (Zheng et al., 2006). Strains related to human infection can be found in GI, GII, and GIV with most of the human-related strains in GI and GII (Patel et al., 2009; Zheng et al., 2006). Multiple porcine noroviruses have been placed in GII (Sugieda et al., 1998; Wang et al., 2005). Bovine noroviruses have been placed in GIII, while murine noroviruses in GV. Noroviruses from GIV have been detected in a lion cub and dogs (Martella et al., 2008, 2007). Noroviruses display a wide degree of genetic variability. The members within a genogroup differ by 45 – 61% in their capsid gene sequences, members within a genotype differ by 14 – 44%, and strains within a genotype differ by 0 – 14% (Zheng et al., 2006). This amount of

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intra-genus variation is high even when compared to genera of other plus-strand RNA virus families.

2.5.3.2 Pathogenesis

Noroviruses are now recognized as the leading cause of gastroenteritis, responsible for 58% of all the foodborne illnesses caused by 31 identified pathogens in the US (Scallan et al., 2011). Although Norovirus disease outbreaks are reported year-round, they peak during months with cold weather in temperate climates (Mounts et al., 2000). Clinical norovirus infection generally has an incubation period of 24 – 48 h, characterized by acute onset of nausea, vomiting, abdominal cramp, myalgia, and non-bloody diarrhea. Norovirus is extremely contagious, with an estimated infectious dose as low as 18 viral particles (Teunis et al., 2008). Although symptoms usually resolve without treatment after 1 – 3 days, more severe and prolonged courses of illness lasting 4 – 6 days can occur, particularly among young children, elderly persons, and hospitalized patients (CDC, 2011). For example, infants and young children can develop more severe gastroenteritis following norovirus infection, with symptoms lasting up to six weeks (Karst, 2010). It has been estimated that HuNoV infections is responsible for 26% of hospitalizations and 11% of deaths caused by foodborne illnesses in the US (Scallan et al., 2011). The estimated annual cost of norovirus infection including factors such as medical costs and loss of work is $2.9 billion USD (Scharff, 2012).

The most important mode of transmission of HuNoVs is the fecal-oral route. Transmission is typically through contaminated food, water, or the environment, or person-to-person contact. Foods implicated in outbreaks of HuNoV gastroenteritis are usually contaminated either directly with fecal matter at the source (e.g., shellfish

harvested from sewage-contaminated waters or berries irrigated with sewage) or by food-handlers carrying HuNoVs (Bresee et al., 2002; Parashar and Monroe, 2001). Transmission through infectious vomit, both by mechanical transmission from environmental surfaces (i.e., through hand/mouth contact) and aerosolization, might account for the rapid and extensive spread of disease outbreaks in closed settings, such as hospitals, hotels, cruise ships, and day-care centers (Patel et al., 2009).

HuNoV is shed in high quantities in the stool and vomitus of infected people. The virus can be detected in stool for an average of 4 weeks following infection, although peak viral shedding occurs 2 – 5 days after infection, with a viral load of approximately 100 billion viral copies per gram of feces (Atmar et al., 2008). Furthermore, up to 30% of norovirus infections are asymptomatic, and asymptomatic persons can shed virus, albeit at lower titers than symptomatic persons (CDC, 2011).

HuNoVs are very contagious and can infect people of all ages who consume contaminated food or water or who have close contact with infected persons (Rockx et al., 2002). Currently, there is no FDA-approved vaccine available to prevent norovirus illnesses in humans. Immunity to HuNoVs is complicated by the heterogeneous responses of the human population and the transient nature of immunity in some individuals. In human volunteer studies, approximately 13 – 40% of volunteers never became infected and only 50% developed illness (Parrino et al., 1977; Wyatt et al., 1974). It was later suggested that host genotype is a prominent factor in the development of HuNoV infection since HuNoV infection depends on the presence of specific HBGA receptors in the gut of susceptible hosts (Lindesmith et al., 2003). The combination of the strain-specific binding and the variable expression of the HBGA receptors may explain the varying host susceptibility observed in HuNoV outbreaks

and volunteer studies (Patel et al., 2009). Moreover, no long-term immunity has been observed after repeated infection in adults and immunity to one strain does not provide good protection from infection with other heterogeneous strains (Fankhauser et al., 2002; Matsui and Greenberg, 2000; Rockx et al., 2002).

Taken together, several characteristics of HuNoVs facilitate their spread in epidemics, which include: (1) the low infectious dose of HuNoVs; (2) prolonged duration of viral shedding increasing the risk of secondary spread; (3) the stability of the virus in the environment at a wide range of temperatures (from freezing to 60 °C); and (4) lack of complete cross-protection against the diverse norovirus strains and inadequate long-term immunity (Patel et al., 2009).

2.5.3.3   Challenges for human norovirus research

Research on HuNoV has been severely hampered since HuNoV cannot be grown in cell culture and no suitable small animal model has been established for *in vivo* studies. Great efforts have been made to cultivate HuNoVs in tissue culture using traditional methods. Duizer et al. (2004) systematically evaluated a variety of cell lines and laboratory methods to cultivate HuNoV using gastric cells, duodenal cells, and small intestinal enterocyte-like cells but none of the cell culture combinations were successful. Straub et al. (2007) first reported that HuNoV can infect and replicate in a physiologically relevant 3-dimensional (3-D), organoid model of human small intestinal epithelium. Although these results are very encouraging, the robustness of virus replication and the amount of newly synthesized viruses have been questioned (Chan et al., 2007).

Researchers have recently demonstrated that human HBGAs may act as receptors for norovirus infection (Tan and Jiang, 2005). This finding has a significant

impact on current and future norovirus research with regard to pathogenesis, immunology, and the epidemiology of norovirus disease (Hutson et al., 2003; Lindesmith et al., 2003, 2008). Recently, Jones et al., (2014) reported that human and mouse noroviruses-infected B cells *in vitro* in the presence of HBGA-expressing enteric bacteria. The author further claimed that the use of B cells as a cellular target of noroviruses and enteric bacteria as a stimulatory factor for norovirus infection can potentially lead to the development of an *in vitro* infection model for HuNoVs. However, further validation on the robustness of this cell culture system is needed.

The stability and susceptibility of HuNoV to food processing technologies are not still well understood due to the lack of an infectivity assay. Most studies therefore had to rely on the use of molecular detection methods such as such as RT-PCR and cultivable HuNoV surrogates. It is known that the majority of HuNoV strains including GI.1 and GII.4 strains can bind to the porcine intestinal tract and to porcine gastric mucin (Tian et al., 2010, 2008, 2007), which chemically mimics the natural HBGA receptors found in the human intestinal tract (Tan and Jiang, 2005; Tian et al., 2007). Recently, an alternative method for evaluation of HuNoV inactivation, known as the porcine gastric mucin-magnetic bead (PGM-MB) binding assay, has been developed to assess the inactivation of norovirus (Dancho et al., 2012; Kingsley et al., 2014). In this assay, porcine gastric mucin is conjugated to magnetic beads to facilitate the extraction of norovirus from food and other matrices for subsequent RT-PCR assay. It was assumed that an inactivated virus with damaged capsid cannot bind to receptor-like PGM-MB. Therefore, only norovirus virions with intact capsid that are able to bind to PGM-MB would be extracted for further RT-PCR detection. Some promising results have been reported using this technique to estimate the inactivation

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of HuNoV (Dancho et al., 2012; Kingsley et al., 2014; Ye et al., 2014). Therefore, before a practical animal model or a cell culture system is developed for HuNoV, this method can potentially offer some quantitative information about the inactivation of non-cultivable HuNoV for certain treatments.

2.5.3.4   Human norovirus surrogates

To date, research on HuNoV has to rely on proper surrogates since HuNoV is non-cultivatable. Some common surrogates for HuNoVs studies have included murine norovirus (MNV), feline calicivirus (FCV), bacteriophage MS2, and Tulane virus which was discovered in 2008 (Farkas et al., 2008).

Murine norovirus

Murine norovirus (MNV) was first isolated from brain tissue of immunocompromised mice in 2003 (Karst et al., 2003). Wobus et al. (2004) developed a cell-culture based system for MNV, which was the first successful *in vitro* cultivation of a norovirus. Since then, MNV has been used as a surrogate to study the pathogenesis, immunology, and replication of HuNoVs, and a great amount of data have been generated (Baert et al., 2008; Cannon et al., 2006; Hirneisen and Kniel, 2013; Huang et al., 2014; Park et al., 2011; Wei et al., 2010b; Wobus et al., 2006).

MNV is a most important surrogate for the study of noroviruses as it shares many biological and molecular properties with HuNoV. MNV-1 has the size $(28 - 35$ nm in diameter), shape (icosahedral), and buoyant density $(1.36 \pm 0.04$ g/cm^3) characteristic of HuNoVs (Wobus et al., 2006). Although MNV-1 was originally isolated by serial passage from the brain tissue of severely immunocompromised mice, it is now clear that this virus is an effective enteric pathogen (Karst et al., 2003). MNV causes a lethal infection in mice featuring hepatitis, pneumonia or inflammation of the

nervous system (Karst et al., 2003). While these symptoms of MNV are different from those of HuNoV, MNV is shed in mouse feces and commonly transmitted via the fecal-oral route. From a molecular point of view, MNV-1 shares many biochemical and genetic features with HuNoVs. MNV is a member of the genus *Norovirus*, included in the genogroup GV of norovirus and it is the most genetically related of all the cultivable HuNoV surrogates.

However, the limitations of this model are that MNV differs in several aspects from HuNoV such as clinical manifestations, host receptors, pathogenesis, and infected cell types (Tan and Jiang, 2010). MNV uses sialic acid as a functional receptor and has tissue tropism for macrophages and dendritic cells (Taube et al., 2009). In contrast, HuNoVs use HBGAs as cellular receptors and infect digestive epithelial cells *in vivo* (Tan and Jiang, 2005; Tan et al., 2008). Additionally, MNV does not cause the clinical symptoms of gastroenteritis that the human counterpart does, such as projectile vomiting and/or explosive, watery diarrhea since mice lack an emetic reflex (Wobus et al., 2006).

As a surrogate, MNV is relatively resistant to acid and heat, and highly stable and persistent in the environment (Cannon et al., 2006; Li et al., 2012; Wobus et al., 2006). Cannon et al. (2006) directly compared the viabilities of MNV and feline FCV, as surrogates for HuNoV. It was found that MNV was stable across the pH range of 2 – 10, whereas FCV was rapidly inactivated at a pH less than 3 and greater than 9. FCV was more stable than MNV at 56 °C, but both viruses exhibited similar inactivation at 63 and 72°C. Long-term persistence studies found that MNV was more stable than FCV at room temperature when both viruses were suspended in a fecal matrix and

inoculated onto stainless steel coupons (Cannon et al., 2006). Taken together, the study showed MNV to be a better surrogate for HuNoV than the previously used FCV.

Tulane virus

The Tulane virus (TV), also known as the monkey calicivirus, was isolated from the stools of rhesus macaques at the Tulane National Primate Research Center (Farkas et al., 2008). TV exhibits a typical calicivirus morphology, with a diameter of 36 nm, and has a buoyant density of 1.37 g/mL. According to these physicochemical and genetic characteristics, TV was proposed to be placed in a new calicivirus genus, *Recovirus*. TV replicates *in vitro* in rhesus monkey kidney (LLC-MK2) cells and causes typical cytopathic effects. Genomic sequence analysis showed that TV was closely related to HuNoV (Farkas et al., 2010). More importantly, similar to HuNoV, TV recognizes type A and B HBGAs as receptors for infection (Farkas et al., 2010). This property makes TV a potentially useful surrogate for HuNoVs studies. However, a weakness of this model is that TV belongs to a unique genus separate from the *Norovirus* genus and it remains unknown whether TV causes gastroenteritis like HuNoV (Tan and Jiang, 2010).

2.6 Intervention Methods for Berry Decontamination

A variety of processing technologies have been evaluated to reduce bacterial and viral pathogens on different berries, which include washing with/without various sanitizers (K. Bialka and Demirci, 2007; Lukasik et al., 2003; Pangloli and Hung, 2013; Predmore and Li, 2011; Udompijitkul et al., 2007), exposure to gaseous chemicals (Bialka and Demirci, 2007; Han and Selby, 2004; Sy et al., 2005), refrigeration/frozen storage (Butot et al., 2008; Knudsen et al., 2001), irradiation (Bidawid et al., 2000; Yu et al., 1995), UV/Pulsed light (Bialka and Demirci, 2008;

Bialka and Demirci, 2007; Fino and Kniel, 2008; Huang and Chen, 2014) and HPP

(Kingsley et al., 2005; Lou et al., 2011).

Today, chlorine (as sodium or calcium hypochlorite) of 50 – 200 ppm is still

the primary postharvest sanitizing agent used by the fresh produce industry because of

its broad antimicrobial activity and low cost. Free chlorine, which is the main

component that has antimicrobial effect, is defined as the concentration of residual

chlorine in water present as dissolved gas (Cl_2), hypochlorous acid (HOCl), and/or

hypochlorite ion (OCl^-). At a pH of 5 – 7, free chlorine is most effective as HOCl is

the predominant form. However, chlorine is known to react with organic matter, which

can significantly diminish its antimicrobial efficacy. In addition, high organic load in

water can also result in formation of noxious chlorine by-products such as

trihalomethanes and chlorine off-gassing problems (Luo et al., 2012). Therefore, there

is a sustained effort to find chlorine-alternatives and a number of studies have been

published on the effectiveness of washing with different sanitizers. Gurtler et al.

(2014) tested 27 antimicrobial treatments to reduce *E. coli* O157:H7 and *Salmonella*

on fresh strawberries and found that four treatments including 1% acetic acid+1%

H_2O_2, 30% ethanol+1% H_2O_2, 90 ppm peracetic acid, and 1% lactic acid+1% H_2O_2

achieved > 3-log reduction. Lukasik et al. (2003) investigated the effects of water and

a range of sanitizers on the reduction of poliovirus 1, bacteriophages, *Salmonella* and

E. coli O157:H7 on strawberries. Washing strawberries with water for 2 min at 22 or

43 °C reduced the tested microorganisms by < 1 log. Approximately 2-log reductions

of bacteria and viruses were obtained by washing with sodium hypochlorite (50-300

ppm free chlorine) and various commercial sanitizers. Predmore and Li (2011)

evaluated the efficacy of a combination of surfactants and sanitizers on removal of

MNV-1 from various produce. Significant increase in efficacy of washing solution was observed by adding 50 ppm of surfactant.

Gaseous decontamination methods have shown some promise for decontamination of small fruits in recent years. ClO_2 is an oxidizing agent that is extremely soluble in water. It is up to five times more soluble than chlorine and has 2.5 times the oxidation capacity of chlorine, but unlike chlorine it does not react with ammonia or organic compounds to form trihalomethanes and is less affected by pH. The disadvantage of ClO_2 is that it is unstable, and it must be generated on site and can be explosive when concentrated (Beuchat, 1998). Several studies reported the efficacy of gaseous ClO_2 in reducing pathogenic bacteria on berries. A continuous treatment of strawberries with 3 mg/liter ClO_2 for 10 min achieved a > 5-log reduction for both *E. coli* O157:H7 and *L. monocytogenes* (Han and Selby, 2004). In another study, treatment with 8.0 mg/L of chlorine dioxide gas for 120 min reduced *Salmonella* by 3.7, 4.4 and 1.5 log CFU/g from blueberries, strawberries and raspberries, respectively (Sy et al., 2005). Ozone is a strong broad-spectrum oxidizing agent, partially soluble in water and is active against a wide range of microorganisms through the oxidation of cellular membranes (Hirneisen et al., 2010). Bialka and Demirci, (2007) reported a 3.8- and 3.6-log reduction of *E. coli* O157:H7 and *Salmonella* from raspberries by gaseous ozone; however, the treatment time was very long (>2 h).

The effects of refrigeration and frozen storage on the survival of various pathogens have been studied. Knudsen et al. (2001) reported that populations of *Salmonella* and *E. coli* O157:H7 remained constant on cut surfaces of strawberries but decreased by 1 – 2 log units on intact surfaces during storage at 5 °C for 7 days. Thirty

days of frozen storage at -20 °C reduced the population of *E. coli* O157:H7 by 0.7 – 2.2 log units. Butot et al. (2008) investigated the effects of frozen storage on the survival of HAV, HuNoV, rotavirus and FCV in strawberries, raspberries and blueberries. Three-month frozen storage had very limited effects on the survival of HAV and rotavirus in all types of berries.

Irradiation is approved for use in the United States for several food commodities (21CFR179.26). There are different types of ionizing radiation including X-rays, gamma-rays, and beta-rays. Ionizing radiation causes breaks in both RNA and DNA. Due to the good penetration ability of ionizing radiation, food products can be processed in the package, which eliminates the possibility of post-process contamination (Hirneisen et al., 2010). Bidawid et al. (2000) found that Gamma irradiation at a dose of 3 kGy was needed to achieve a 1-log reduction of HAV on lettuce and strawberries. Irradiation at a dose of 10 kGy reduced HAV on strawberries by about 3 log units. Yu et al. (1995) reported that electron irradiation of strawberries at doses of 1 and 2 kGy can extend shelf life by 2 and 4 days, respectively; however, a decrease in firmness and increase in off-flavors were observed during storage of the strawberries.

Ultraviolet light is electromagnetic radiation with wavelengths in the range of 100 – 400 nm. UV light can be further divided into different ranges including UVC (100 – 280 nm), UVB (280 – 320 nm), and UVA (320 – 400 nm). The germicidal effect of UV light is primarily due to the dimerization of pyrimidine bases in the DNA/RNA of microorganisms (Giese and Darby, 2000; Mitchell et al., 1992). UV light treatment of strawberries was studied by Fino and Kniel (2008). At a dose of 240 mJ/cm^2, HAV, aichivirus and FCV on strawberries were reduced by 2.6, 1.9 and 2.3

log TCIC$_{50}$/mL, respectively. Similar results were reported for UV inactivation of bacterial pathogens by Kim & Hung (2012), who reported that UV treatment at 20 mW/cm^2 for 10 min reduced 2.14 and > 4.05 log CFU/g of *E. coli* O157:H7 on the calyx and skin of blueberries, respectively.

2.7   High-hydrostatic Pressure

High pressure processing (HPP), also described as high hydrostatic pressure (HHP), or ultra-high pressure (UHP) processing, is a process subjecting liquid and solid foods, with or without packaging, to pressures between 100 and 800 MPa (Farkas and Hoover, 2000). HPP is recognized as one of the most promising nonthermal technologies that ensure food safety, food quality, and extend shelf life by eliminating pathogenic and spoilage microorganisms with minimal effects on the organoleptic and nutritional properties of foods (Yordanov and Angelova, 2010). Although lethal to microorganisms, pressure treatment does not impact covalent bonds and has a minimal effect on food chemistry. Therefore, HPP provides a means for retaining food quality while avoiding the need for excessive thermal treatments or chemical preservatives.

A typical HPP process uses food products packaged in a high-barrier, flexible pouch or a plastic container. The packages are loaded into the high-pressure chamber. The vessel is sealed and the vessel is filled with a pressure-transmitting fluid such as water, silicone oil, ethanol or glycol. The ability of the pressure-transmitting fluid to protect the inner vessel surface from corrosion, the specific HHP system to be used, the process temperature range and the viscosity of the fluid under pressure are some of the factors determine the selection of the medium (Hogan et al., 2005). Foods are then pressurized by the use of a high-pressure pump, which injects additional quantities of

fluid. The packages of food, surrounded by the pressure-transmitting fluid, are subjected to the same pressure as exists in the vessel itself. After holding the product for the desired time at the target pressure, the vessel is decompressed by releasing the pressure-transmitting fluid (Farkas and Hoover, 2000).

Studies of the effects of high pressures on foods date back over a century. In 1899, Bert Hite at the Agricultural Research Station in Morgantown, West Virginia, USA, designed and constructed a high-pressure unit to pasteurize milk and other food products. It was demonstrated that pressurization at 658 MPa for 10 min destroyed microorganisms in milk, fruit juice, meat, and fruits (Hite, 1899). However, it then took almost eighty years for HPP to be used for food processing. The first high pressure processed foods were introduced to the Japanese market in 1990 by Meidi-ya, who have been marketing a line of jams, jellies, and sauces packaged and processed without application of heat (Thakur and Nelson, 1998). In the US, commercially available foods treated by HPP include fruit smoothies, guacamole, oysters, ready-to-eat meals with meat and vegetables, ham, chicken strips, fruit juices and salsa.

Although the potential for HPP of foods has been discovered since the late nineteenth century, its potential and value in food safety and preservation have only been truly appreciated in the food industry in the last 30 years. Currently, industrial HPP treatment is usually in a batch or semi-continuous process. Avure Technologies, NC Hyperbaric, and Uhde are major suppliers of commercial scale pressure equipment (Balasubramaniam et al., 2008). The selection of equipment depends on the kind of food product to be processed. Solid food products or foods with large solid particles can only be treated in a batch mode whereas, liquids, slurries and other pumpable products have the additional option of semi-continuous production (Ting and

Marshall, 2002). Commercial batch HPP vessels have internal volumes ranging from 30 to more than 600 liters. Commercial-scale, high pressure processing systems cost approximately $500,000 to $2.5 million, depending on equipment capacity and extent of automation (Ramaswamy et al., 2011). HPP treatment costs are quoted as ranging from 4 – 10 cents/lb, including operating cost and depreciation, and are not 'orders of magnitude' higher than thermal processing, as is often thought (Saiz et al., 2008). As the demand for HPP equipment grows, it is foreseeable that technical innovation is going to further reduce the capital and operating costs of HPP.

2.7.1   Principles of HHP

Two basic principles describe the effect of HHP. The first principle is called Le Chatelier's principle. According to this principle, any phenomenon (phase transition, chemical reaction, change in molecular configuration) accompanied by a decrease in volume can be enhanced by pressure (and vice versa). Accordingly, pressure shifts the system to that of lowest volume (Farkas and Hoover, 2000). The second principle is the Isostatic Principle, which states that pressure is instantaneously and uniformly distributed throughout a sample under pressure, whether the sample is in direct contact with the pressure medium or hermetically sealed in a flexible package that transmits pressure. In other words, pressure is transmitted in a uniform (isostatic) and quasi-instantaneous manner throughout the sample during the pressurization, in contrast to thermal processing, and the process time is independent of sample size and shape, assuming uniform thermal distribution within the sample (Balasubramaniam et al., 2008). The principle of isostatic processing is presented in Figure 2.1.
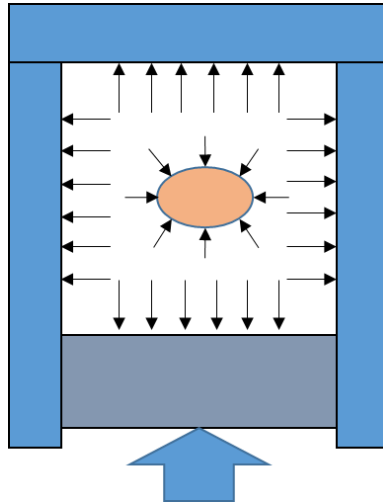
Figure 2.1 Principle of isostatic processing

However, care must be taken to understand the interdependence of pressure and temperature during HPP. Although HPP is classified as a nonthermal process, the adiabatic heating effect can substantially increase the temperature of both pressure medium and food samples. Both pure water and most moist foods subjected to a 600 MPa treatment at ambient temperature will experience about a 15% reduction in volume (Balasubramaniam et al., 2008). During pressurization, the temperature of foods changes as a result of physical compression/decompression. In a perfectly insulated (adiabatic) system, the product will return to its initial temperature upon decompression; however, in many cases, the product will return to a temperature slightly lower than its initial temperature due to heat losses to the walls of the pressure vessel (Farkas and Hoover, 2000). The temperature increase due to adiabatic heating under pressure is dependent on factors such as food composition, initial temperature and final pressure level. For water and most foods, the work of compression during HPP treatment will increase the temperature of foods approximately 3 °C per 100 MPa

(Farkas and Hoover, 2000). However, foods containing more fats and oils usually show higher compression heating values (8 – 9 °C per 100 MPa) and proteins and carbohydrates have intermediate compression heat values (Balasubramaniam et al., 2008; Rasanayagam et al., 2003).

2.7.2   Factors affecting microbial sensitivity to HHP

2.7.2.1   Type of microorganism

In general, Gram-positive bacteria tend to be more resistant to pressure than Gram-negatives and cocci are more resistant than rod-shaped bacteria. The more developed (evolutionarily) the life form, the more sensitive it is to pressure (Farkas and Hoover, 2000). However, there are notable exceptions to these general rules. Certain strains of *E. coli* O157:H7, for example, can be exceptionally pressure resistant. An *E. coli* O157:H7 strain isolated from a major hamburger patty outbreak in the US showed less than 1 log reduction when treated in laboratory medium at 500 MPa at < 45 °C for 30 min (Benito et al., 1999). This strain was also more resistant to heat, acid, oxidative and osmotic stresses than a pressure-sensitive strain.

Elimination of bacterial endospores from food probably represents the greatest food processing and food-safety challenge to the food industry. It is well-established that spores are very resistant to a range of physical treatments such as irradiation and heat, and can survive treatments of more than 1000 MPa (Patterson, 2005). It was found that relatively low pressures (below 200 MPa) can trigger spore germination (Gould and Sale, 1970). Pressure-induced germination sensitizes spores to the HPP treatment. Therefore, one strategy to inactivate spores is to use cycled HPP, in which the first cycle is to germinate spores and is followed by further cycles to inactivate the

cells. However, the problems of superdormancy and an inability to achieve 100%

germination hamper this method (Black et al., 2010). Another approach to the problem

of the pressure resistance of bacterial spores is to combine high temperature along

with pressure treatment, which is usually called pressure-assisted thermal processing

(PATS). This approach is now being widely used for the commercial production of

shelf-stable foods that have a pH greater than 4.5. The temperature, pressure level,

treatment time and time interval between the cycles can be varied depending on the

product but are designed to give greater than the equivalent of a 12D process for

spores of *C. botulinum* (Patterson, 2005).

Yeasts are generally not associated with food-borne disease but are important

in spoilage. Treatment at pressures less than 400 MPa for a few minutes is usually

sufficient to inactivate most yeasts (Hogan et al., 2005). This is one reason why

pressure treatment of fruit products to extend shelf-life is particularly successful.

There is relatively little information on the pressure sensitivity of molds but it has

been shown that vegetative forms are relatively sensitive, while ascospores are more

resistant (Butz et al., 1996; Voldřich et al., 2004). Butz et al. (1996) examined

responses of heat-resistant molds to HPP (300 to 800 MPa) used in combination with

different treatment temperatures (10 to 70 °C). All the vegetative forms were

inactivated by exposure to 300 MPa at 25 °C within a few minutes; however,

ascospores required treatment at higher pressures.

The pressure sensitivity of viruses is highly variable and cannot readily be

predicted based on genetic classification. Kingsley et al. (2004) investigated the

pressure sensitivity of several picornaviruses including Aichi virus, human

parechovirus-1, and the coxsackievirus strains A9 and B5 and found that different

picornaviruses had widely variable pressure inactivation thresholds and even different virus stains could behave dramatically differently in respond to pressure. For example, a 5-min HPP treatment resulted in 3.4-, 6.5-, and 7.6-log tissue culture infectious dose (50%) ($TCID_{50}$) reductions of coxsackievirus A9 at 400, 500, and 600 MPa, respectively. However, coxsackievirus B5 remained fully infectious even after a 5-min pressure treatment at 600 MPa. The reason for this difference is not fully understood but one hypothesis is that it relates to the receptor-binding mechanisms (Kingsley et al., 2004).

2.7.2.2   Composition, pH, water activity, and temperature of the food

The chemical composition, pH and a_w of the substrate during treatment can have a significant effect on the response of microorganisms to pressure. Certain food constituents such as proteins, carbohydrates and lipids can have a protective effect (Simpson and Gilmour, 1997). A low water activity protects microorganisms against the effects of pressure; however, the nature of the solute is important as a different solute provides different degree of protection (Patterson, 2005). Pressure inactivation of bacterial cells generally is enhanced by exposure to acidic pH (Farkas and Hoover, 2000). Linton et al. (1999) showed that initial pH had a significant effect on inactivation rates of *E. coli* O157:H7 in orange juice. As pH was lowered, the cells were more susceptible to pressure inactivation and sublethally injured cells failed to repair and died more rapidly during subsequent storage of the juice. In addition, the pH of acidic solutions decreases as pressure increases and it has been estimated that in apple juice, there is a pH drop of 0.2 per 100 MPa (Heremans, 1995).

2.7.2.3 Temperature, pressure and holding time

In general, increasing the pressure, time or temperature of the pressure process will increase the number of microorganisms inactivated (with bacterial endospores the exception) (Hogan et al., 2005). Temperature during pressure treatment can have a significant effect on microbial survival. Increased inactivation is usually observed at temperatures above or below 20 °C (Farkas and Hoover, 2000). The combination of elevated temperatures (< 50 °C) with pressure has also been suggested as a practical way to overcome the problem of pressure-resistant strains of vegetative cells. Patterson and Kilpatrick (1998) reported approximately a 6-log reduction of a pressure-resistant *E. coli* O157:H7 in poultry mince and a 5-log reduction in milk using a treatment of 400 MPa at 50 °C for 15 min. Neither heat nor pressure alone could achieve this level of inactivation. In addition, there is a minimum critical pressure below which microbial inactivation by HPP cannot be seen regardless of process time. Some important processing parameters in HPP include the come-up times (period necessary for the HPP unit to reach target pressure), pressure-release times, and changes in temperature due to compression. It is obvious that long come-up time and pressure-release time can add appreciably to the total process time and affect both the quality of treated foods and the inactivation kinetics of microorganisms (Farkas and Hoover, 2000; Hogan et al., 2005). Therefore, consistency and control of these times are important in HPP development.

2.7.3 Mechanisms of bacterial inactivation by HHP

Although the intricate mechanistic details of microbial inactivation still remain uncertain, several theories have been proposed over the years in an attempt to shed light on the mechanism of microbial inactivation by HPP. Pressure-induced damage to

microorganisms can include cell membrane disruption, ribosomal destruction, inactivation of enzymes, inactivation of membrane-bound transport systems, and damage to the proton efflux system (Black et al., 2010).

The cell membrane is generally acknowledged to be the primary site of pressure-induced damage in microorganisms, which results in leakage of intracellular components and loss of homeostasis (Farkas and Hoover, 2000). Evidence of physical damage to the cell membrane has been demonstrated as leakage of ATP or UV-absorbing material from pressure-treated bacterial cells (Smelt et al., 1994) or increased uptake of fluorescent dyes such as propidium iodide that do not normally penetrate membranes of healthy cells (Benito et al., 1999). Manas and Mackey (2004) proposed that exponential-phase cells are inactivated under high pressure by irreversible damage to the cell membrane while stationary-phase cells have a more robust cytoplasmic membrane that can better withstand pressure treatment. Wouters et al. (1998) observed reduced activity of the integral membrane protein F(0)F(1) ATPase in *Lactobacillus plantarum* treated at 250 MPa in addition to impairment of acid reflux and maintenance of intracellular pH.

In addition to membrane damage, enzyme inactivation and protein denaturation are other important factors in the pressure inactivation of microorganisms. Ritz et al. (2000) analyzed the effect of HPP on the membrane proteins of the *Salmonella* Typhimurium. Outer membrane protein content was drastically modified after HPP treatments. In some cases, except for the major proteins OmpA and LamB, other outer membrane proteins appeared to be completely denatured, while a few were resistant. In another study, Ulmer et al. (2000) determined the pressure inactivation of *Lactobacillus plantarum* TMW1.460, a beer-spoiling

organism. It was shown that HPP at 200 MPa caused inactivation of HorA, an ATP binding cassette-type multidrug resistance transporter conferring resistance to hops compounds during beer storage. The inactivation of this protein also resulted in reduced resistance of *L. plantarum* to hops during beer storage.

The ribosome has been suggested as a potential target of HPP inactivation of microorganisms. Niven et al. (1999) analyzed of the effects of HPP treatment on ribosomes in living *E. coli* cells. A close correlation was observed between loss of cell viability and ribosome damage in cells subjected to pressures of 50-250 MPa for 20 min. In pressure-treated cells, the remaining ribosomes had adopted a less stable conformation. However, this change in ribosome conformation had no apparent effect on cell survival, as viability continued to decrease. Alpas et al. (2003) used differential scanning calorimetry to compare the pressure sensitivity of *Staphylococcus aureus* and *E. coli* O157:H7. They found that ribosomal denaturation coincided with cell death.

Taken together, HHP-induced inactivation of microorganisms can occur as a result of one destructive event to the membrane or protein moieties, or more likely, as a result of a combination of pressure-induced effects (Black et al., 2010).

2.7.4   Mechanisms of viral inactivation by HHP

It has not been extensively elucidated how HPP actually inactivates foodborne viruses, but all indications are that high pressure can impact viruses by altering the virus capsid or protein coat (Kingsley, 2013). Virus particles consist of the genetic material made from either DNA or RNA, the protein capsid surrounding the genetic material, and in some cases a lipid bilayer envelope that surrounds the protein capsid. Therefore, it is presumable that viruses can be inactivated by attacking one or multiple

of these components. As high pressure generally does not disrupt covalent bonds, it is generally believed that high pressure does not damage the primary structure of nucleic acids, such as the RNA encoded within these viruses. Thus, high pressure must cause a protein-mediated effect that prevents virus attachment, penetration of the host cell, or uncoating once the virus has entered the cell (Kingsley, 2013). Some recent studies have shed some light on the potential mechanism of pressure inactivation of nonenveloped viruses. Tang et al. (2010) has shown that pressure treatment at 400 MPa for 5 min significantly reduced the receptor binding ability of MNV-1. Pressure-treated MNV-1 lost its infectivity but retained the integrity of its capsid and RNA genome. A subsequent study by Lou et al. (2011) demonstrated that 600 MPa is needed to destroy the integrity of the MNV-1 capsid. Lou et al. (2011) also pointed out that disruption of viral capsid structure, but not degradation of viral genomic RNA, is the primary mechanism of virus inactivation by HPP. However, HPP does not degrade viral capsid protein, and the pressurized capsid protein was still antigenic (Lou et al., 2011).

2.8   Pulsed Light

2.8.1   Principles of PL

Pulsed light (PL), also known as pulsed UV light, high intensity broad spectrum pulsed light and pulsed white light, is an emerging nonthermal technology for rapid inactivation of microorganisms on food surfaces, equipment and food packaging materials. During the pulse, the PL system can deliver a spectrum that is at least 20,000 times more intense than sunlight at the earth's surface (Dunn et al., 1995). The wavelength distribution ranges from 100 to 1100 nm, including UV (100 – 400

nm), visible light (400 – 700 nm), and infrared (700 – 1,100 nm) (Oms-Oliu et al., 2008). In 1996, this technology was adopted by the food industry when it was approved for the use of production, processing and handling of foods by the US Food and Drug Administration (21 CFR 179.41) on the condition that the light pulses of wavelengths is between 200 and 1000 nm, with a pulse width not exceeding 2 µs and a cumulative treatment of less than 12 J/cm^2. Pulses of light used for food processing applications typically emit 1 to 20 flashes per second at an energy density in the range of about 0.01 to 50 J/cm^2 at the surface (Oms-Oliu et al., 2008).

To date, at least two commercial companies produce disinfection systems based on PL. One is SteriBeam Systems from Germany, the other is Xenon Corporation from the USA. The PL unit used in our project was a bench top model, Steripulse-XL RS-3000, purchased from Xenon Corp. The PL unit consists of a controller module, a treatment chamber and an air cooling module. The control module is used to generate a high voltage, which will be used to charge the PL lamp. Basically, AC power is first converted to high-voltage DC power, which is then used to charge a capacitor. During the pulse, a high-voltage switch discharges the energy in the capacitor into a lamp. The control module also controls pulse width, frequency and peak power. The treatment chamber is usually built of stainless steel. It has a shelf to hold the samples, which can be displaced vertically, allowing adjustment of the distance between the target and light source. The light source is a linear Xenon flash lamp located at the top center of the chamber, inside the lamp housing. By using an air blower, a filtered air stream can flow around the lamp serving two functions: dissipating the heat generated by the lamp, and avoiding the accumulation of high

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levels of toxic ozone produced by the shortest wavelengths (Gómez-López et al., 2007). A schematic diagram of a bench top PL unit is shown in Figure 2.2.
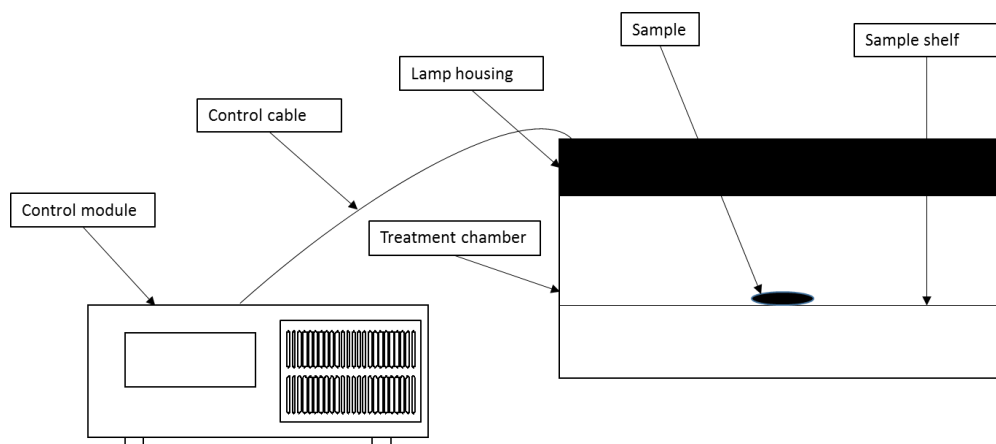
Figure 2.2 Schematic diagram of a bench top PL unit.

In our PL unit, the lamp was capable of generating PL in the wavelength of 200 – 1100 nm, with 40% of the energy being in the UV region (Hsu and Moraru, 2011). The pulses were generated at a rate of 3 pulses/s with a pulse width of 360 μs. According to the manufacturer's specifications, each pulse delivers 1.27 J/cm^2 for an input of 3800 V at 19.3 mm from the quartz window of the lamp.

UV disinfection systems as applied, for instance, to water treatments, have traditionally used low or medium pressure mercury lamps as the source of germicidal radiation. Low pressure mercury lamps generally provide monochromatic radiation at 254 nm, whereas the germicidal wavelength range of medium pressure mercury UV lamps is between 200 and 300 nm (Oms-Oliu et al., 2008). PL is considered an alternative to continuous ultraviolet light treatments for solid and liquid foods due to

several advantages. A comparison of continuous UV and pulse light lamps is shown in Table 2.2.

Table 2.2 Main features of continuous UV and pulsed light

Feature	Continuous UV	Pulsed light
Wavelength	254 nm	200 – 1100 nm (typical)
Peak power	Low (100 – 1000 kW)	High (up to 35 mW)
Treatment time	Long	Short
Inactivation mechanism	Photochemical damage	Photochemical; photophysical and photothermal
Cost	Low	Relatively higher
Mercury	Commonly used as source	Mercury free

2.8.2 Microbial inactivation by PL

An accumulating number of studies have demonstrated a high efficiency of PL on inactivating bacteria, mold spores, and viruses. Rowan et al. (1999) showed approximately 6-log reductions of *L. monocytogenes*, *E. coli* O157:H7, *Salmonella* Enteritidis, *Pseudomonas aeruginosa*, *Bacillus cereus*, and *Staphylococcus aureus* on agar plates by using 200 light pulses with high UV content. Bialka and Demirci (2008) reported that PL treatment of raspberries at 72 J/cm^2 reduced *E. coli* O157 and *Salmonella* by 3.9 and 3.4 log CFU/g. A 3.3 – 4.3 log reduction of *E. coli* O157:H7 and *Salmonella* was achieved on strawberries by PL treatment at 64.8 J/cm^2 but severe damage to the calyx of fruits was reported. Sauer and Moraru (2009) reported that PL could effectively inactivate *E. coli* O157:H7 from liquids with different levels of clarity. In their study, *E. coli* O157:H7 was reduced by 5.76 and 7.15 log in turbulent apple cider and juice, respectively, after a PL treatment at 12 J/cm^2. Ramos-Villarroel

et al. (2011) investigated the impact of PL on inactivation of *L. innocua* and *E. coli* in fresh-cut avocado. PL treatment at 12 J/cm^2 reduced *L. innocua* and *E. coli* by ~3 log units.

While PL inactivation of bacteria pathogens has been well documented, studies on PL inactivation of viruses in foods are still limited. Roberts and Hope (2003) investigated the PL inactivation of a range of enveloped, i.e. Sindbis, herpes simplex virus type 1 and non-enveloped viruses, i.e. encephalomyocarditis, polio virus type 1, hepatitis A (HAV), bovine parvovirus and canine parvovirus. A total dose of 1 J/cm^2 was sufficient to inactivate 4.8-7.2 logs of viruses in phosphate buffered saline. Jean et al. (2011) evaluated the PL inactivation of MNV and HAV in liquid suspension and on stainless steel and polyvinyl chloride disks. A 5-log reduction was reported for a 2-s treatment in the absent of fetal bovine serum (FBS) in different conditions, but a lower reduction rate of viruses was observed for the same treatment in the presence of FBS (~3 logs). Daryany et al. (2009) also reported similar findings that FBS can reduce the efficacy of PL treatment in liquid. Lamont et al. (2007) investigated the inactivation effect of PL on poliovirus and adenovirus in PBS suspension, where 10 and 200 pulses were needed to obtain a > 4-log reduction of poliovirus and adenovirus, respectively.

2.8.3   Factors affecting the efficacy of PL

The most important factor determining the efficacy of PL is the fluence (J/cm^2), the energy received from the lamp by the sample per unit area during the treatment. Therefore, the energy emitted by the flash lamp is different from the energy incident on the sample. Factors such as distance from light source to target, propagation vehicle (air, water, fruit juice), and surface topography affect the level of energy that ultimately reaches the target (Gómez-López et al., 2007).

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Generally, PL fluence decays away from the lamp source due to light absorption and scattering phenomena. Hsu and Moraru (2011) quantified and mapped the spatial distribution of both total and PL fluence both in air and in liquid substrates including Butterfield's phosphate buffer (BPB), tryptic soy broth (TSB), and apple juice (clarified apple cider) with different optical properties. Fluence was measured at incremental distances from the lamp, along the x-, y- and z-axes. Their result showed that PL fluence decreased with increasing distance from the lamp, in all three directions. Colored liquids tended to absorb more total energy than clearer liquids, thereby decays of PL fluence in TSB and apple juice were much pronounced than BPB.

Surface topography has a significant effect in light-based treatments such as with UV radiation or PL. Woodling and Moraru (2005) investigated the effect of surface topography on PL inactivation using stainless steel of different surface roughness. The stainless steel coupons were inoculated with *L. innocua* and subjected to various doses of PL treatments. Their results showed that the inactivation levels for the two smoothest surfaces were not statistically different from each other, but were significantly lower than for the two roughest surfaces. The experimental data also suggested that, in addition to topography, reflectivity and surface hydrophobicity had also limited the effectiveness of PL treatment.

Food composition also may affect the efficacy of PL treatment. Gómez-López et al. (2005) treated *Photobacterium phosphoreum*, *L. monocytogenes* and *Candida lambica* inoculated onto surfaces of agars supplemented with several food components and demonstrated that proteins and oil decreased the decontaminant efficacy of PL, whereas water and starch did not. Roberts and Hope (2003) also found that the

addition of protein to a buffered saline solution decreased the efficacy of virus inactivation.

2.8.4   Mechanisms of microbial inactivation by PL

The mechanisms involved in microbial inactivation by PL are not yet fully elucidated, but it is generally accepted that the rich broad-spectrum UV region is the most important part of PL spectrum that contribute to microbial inactivation. In addition, it has been suggest that both the visible and infrared regions, combined with the high peak power of pulsed light, contribute to microbial inactivation, due to photothermal and photophysical mechanisms (Elmnasser et al., 2007; Wekhof, 2001, 2000; Kramer and Muranyi, 2013; Takeshita et al., 2003).

2.8.4.1   Photochemical mechanism

UV is regarded as the most important part of the PL spectrum. The germicidal effect of UV light has been attributed primarily due to a photochemical transformation of pyrimidine bases in the DNA/RNA of bacteria, viruses, and other pathogens to form dimers (Giese and Darby, 2000; Mitchell et al., 1992). Without sufficient repair mechanisms, such damage results in mutations, impaired replication and gene transcription, and ultimately the death of the organism (Elmnasser et al., 2007). Though cyclobutyl pyrimidine dimer formation is the main inactivation mechanism, there are other photoproducts formed during UV exposure including pyrimidine pyrimidinone [6-4]-photoproduct, Dewar pyrimidinone, adenine-thymine heterodimer, cytosine photohydrate, thymine photohydrates, single-strand break, and DNA-protein cross-linking (Demirci and Krishnamurthy, 2011). Wang et al. (2005) showed that the germicidal efficiency of pulsed UV on *E. coli* is a function of wavelength within the

range 230 – 300 nm, with a maximum effect at 270 nm. No inactivation can be observed above 300 nm. Woodling and Moraru (2007) treated stainless-steel coupons inoculated with *Listeria innocua* with PL and compared the effects of the full spectrum (180 to 1,100 nm) with the effects obtained when only certain regions of UV, visible, and near-infrared light were used. At a fluence of about 6 J/cm^2, the full-spectrum, PL treatment resulted in a 4.08-log reduction of *L. innocua* on coupon surface, the removal of wavelength < 200 nm diminished the reduction to only 1.64 log, and total elimination of UV light resulted in no lethal effects on *L. innocua*. Their data demonstrated that the portions of the PL spectrum responsible for bacterial inactivation were located in the range < 300 nm (the UV-B and UV-C ranges), with some bacterial inactivation taking place as a result to exposure to wavelength between 300 and 400 nm (UV-A) and no inactivation observed upon exposure to visible and near-infrared light (wavelength > 400 nm).

Photoreactivation is a well-known phenomenon in the continuous UV treatment field, which means the reversal of UV damage in bacteria by illumination with visible light. It is catalyzed by the enzyme photolyase, which uses light energy to split UV-induced cyclobutane dimers in damaged DNA through a radical mechanism (Walker, 1984). However, the enzymatic repair of DNA was not observed after PL treatment. The degree of damage caused by PL was thought to be too severe for repair mechanisms to operate (Elmnasser et al., 2007). It is conceivable that the DNA repair system itself is inactivated, as well as other enzymatic functions (Dunn et al., 1995; Mcdonald et al., 2000; Smith et al., 2002).

2.8.4.2 Photothermal mechanism

The lethal effect of PL treatment can also be due to a photothermal mechanism. Wekhof (2000) proposed that with an energy exceeding 0.5 J/cm^2, disinfection is achieved through bacterial disruption during temporary overheating resulting from the absorption of all UV light from a flash lamp. This overheating was thought to be due to a difference in UV light absorption by bacterial cells and that of a surrounding medium. Water in bacteria was vaporized by the heat, generating a small steam flow that induces membrane disruption (Takeshita et al., 2003). Moreover, Wekhof (2001) showed a ruptured top of treated *Aspergillus niger* spores evidently punctured by an escape of overheated contents of the spore, which became empty after such an internal 'explosion' resulted in an 'evacuation' of its contents during the PL treatment.

2.8.4.3 Photophysical mechanism

The impact of PL on proteins, membranes, and cellular material is reported in several studies and is thought to be another potential inactivation mechanism. Takeshita et al. (2003) compared the damage of yeast cells induced by both continuous UV and PL. DNA damage, such as formation of single strand breaks and pyrimidine dimers was observed in yeast cells after PL treatment, which were essentially the same as observed with continuous UV. However, they only observed increased concentration of eluted protein and structural change in the PL-irradiated yeast cells, which suggested cell membrane damage induced by PL treatment. A similar phenomenon was observed by Cheigh et al. (2012) who compared the microbial inactivation of *L. monocytogenes* and *E. coli* O157:H7 and cell damage induced by PL and continuous UV-C. Transmission electron microscopy showed that

bacterial cell structures were destroyed by PL treatment but not by UV-C treatment. Kramer and Muranyi (2014) recently demonstrated the cellular impact of PL treatments on *L. innocua* and *E. coli*. Their reported that the shutdown of cellular functions such as the depolarization of cell membranes, the loss of metabolic, esterase and pump activities or the occurrence of membrane damage occurred at considerably higher fluences than loss of cultivability. The loss of cultivability was on the other hand directly accompanied by the formation of reactive oxygen species and DNA damage. Wuytack et al. (2003) concluded that PL inactivation should be regarded as a multi-target process, where structural changes to nucleic acid would be a major factor, and damage to membranes, proteins and other macromolecules plays a minor role.

REFERENCES

Alpas, H., Lee, J., Bozoglu, F., Kaletunç, G., 2003. Evaluation of high hydrostatic pressure sensitivity of Staphylococcus aureus and Escherichia coli O157:H7 by differential scanning calorimetry. Int. J. Food Microbiol. 87, 229–37.

Atmar, R.L., Opekun, A.R., Gilger, M.A., Estes, M.K., Crawford, S.E., Neill, F.H., Graham, D.Y., 2008. Norwalk virus shedding after experimental human infection. Emerg. Infect. Dis. 14, 1553–7. doi:10.3201/eid1410.080117

Azar Daryany, M.K., Hosseini, S.M., Raie, M., Fakharie, J., Zareh, A., 2009. Study on continuous (254 nm) and pulsed UV (266 and 355 nm) lights on BVD virus inactivation and its effects on biological properties of fetal bovine serum. J. Photochem. Photobiol. B. 94, 120–4. doi:10.1016/j.jphotobiol.2008.10.009

Baert, L., Mattison, K., Loisy-Hamon, F., Harlow, J., Martyres, A., Lebeau, B., Stals, A., Van Coillie, E., Herman, L., Uyttendaele, M., 2011. Review: norovirus prevalence in Belgian, Canadian and French fresh produce: a threat to human health? Int. J. Food Microbiol. 151, 261–9. doi:10.1016/j.ijfoodmicro.2011.09.013

Baert, L., Uyttendaele, M., Stals, A., VAN Coillie, E., Dierick, K., Debevere, J., Botteldoorn, N., 2009. Reported foodborne outbreaks due to noroviruses in Belgium: the link between food and patient investigations in an international context. Epidemiol. Infect. 137, 316–25. doi:10.1017/S0950268808001830

Baert, L., Uyttendaele, M., Van Coillie, E., Debevere, J., 2008. The reduction of murine norovirus 1, B. fragilis HSP40 infecting phage B40-8 and *E. coli* after a mild thermal pasteurization process of raspberry puree. Food Microbiol. 25, 871–4. doi:10.1016/j.fm.2008.06.002

Balasubramaniam, V.M., Farkas, D., Turek, E.J., 2008. Preserving Foods through High-Pressure Processing. Food Technol. 62, 32–38.

Belliot, G., Sosnovtsev, S. V, Mitra, T., Hammer, C., Garfield, M., Green, K.Y., 2003. In vitro proteolytic processing of the MD145 norovirus ORF1 nonstructural polyprotein yields stable precursors and products similar to those detected in calicivirus-infected cells. J. Virol. 77, 10957–74.

Benito, A., Ventoura, G., Casadei, M., Robinson, T., Mackey, B., 1999. Variation in resistance of natural isolates of *Escherichia coli* O157 to high hydrostatic pressure, mild heat, and other stresses. Appl. Environ. Microbiol. 65, 1564–9.

Berger, C.N., Sodha, S. V, Shaw, R.K., Griffin, P.M., Pink, D., Hand, P., Frankel, G., 2010. Fresh fruit and vegetables as vehicles for the transmission of human pathogens. Environ. Microbiol. 12, 2385–97. doi:10.1111/j.1462-2920.2010.02297.x

Bertolotti-Ciarlet, A., Crawford, S.E., Hutson, A.M., Estes, M.K., 2003. The 3' end of Norwalk virus mRNA contains determinants that regulate the expression and stability of the viral capsid protein VP1: a novel function for the VP2 protein. J. Virol. 77, 11603–15.

Beuchat, L.R., 1998. Surface decontamination of fruits and vegetables eaten raw : a review. Geneva : World Health Organization.

Beuchat, L.R., 2002. Ecological factors influencing survival and growth of human pathogens on raw fruits and vegetables. Microbes Infect. 4, 413–423. doi:10.1016/S1286-4579(02)01555-1

Beuchat, L.R., Ryu, J.H., 1997. Produce handling and processing practices. Emerg. Infect. Dis. 3, 459–65. doi:10.3201/eid0304.970407

Bhunia, A., 2008. Foodborne microbial pathogens : mechanisms and pathogenesis. Springer, New York.

Bialka, K., Demirci, A., 2007. Efficacy of aqueous ozone for the decontamination of *Escherichia coli* O157: H7 and *Salmonella* on raspberries and strawberries. J. Food Prot. 70, 1088–1092.

Bialka, K.L., Demirci, A., 2007. Decontamination of *Escherichia coli* O157:H7 and *Salmonella enterica* on blueberries using ozone and pulsed UV-light. J. Food Sci. 72, M391–6. doi:10.1111/j.1750-3841.2007.00517.x

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. J. Food Sci. 73, M201–M207. doi:10.1111/j.1750-3841.2008.00743.x

Bidawid, S., Farber, J.., Sattar, S.., 2000. Inactivation of hepatitis A virus (HAV) in fruits and vegetables by gamma irradiation. Int. J. Food Microbiol. 57, 91–97. doi:10.1016/S0168-1605(00)00235-X

Black, E.P., Stewart, C.M., Hoover, D.G., 2010. Microbiological Aspects of High-Pressure Food Processing, in: Zhang, H.Q., Barbosa-Cánovas, G. V., Balasubramaniam, V.M., Dunne, C.P., Farkas, D.F., Yuan, J.T.C. (Eds.), Nonthermal Processing Technologies for Food. Wiley-Blackwell, Oxford, UK, pp. 51–71. doi:10.1002/9780470958360

Boriss, H., Brunke, H., Kreith, M., 2014. Commodity Strawberry Profile URL http://www.agmrc.org/commodities__products/fruits/strawberries/commodity-strawberry-profile/ (accessed 12.11.14).

Bower, C., 2007. Postharvest handling, storage, and treatment of fresh market berries, in: Zhao, Y. (Ed.), Berry Fruit: Value-Added Products for Health Promotion. CRC Press, pp. 261–288.

Brackett, R., 1999. Incidence, contributing factors, and control of bacterial pathogens in produce. Postharvest Biol. Technol. 15, 305–311. doi:10.1016/S0925-5214(98)00096-9

Brassard, J., Gagné, M.-J., Généreux, M., Côté, C., 2012. Detection of human food-borne and zoonotic viruses on irrigated, field-grown strawberries. Appl. Environ. Microbiol. 78, 3763–6. doi:10.1128/AEM.00251-12

Brennan, R., Caligari, P., Clark, J., Brás de Oliveira, P., Finn, C., Hancock, J., Jarret, D., Lobos, G., Raffle, S., Simpson, D., 2014. Berry crops, in: Dixon, G. (Ed.), Horticulture. Volume 1, Production Horticulture : Plants for People and Places. Springer, pp. 301–322.

Bresee, J.S., Widdowson, M.-A., Monroe, S.S., Glass, R.I., 2002. Foodborne viral gastroenteritis: challenges and opportunities. Clin. Infect. Dis. 35, 748–53. doi:10.1086/342386

Brown, G.K., Schulte, N.L., Timm, E.J., Beaudry, R.M., Peterson, D.L., 1996. Estimates of mechanization effects on fresh blueberry quality. Appl. Eng. Agric. 12, 21 – 26.

Butot, S., Putallaz, T., Sánchez, G., 2007. Procedure for rapid concentration and detection of enteric viruses from berries and vegetables. Appl. Environ. Microbiol. 73, 186–92. doi:10.1128/AEM.01248-06

Butot, S., Putallaz, T., Sánchez, G., 2008. Effects of sanitation, freezing and frozen storage on enteric viruses in berries and herbs. Int. J. Food Microbiol. 126, 30–5. doi:10.1016/j.ijfoodmicro.2008.04.033

Butz, P., Funtenberger, S., Haberditzl, T., Tauscher, B., 1996. High pressure inactivation of byssochlamys nivea ascospores and other heat resistant moulds. Food Sci. Technol. 29, 404–410. doi:10.1006/fstl.1996.0062

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. Epidemiol. Infect. 131, 745–751.

Cannon, J.L., Papafragkou, E., Park, G.W., Osborne, J., Jaykus, L.-A., Vinje, J., 2006. Surrogates for the study of norovirus stability and inactivation in the environment: A comparison of murine norovirus and feline calicivirus. J. Food Prot. 69, 2761–2765.

Cao, S., Lou, Z., Tan, M., Chen, Y., Liu, Y., Zhang, Z., Zhang, X.C., Jiang, X., Li, X., Rao, Z., 2007. Structural basis for the recognition of blood group trisaccharides by norovirus. J. Virol. 81, 5949–57. doi:10.1128/JVI.00219-07

CDC, 1997. Outbreak of *Cyclosporiasis* -- Northern Virginia-Washington, D.C.-Baltimore, Maryland, Metropolitan Area, 1997.

CDC, 2010. CDC Reports 1 in 6 Get Sick from Foodborne Illnesses Each Year. URL http://www.cdc.gov/media/pressrel/2010/r101215.html (accessed 12.12.14).

CDC, 2011. Updated Norovirus Outbreak Management and Disease Prevention Guidelines.

CDC, Foodborne Outbreak Online Database (FOOD). URL http://wwwn.cdc.gov/foodborneoutbreaks/ (accessed 12.12.14)

CFR - Code of Federal Regulations, 1996. 21 CFR 179.41.

CFR - Code of Federal Regulations, 2014. 21 CFR 179.26.

Chan, M.C.W., Wong, Y.P., Leung, W.K., 2007. Cell culture assay for human noroviruses. Emerg. Infect. Dis. 13, 1117; author reply 1117–8. doi:10.3201/eid1307.070131

Cheigh, C.-I., Park, M.-H., Chung, M.-S., Shin, J.-K., Park, Y.-S., 2012. Comparison of intense pulsed light- and ultraviolet (UVC)-induced cell damage in *Listeria monocytogenes* and *Escherichia coli* O157:H7. Food Control 25, 654–659. doi:10.1016/j.foodcont.2011.11.032

Cody, S.H., Glynn, M.K., Farrar, J.A., Cairns, K.L., Griffin, P.M., Kobayashi, J., Fyfe, M., Hoffman, R., King, A.S., Lewis, J.H., Swaminathan, B., Bryant, R.G., Vugia, D.J., 1999. An outbreak of *Escherichia coli* O157:H7 infection from unpasteurized commercial apple juice. Ann. Intern. Med. 130, 202–9.

Cooley, M., Carychao, D., Crawford-Miksza, L., Jay, M.T., Myers, C., Rose, C., Keys, C., Farrar, J., Mandrell, R.E., 2007. Incidence and tracking of *Escherichia coli* O157:H7 in a major produce production region in California. PLoS One 2, e1159. doi:10.1371/journal.pone.0001159

Cspinet, n.d. Outbreak Alert! Database. URL http://www.cspinet.org/foodsafety/outbreak/pathogen.php (accessed 12.12.14).

Dancho, B.A., Chen, H., Kingsley, D.H., 2012. Discrimination between infectious and non-infectious human norovirus using porcine gastric mucin. Int. J. Food Microbiol. 155, 222–6. doi:10.1016/j.ijfoodmicro.2012.02.010

Davis, H., Taylor, J.P., Perdue, J.N., Stelma, G.N., Humphreys, J.M., Rowntree, R., Greene, K.D., 1988. A shigellosis outbreak traced to commercially distributed shredded lettuce. Am. J. Epidemiol. 128, 1312–21.

Dawson, D., 2005. Foodborne protozoan parasites. Int. J. Food Microbiol. 103, 207–27. doi:10.1016/j.ijfoodmicro.2004.12.032

Demirci, A., Krishnamurthy, K., 2011. Pulsed Ultraviolet Light, in: Zhang, H.Q., Barbosa-Cánovas, G. V., Balasubramaniam, V.M., Dunne, C.P., Farkas, D.F., Yuan, J.T.C. (Eds.), Nonthermal Processing Technologies for Food. Wiley-Blackwell, pp. 249–261.

Deuel, C.L., Plotto, A., 2004. Strawberries and Raspberries, in: Barrett, D.M.., Somogyi, L.P., Ramaswamy, H.S.. (Eds.), Processing Fruits. CRC Press, pp. 531–561.

Donaldson, E.F., Lindesmith, L.C., Lobue, A.D., Baric, R.S., 2008. Norovirus pathogenesis: mechanisms of persistence and immune evasion in human populations. Immunol. Rev. 225, 190–211. doi:10.1111/j.1600-065X.2008.00680.x

Doyle, M.P., Erickson, M.C., 2008. Summer meeting 2007 - the problems with fresh produce: an overview. J. Appl. Microbiol. 105, 317–30. doi:10.1111/j.1365-2672.2008.03746.x

Duizer, E., Schwab, K.J., Neill, F.H., Atmar, R.L., Koopmans, M.P.G., Estes, M.K., 2004. Laboratory efforts to cultivate noroviruses. J. Gen. Virol. 85, 79–87.

Dunn, J., Ott, T., Clark, W., 1995. Pulsed-Light Treatment of Food and Packaging. Food Technol. 49, 95–98.

Elmnasser, N., Guillou, S., Leroi, F., Orange, N., Bakhrouf, a, Federighi, M., 2007. Pulsed-light system as a novel food decontamination technology: a review. Can. J. Microbiol. 53, 813–21. doi:10.1139/W07-042

Erickson, M.C., Webb, C.C., Diaz-Perez, J.C., Phatak, S.C., Silvoy, J.J., Davey, L., Payton, A.S., Liao, J., Ma, L., Doyle, M.P., 2010. Infrequent internalization of *Escherichia coli* O157:H7 into field-grown leafy greens. J. Food Prot. 73, 500–6.

Fankhauser, R.L., Monroe, S.S., Noel, J.S., Humphrey, C.D., Bresee, J.S., Parashar, U.D., Ando, T., Glass, R.I., 2002. Epidemiologic and molecular trends of "Norwalk-like viruses" associated with outbreaks of gastroenteritis in the United States. J. Infect. Dis. 186, 1–7. doi:10.1086/341085

Farkas, D.F., Hoover, D.G., 2000. Kinetics of microbial inactivation for alternative food processing technologies - HPP. J. Food Sci. 65, 47–64.

Farkas, T., Cross, R.W., Hargitt, E., Lerche, N.W., Morrow, A.L., Sestak, K., 2010. Genetic diversity and histo-blood group antigen interactions of rhesus enteric caliciviruses. J. Virol. 84, 8617–25. doi:10.1128/JVI.00630-10

Farkas, T., Sestak, K., Wei, C., Jiang, X., 2008. Characterization of a rhesus monkey calicivirus representing a new genus of Caliciviridae. J. Virol. 82, 5408–16. doi:10.1128/JVI.00070-08

FDA, 2001. FDA Survey of Imported Fresh Produce FY 1999 Field Assignment. URL http://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatory Information/ProducePlantProducts/ucm118891.htm (accessed 12.12.14).

FDA, 2007. FDA/CFSAN - Food Safety A to Z Reference Guide. URL http://web.archive.org/web/20090302092542/http://www.cfsan.fda.gov/~dms/a 2z-s.html (accessed 12.22.14).

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. J. Food Prot. 71, 908–913.

Fratamico, P.M., Smith, J.L., 2006. *Escherichia coli*, in: Cliver, D., Potter, M., Riemann, H. (Eds.), Foodborne Infections and Intoxications. Academic Press, pp. 205–239.

Friedman, D.S., Heisey-Grove, D., Argyros, F., Berl, E., Nsubuga, J., Stiles, T., Fontana, J., Beard, R.S., Monroe, S., McGrath, M.E., Sutherby, H., Dicker, R.C., DeMaria, A., Matyas, B.T., 2005. An outbreak of norovirus gastroenteritis associated with wedding cakes. Epidemiol. Infect. 133, 1057–63. doi:10.1017/S0950268805004760

Giese, N., Darby, J., 2000. Sensitivity of microorganisms to different wavelengths of UV light: implications on modeling of medium pressure UV systems. Water Res. 34, 4007–4013. doi:10.1016/S0043-1354(00)00172-X

Girard, K., Sinha, N., 2006. Cranberry, Blueberry, Currant, and Gooseberry, in: Hui, Y.H. (Ed.), Handbook of Fruits and Fruit Processing. BLACKWELL PUBLISHING, pp. 369–390.

Gómez-López, V.M., Devlieghere, F., Bonduelle, V., Debevere, J., 2005. Intense light pulses decontamination of minimally processed vegetables and their shelf-life. Int. J. Food Microbiol. 103, 79–89. doi:10.1016/j.ijfoodmicro.2004.11.028

Gómez-López, V.M., Ragaert, P., Debevere, J., Devlieghere, F., 2007. Pulsed light for food decontamination: a review. Trends Food Sci. Technol. 18, 464–473. doi:10.1016/j.tifs.2007.03.010

Gould, G.W., Sale, A.J.H., 1970. Initiation of Germination of Bacterial Spores by Hydrostatic Pressure. J. Gen. Microbiol. 60, 335–346. doi:10.1099/00221287-60-3-335

Gould, L.H., Walsh, K.A., Vieira, A.R., Herman, K., Williams, I.T., Hall, A.J., Cole, D., 2013. Surveillance for foodborne disease outbreaks - United States, 1998-2008. MMWR Surveill. Summ. 62, 1–34.

Gray, J.T., Fedorka-Cray, P.J., 2002. *Salmonella*, in: Cliver, D.O., Riemann, H.P. (Eds.), Foodborne Diseases. Academic Press, pp. 55–67.

Green, K.Y., Ando, T., Balayan, M.S., Berke, T., Clarke, I.N., Estes, M.K., Matson, D.O., Nakata, S., Neill, J.D., Studdert, M.J., Thiel, H.J., 2000. Taxonomy of the caliciviruses. J. Infect. Dis. 181 Suppl , S322–30. doi:10.1086/315591

Gurtler, J.B., Bailey, R.B., Jin, T.Z., Fan, X., 2014. Reduction of an *E. coli* O157:H7 and *Salmonella* composite on fresh strawberries by varying antimicrobial washes and vacuum perfusion. Int. J. Food Microbiol. 189, 113–8. doi:10.1016/j.ijfoodmicro.2014.08.005

Han, Y., Selby, T., 2004. Decontamination of strawberries using batch and continuous chlorine dioxide gas treatments. J. Food Prot. 67, 2450–2455.

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. Compr. Rev. Food Sci. Food Saf. 2, 78–141. doi:10.1111/j.1541-4337.2003.tb00031.x

Heremans, K., 1995. High pressure effects on biomolecules, in: Ledward, D.A., Johnston, D.E., Earnshaw, R.G., Hasting, A.P.M. (Eds.), High Pressure Processing of Foods. Nottingham University Press., pp. 81–97.

Herwaldt, B., Beach, M., 1999. The return of *Cyclospora* in 1997: Another outbreak of cyclosporiasis in North America associated with imported raspberries. Ann. Intern. Med. 130, 210–220.

Herwaldt, B.L., Ackers, M.L., 1997. An outbreak in 1996 of cyclosporiasis associated with imported raspberries. The *Cyclospora* Working Group. N. Engl. J. Med. 336, 1548–56. doi:10.1056/NEJM199705293362202

Hirneisen, K.A., Black, E.P., Cascarino, J.L., Fino, V.R., Hoover, D.G., Kniel, K.E., 2010. Viral Inactivation in Foods: A review of traditional and novel food-processing technologies. Compr. Rev. Food Sci. Food Saf. 9, 3–20. doi:10.1111/j.1541-4337.2009.00092.x

Hirneisen, K.A., Kniel, K.E., 2013. Comparing human norovirus surrogates: murine norovirus and Tulane virus. J. Food Prot. 76, 139–43. doi:10.4315/0362-028X.JFP-12-216

Hite, B., 1899. The effect of pressure in the preservation of milk : a preliminary report. West Virginia Agricultural Experiment Station, Morgantown W.V.

Hogan, E., Kelly, A.L., Sun, D., 2005. Emerging Technologies for Food Processing, in: Sun, D.W. (Ed.), Emerging Technologies for Food Processing. Elsevier, pp. 3–32. doi:10.1016/B978-012676757-5/50003-7

Hsu, L., Moraru, C.I., 2011. Quantifying and mapping the spatial distribution of fluence inside a pulsed light treatment chamber and various liquid substrates. J. Food Eng. 103, 84–91. doi:10.1016/j.jfoodeng.2010.10.002

Huang, R., Li, X., Huang, Y., Chen, H., 2014. Strategies to enhance high pressure inactivation of murine norovirus in strawberry puree and on strawberries. Int. J. Food Microbiol. 185, 1–6. doi:10.1016/j.ijfoodmicro.2014.05.007

Huang, Y., Chen, H., 2014. A novel water-assisted pulsed light processing for decontamination of blueberries. Food Microbiol. 40, 1–8.

Hutin, Y.J., Pool, V., Cramer, E.H., Nainan, 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. N. Engl. J. Med. 340, 595–602. doi:10.1056/NEJM199902253400802

Hutson, A.M., Atmar, R.L., Marcus, D.M., Estes, M.K., 2003. Norwalk virus-like particle hemagglutination by binding to h histo-blood group antigens. J. Virol. 77, 405–15.

Jay, M.T., Cooley, M., Carychao, D., Wiscomb, G.W., Sweitzer, R.A., Crawford-Miksza, L., Farrar, J.A., Lau, D.K., O'Connell, J., Millington, A., Asmundson, R. V, Atwill, E.R., Mandrell, R.E., 2007. *Escherichia coli* O157:H7 in feral swine near spinach fields and cattle, central California coast. Emerg. Infect. Dis. 13, 1908–11. doi:10.3201/eid1312.070763

Jean, J., Morales-Rayas, R., Anoman, M.-N., Lamhoujeb, S., 2011. Inactivation of hepatitis A virus and norovirus surrogate in suspension and on food-contact surfaces using pulsed UV light (pulsed light inactivation of food-borne viruses). Food Microbiol. 28, 568–72. doi:10.1016/j.fm.2010.11.012

Jiang, X., Wang, M., Wang, K., Estes, M.K., 1993. Sequence and genomic organization of Norwalk virus. Virology 195, 51–61. doi:10.1006/viro.1993.1345

Jones, M.K., Watanabe, M., Zhu, S., Graves, C.L., Keyes, L.R., Grau, K.R., Gonzalez-Hernandez, M.B., Iovine, N.M., Wobus, C.E., Vinje, J., Tibbetts, S.A., Wallet, S.M., Karst, S.M., 2014. Enteric bacteria promote human and mouse norovirus infection of B cells. Science. 346, 755–759. doi:10.1126/science.1257147

Joseph, S.W., Ingram, D.T., Kaper, J.B., 2002. The epidemiology, pathogenicity and microbiology of foodborne *Escherichia coli* O157:H7. Rev. Med. Microbiol. 13, 53–62.

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. J. Virol. 10, 1075–1081.

Karst, S.M., 2010. Pathogenesis of noroviruses, emerging RNA viruses. Viruses 2, 748–81. doi:10.3390/v2030748

Karst, S.M., Wobus, C.E., Lay, M., Davidson, J., Virgin, H.W., 2003. STAT1-dependent innate immunity to a Norwalk-like virus. Science 299, 1575–8. doi:10.1126/science.1077905

Katz, D., 1999. Research article. Cyclosporiasis associated with imported raspberries, Florida, 1996. Public Health Rep. 114, 427–438. doi:10.1093/phr/114.5.427

Kim, C., Hung, Y.-C., 2012. Inactivation of *E. coli* O157:H7 on blueberries by electrolyzed water, ultraviolet light, and ozone. J. Food Sci. 77, M206–11. doi:10.1111/j.1750-3841.2011.02595.x

Kingsley, D.H., 2013. High pressure processing and its application to the challenge of virus-contaminated foods. Food Environ. Virol. 5, 1–12. doi:10.1007/s12560-012-9094-9

Kingsley, D.H., Chen, H., Hoover, D.G., 2004. Inactivation of selected picornaviruses by high hydrostatic pressure. Virus Res. 102, 221–4. doi:10.1016/j.virusres.2004.01.030

Kingsley, D.H., Guan, D., Hoover, D.G., 2005. Pressure inactivation of hepatitis A virus in strawberry puree and sliced green onions. J. Food Prot. 68, 1748–1751.

Kingsley, D.H., Vincent, E.M., Meade, G.K., Watson, C.L., Fan, X., 2014. Inactivation of human norovirus using chemical sanitizers. Int. J. Food Microbiol. 171, 94–9. doi:10.1016/j.ijfoodmicro.2013.11.018

Kniel, K.E., Shearer, A.E.H., 2009. Berry contamination: outbreaks and contamination issues, in: Sapers, G.M., Solomon, E.B., Matthews, K.R. (Eds.), The Produce Contamination Problem. Elsevier, pp. 271–305. doi:10.1016/B978-0-12-374186-8.00012-4

Knudsen, D.M., Yamamoto, S.A., Harris, L.J., 2001. Survival of *Salmonella* spp . and *Escherichia coli* O157 : H7 on fresh and frozen strawberries 64, 1483–1488.

Konowalchuk, J., Speirs, J.I., Stavric, S., 1977. Vero response to a cytotoxin of *Escherichia coli*. Infect. Immun. 18, 775–9.

Kramer, B., Muranyi, P., 2013. Effect of pulsed light on structural and physiological properties of *Listeria innocua* and *Escherichia coli*. J. Appl. Microbiol. 116, 596–611.

Laidler, M.R., Tourdjman, M., Buser, G.L., Hostetler, T., Repp, K.K., Leman, R., Samadpour, M., Keene, W.E., 2013. *Escherichia coli* O157:H7 infections associated with consumption of locally grown strawberries contaminated by deer. Clin. Infect. Dis. 57, 1129–34. doi:10.1093/cid/cit468

Lamont, Y., Rzezutka, A., Anderson, J.G., MacGregor, S.J., Given, M.J., Deppe, C., Cook, N., 2007. Pulsed UV-light inactivation of poliovirus and adenovirus. Lett. Appl. Microbiol. 45, 564–7. doi:10.1111/j.1472-765X.2007.02261.x

Lang, N.L., Smith, S.R., 2007. Influence of soil type, moisture content and biosolids application on the fate of *Escherichia coli* in agricultural soil under controlled laboratory conditions. J. Appl. Microbiol. 103, 2122–31. doi:10.1111/j.1365-2672.2007.03490.x

León-Félix, J., Martínez-Bustillos, R.A., Báez-Sañudo, M., Peraza-Garay, F., Chaidez, C., 2010. Norovirus contamination of bell pepper from handling during harvesting and packing. Food Environ. Virol. 2, 211–217. doi:10.1007/s12560-010-9048-z

Li, J., Predmore, A., Divers, E., Lou, F., 2012. New interventions against human norovirus: progress, opportunities, and challenges. Annu. Rev. Food Sci. Technol. 3, 331–52. doi:10.1146/annurev-food-022811-101234

Lindesmith, L., Moe, C., Marionneau, S., Ruvoen, N., Jiang, X., Lindblad, L., Stewart, P., LePendu, J., Baric, R., 2003. Human susceptibility and resistance to Norwalk virus infection. Nat. Med. 9, 548–53. doi:10.1038/nm860

Lindesmith, L.C., Donaldson, E.F., Lobue, A.D., Cannon, J.L., Zheng, D.P., Vinje, J., Baric, R.S., 2008. Mechanisms of GII.4 norovirus persistence in human populations. PLoS Med. 5, e31. doi:10.1371/journal.pmed.0050031

Linton, M., McClements, J.M., Patterson, M.F., 1999. Survival of *Escherichia coli* O157:H7 during storage in pressure-treated orange juice. J. Food Prot. 62, 1038–40.

Lou, F., Neetoo, H., Chen, H., Li, J., 2011. Inactivation of a human norovirus surrogate by high-pressure processing: effectiveness, mechanism, and potential application in the fresh produce industry. Appl. Environ. Microbiol. 77, 1862–71. doi:10.1128/AEM.01918-10

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. J. Food Prot. 66, 188–93.

Luo, Y., Nou, X., Millner, P., Zhou, B., Shen, C., Yang, Y., Wu, Y., Wang, Q., Feng, H., Shelton, D., 2012. A pilot plant scale evaluation of a new process aid for enhancing chlorine efficacy against pathogen survival and cross-contamination during produce wash. Int. J. Food Microbiol. 158, 133–9. doi:10.1016/j.ijfoodmicro.2012.07.008

Lynch, M.F., Tauxe, R. V, Hedberg, C.W., 2009. The growing burden of foodborne outbreaks due to contaminated fresh produce: risks and opportunities. Epidemiol. Infect. 137, 307–15. doi:10.1017/S0950268808001969

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 Environ. Virol. 5, 162–168. doi:10.1007/s12560-013-9118-0

Manas, P., Mackey, B.M., 2004. Morphological and physiological changes induced by high hydrostatic pressure in exponential- and stationary-phase cells of *Escherichia coli*: relationship with cell death. Appl. Environ. Microbiol. 70, 1545–1554. doi:10.1128/AEM.70.3.1545-1554.2004

Martella, V., Campolo, M., Lorusso, E., Cavicchio, P., Camero, M., Bellacicco, A.L., Decaro, N., Elia, G., Greco, G., Corrente, M., Desario, C., Arista, S., Banyai, K., Koopmans, M., Buonavoglia, C., 2007. Norovirus in captive lion cub (Panthera leo). Emerg. Infect. Dis. 13, 1071–3. doi:10.3201/eid1307.070268

Martella, V., Lorusso, E., Decaro, N., Elia, G., Radogna, A., D'Abramo, M., Desario, C., Cavalli, A., Corrente, M., Camero, M., Germinario, C.A., Bányai, K., Di Martino, B., Marsilio, F., Carmichael, L.E., Buonavoglia, C., 2008. Detection and molecular characterization of a canine norovirus. Emerg. Infect. Dis. 14, 1306–8. doi:10.3201/eid1408.080062

Matsui, S.M., Greenberg, H.B., 2000. Immunity to calicivirus infection. J. Infect. Dis. 181 Suppl , S331–5. doi:10.1086/315587

Mattison, K., 2011. Norovirus as a foodborne disease hazard. Adv. Food Nutr. Res. 62, 1–39. doi:10.1016/B978-0-12-385989-1.00001-6

McDonald, K.F., Curry, R.D., Clevenger, T.E., Brazos, B.J., Unklesbay, K., Eisenstark, A., Baker, S., Golden, J., Morgan, R., 2000. The development of photosensitized pulsed and continuous ultraviolet decontamination techniques for surfaces and solutions. IEEE Trans. Plasma Sci. 28, 89–96.

Millner, P.D., 2009. Manure Management, in: Sapers, G.M., Solomon, E.B., Matthews, K.R. (Eds.), The produce contamination problem. Elsevier, pp. 79–104. doi:10.1016/B978-0-12-374186-8.00004-5

Mitchell, D.L., Jen, J., Cleaver, J.E., 1992. Sequence specificity of cyclobutane pyrimidine dimers in DNA treated with solar (ultraviolet B) radiation. Nucleic Acids Res. 20, 225–9.

Molbak, K., Olsen, J.E., Wegener, H.C., 2006. *Salmonella* infections, in: Cliver, D., Potter, M., Riemann, H. (Eds.), Foodborne Infections and Intoxications. Academic Press, pp. 57–115.

Mootian, G., Wu, W.-H., Matthews, K.R., 2009. Transfer of *Escherichia coli* O157:H7 from soil, water, and manure contaminated with low numbers of the pathogen to lettuce plants. J. Food Prot. 72, 2308–12.

Mounts, A.W., Ando, T., Koopmans, M., Bresee, J.S., Noel, J., Glass, R.I., 2000. Cold weather seasonality of gastroenteritis associated with Norwalk-like viruses. J. Infect. Dis. 181 Suppl , S284–7. doi:10.1086/315586

Nicholson, F., Groves, S., Chambers, B., 2005. Pathogen survival during livestock manure storage and following land application. Bioresour. Technol. 96, 135–143. doi:10.1016/j.biotech.2004.02.030

Niu, M.T., Polish, L.B., Robertson, B.H., Khanna, B.K., Bradley, A., Shapiro, C.N., Miller, M.A., Smith, J.D., Gedrose, J.K., Miriam, J., Margolis, H.S., Woodruff, B.A., Alter, M.J., 1992. Multistate outbreak of hepatitis A associated with frozen strawberries. J. Infect. Dis. 166, 518–24.

Niven, G.W., Miles, C.A., Mackey, B.M., 1999. The effects of hydrostatic pressure on ribosome conformation in *Escherichia coli*: an *in vivo* study using differential scanning calorimetry. Microbiology 145, 419–425. doi:10.1099/13500872-145-2-419

Nunez-Barrios, A., Nesmith, D.S., Chinnan, M., Prussia, S.E., 2005. Dynamics of rabbiteye blueberry fruit quality in response to harvest method and postharvest handling temperature. Small Fruits Rev. 4, 73–81. doi:10.1300/J301v04n02_08

Okafo, C.N., Umoh, V.J., Galadima, M., 2003. Occurrence of pathogens on vegetables harvested from soils irrigated with contaminated streams. Sci. Total Environ. 311, 49–56. doi:10.1016/S0048-9697(03)00057-3

Olaimat, A.N., Holley, R.A., 2012. Factors influencing the microbial safety of fresh produce: a review. Food Microbiol. 32, 1–19. doi:10.1016/j.fm.2012.04.016

Oms-Oliu, G., Martín-Belloso, O., Soliva-Fortuny, R., 2008. Pulsed light treatments for food preservation. A review. Food Bioprocess Technol. 3, 13–23. doi:10.1007/s11947-008-0147-x

Orskov, F., Orskov, I., 1992. *Escherichia coli* serotyping and disease in man and animals. Can. J. Microbiol. 38, 699–704.

Pangloli, P., Hung, Y.-C., 2013. Reducing microbiological safety risk on blueberries through innovative washing technologies. Food Control 32, 621–625. doi:10.1016/j.foodcont.2013.01.052

Parashar, U.D., Monroe, S.S., 2001. "Norwalk-like viruses" as a cause of foodborne disease outbreaks. Rev. Med. Virol. 11, 243–52.

Paredes-López, O., Cervantes-Ceja, M.L., Vigna-Pérez, M., Hernández-Pérez, T., 2010. Berries: improving human health and healthy aging, and promoting quality life--a review. Plant foods Hum. Nutr. 65, 299–308. doi:10.1007/s11130-010-0177-1

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. Lett. Appl. Microbiol. 52, 162–7. doi:10.1111/j.1472-765X.2010.02982.x

Park, S., Worobo, R.W., Durst, R.A., 2001. *Escherichia coli* O157:H7 as an emerging foodborne pathogen: a literature review. Crit. Rev. Biotechnol. 21, 27–48. doi:10.1080/20013891081674

Parrino, T.A., Schreiber, D.S., Trier, J.S., Kapikian, A.Z., Blacklow, N.R., 1977. Clinical immunity in acute gastroenteritis caused by Norwalk agent. N. Engl. J. Med. 297, 86–9. doi:10.1056/NEJM197707142970204

Patel, M.M., Hall, A.J., Vinjé, J., Parashar, U.D., 2009. Noroviruses: a comprehensive review. J. Clin. Virol. 44, 1–8. doi:10.1016/j.jcv.2008.10.009

Patterson, M.F., 2005. Microbiology of pressure-treated foods. J. Appl. Microbiol. 98, 1400–9. doi:10.1111/j.1365-2672.2005.02564.x

Patterson, M.F., Kilpatrick, D.J., 1998. The combined effect of high hydrostatic pressure and mild heat on inactivation of pathogens in milk and poultry. J. Food Prot. 61, 432–6.

Predmore, A., Li, J., 2011. Enhanced removal of a human norovirus surrogate from fresh vegetables and fruits by a combination of surfactants and sanitizers. Appl. Environ. Microbiol. 77, 4829–38. doi:10.1128/AEM.00174-11

Pui, C.F., Wong, W.C., Chai, L.C., Tunung, R., Jeyaletchumi, P., Noor Hidayah, M.N., Ubong, A., Farinazleen, M.G., Cheah, Y.K., Son, R., 2011. *Salmonella*: A foodborne pathogen. Int. Food Res. J.

Ramaswamy, R., Balasubramaniam, V. (Bala), Kaletunç, G., 2011. High pressure processing fact sheet for food processors, in: Zhang, H.Q., Barbosa-Cánovas, G. V., Balasubramaniam, V.M., Dunne, C.P., Farkas, D.F., Yuan, T.C. (Eds.), Nonthermal Processing Technologies for Food. Wiley, p. 596.

Ramos-Villarroel, a. Y., Martín-Belloso, O., Soliva-Fortuny, R., 2011. Bacterial inactivation and quality changes in fresh-cut avocado treated with intense light pulses. Eur. Food Res. Technol. 233, 395–402. doi:10.1007/s00217-011-1533-6

Rangel, J.M., Sparling, P.H., Crowe, C., Griffin, P.M., Swerdlow, D.L., 2005. Epidemiology of *Escherichia coli* O157:H7 outbreaks, United States, 1982-2002. Emerg. Infect. Dis. 11, 603–9. doi:10.3201/eid1104.040739

Rasanayagam, V., Balasubramaniam, V.M., Ting, E., Sizer, C.E., Bush, C., Anderson, C., 2003. Compression heating of selected fatty food materials during high-pressure processing. J. Food Sci. 68, 254–259. doi:10.1111/j.1365-2621.2003.tb14148.x

Riley, L.W., Remis, R.S., Helgerson, S.D., McGee, H.B., Wells, J.G., Davis, B.R., Hebert, R.J., Olcott, E.S., Johnson, L.M., Hargrett, N.T., Blake, P.A., Cohen, M.L., 1983. Hemorrhagic colitis associated with a rare *Escherichia coli* serotype. N. Engl. J. Med. 308, 681–5. doi:10.1056/NEJM198303243081203

Ritz, M., Freulet, M., Orange, N., Federighi, M., 2000. Effects of high hydrostatic pressure on membrane proteins of *Salmonella* typhimurium. Int. J. Food Microbiol. 55, 115–119. doi:10.1016/S0168-1605(00)00165-3

Roberts, P., Hope, A., 2003. Virus inactivation by high intensity broad spectrum pulsed light. J. Virol. Methods 110, 61–65. doi:10.1016/S0166-0934(03)00098-3

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. Clin. Infect. Dis. 35, 246–53. doi:10.1086/341408

Rowan, N.J., MacGregor, S.J., Anderson, J.G., Fouracre, R.A., McIlvaney, L., Farish, O., 1999. Pulsed-light inactivation of food-related microorganisms. Appl. Envir. Microbiol. 65, 1312–1315.

Saiz, A.H., Mingo, S.T., Balda, F.P., Samson, C.T., 2008. Advances in design for successful commercial high pressure food processing. Food Aust. 60, 154–156.

Sandvig, K., Garred, O., Prydz, K., Kozlov, J. V, Hansen, S.H., van Deurs, B., 1992. Retrograde transport of endocytosed Shiga toxin to the endoplasmic reticulum. Nature 358, 510–2. doi:10.1038/358510a0

Sarvikivi, E., Roivainen, M., Maunula, L., Niskanen, T., Korhonen, T., Lappalainen, M., Kuusi, M., 2012. Multiple norovirus outbreaks linked to imported frozen raspberries. Epidemiol. Infect. 140, 260–7. doi:10.1017/S0950268811000379

Sauer, A., Moraru, C.I., 2009. Inactivation of *Escherichia coli* ATCC 25922 and *Escherichia coli* O157: H7 in apple juice and apple cider, using pulsed light treatment. J. Food Prot. 72, 937–944.

Scallan, E., Hoekstra, R.M., Angulo, F.J., Tauxe, R. V, Widdowson, M.-A., Roy, S.L., Jones, J.L., Griffin, P.M., 2011. Foodborne illness acquired in the United States--major pathogens. Emerg. Infect. Dis. 17, 7–15. doi:10.3201/eid1701.091101p1

Scharff, R.L., 2012. Economic burden from health losses due to foodborne illness in the United States. J. Food Prot. 75, 123–31. doi:10.4315/0362-028X.JFP-11-058

Seeram, N.P., 2008. Berry fruits: compositional elements, biochemical activities, and the impact of their intake on human health, performance, and disease. J. Agric. Food Chem. 56, 627–9. doi:10.1021/jf071988k

Simpson, R.K., Gilmour, A., 1997. The effect of high hydrostatic pressure on *Listeria monocytogenes* in phosphate-buffered saline and model food systems. J. Appl. Microbiol. 83, 181–8.

Sinha, N., 2006. Strawberries and raspberries, in: Hui, Y. (Ed.), Handbook of fruits and fruit processing. Blackwell publishing, Ames, Iowa, pp. 581–595.

Smelt, J.P.P.M., Rijke, A.G.F., Hayhurst, A., 1994. Possible mechanism of high pressure inactivation of microorganisms. High Press. Res. 12, 199–203.

Smith, W.L., Lagunas-Solar, M.C., Cullor, J.S., 2002. Use of pulsed ultraviolet laser light for the cold pasteurization of bovine milk. J. Food Prot. 65, 1480–2.

Sosnovtsev, S. V, Belliot, G., Chang, K.-O., Prikhodko, V.G., Thackray, L.B., Wobus, C.E., Karst, S.M., Virgin, H.W., Green, K.Y., 2006. Cleavage map and proteolytic processing of the murine norovirus nonstructural polyprotein in infected cells. J. Virol. 80, 7816–31. doi:10.1128/JVI.00532-06

Straub, T.M., Bentrup, K.H. zu, Orosz-Coghlan, P., Dohnalkova, A., Mayer, B.K., Bartholomew, R.A., Valdez, C.O., Bruckner-Lea, C.J., Gerba, C.P., Abbaszadegan, M., Nickerson, C.A., Höner zu Bentrup, K., 2007. In vitro cell culture infectivity assay for human noroviruses. Emerg. Infect. Dis. 13, 396–403. doi:10.3201/eid1303.060549

Strik, B.C., 2007. Berry crops: Worldwide area and production systems, in: Zhao, Y. (Ed.), Berry Fruit: Value-Added Products for Health Promotion. CRC Press, pp. 4–45.

Sugieda, M., Nagaoka, H., Kakishima, Y., Ohshita, T., Nakamura, S., Nakajima, S., 1998. Detection of Norwalk-like virus genes in the caecum contents of pigs. Arch. Virol. 143, 1215–21.

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. J. Food Prot. 68, 1165–1175.

Takeshita, K., Shibatoa, J., Sameshimaa, T., Fukunagab, S., Isobec, S., Ariharad, K., Itohd, M., 2003. Damage of yeast cells induced by pulsed light irradiation. Int. J. Food Microbiol. 85, 151–158. doi:10.1016/S0168-1605(02)00509-3

Tan, M., Fang, P., Chachiyo, T., Xia, M., Huang, P., Fang, Z., Jiang, W., Jiang, X., 2008. Noroviral P particle: structure, function and applications in virus-host interaction. Virology 382, 115–23. doi:10.1016/j.virol.2008.08.047

Tan, M., Jiang, X., 2005. Norovirus and its histo-blood group antigen receptors: an answer to a historical puzzle. Trends Microbiol. 13, 285–93. doi:10.1016/j.tim.2005.04.004

Tan, M., Jiang, X., 2010. Norovirus gastroenteritis, carbohydrate receptors, and animal models. PLoS Pathog. 6, e1000983. doi:10.1371/journal.ppat.1000983

Tang, Q., Li, D., Xu, J., Wang, J., Zhao, Y., Li, Z., Xue, C., 2010. Mechanism of inactivation of murine norovirus-1 by high pressure processing. Int. J. Food Microbiol. 137, 186–9. doi:10.1016/j.ijfoodmicro.2009.10.033

Taube, S., Perry, J.W., Yetming, K., Patel, S.P., Auble, H., Shu, L., Nawar, H.F., Lee, C.H., Connell, T.D., Shayman, J.A., Wobus, C.E., 2009. Ganglioside-linked terminal sialic acid moieties on murine macrophages function as attachment receptors for murine noroviruses. J. Virol. 83, 4092–101. doi:10.1128/JVI.02245-08

Teunis, P.F.M., Moe, C.L., Liu, P., Miller, S.E., Lindesmith, L., Baric, R.S., Le Pendu, J., Calderon, R.L., 2008. Norwalk virus: how infectious is it? J. Med. Virol. 80, 1468–76. doi:10.1002/jmv.21237

Thakur, B., Nelson, P., 1998. High-pressure processing and preservation of food. Food Rev. Int. 14, 427–447.

Tian, P., Engelbrektson, A., Mandrell, R., 2008. Two-log increase in sensitivity for detection of norovirus in complex samples by concentration with porcine gastric mucin conjugated to magnetic beads. Appl. Environ. Microbiol. 74, 4271–6. doi:10.1128/AEM.00539-08

Tian, P., Jiang, X., Zhong, W., Jensen, H.M., Brandl, M., Bates, A.H., Engelbrektson, A.L., Mandrell, R., 2007. Binding of recombinant norovirus like particle to histo-blood group antigen on cells in the lumen of pig duodenum. Res. Vet. Sci. 83, 410–8. doi:10.1016/j.rvsc.2007.01.017

Tian, P., Yang, D., Jiang, X., Zhong, W., Cannon, J.L., Burkhardt, W., Woods, J.W., Hartman, G., Lindesmith, L., Baric, R.S., Mandrell, R., 2010. Specificity and kinetics of norovirus binding to magnetic bead-conjugated histo-blood group antigens. J. Appl. Microbiol. 109, 1753–62. doi:10.1111/j.1365-2672.2010.04812.x

Ting, E.Y., Marshall, R.G., 2002. Production issues related to UHP food, in: Welti-Chanes, J., Aguilera, J.M. (Eds.), Engineering and Food for the 21st Century. CRC Press, pp. 727–738.

Udompijitkul, P., Daeschel, M.A., Zhao, Y., 2007. Antimicrobial effect of electrolyzed oxidizing water against *Escherichia coli* O157:H7 and *Listeria monocytogenes* on fresh strawberries (*Fragaria x ananassa*). J. Food Sci. 72, M397–M406. doi:10.1111/j.1750-3841.2007.00531.x

Ulmer, H.M., Ganzle, M.G., Vogel, R.F., 2000. Effects of high pressure on survival and metabolic activity of *Lactobacillus plantarum* TMW1.460. Appl. Environ. Microbiol. 66, 3966–3973. doi:10.1128/AEM.66.9.3966-3973.2000

USDA, 2002. Organic production and handling standards. URL: http://www.ams.usda.gov/AMSv1.0/getfile?dDocName=STELDEV3004445 (accessed 12.12.14)

USDA-ERS, 2014a. USDA Economic Research Service - Yearbook Tables. URL: http://www.ers.usda.gov/data-products/fruit-and-tree-nut-data/yearbook-tables.aspx (accessed 12.12.14)

USDA-ERS, 2014b. Oranges and apples are America's top fruit choices. URL: http://www.ers.usda.gov/data-products/chart-gallery/detail.aspx?chartId=40075&ref=collection&embed=True&widgetId=39734 (accessed 12.12.14)

Voldřich, M., Dobiáš, J., Tichá, L., Čeřovský, M., Krátká, J., 2004. Resistance of vegetative cells and ascospores of heat resistant mould *Talaromyces avellaneus* to the high pressure treatment in apple juice. J. Food Eng. 61, 541–543. doi:10.1016/S0260-8774(03)00224-3

Wahlström, H., Tysén, E., Olsson Engvall, E., Brändström, B., Eriksson, E., Mörner, T., Vågsholm, I., 2003. Survey of Campylobacter species, VTEC O157 and *Salmonella* species in Swedish wildlife. Vet. Rec. 153, 74–80.

Walker, G.C., 1984. Mutagenesis and inducible responses to deoxyribonucleic acid damage in Escherichia coli. Microbiol. Rev. 48, 60–93.

Wang, Q.-H., Han, M.G., Cheetham, S., Souza, M., Funk, J.A., Saif, L.J., 2005. Porcine noroviruses related to human noroviruses. Emerg. Infect. Dis. 11, 1874–81. doi:10.3201/eid1112.050485

Wang, T., Macgregor, S.J., Anderson, J.G., Woolsey, G. a, 2005. Pulsed ultra-violet inactivation spectrum of *Escherichia coli*. Water Res. 39, 2921–5. doi:10.1016/j.watres.2005.04.067

Warriner, K., Huber, A., Namvar, A., Fan, W., Dunfield, K., 2009. Recent advances in the microbial safety of fresh fruits and vegetables. Adv. Food Nutr. Res. 57, 155–208. doi:10.1016/S1043-4526(09)57004-0

Wei, J., Jin, Y., Sims, J.T., Kniel, K.E., 2010a. Fate of human enteric viruses during dairy manure-based composting. J. Food Prot. 73, 1543–7.

Wei, J., Jin, Y., Sims, T., Kniel, K.E., 2009. Survival of human adenovirus 41 in land-applied manure and biosolids. Food Environ. Virol. 1, 148–154. doi:10.1007/s12560-009-9021-x

Wei, J., Jin, Y., Sims, T., Kniel, K.E., 2010b. Survival of murine norovirus and hepatitis A virus in different types of manure and biosolids. Foodborne Pathog. Dis. 7, 901–6. doi:10.1089/fpd.2009.0490

Wekhof, A., 2000. Disinfection with flash lamps. J. Pharm. Sci. Technol. / PDA 54, 264–76.

Wekhof, A., 2001. Pulsed UV Disintegration (PUVD): a new sterilisation mechanism for packaging and broad medical-hospital applications ., The first international conference on Ultraviolet technologies.

WHO, 2002. WHO | The world health report 2002 - Reducing Risks, Promoting Healthy Life. World Health Organization. URL: http://www.who.int/whr/2002/en/ (accessed 12.12.14)

Wobus, C.E., Karst, S.M., Thackray, L.B., Chang, K.-O., Sosnovtsev, S. V, Belliot, G., Krug, A., Mackenzie, J.M., Green, K.Y., Virgin, H.W., 2004. Replication of Norovirus in cell culture reveals a tropism for dendritic cells and macrophages. PLoS Biol. 2, e432. doi:10.1371/journal.pbio.0020432

Wobus, C.E., Thackray, L.B., Virgin, H.W., 2006. Murine norovirus: a model system to study norovirus biology and pathogenesis. J. Virol. 80, 5104–12. doi:10.1128/JVI.02346-05

Woodling, S.E., Moraru, C.I., 2005. Influence of surface topography on the effectiveness of pulsed light treatment for the inactivation of *Listeria innocua* on stainless-steel surfaces. J. Food Sci. 70, m345–m351. doi:10.1111/j.1365-2621.2005.tb11478.x

Woodling, S.E., Moraru, C.I., 2007. Effect of spectral range in surface inactivation of *Listeria innocua* using broad-spectrum pulsed light. J. Food Prot. 70, 909–916.

Wouters, P.C., Glaasker, E., Smelt, J.P., 1998. Effects of high pressure on inactivation kinetics and events related to proton efflux in *Lactobacillus plantarum*. Appl. Environ. Microbiol. 64, 509–14.

Wuytack, E.Y., Phuong, L.D.T., Aertsen, A., Reyns, K.M.F., Marquenie, D., De Ketelaere, B., Masschalck, B., Van Opstal, I., Diels, A.M.J., Michiels, C.W., 2003. Comparison of sublethal injury induced in *Salmonella enterica* serovar Typhimurium by heat and by different nonthermal treatments. J. Food Prot. 66, 31–7.

Wyatt, R.G., Dolin, R., Blacklow, N.R., DuPont, H.L., Buscho, R.F., Thornhill, T.S., Kapikian, A.Z., Chanock, R.M., 1974. Comparison of three agents of acute infectious nonbacterial gastroenteritis by cross-challenge in volunteers. J. Infect. Dis. 129, 709–14.

Ye, M., Li, X., Kingsley, D.H., Jiang, X., Chen, H., 2014. Inactivation of human norovirus in contaminated oysters and clams by high hydrostatic pressure. Appl. Environ. Microbiol. 80, 2248–53. doi:10.1128/AEM.04260-13

Yordanov, D.G., Angelova, G.V., 2010. High pressure processing for foods preserving. Biotechnol. Biotechnol. Equip. 24, 1940–1945. doi:10.2478/V10133-010-0057-8

Yu, L., Reitmeier, C.A., Gleason, M.L., Nonnecke, G.R., Olson, D.G., Gladon, R.J., 1995. Quality of Electron Beam Irradiated Strawberries. J. Food Sci. 60, 1084–1087. doi:10.1111/j.1365-2621.1995.tb06297.x

Zhang, G., Ma, L., Beuchat, L.R., Erickson, M.C., Phelan, V.H., Doyle, M.P., 2009. Lack of internalization of *Escherichia coli* O157:H7 in lettuce (*Lactuca sativa L.*) after leaf surface and soil inoculation. J. Food Prot. 72, 2028–37.

Zheng, D.-P., Ando, T., Fankhauser, R.L., Beard, R.S., Glass, R.I., Monroe, S.S., 2006. Norovirus classification and proposed strain nomenclature. Virology 346, 312–23. doi:10.1016/j.virol.2005.11.015

Chapter 3

INACTIVATION OF *ESCHERICHIA COLI* O157:H7 AND *SALMONELLA* SPP. IN STRAWBERRY PUREE BY HIGH HYDROSTATIC PRESSURE WITH/WITHOUT SUBSEQUENT FROZEN STORAGE

ABSTRACT

The objectives of this study were to investigate the survival of *E. coli* O157:H7 and *Salmonella* spp. in frozen strawberry puree and to assess the application of high pressure processing (HPP) to decontaminate strawberry puree from both pathogens. Fresh strawberry puree was inoculated with high (~6 log CFU/g) and low (~3 log CFU/g) levels of *E. coli* O157:H7 or *Salmonella* spp. and stored at -18 °C for 12 weeks. Both pathogens were able to persist for at least 4 weeks and samples with high inoculums were still positive for both pathogens after 12 weeks. Pressure treatment of 450 MPa for 2 min at 21 °C was able to eliminate both pathogens in strawberry puree. Frozen storage at -18 °C after pressure treatment substantially enhanced the inactivation of both pathogens and 4-8 days of frozen storage was able to reduce the pressure level needed for elimination of both pathogens to 250-300 MPa. Natural yeasts and molds in strawberry puree were effectively reduced by pressure of 300 MPa for 2 min at 21 °C. No adverse impacts on physical properties such as color, soluble solids content, pH and viscosity of strawberry puree was found for pressure-treated samples. Therefore, the treatment of 300 MPa for 2 min at 21 °C followed by 4 days frozen storage at -18 °C was recommended for the minimal processing of strawberry

puree with great retention of fresh-like sensory properties. HPP could be a promising alternative to traditional thermal processing for berry purees.

3.1 Introduction

As one of the most popular fruits, strawberry is rich in a variety of bioactive compounds that have been shown to demonstrate anticancer activity, anti-inflammatory effect, preventative effect on heart disease and potential benefits to the aging brain (Hannum, 2004). As a result, there is an increasing consumption demand of strawberry over the years. From 1970 to 2009, the production of strawberries in the U.S. has increased from 496 million to 2801.3 million lbs. with a total value about $2.1 billion dollars, which makes United States the largest producer of strawberries in the world (Economic Research Service, 2010).

However, concerns about the microbial safety of strawberries have been raised in recent years due to the implication of strawberries, and some other berry fruits in several large foodborne outbreaks. Both strawberries and raspberries have been associated with outbreaks of hepatitis A virus (HAV) and human norovirus (Friedman et al., 2005; Hutin et al., 1999; Korsager et al., 2005; Niu et al., 1992; Ransay & Upton, 1989; Reid & Robinson, 1987; Sarvikivi et al., 2012). Contaminated blueberries were reported to be the source of an outbreak of hepatitis A in 2003 (Calder et al., 2003). Fresh raspberries and blackberries imported from Guatemala were associated with several large outbreaks of *Cyclospora cayetanensis* (Dawson, 2005; Herwaldt et al., 1997; Herwaldt et al., 1999; Katz et al., 1999). A survey conducted by the U.S. Food and Drug Administration (FDA) in 1999 found that 1 out of 143 imported strawberries samples was tested positive for *Salmonella* (FDA, 2001).

Recently, fresh strawberries from a farm in Oregon were linked with an *Escherichia coli* O157:H7 outbreak, which caused at least 15 people sick including one death. Deer dropping in strawberry field was confirmed as the source of *E. coli* O157:H7 (Oregon Health Authority, 2011).

Strawberries are usually sold as fresh or processed into frozen berries or puree. As an intermediate product, strawberry puree is an important industrial ingredient and is widely used in a variety of foods such as yoghurt, jams or jellies, popsicle, smoothies, snacks, and different drinks. To extend shelf life and inactivate pathogens, a thermal treatment (88 °C for 1.5-2 min) is usually required. However, in order to maintain fresh berry flavor and nutritional content as increasingly demanded by consumers, the pasteurization processes are sometimes mitigated and even omitted (Deuel & Plotto, 2004). Unfortunately, those mitigated thermal pasteurization process are sometimes not sufficient to eliminate pathogens (Baert et al., 2008). It is well documented that some strains of *E. coli* O157:H7 and *Salmonella* are very acid tolerant, and can survive and sometimes grow in acidic conditions (Conner & Kotrola, 1995; Janisiewicz et al., 1999; Knudsen et al., 2001; Ma et al., 2010; Oyarzabal et al., 2003). Actually, those pathogens have already been involved in several outbreaks linked to acidic fruits and fruit juices (Besser et al., 1993; Hilborn et al., 2000; Jain et al., 2009).

High pressure processing (HPP) or high hydrostatic pressure has been recognized as a pasteurization process without the use of high temperatures (≥70 °C) that can dramatically alter the sensory and nutritional qualities of food products (Kingsley et al., 2005). HPP has been showed to have little detrimental effect on nutrient components and have good retention of product flavor quality (Butz et al.,

2003; Sanchez-Moreno et al., 2003). In some cases, HPP can also be used to inactivate undesirable spoilage enzymes (San Martin et al., 2002). Therefore, development of a nonthermal processing that can preserve the nutritional and organoleptic quality of fresh strawberry puree while improving microbiological safety would greatly benefit the food industry.

In this study, the objectives were to investigate the survival of *E. coli* O157:H7 and *Salmonella* spp. in frozen strawberry puree and to assess the application of HPP as a nonthermal processing method to decontaminate strawberry puree from both pathogens with minimal adverse effect on its physical and sensorial properties.

3.2   Materials and Methods

3.2.1   Bacterial strains and inoculum preparation

Five *E. coli* O157:H7 strains and four *Salmonella enterica* strains of different serotypes were used in this study. *E. coli* O157:H7 strains were kindly provided by Dr Joerger and Dr. Kniel, University of Delaware and *Salmonella* strains were kindly provided by Dr. Gurtler, U.S. Department of Agriculture. Detailed strain information is shown in Table 3.1. The individual wild-type strains were adapted to grow in the presence of 50 µg/mL of 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 (Difco) and 50 µg/mL of nalidixic acid (TSAYE-N) as described previously (Huang & Chen, 2011). For individual culture, single colonies of each strain were subcultured in tryptic soy broth (Difco) supplemented with 0.6% yeast extract and 50 µg/mL of nalidixic acid (TSBYE-N) for 2 consecutive 24-h periods. Equal volumes of individual cultures were then mixed to

form a 5-strain cocktail of *E. coli* O157:H7 and a 4-strain cocktail of *Salmonella*.

Dilutions of the cocktails were prepared by diluting appropriate volumes of the culture

cocktail into sterile 0.1% peptone water (Difco).

Table 3.1 Bacterial strain information

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

3.2.2 Survival of *E. coli* O157:H7 and *Salmonella* spp. in strawberry puree during frozen storage

Fresh strawberries were purchased from local grocery stores the day before

each experiment and stored at 4 °C until use. Strawberries were washed by tap water

and dried in a strainer. Calyxes of the strawberries were removed with a flame-

sterilized stainless knife and strawberries were blended in a food blender at high

speed. The resulting puree was then inoculated with diluted *E. coli* O157:H7 or

Salmonella cocktails to high (~6 log CFU/g) and low (~3 log CFU/g) inoculation

levels and pummeled in a stomacher (Seward 400, Seward, London, U.K.) for 30 s to

homogenize. Inoculated puree was then packaged in small sterile stomacher bags (~7

g/bag) and immediately stored at -18 °C. The counts of the two pathogens in the puree

samples were determined at 0 h (before frozen storage), 8 h and on days 1, 3, 7, 14, 28, 56 and 84 as described in Section 3.2.6.

3.2.3 Pressure inactivation of *E. coli* O157:H7 and *Salmonella* spp. in strawberry puree

Strawberry puree was inoculated with *E. coli* O157:H7 or *Salmonella* as described above and placed into individual sterile stomacher bags, double-sealed and double-bagged (~7 g/bag). Samples were then pressure-treated at levels ranging from 200-500 MPa (in 50 MPa increments) for 2 min at 21 °C (initial sample temperature) using a high pressure unit with temperature control (Model Avure PT-1, Avure Technologies, Kent, WA). The pressure-come-up rate was approximately 22 MPa/s. The pressure-release was almost immediate (<4 s). Pressurization time reported in this study did not include the pressure come-up or release times. After pressure treatments, the samples were immediately subjected to microbiological analyses as described in Section 3.2.6.

3.2.4 Effect of frozen storage after HPP on the inactivation of *E. coli* O157:H7 and *Salmonella* spp. in strawberry puree

Strawberry puree was inoculated with *E. coli* O157:H7 or *Salmonella* and placed into individual sterile stomacher bags as described above. Samples were then treated at 250-300 MPa for 2 min at 21 °C and immediately stored at -18 °C for up to 8 days before the bacterial pathogens were enumerated as described in Section 3.2.6.

3.2.5 Effect of HPP on the physical properties and yeasts and molds counts of strawberry puree

To determine whether HPP treatment had any negative effects on the quality of strawberry puree, color, pH, soluble solids content (SSC), viscosity, and natural yeasts

and molds counts of strawberry puree were analyzed. Uninoculated samples of strawberry puree (~7 g/bag) were packaged and treated at 250 and 300 MPa for 2 min at 21 °C (initial sample temperature) using the pressure unit mentioned above and immediately subjected to frozen storage at -18 °C for up to 8 days. A chromameter (Minolta CR-400, Minolta, Osaka, Japan) was used to measure the color of untreated and pressure-treated strawberry puree. Color parameters were quantified in the Hunter *L, a* and *b* color space where *L* refers to lightness, ranging from 0 (blackness) to 100 (whiteness), positive *a* means red and negative *a* green, and positive *b* means yellow and negative *b* blue. SSC (expressed as °Brix) was measured with a refractometer (Reichert Abbe Mark II, Reichert, NY, USA) and pH values of puree samples with a pH meter (pHTestr 20, Eutech Instruments, Thermo Scientific). Yeasts and molds were enumerated on dichloran rose Bengal chloramphenicol agar (DRBC) (Difco). The plates were incubated at 25 °C for 3-5 days. Since a larger volume of strawberry puree was needed for viscosity measurement than other tests, samples for viscosity analysis were treated using a 2-L pressure unit (Avure Technologies, Kent, WA, USA) at the Eastern Regional Research Center of U.S. Department of Agriculture. The viscosity of strawberry puree was measured with a digital viscometer (DV-E, Brookfield, MA, USA). Temperatures of samples were equilibrated at 21 °C in a water bath and filled in a 600-mL glass beak according to the manufacturer's instruction. In the test setup, the shear rate was 10 RPM and all the measurements were performed with spindle S63 at 21 °C. All the samples were measured in triplicate.

3.2.6   Microbiological analysis

Pouches containing treated or untreated samples were aseptically opened and 5 g of samples were transferred into a sterile filter bag (Whirl-Pak, Nasco, USA)

containing 45 mL TSBYE-N. The mixture (pH 6.35) was pummeled in a stomacher for 2 min at 260 rpm. The homogenate was then serially diluted in sterile 0.1% peptone water and surface-plated on TSAYE-N followed by incubation at 35 °C for 72 h. Presumptive colonies of *E. coli* O157:H7 or *Salmonella* formed on the plates were counted. The strawberry puree samples were also directly enriched in 45 mL of TSBYE-N and incubated for 48 h at 35 °C to allow resuscitation of sub-lethally injured cells. Enriched samples were then streaked onto Sorbitol Macconkey (Difco) supplemented with 50 μg/mL of nalidixic acid (SMAC-N) for *E. coli* O157:H7 or Xylose Lysine Desoxycholate agar (Difco) supplemented with 50 μg/mL of nalidixic acid (XLD-N) for *Salmonella*.

3.2.7   Statistical analysis

At least three independent trials were conducted for all the experiments. Colony counts were converted to log CFU/g and means and standard deviations were calculated. Statistical analyses were conducted using JMP (SAS Cary, NC, USA). One-way analysis of variance and Tukey's one-way multiple comparisons were used to determine significant differences among treatments ($P < 0.05$).

3.3   Results and Discussion

3.3.1   Survival of *E. coli* O157:H7 and *Salmonella* spp. in strawberry puree during frozen storage

Cocktails of *E. coli* O157:H7 and *Salmonella* were used in our study to test their persistence in frozen unpasteurized strawberry puree (pH 3.6; 8.8 °Brix) during a 12-week frozen storage at −18 °C because strains of *E. coli* O157:H7 and serotypes of *Salmonella* have been shown to vary in their ability to survive in low pH foods

(Sharma et al., 2001; Yamamoto & Harris, 2001). Our results showed that both pathogens were unable to grow in strawberry puree, but they were able to survive for a long period of time (Figure 3.1). For both pathogens, there was a sharp decline in bacterial population in the first 3 days and a slower but steady decline of bacterial counts was observed during the following 12 weeks. The tailing effect observed in the survival curve could be due to differences in resistance to acid/frozen storage among the strains included in the cocktails. For samples with low initial inoculation level (~3 log CFU/g), *E. coli* O157:H7 and *Salmonella* were not detectable by enrichment (detection limit of 1 CFU/5g) after 8 and 12 weeks frozen storages, respectively. However, in strawberry puree with high initial inoculation level (~6 log CFU/g), those pathogens were still viable even after 12 weeks frozen storage.
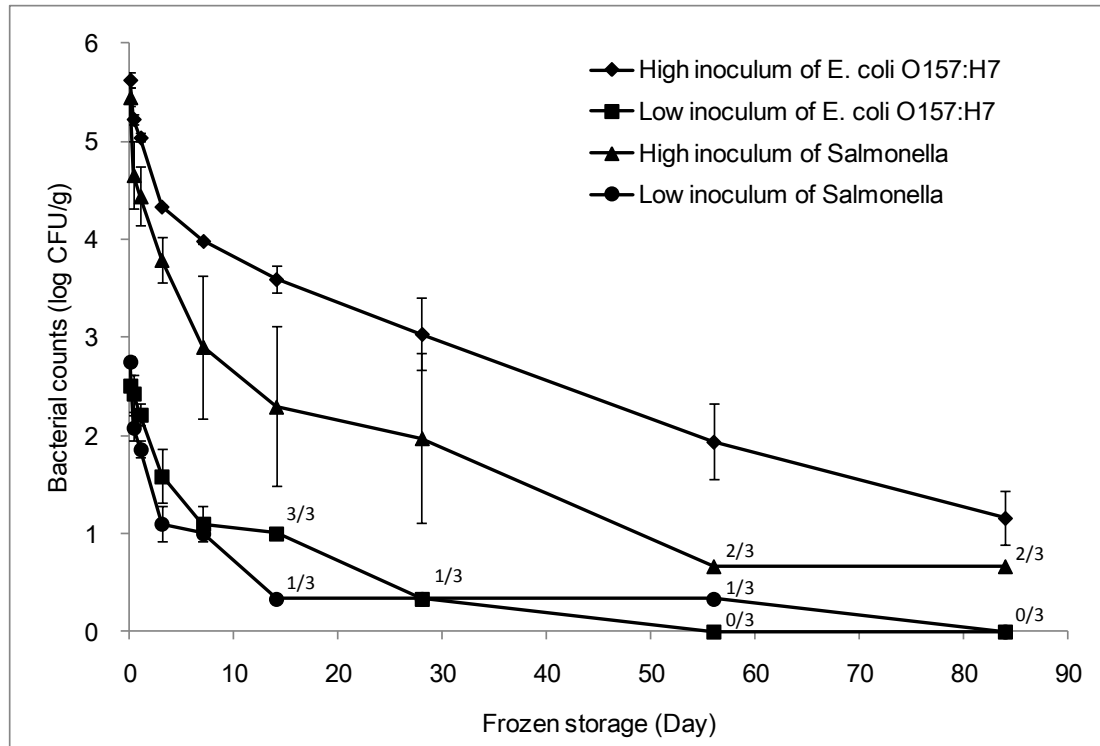
Figure 3.1 Survival curves of *E. coli* O157:H7 and *Salmonella* spp. in strawberry
puree during 12-week frozen storage. Strawberry puree inoculated with
high and low levels of the two pathogens were stored at -18 °C Error bars
shown in figures represent one standard deviation. Enrichment was
conducted when the bacterial counts were below the detection limit by
the plating method (1 log CFU/g). Numbers in fraction represent the
number of samples testing positive after enrichment out of a total of 3
trials.

The data clearly demonstrated that the two pathogens were capable of

surviving in frozen and acidic food for long period of time, which is in agreement with

previous studies. Knudsen et al. (2001) reported that *E coli* O157:H7 and *Salmonella*

could survive on the surface of frozen strawberry for at least 1 month. Oyarzabal et al.

(2003) demonstrated that *E. coli* O157:H7, *Listeria monocytogenes*, and *Salmonella* in

apple, orange, pineapple and white grape juice concentrates and banana puree could be

recovered from all the five products through 12 weeks storage at -23 °C. Our results

suggested that the ability of *E. coli* O157:H7 and *Salmonella* to survive in frozen

strawberry puree cannot be underestimated and a proper pasteurization process is

needed. Otherwise, contaminated strawberry puree would pose potential health risks

for consumers considering that those bacterial pathogens have low infectious doses

and can cause severe diseases (Kothary & Babu, 2001).

3.3.2 Effect of HPP on the inactivation of *E. coli* O157:H7 and *Salmonella* spp. in strawberry puree

Our results for the high-pressure inactivation of *E. coli* O157:H7 and

Salmonella spp. are shown in Table 3.2. The efficacy of pressure inactivation varied as

a function of the pressure intensity for both bacterial pathogens. A steady decrease in

the survival population of *E. coli* O157:H7 and *Salmonella* spp. was observed at

increasing pressure levels. Pressure treatments of 450 and 350 MPa for 2 min at 21 °C

were able to eliminate *E. coli* O157:H7 in strawberry puree with high (~6 log CFU/g)

and low (~3 log CFU/g) inoculation levels, respectively. *Salmonella* was shown to be

more sensitive to pressure treatment. When strawberry puree with low inoculation

level was treated with 300 MPa for 2 min, *Salmonella* was undetectable in all samples

by enrichment method. Pressure treatment at 350 MPa for 2 min was able to eliminate

Salmonella in strawberry puree with high inoculation level (6 log CFU/g). It has been

reported in some other studies that HPP was able to inactivate bacterial pathogens in

different types of fruit juices, but results may be different between these studies due to

the use of different strains, food matrixes, treatment temperatures and times. Jordan et

al. (2001) reported that pressure treatment of 500 MPa for 5 min at 20 °C could

achieve an immediate 5-log reduction of *E. coli* O157:H7 in apple juice (pH 3.5) and

tomato juice (pH 4.1), but only about a 1-2 log reduction in orange juice (pH 3.8). *E. coli* O157:H7 and *Listeria innocua* were inactivated by 4 logs from kiwifruit juice by pressure treatment of 300 MPa for 5 min at 20 °C, while only 1 log reduction was achieved in pineapple juice (Buzrul et al., 2008).

Table 3.2 Pressure inactivation of *E. coli* O157:H7 and *Salmonella* spp. in strawberry puree. Strawberry puree inoculated with high and low levels of the two pathogens was treated at 200 - 500 MPa for 2 min at 21 °C.

Pressure (MPa)	*E. coli* O157:H7 (log CFU/g)		*Salmonella* (log CFU/g)	
	Low inoculum	High inoculum	Low inoculum	High inoculum
Control	2.5 ± 0.0a	5.4 ± 0.1a	2.9 ± 0.2a	6.0 ± 0.1a
200	1.7 ± 0.1b	4.5 ± 0.3ab	2.2 ± 0.1b	5.1 ± 0.1b
250	1.4 ± 0.3b	4.1 ± 0.5ab	1.5 ± 0.4c	3.6 ± 0.2c
300	<1(2/3)c	3.5 ± 0.8bc	<1(0/3)d	<1(1/3)d
350	<1(0/3)c	2.3 ± 0.8c	<1(0/3)d	<1(0/3)d
400	<1(0/3)c	<1(1/3)d	<1(0/3)d	<1(0/3)d
450	<1(0/3)c	<1(0/3)d	<1(0/3)d	<1(0/3)d
500	<1(0/3)c	<1(0/3)d	<1(0/3)d	<1(0/3)d

Data represent mean survival population (log CFU/g) of three replicates ± 1 standard deviations.

Numbers in parentheses represent the number of samples shown positive after enrichment out of a total of 3 trials.

The limits of detection were 1 log CFU/g for plating method and 1 CFU/5 g for the enrichment method.

Data in the same column followed by the same lowercase letter are not significantly different (P > 0.05).

3.3.3 Effect of frozen storage after HPP on the inactivation of *E. coli* O157:H7 and *Salmonella* spp. in strawberry puree

HPP is able to effectively inactivate bacterial pathogens in strawberry puree, which makes HPP a potential alternative to traditional thermal treatments. However, the high capital costs of high pressure equipments have hindered the application of this technology in food industry. Since the capital costs increase exponentially with operating pressure level and the processing costs are also closely related to it, it is economically beneficial to use lower levels of pressure in combination with other processing techniques to achieve the same level of pathogen inactivation.

In this study, the effect of a short period of frozen storage after HPP on the inactivation of *E. coli* O157:H7 and *Salmonella* was evaluated. Inoculated strawberry puree (~3 or 6 log CFU/g) was pressure-treated at 200-300 MPa for 2 min at 21 °C and immediately stored at -18 °C for 0 (before frozen storage), 2, 4 or 8 days. As shown in Figures 3.2 and 3.3, a significant decrease in the population of both pathogens was observed during the subsequent frozen storage after HPP treatment. The inactivation achieved during the frozen storage was sometimes rather substantial compared to the inactivation caused by pressure treatment alone. For example, pressure treatment at 200 MPa for 2 min only reduced *E. coli* O157:H7 by 1.2 log and a further 3.6 log reduction was achieved during the 8-day frozen storage (Figure 3.2). In the control sample, 8 days of frozen storage reduced *E. coli* O157:H7 by 2.3 log units; therefore, an extra 1.3 log reduction of *E. coli* O157:H7 was achieved by the synergistic effect of HPP and subsequent frozen storage.
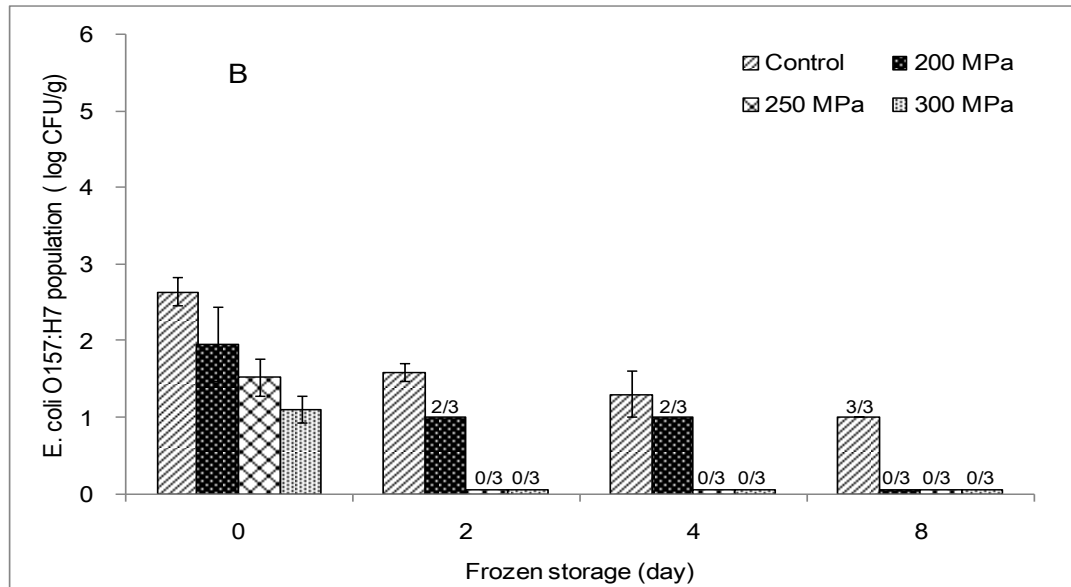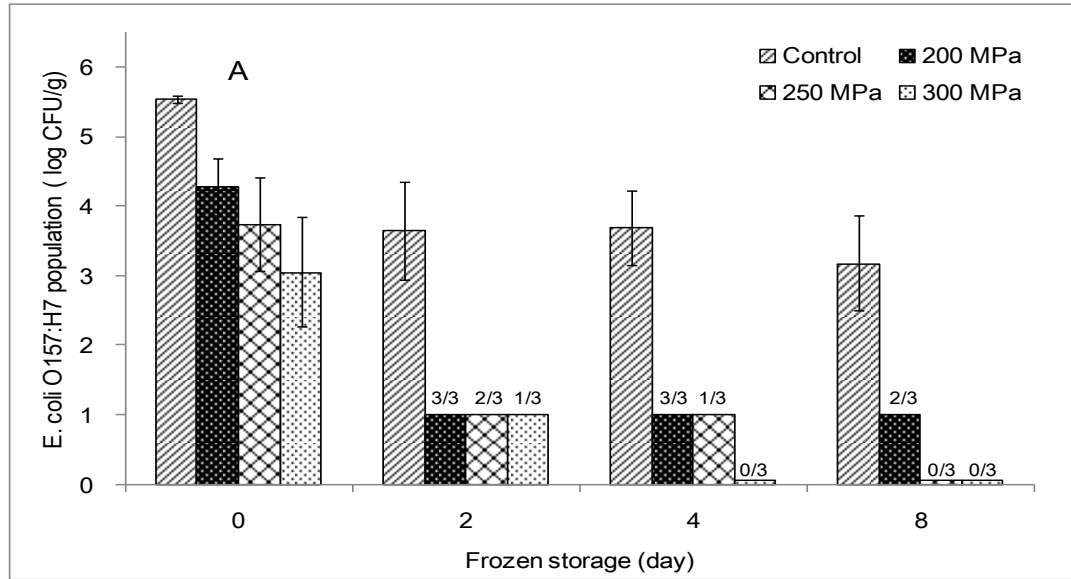
Figure 3.2 Populations of *E. coli* O157:H7 in the control and pressure-treated
strawberry puree with high (A) and low (B) inoculation levels during 8
days frozen storage. Inoculated samples were treated at 200-300 MPa for
2 min at 21 °C and then stored at -18 °C. Error bars shown in figures
represent one standard deviation. Enrichment was conducted when the
bacterial counts were below the detection limit by the plating method (1
log CFU/g). Numbers in fraction represent the number of samples testing
positive after enrichment out of a total of 3 trials.

Compared to the pressure level (450 MPa) required for elimination of ~5.5 log CFU/g *E. coli* O157:H7 in strawberry puree by HPP alone, only 250 MPa was needed if the pressure treatment was followed by 8 days frozen storage at -18 °C. Similarly, for samples inoculated with ~6 log CFU/g *Salmonella*, pressure at 350 MPa for 2 min at 21 °C was needed to get a complete kill by HPP alone (Figure 3.3). If combined with subsequent 8 days frozen storage, only 200 MPa was needed to achieve a complete elimination. Similar phenomenon was also observed for samples with low inoculation levels of *E. coli* O157:H7 and *Salmonella*. Those data indicate that a subsequent frozen storage after HPP could significantly enhance the inactivation of both pathogens in strawberry puree. This is in general agreement with the results reported by other researchers who observed the death of pressure-treated bacterial cells during subsequent storage under acidic conditions (Buzrul et al., 2008; Garcia-Graells et al., 1998; Jordan et al., 2001; Linton et al., 1999) although none of those studies used temperature below 0 °C for storage. Some previous studies have suggested that HPP can disrupt bacterial cell membrane and cause leakage of cytoplasm (Guerrero-Beltran et al., 2005). Membrane disruption may allow the entry of antimicrobials into the cells more easily than in the absence of HPP. It was also speculated that the pressure treatment may cause sublethal injury to a large proportion of the cells (Garcia-Graells et al., 1998), which could become more susceptible, in our case, to the natural antimicrobials and organic acids in strawberry puree. This could partially explain why there was a considerable further inactivation during the frozen storage after pressure inactivation. On the other hand, the low pH of strawberry puree and the frozen storage are certainly another two important factors that caused death of those pathogens. Since the treatment of 250 MPa followed by 8 days of frozen storage

and the treatment of 300 MPa followed by 4 days frozen storage were as effective as pressure treatment of 450 MPa alone in elimination of both pathogens at high inoculation level, these two pressure levels were chosen for the following study.
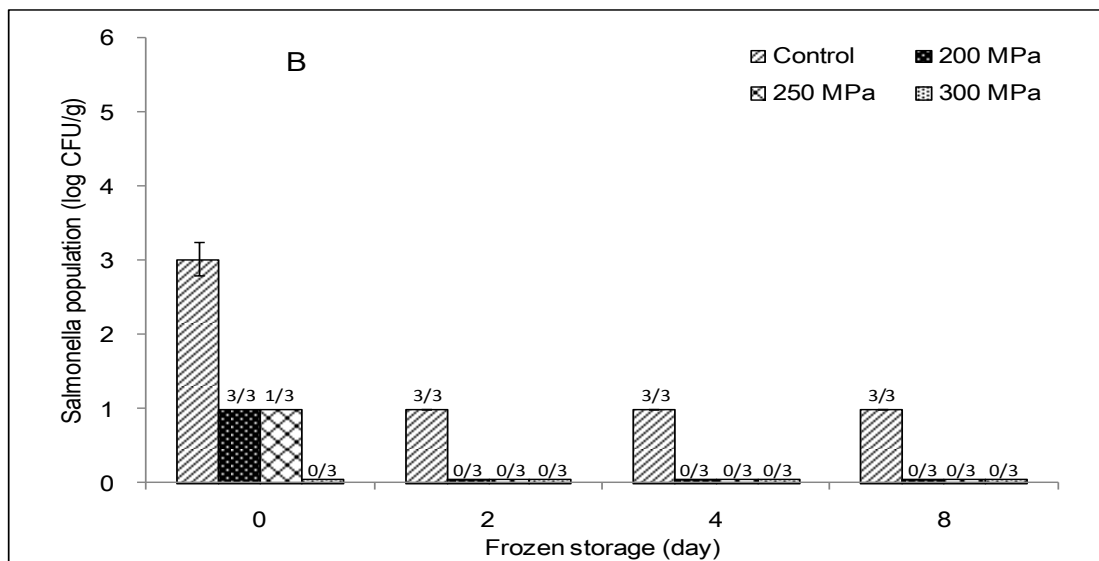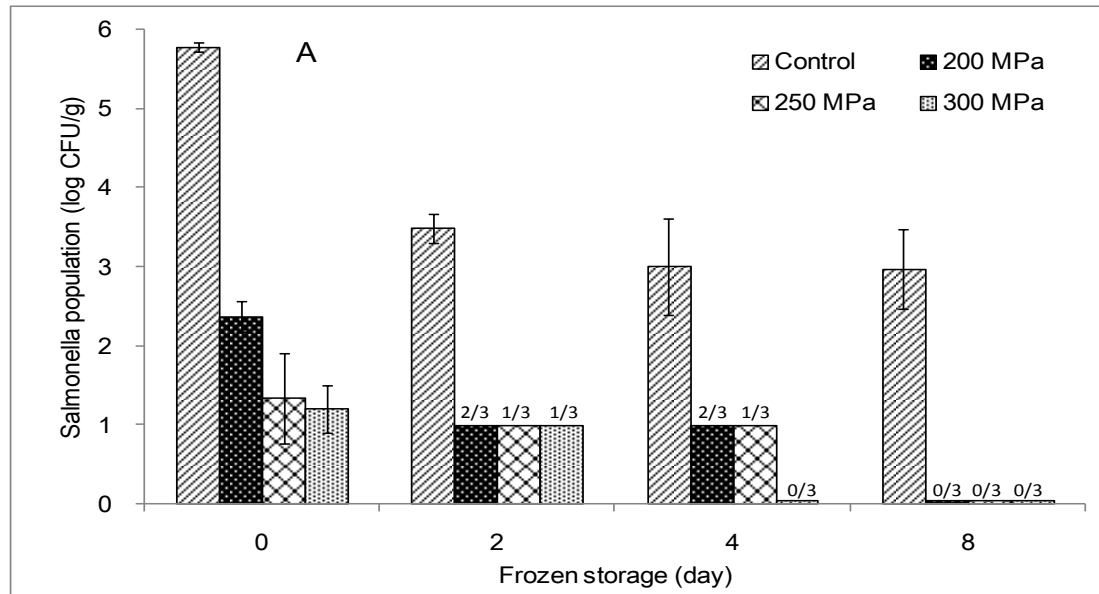
Figure 3.3 Populations of *Salmonella* spp. in the control and pressure-treated
strawberry puree with high (A) and low (B) inoculation levels during 8
days frozen storage. Inoculated samples were treated at 200-300 MPa for
2 min at 21 °C and then stored at -18 °C. Error bars shown in figures
represent one standard deviation. Enrichment was conducted when the
bacterial counts were below the detection limit by the plating method (1
log CFU/g). Numbers in fraction represent the number of samples testing
positive after enrichment out of a total of 3 trials.

3.3.4 Effect of HPP on the physical properties and yeasts and molds counts of strawberry puree

The effect of HPP on the physical properties of strawberry puree is shown in Table 3.3. The evolution of these parameters and yeasts and molds counts during 8 days frozen storage was also monitored. The consistency of strawberry puree expressed in viscosity is important for the quality of strawberry puree. The effect of HPP on viscosity of strawberry puree was compared with untreated puree. Our results showed that viscosity of strawberry puree was positively correlated to pressure level. Viscosities of puree samples stored at -18 °C for 4 or 8 days were significant lower than those on day 0 (before frozen storage) for both control and pressurized samples. There was no significant difference between samples from day 4 and day 8. Moreover, after 4 or 8 days frozen storage, viscosities of pressure-treated samples became very close to that of freshly-made puree. Some other studies also reported that high pressure treatment can change the rheological properties of fruit products, although the effect depends on the type of fruit and vegetable as well. Viscosity of mango pulp increased after pressure treatment at 100-200 MPa, while a reduction in viscosity was observed after pressure treatment at 300 and 400 MPa (Ahmed et al., 2005). Navel orange juice treated at 600 MPa for 4 min at 40 °C resulted in a higher viscosity than thermal treated one (Polydera et al., 2005). Landl et al. (2010) found that pressure treatment at 400 and 600 MPa increase the viscosity of apple puree. They also suggested that viscosity increase after HPP was attributed to an increase in the linearity of the cell walls and volumes of particles due to the permeabilization of cell walls.

Table 3.3 Physical properties and yeasts and molds counts of the control and pressure-treated strawberry puree during 8 days frozen storage. Uninoculated strawberry puree was treated at 250 - 300 MPa for 2 min at 21 °C and then stored at -18 °C.

Treatment	Day	Y&M (log CFU/g)	Viscosity (Pa s)	SSC(°Brix)	pH	Color		
						L	a	b
Control	0	4.3 ± 0.0^{aA}	5.1 ± 0.0^{cA}	8.8 ± 0.3^{aA}	3.57 ± 0.03^{aA}	30.7 ± 0.2^{aA}	17.3 ± 0.5^{aA}	5.6 ± 0.1^{aA}
	4	4.1 ± 0.1^{aAB}	4.7 ± 0.0^{cB}	8.6 ± 0.3^{aA}	3.59 ± 0.02^{aA}	30.4 ± 1.6^{aA}	16.4 ± 2.9^{aA}	5.4 ± 1.0^{aA}
	8	3.8 ± 0.2^{aB}	4.7 ± 0.0^{cB}	8.9 ± 0.1^{aA}	3.60 ± 0.01^{aA}	31.1 ± 0.3^{aA}	17.5 ± 0.8^{aA}	5.8 ± 0.2^{aA}
250 MPa	0	1.9 ± 0.3^{bA}	5.8 ± 0.0^{bA}	9.0 ± 0.1^{aA}	3.60 ± 0.01^{aA}	31.8 ± 0.6^{aA}	19.6 ± 1.1^{aA}	6.3 ± 0.5^{aA}
	4	2.1 ± 0.1^{bA}	4.9 ± 0.0^{bB}	8.4 ± 0.3^{aB}	3.61 ± 0.01^{aA}	31.0 ± 0.3^{aA}	17.3 ± 0.5^{aB}	5.6 ± 0.2^{aA}
	8	1.9 ± 0.3^{bA}	4.8 ± 0.0^{bB}	8.7 ± 0.2^{aAB}	3.62 ± 0.02^{aA}	31.1 ± 0.1^{aA}	17.4 ± 0.2^{aB}	5.7 ± 0.2^{aA}
300 MPa	0	$<1^{cA}$	6.1 ± 0.0^{aA}	8.6 ± 0.1^{aA}	3.59 ± 0.01^{aA}	31.7 ± 0.6^{aA}	19.5 ± 0.9^{bA}	6.3 ± 0.4^{aA}
	4	$<1^{cA}$	5.3 ± 0.0^{aB}	8.5 ± 0.1^{aA}	3.59 ± 0.01^{aA}	31.4 ± 0.8^{aA}	18.4 ± 1.3^{aA}	5.9 ± 0.5^{aA}
	8	$<1^{cA}$	5.3 ± 0.0^{aB}	8.6 ± 0.2^{aA}	3.62 ± 0.01^{aB}	31.8 ± 1.0^{aA}	18.8 ± 1.8^{aA}	6.1 ± 0.7^{aA}

Y&M = yeasts and molds. The limits of detection were 1 log CFU/g for plating method

Data represent mean value of three replicates ± 1 standard deviations.

Data in the same column and storage day followed by the same lowercase letter are not significant different (P > 0.05).

Data in the same column and treatment category followed by the same uppercase letter are not significantly different (P > 0.05).

The control sample had a SSC of 8.8 °Brix and pH of 3.57. Those two parameters were rather stable during the 8-day frozen storage and HPP treatment did not affect either of them. Color is another important parameter for strawberry puree. Generally, HPP at low and moderate temperatures has a limited effect on pigments responsible for the color of fruits and vegetables (Oey et al., 2008). In this study, no significant difference in CIE $L*a*b$ parameters were found for all the treatments compared to control. Those parameters were also very stable during the 8-day frozen storage at -18 °C afterwards. Our findings were supported by Zabetakis et al. (2000), who demonstrated that pelargonidin-3-glucoside and pelargonidin-3-rutinoside, two flavonoid pigments responsible for the color of strawberry, are quiet stable during pressure treatment at 800 MPa (18-22 °C/15 min) and the subsequent 1-week frozen storage at -25 °C. Ahmed et al. (2005) also reported that HPP had great color retention for mango pulp, which they thought was the best example of an unchanged quality attribute for HPP.

Yeasts and molds are predominant fungi involved in fruits and fruit products, which can cause spoilage, producing off flavors and discoloration of fruit products (Tournas et al., 2006). Therefore, the survival of yeasts and molds in strawberry puree during and after pressure treatment was studied (Table 3.3). The initial level of yeasts and molds in untreated strawberry puree was about 4.3 log CFU/g. The inactivation of yeasts and molds was positively correlated to the pressure level. More than 3 logs of yeasts and molds were inactivated by treatment at 300 MPa for 2 min at 21 °C; while only 0.5 log reduction was achieved with treatment at 200 MPa (Data not shown). This result is in general agreement with some previous studies. Ogawa et al. (1990) tested 9 species of yeasts and molds and found that pressure level required for a 5-log

reduction differed between fungal species and 400 MPa for 5 min was required to get a 5-log reduction of a pressure-resistant yeast in juice. Hocking et al. (2006) reported that a 3-4 log reduction was achieved for both *Saccharomyces cerevisiae* and *Pichia anomala* after 60 s pressurization at 400 MPa, with a 4-5 log reduction after 120 s at 400 MPa. Unlike bacterial pathogens which died quite rapidly during the first few days of frozen storage, the population of yeasts and molds in the control strawberry puree samples (without pressure treatment) was almost not affected by the 8 days frozen storage. Frozen storage also did not cause any further inactivation of yeasts and molds in pressure-treated samples although substantial inactivation of bacterial pathogens was observed during post-pressure storage. Therefore, a pressure treatment of 300 MPa for 2 min at 21 °C was needed to reduce natural yeasts and molds count to < 1 log.

3.4 Conclusions

This study highlighted a practical way of application of HPP at relative low pressure for processing strawberry puree with minimal impact on its physical properties. Our results show that both *E. coli* O157:H7 and *Salmonella* were able to persist in frozen strawberry puree for a long period of time. High pressure processing of strawberry puree at 200-500 MPa for 2 min at 21 °C brought about different levels of inactivation of pathogens depending on the pressure level. The use of short term post-pressure frozen storage was able to effectively lower the pressure level to 250-300 MPa for elimination of both pathogens in strawberry puree. In addition, HPP (250-300 MPa) had no adverse impact on physical properties of strawberry puree such as color, SSC, pH and viscosity. Pressurization of 300 MPa for 2 min effectively reduced natural yeasts and molds in strawberry puree to < 1 log. Therefore, HPP of

300 MPa for 2 min at 21 °C followed by 4 days frozen storage may be a viable decontamination method for the minimal processing of strawberry puree with great retention of fresh-like sensory properties.

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REFERENCES

Ahmed, J., Ramaswamy, H., Hiremath, N., 2005. The effect of high pressure treatment on rheological characteristics and colour of mango pulp. International Journal of Food Science and Technology 40, 885-895.

Baert, L., Uyttendaele, M., Van Coillie, E., Debevere, J., 2008. The reduction of murine norovirus 1, B-fragilis HSP40 infecting phage B40-8 and *E. coli* after a mild thermal pasteurization process of raspberry puree. Food Microbiology 25, 871-874.

Besser, R.E., Lett, S.M., Weber, J.T., Doyle, M.P., Barrett, T.J., Wells, J.G., Griffin, P.M., 1993. An outbreak of diarrhea and hemolytic uremic syndrome from *Escherichia coli* O157:H7 in fresh pressed apple cider. Journal of the American Medical Association 269, 2217-2220.

Butz, P., FernandezGarcia, A., Lindauer, R., Dieterich, S., Bognar, A., Tauscher, B., 2003. Influence of ultra high pressure processing on fruit and vegetable products. Journal of Food Engineering 56, 233-236.

Buzrul, S., Alpas, H., Largeteau, A., Demazeau, G., 2008. Inactivation of *Escherichia coli* and *Listeria innocua* in kiwifruit and pineapple juices by high hydrostatic pressure. International journal of food microbiology 124, 275-278.

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 and infection 131, 745-751.

Conner, D.E., Kotrola, J.S., 1995. Growth and survival of *Escherichia coli* O157:H7 under acidic conditions. Applied and Environmental Microbiology 61, 382-385.

Dawson, D., 2005. Foodborne protozoan parasites. International Journal of Food Microbiology 103, 207-227.

Deuel, C.L., Plotto, A., 2004. Chapter 22 Strawberrie and Raspberries, In D. Barrett, L. Somogyi, H. Ramaswamy (Eds.), Processing fruits: Science and Technology. CRC Press,pp. 542.

Economic Research Service. (2010). *U.S. strawberry production, utilization, prices, and values, 1970-2009.* Retrieved 3/8, 2012, from http://usda01.library.cornell.edu/usda/ers/95003/table01.xls

Food and Drug Administration. (2001). *FDA survey of imported fresh produce FY 1999 field assignment.* Retrieved 3/29, 2012, from http://www.fda.gov/Food/FoodSafety/Product-SpecificInformation/FruitsVegetablesJuices/GuidanceComplianceRegulatoryInformation/ucm118891.htm

Friedman, D., Heisey-Grove, D., Argyros, F., Berl, E., Nsubuga, J., Stiles, T., Fontana, J., Beard, R., Monroe, S., McGrath, M., Sutherby, H., Dicker, R., DeMaria, A., Matyas, B., 2005. An outbreak of norovirus gastroenteritis associated with wedding cakes. Epidemiology and infection 133, 1057-1063.

Garcia-Graells, C., Hauben, E., Michiels, C., 1998. High-pressure inactivation and sublethal injury of pressure-resistant *Escherichia coli* mutants in fruit juices. Applied and Environmental Microbiology 64, 1566-1568.

Guerrero-Beltran, J., Barbosa-Canovas, G., Swanson, B., 2005. High hydrostatic pressure processing of fruit and vegetable products. Food Reviews International 21, 411-425.

Hannum, S., 2004. Potential impact of strawberries on human health: A review of the science. Critical reviews in food science and nutrition 44, 1-17.

Herwaldt, B.L., Ackers, M.L., *Cyclospora* Working Group, 1997. An outbreak in 1996 of cyclosporiasis associated with imported raspberries. New England Journal of Medicine 336, 1548-1556.

Herwaldt, B.L., Beach, M.J., *Cyclospora* Working Group, 1999. The return of *Cyclospora* in 1997: Another outbreak of cyclosporiasis in North America associated with imported raspberries. Annals of Internal Medicine 130, 210-220.

Hilborn, E., Mshar, P., Fiorentino, T., Dembek, Z., Barrett, T., Howard, R., Cartter, M., 2000. An outbreak of *Escherichia coli* O157:H7 infections and haemolytic uraemic syndrome associated with consumption of unpasteurized apple cider. Epidemiology and infection 124, 31-36.

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., Pool, V., Cramer, E., Nainan, O., Weth, J., Williams, I., Goldstein, S., Gensheimer, K., Bell, B., Shapiro, C., Alter, M., Margolis, H., Natl Hepatitis A Invest Team, 1999. A multistate, foodborne outbreak of hepatitis A. New England Journal of Medicine 340, 595-602.

Hocking, A., Begum, M., Stewart, C.,2006. Inactivation of fruit spoilage yeasts and moulds using high pressure processing. Advances in Food Mycology 571, 239-246.

Jain, S., Bidol, S.A., Austin, J.L., Berl, E., Elson, F., LeMaile-Williams, M., Deasy,Marshall,,III, Moll, M.E., Rea, V., Vojdani, J.D., Yu, P.A., Hoekstra, R.M., Braden, C.R., Lynch, M.F., 2009. Multistate Outbreak of *Salmonella* Typhimurium and Saintpaul infections associated with unpasteurized orange juice-United States, 2005. Clinical Infectious Diseases 48, 1065-1071.

Janisiewicz, W., Conway, W., Brown, M., Sapers, G., Fratamico, P., Buchanan, R., 1999. Fate of *Escherichia coli* O157:H7 on fresh-cut apple tissue and its potential for transmission by fruit flies. Applied and Environmental Microbiology 65, 1-5.

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.

Katz, D., Kumar, S., Malecki, J., Lowdermilk, M., Koumans, E.H.A., Hopkins, R., 1999. Cyclosporiasis associated with imported raspberries, Florida, 1996. Public health reports 114, 427-438.

Kingsley, D., Guan, D., Hoover, D., 2005. Pressure inactivation of hepatitis A virus in strawberry puree and sliced green onions. Journal of food protection 68, 1748-1751.

Knudsen, D., Yamamoto, S., Harris, L., 2001. Survival of *Salmonella* spp. and *Escherichia coli* O157:H7 on fresh and frozen strawberries. Journal of Food Protection 64, 1483-1488.

Korsager, B., Hede, S., Boggild, H., Bottiger, B.E., Molbak, K., 2005. Two outbreaks of norovirus infections associated with the consumption of imported frozen raspberries, Denmark, May-June 2005. Euro surveillance 10, E050623.1.

Kothary, M.H., Babu, U.S., 2001. Infective dose of foodborne pathogens in volunteers: a review. Journal of Food Safety 21, 49-68.

Landl, A., Abadias, M., Sarraga, C., Vinas, I., Picouet, P.A., 2010. Effect of high pressure processing on the quality of acidified Granny Smith apple puree product. Innovative Food Science & Emerging Technologies 11, 557-564.

Linton, M., McClements, J., Patterson, M., 1999. Survival of *Escherichia coli* O157:H7 during storage in pressure-treated orange juice. Journal of food protection 62, 1038-1040.

Ma, L., Zhang, G., Gerner-Smidt, P., Tauxe, R.V., Doyle, M.P., 2010. Survival and growth of *Salmonella* in salsa and related ingredients. Journal of food protection 73, 434-444.

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., Margolis, H.S., 1992. Multistate outbreak of hepatitis-A associated with frozen strawberries. Journal of Infectious Diseases 166, 518-524.

Oey, I., Lille, M., Van Loey, A., Hendrickx, M., 2008. Effect of high-pressure processing on colour, texture and flavour of fruit- and vegetable-based food products: a review. Trends in Food Science & Technology 19, 320-328.

Ogawa, H., Fukuhisa, K., Kubo, Y., Fukumoto, H.,1990. Pressure Inactivation of Yeasts, Molds, and Pectinesterase in Satsuma Mandarin Juice - Effects of Juice Concentration, pH, and Organic-Acids, and Comparison with Heat Sanitation. Agricultural and Biological Chemistry 54.

Oregon Health Authority. (2011). *Lab tests confirm source of E. coli O157 from deer droppings in strawberry fields in NW Oregon.* Retrieved 3/29, 2012, from http://www.oregon.gov/OHA/news/2011/2011-0817a.pdf?ga=t

Oyarzabal, O., Nogueira, M., Gombas, D., 2003. Survival of *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella* in juice concentrates. Journal of food protection 66, 1595-1598.

Polydera, A., Stoforos, N., Taoukis, P., 2005. Quality degradation kinetics of pasteurised and high pressure processed fresh navel orange juice: nutritional parameters and shelf life. Innovative Food Science & Emerging Technologies 6, 1-9.

Ransay, C.N., Upton, P.A., 1989. Hepatitis-A and frozen raspberries. Lancet 1, 43-44.

Reid, T.M.S., Robinson, H.G., 1987. Frozen raspberries and Hepatitis-A. Epidemiology and Infection 98, 109-112.

San Martin, M., Barbosa-Canovas, G., Swanson, B., 2002. Food processing by high hydrostatic pressure. Critical reviews in food science and nutrition 42, 627-645.

Sanchez-Moreno, C., Plaza, L., De Ancos, B., Cano, M., 2003. Vitamin C, provitamin A carotenoids, and other carotenoids in high-pressurized orange juice during refrigerated storage. Journal of Agricultural and Food Chemistry 51, 647-653.

Sarvikivi, E., Roivainen, M., Maunula, L., Niskanen, T., Korhonen, T., Lappalainen, M., Kuusi, M., 2012. Multiple norovirus outbreaks linked to imported frozen raspberries. Epidemiology and Infection 140, 260-267.

Sharma, M., Beuchat, L., Doyle, M., Chen, J., 2001. Survival of *Salmonellae* in pasteurized, refrigerated calcium-fortified orange juice. Journal of food protection 64, 1299-1304.

Tournas, V., Heeres, J., Burgess, L., 2006. Moulds and yeasts in fruit salads and fruit juices. Food Microbiology 23, 684-688.

Yamamoto, S., Harris, L.,2001. The effects of freezing and thawing on the survival of *Escherichia coli* O157:H7 in apple juice. International Journal of Food Microbiology 67, 89-96.

Zabetakis, I., Leclerc, N., Kajda, P., 2000. The effect of high hydrostatic pressure on the strawberry anthocyanins. Journal of Agricultural and Food Chemistry 48, 2749-2754.

Chapter 4

A NOVEL WATER-ASSISTED PULSED LIGHT PROCESSING FOR DECONTAMINATION OF BLUEBERRIES

(A manuscript published in Food Microbiology)

ABSTRACT

Sample heating and shadowing effect have limited the application of pulsed light (PL) technology for decontamination of fresh produce. In this study, a novel setup using water-assisted PL processing was developed to overcome these limitations. Blueberries inoculated with *Escherichia coli* O157:H7 or *Salmonella* were either treated with PL directly (dry PL treatment) or immersed in agitated water during the PL treatment (wet PL treatment) for 5–60s. Although both pathogens were effectively inactivated by the dry PL treatments, the appearance of the blueberries was adversely affected and a maximum temperature of 64.8 °C on the blueberry surface was recorded. On the other hand, the visual appearance of blueberries remained unchanged after wet PL treatments and sample heating was significantly reduced. The wet PL treatments were more effective than chlorine washing on inactivating both pathogens. After a 60-s wet PL treatment, the populations of *E. coli* O157:H7 inoculated on calyx and skin of blueberries were reduced by 3.0 and > 5.8 log CFU/g, respectively. *Salmonella* on blueberry calyx and skin was reduced by 3.6 and > 5.9 log

CFU/g, respectively. No viable bacterial cells were recovered from the water used in the wet PL treatments, demonstrating that this setup could prevent the risk of cross-contamination during fresh produce washing. Our results suggest that this new water-assisted PL treatment could be a potential non-chemical alternative (residue free) to chlorine washing since it is both more effective and environmentally friendly than chlorine washing.

4.1 Introduction

Fresh berries are highly valued for their high antioxidant and vitamin content. Many bioactive compounds in berries have been shown to provide significant health benefits (Hannum, 2004; Szajdek and Borowska, 2008). As a result, the consumption of berries has been increasing rapidly in recent years in the United States. From 2000 to 2010, a five-fold increase was observed for the consumption of fresh blueberries (ERS, 2012) and the U.S. has become the largest producer of blueberries in the world (FAO, 2012).

Unfortunately, these small fruits are susceptible to contamination by various pathogenic microorganisms since they are constantly exposed to soils and irrigation water in the fields and human contacts during harvesting. Moreover, berries destined for fresh market are usually picked by hands and not washed before sale for the sake of fruit quality and shelf-life. Recently, the implication of blueberries and other berries in several foodborne outbreaks has raised concerns about their microbial safety. In 2003, contaminated blueberries were reported to be the source of an outbreak of hepatitis A virus (Calder et al., 2003). A multistate outbreak of *Salmonella* Muenchen

reported in 2009 was traced back to consumption of blueberries, which caused 14 cases of illnesses (CDC, 2013). In 1999, the U.S. Food and Drug Administration (FDA) initiated a survey focused on high-volume imported fresh produce; *Salmonella* was isolated from 1 of 143 samples of strawberries imported into the U.S. (FDA, 2001). In 2011, fresh strawberries from a farm in Oregon were linked with an *Escherichia coli* O157:H7 outbreak, which caused at least 15 illnesses including one death. Deer droppings in the strawberry field were later confirmed as the source of contamination (Oregon Health Authority, 2011). Therefore, effective intervention methods with minimal effect on the quality and shelf-life of blueberries are urgently needed.

Pulsed light (PL) is an emerging nonthermal technology that utilizes short, intense pulses of broad spectrum light (wavelength = 180–1100 nm) to inactivate microorganisms (Gomez-Lopez et al., 2007). This technology was adopted by the U.S. FDA for food processing in 1996 (FDA, 1996). The efficacy of PL for inactivating bacteria, fungi and viruses *in vitro* is well documented (Anderson et al., 2000; Roberts and Hope, 2003; Rowan et al., 1999). Use of PL to process food products such as apple juice, milk, minimally processed vegetables, berries, alfalfa seeds, hot dogs and salmon fillets have been studied with the intention of extending shelf-life and/or inactivating pathogens (Oms-Oliu et al., 2010). The primary mechanism of microbial inactivation is the photochemical dimerization of nucleic acids in microorganisms, caused mainly by UV-C part of the PL spectrum (Gomez-Lopez et al., 2007). This dimerization inhibits transcription and replication, thus resulting in cellular inactivation. In addition, it has also been shown that both the visible and infrared

regions of PL in combination with its high peak power also contribute to the killing effect on microorganisms (Elmnasser et al., 2007).

The application of PL to enhance the safety and/or shelf-life of fresh produce, including berries, has been studied previously (Bialka and Demirci, 2007; Gomez et al., 2012; Ramos-Villarroel et al., 2011; Ramos-Villarroel et al., 2012). Currently there are two main challenges that limit the PL application in the fresh produce industry. One issue is that PL treatment causes substantial heating of the samples, which might damage the quality of fresh produce. Another issue is that microorganisms on an opaque food surface must directly face the PL-strobe to be inactivated due to the shallow penetration depth of PL. In addition, samples positioned in different parts of the PL chamber might be exposed to different doses of PL. To overcome these two limitations, we developed a water-assisted PL system in which blueberry samples were immersed in agitated water during PL treatment. With this new system, the temperature increases of water and the samples were minimized due to the large specific heat of water. Moreover, the blueberry samples could randomly move and rotate in the agitated water, thus allowing more uniform PL exposure of all the blueberry surfaces.

The objectives of this study were to evaluate the efficacy of PL on the inactivation of *E. coli* O157:H7 and *Salmonella* on blueberries and to use this novel setup to minimize the adverse effects of PL on the physical and sensorial properties of blueberries.

4.2 Materials and Methods

4.2.1 Bacterial strains and inoculum preparation

Five *E. coli* O157:H7 strains and four *Salmonella enterica* strains of different serotypes were used in this study. The *E. coli* O157:H7 strains were kindly provided by Dr. Joerger and Dr. Kniel, University of Delaware; and the *Salmonella* strains by Dr. Gurtler, U.S. Department of Agriculture. Detailed strain information is shown in Table 4.1.

Table 4.1 Bacterial strain information

Species	Serotype	Strain	Origin
E. coli	O157:H7	H1730	Lettuce outbreak
E. coli	O157:H7	cider	Cider outbreak
E. coli	O157:H7	250	Sprout outbreak
E. coli	O157:H7	251	Lettuce outbreak
E. coli	O157:H7	J58	Lettuce isolate
S. enterica	St. Paul	02-517-1	Cantaloupe outbreak
S. enterica	Newport	H1275	Sprout outbreak
S. enterica	Montevideo	G4639	Tomato outbreak
S. enterica	Stanley	HO588	Sprout outbreak

The individual wild-type strains were adapted to become nalidixic-acid-resistant as described by Huang et al. (2013). The cultures were maintained on tryptic soy agar (Difco Laboratories, Sparks, MD) supplemented with 0.6% yeast extract (Difco) and 50 µg/mL of nalidixic acid (Fisher Scientific, Hampton, NH) (TSAYE-N) at 10 °C. Individual cultures were grown in 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 for another 24-h incubation at

35 °C. One mL of each culture was mixed to form a 5-strain cocktail of *E. coli* O157:H7 or a 4-strain cocktail of *Salmonella*. Bacterial cells were harvested by centrifugation at $2450 \times g$ for 10 min (Centra CL2, Centrifuge, Thermo Scientific). The supernatant was discarded and the pellet was resuspended in 1 mL of sterile 0.1% peptone water (Fisher Scientific) to yield a final concentration of ~10^9 CFU/mL.

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. All the berries were UV-treated (254 nm) in a biosafety hood for 10 min to reduce the impact of background microflora before each experiment. The blueberries were then spot-inoculated with 25 μl of the 5-strain cocktail of *E. coli* O157:H7 or the 4-strain cocktail of *Salmonella*. Inoculum was deposited on either the skin or calyx tissue of blueberries in small droplets to simulate two contamination conditions. After inoculation, the samples were air-dried in a biosafety hood at 22 ± 2 °C for 2 h to facilitate bacterial attachment. The final inoculation levels of *E. coli* O157:H7 and *Salmonella* on berries were 6.8–7.0 log CFU/g.

4.2.3 PL treatment

The PL treatments were performed with a laboratory scale PL system (Steripulse-XL RS-3000, Xenon Corp.,Wilmington, MA), which consisted of a controller module, a treatment chamber and an air cooling module. The lamp was capable of generating PL in the wavelength of 180–1100 nm, with 40% of the energy being in the UV region (Hsu and Moraru, 2011). The pulses were generated at a rate of 3 pulses/s with pulse width of 360 μs. According to the manufacturer's

specifications, each pulse delivers 1.27 J/cm^2 for an input of 3800 V at 19.3 mm from the quartz window of the lamp.

In this study, PL treatments were performed in two modes, dry and wet PL treatments. For dry PL treatments, three blueberries were placed on a sterile petri-dish with the inoculation site facing the PL lamp. The samples was placed at the center of the PL chamber and directly illuminated by PL for 5, 15, 30 and 60 s. The distance between the lamp and the quartz window was 5.8 cm and the distance between the top of blueberries and the quartz window was ~15 cm. For wet PL treatments, three blueberries were immersed in 150-mL agitated tap water during the PL treatment in a 1-L glass beaker containing a 2.5-cm stirring bar. An ultra-thin magnetic stirrer (Lab Disc, Fisher Scientific) was placed under the PL chamber to agitate the water in the beaker so that random rotation and movement of blueberries could be achieved. The treatment durations were 5, 15, 30 and 60 s. The distance between the top of blueberries and the quartz window was also ~15 cm and the distance from the top of blueberries to the water surface was about 1 cm. For comparison, blueberries were also washed with 150-mL agitated tap water or 10 ppm chlorinated water for 60 s in the beaker with the stirring bar. The 10 ppm chlorinated water was chosen since it was generally used by the food industry to wash blueberries intended for further processing such as frozen storage. Chlorinated water (pH 6.5) was prepared by adding commercial bleach (Clorox, Oakland, CA, USA) into DI water to obtain 10 ppm of free chlorine. Chlorine concentration was determined by free chlorine micro check test strips (HF Scientific, Ft. Myer, FL).

4.2.4 PL fluence measurements

The broadband energy of each pulse, expressed in J/cm^2, was quantified using a Vega laser power meter (Ophir Optronics Inc., Wilmington, MA) equipped with a pyroelectric energy sensor (PE-50C, Ophir Optronics). The wavelength setting was 300 nm with pulse width of 500 μm. To measure the PL energy received by blueberries for the dry PL treatment, the pyroelectric sensor was placed at the center of the PL chamber bottom, which was ~15 cm from the quartz window. To measure the PL energy received by blueberries for the wet PL treatment, a 1-L glass beaker with its bottom cut was placed on the pyroelectric sensor which was at the center of the PL chamber bottom. Since the sensor could not be immersed in water, a 500-mL quartz beaker (GM Associates, Inc., Oakland, CA) containing 1 cm deep water was placed inside the glass beaker and sat on the sensor. Precautions were taken to allow about 30-s pauses between each measurement to prevent sensor from overheating. All fluence measurements were performed in triplicate.

4.2.5 Temperature measurement

The temperature profile of blueberry surface during 60-s dry and wet PL treatment was monitored in 10-s interval using a K-type thermocouple connected to a thermometer (H84, Omega enineering, Stamford, CT). The thermocouple was placed under the skin of a blueberry. For the dry PL treatment, a blueberry with the thermocouple attached was exposed to PL during the temperature measurement. Since it was impossible to secure the thermocouple to a moving blueberry in agitated water during the wet PL treatment, a blueberry with the thermocouple attached was immersed in un-agitated water and exposed to PL during the temperature measurement.

4.2.6 Microbiological analysis

After PL treatment, each sample consisting of 3 blueberries (~5g) was transferred into a sterile filter bag (Whirl-Pak, Nasco, USA) containing 45 mL of Dey-Engley (D/E) neutralizing broth (Difco) and pummeled in a laboratory stomacher (Seward 400, Seward, London, U.K.) for 2 min at 260 rpm. The homogenate was then serially diluted in sterile 0.1% peptone water and surface-plated on TSAYE-N followed by incubation at 35 °C for 72 h. Presumptive colonies of *E. coli* O157:H7 or *Salmonella* formed on the plates were counted. Blueberry homogenate (10 mL) was also enriched in 40 mL of TSBYE-N and incubated for 48 h at 35 °C to allow resuscitation of sub-lethally injured cells. Enriched samples were then streaked onto Sorbitol MacConkey (Difco) supplemented with 50 µg/mL of nalidixic acid for *E. coli* O157:H7 or Xylose Lysine Desoxycholate agar (Difco) supplemented with 50 µg/mL of nalidixic acid for *Salmonella*. After 24 h of incubation, the presence of growth exhibiting typical morphology of *E. coli* O157:H7 or *Salmonella* was determined by visual inspection. To demonstrate whether cross-contamination could happen during the wet PL treatments, 1 mL water samples was immediately taken from the glass beaker after each wet PL treatment and plated onto TSAYE-N plates. The plates were incubated at 35 °C for 72 h before the counts of *E. coli* O157:H7 and *Salmonella* were determined.

4.2.7 Color measurement

Un-inoculated blueberries were treated with dry or wet PL for 30 s or 60 s. A chroma meter (Minolta CR-400, Minolta, Osaka, Japan) was used to measure the color of untreated and PL-treated blueberries. Three spots of blueberry skin were randomly selected for the color measurements after wet PL treatments. For dry-PL-treated

blueberries, three spots on the surface facing the PL lamp were selected for color measurement. Color parameters were quantified in the Hunter *L*, *a*, *b* color space where *L* refers to lightness, ranging from 0 (blackness) to 100 (whiteness), positive *a* means red and negative *a* green, and positive *b* means yellow and negative *b* blue.

4.2.8   Effect of wet PL treatment on natural yeasts and molds on blueberries

Un-inoculated blueberries (3 berries/sample) were treated with wet PL for 30 s or 60 s as described above. The berries were then air-dried in a biosafety hood for 45 – 60 min and packaged in vented clamshell containers (H312, Highland Packaging, Plant City, FL). The clamshells were stored at 0 °C with 90 – 95% relative humidity as recommended by Mitcham et al. (2013) for up to 8 days. Saturated potassium nitrate (Fisher Scientific) solution was used to control the relative humidity in the environment. A traceable thermo-hygrometer (Fisher Scientific) was used to monitor the relative humidity. On days 0, 4 and 8, the counts of natural yeasts and molds on blueberries were determined by plating samples on dichloran rose Bengal chloramphenicol agar (Difco). The plates were incubated at 25 °C for 3 – 5 days before populations of yeasts and molds were enumerated.

4.2.9   Statistical analysis

At least three independent trials were conducted for all the experiments. Colony counts were converted to log CFU/g and means and standard deviations were calculated. Statistical analyses were conducted using JMP (SAS Cary, NC, USA). One-way analysis of variance and Tukey's one-way multiple comparisons were used to determine significant differences among treatments ($P < 0.05$).

Table 4.2 Reduction of *E. coli* O157:H7 and *Salmonella* on blueberries using dry PL treatments. *E. coli* O157:H7 and *Salmonella* were inoculated on the calyx and skin of blueberries and the blueberries were then treated with dry PL for 5-60 s.

Treatment	PL fluence (J/cm^2)	*E. coli* O157:H7 Calyx	skin	*Salmonella* calyx	skin
PL for 5 s	5.0	2.1 ± 0.1 bA	3.8 ± 0.4 cB	Not done	Not done
PL for 15 s	14.3	2.8 ± 0.6 bA	4.8 ± 0.5 bB	Not done	Not done
PL for 30 s	28.2	3.0 ± 0.6 bA	> 5.7 (3/3) bB	2.6 ± 0.3 aA	4.8 ± 0.6 aB
PL for 60 s	56.1	4.3 ± 0.8 aA	> 6.7 (0/3) aB	4.1 ± 0.5 bA	5.7 ± 0.1 bB

Data represent mean population reduction (log CFU/g) of three replicates ± 1 standard deviations.

The initial populations of *E. coli* O157:H7 and *Salmonella* on blueberries were 6.8 ± 0.2 and 7.0 ± 0.1 log CFU/g, respectively.

Numbers in parentheses represent the number of samples shown positive after enrichment out of a total of 3 trials.

The limits of detection were 1 log CFU/g for the plating method and 1 CFU/g for the enrichment method.

Data in the same column followed by the same lowercase letter are not significantly different (P > 0.05).

Data in the same row and bacteria category followed by the same uppercase letter are not significantly different (P > 0.05).

4.3 Results

4.3.1 Effect of dry and wet PL treatments on the inactivation of *E. coli* O157:H7 and *Salmonella* on blueberries.

Table 4.2 shows the effect of dry PL treatments on inactivation of *E. coli* O157:H7 and *Salmonella* on blueberries. In general, the populations of *E. coli* O157:H7 and *Salmonella* were reduced by the dry PL treatments in a fluence-dependent manner for both inoculation sites. For samples inoculated on the calyx, *E. coli* O157:H7 was reduced by 2.1–4.3 log CFU/g depending on the treatment time or fluence level. Dry PL treatments for 5–60 s resulted in a 3.8- to > 6.7-log (survivors undetectable by the enrichment method) reduction of *E. coli* O157:H7 inoculated on the skin of blueberries. Up to 4.1- and 5.7-log reductions of *Salmonella* on blueberry calyx and skin, respectively, were achieved by a 60-s dry PL treatment. Compared with *E. coli* O157:H7, *Salmonella* showed higher ($P < 0.05$) resistance to the PL treatment when both were inoculated onto the skin of the blueberries. Similar reductions ($P > 0.05$) of *E. coli* O157:H7 and *Salmonella* were obtained when inocula were applied on the calyx.

Results for inactivation of *E. coli* O157:H7 and *Salmonella* by the wet PL treatments are shown in Table 4.3. Generally, the efficacy of the wet PL treatments varied as a function of the treatment time regardless of the inoculation site. Similar to the dry PL treatments, the wet PL treatments resulted in higher log reductions of both pathogens inoculated on the blueberry skin rather than on the calyx. For samples with inoculum on the calyx, the 60-s wet PL treatment resulted in a 3.0-log CFU/g reduction of *E. coli* O157:H7 while the two control treatments, 60-s water washing and 10 ppm chlorine washing, only reduced population of *E. coli* O157:H7 by 0.8 and

2.3 log CFU/g, respectively. For blueberries inoculated on the skin, a 5-s wet PL treatment reduced *E. coli* O157:H7 by 4.5 log CFU/g and longer treatment times, 30 and 60 s, reduced population of *E. coli* O157:H7 below the detection limit of the plating method (1 log CFU/g). The two most effective treatments, 30-s and 60-s wet PL treatments, were therefore selected for the *Salmonella* study and similar results were obtained (Table 4.3). Maximum reductions of 3.7 and > 5.9 log CFU/g (survivors undetectable by the plating method) were observed for blueberries inoculated with *Salmonella* on calyx and skin, respectively, after 60-s wet PL treatments. The 60-s wet PL treatment also exhibited consistently higher, if not significantly, efficacy in inactivating *Salmonella* on both inoculation sites of blueberries compared with the 60-s chlorine washing.

Table 4.3 Reduction of *E. coli* O157:H7 on blueberries using wet PL treatments. *E. coli* O157:H7 and *Salmonella* were inoculated on the calyx and skin of blueberries and the blueberries were then treated with wet PL for 5-60 s or washed with water or chlorine for 60 s.

Treatment	PL fluence (J/cm²)	*E. coli* O157:H7		*Salmonella*	
		calyx	skin	calyx	Skin
PL for 5 s	5.0	1.5 ± 0.4 bA	4.5 ± 0.6 bB	Not done	Not done
PL for 15 s	14.3	2.4 ± 0.4 aA	5.3 ± 0.3 aB	Not done	Not done
PL for 30 s	28.2	2.8 ± 0.6 aA	> 5.8 (3/3) aB	3.1 ± 0.3 abA	3.7 ± 0.8 bA
PL for 60 s	56.1	3.0 ± 0.0 aA	> 5.8 (3/3) aB	3.6 ± 0.1 aA	> 5.9 (4/5) aB
Water washing		0.9 ± 0.1 bA	2.8 ± 0.1 cB	1.7 ± 0.2 cA	3.0 ± 0.4 bB
10 ppm chlorine		2.3 ± 0.2 aA	5.7 ± 0.2 aB	2.7 ± 0.3 bA	5.5 ± 0.6 aB

All of the water sample tested were negative for *E. coli* O157:H7 or *Salmonella*.

Data represent mean population log reduction (log CFU/g) of at least three replicates ± 1 standard deviations.

The initial populations of *E. coli* O157:H7 and *Salmonella* were 6.8 ± 0.2 and 7.0 ± 0.1 log CFU/g, respectively.

Numbers in parentheses represent the number of samples shown positive after enrichment out of a total of at least 3 trials.

The limits of detection were 1 log CFU/g for the plating method and 1 CFU/g for the enrichment method.

Data in the same column followed by the same lowercase letter are not significantly different (P > 0.05).

Data in the same row and bacteria category followed by the same uppercase letter are not significantly different (P > 0.05).

For fresh produce washing, cross-contamination between contaminated items and uncontaminated items via water is a serious concern. To demonstrate whether the wet PL treatments could eliminate the risk of cross-contamination, water samples were taken from the glass beaker for microbial analysis immediately after each PL treatment of inoculated blueberries. The counts of *E. coli* O157:H7 and *Salmonella* were determined by direct plating onto TSAYE-N plates. Negative results were obtained for all the samples, which indicated that the wet PL system would not pose any risk of cross-contamination (Table 4.3).

4.3.2 Effect of dry and wet PL treatments on the quality of blueberries

During PL treatments, samples absorbed the light energy resulting in temperature increases. The temperature profiles of the blueberry surfaces during dry and wet PL treatments are shown in Figure 4.1. A maximum temperature of 64.8 °C on the blueberry surface was recorded after a 60-s dry PL treatment. The increase in temperature during the dry PL treatments was coupled with severe discoloration of blueberries. The blueberry bloom/wax was also degraded by the dry PL treatments resulting in a burnt appearance as shown in Figure 4.2. In contrast, the surface temperatures of blueberries were much lower when the blueberries were immersed in water during the PL treatment; reaching a maximum of 34 °C at the end of the 60-s wet PL treatment. The wet PL treatments also preserved the appearance of blueberries. No noticeable appearance change was observed after the 60-s wet PL treatment as shown in Figure 4.2.
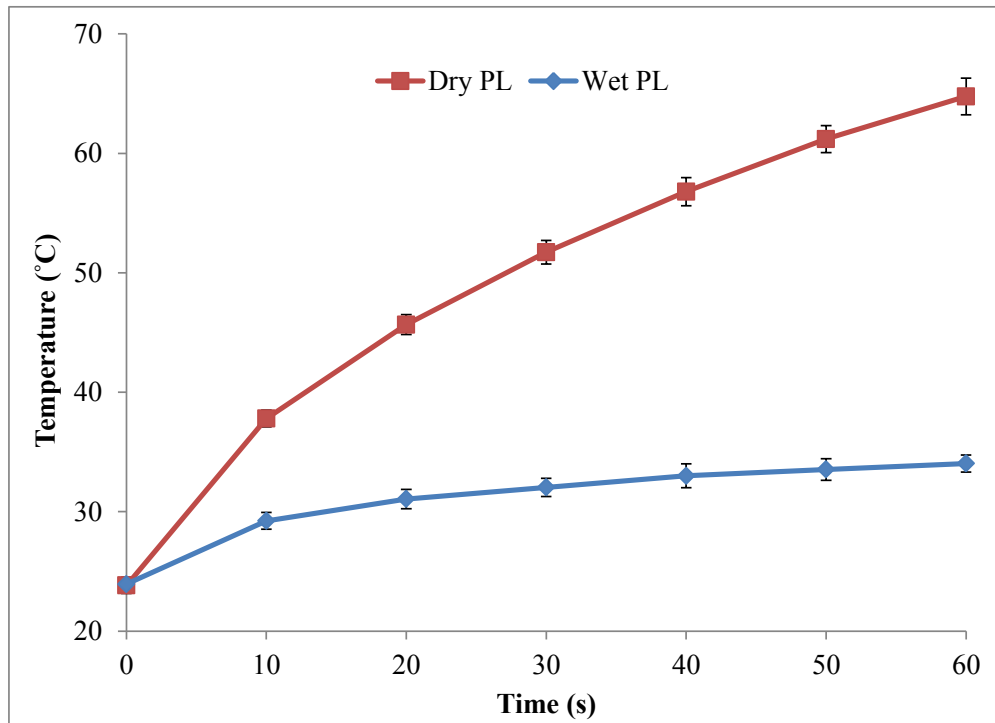
Figure 4.1 Surface temperatures of un-inoculated blueberries during 60-s dry and wet
PL treatments. Data represent mean values of three replicates ± 1
standard deviation.
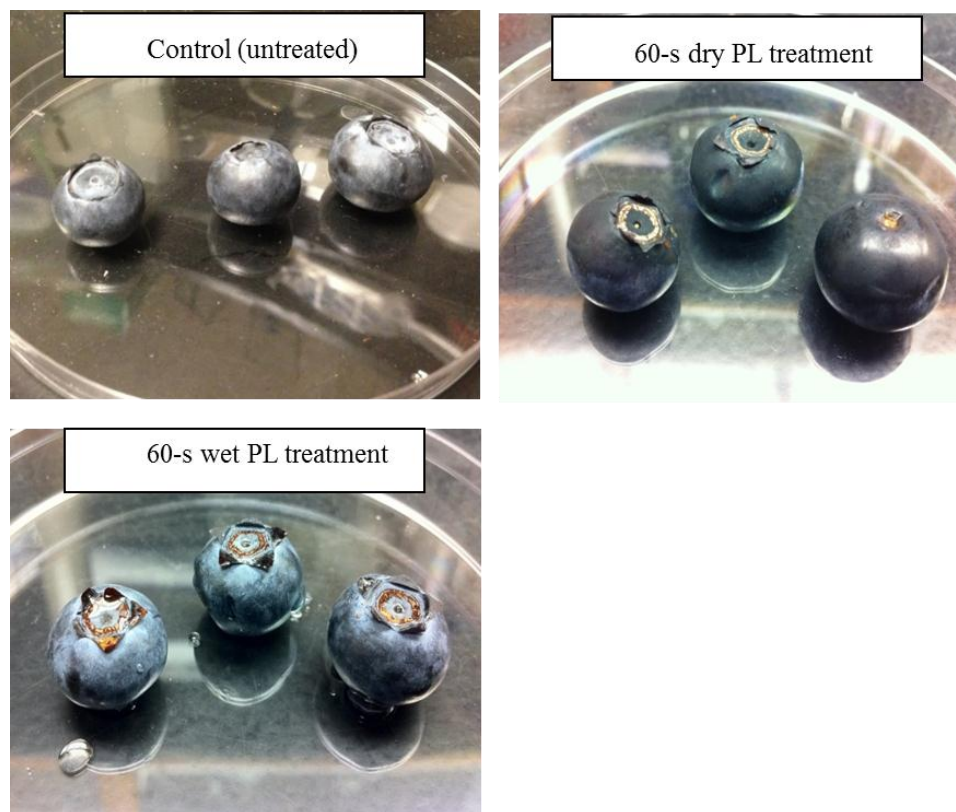
Figure 4.2 Pictures of untreated and PL-treated blueberries.

The visual observation of the blueberry appearance during the dry and wet PL treatments was corroborated by the color measurements as shown in Table 4.4. Significant differences ($P < 0.05$) in the L, a, b values were observed between dry-PL-treated and untreated blueberries, although no significant difference were found between samples treated with PL for different durations. A significant decrease ($P < 0.05$) in the L value and increase in the a and b values was observed for blueberries treated with dry PL compared with untreated samples, which corresponded to the darker appearance as shown in Figure 4.2. On the other hand, there were no significant

differences (P > 0.05) in the *L, a* and *b* values between the untreated and the wet PL-treated samples.

Table 4.4 Hunter *L, a* and *b* color readings of untreated and PL-treated blueberries. Un-inoculated blueberries were treated with dry or wet PL treatments for 30-60 s.

Color	Untreated	30-s Dry PL	60-s Dry PL	30-s Wet PL	60-s Wet PL
L value	37.8 ± 2.1 a	28.7 ± 0.5 b	28.7 ± 0.1 b	36.8 ± 1.3 a	35.5 ± 0.2 a
a value	1.3 ± 0.1 bc	2.2 ± 0.8 ab	2.4 ± 0.2 a	1.1 ± 0.2 c	1.7 ± 0.1 abc
b value	-1.6 ± 0.3 b	1.3 ± 0.1 a	1.5 ± 0.2 a	-1.4 ± 0.2 b	-1.2 ± 0.3 b

Data represent mean color values of three replicates ± 1 standard deviation.

For the same color parameter, values in the same row followed by the same lower case letter are not significantly different (P > 0.05).

L refers to lightness, positive *a* means red and negative *a* green, and positive *b* means yellow and negative *b* blue.

Since the blueberry quality after dry PL treatment was not acceptable, only the wet PL treatment was tested for its impact on the survival of natural yeasts and molds during 8 days of refrigerated storage. The population of yeasts and molds was reduced by 0.5- and 0.8- log CFU/g, respectively, by the 30-s and 60-s wet PL treatments on Day 0 (Table 4.5). During the subsequent 8 days storage at 0°C, The populations of yeasts and molds were very stable, varying between 3.0 and 3.5 log. There were no significant differences in the populations of yeasts and molds between the treated and untreated samples. No significant appearance change was visually observed for the wet PL-treated and untreated samples during the 8 days storage and none became moldy after 8 days of storage.

Table 4.5 Population of yeasts and molds on blueberries during 8-day storage. Un-
        inoculated blueberries were treated with wet PL for 30-60 s and then
        stored for 8 days at 0 °C with 90-95% relative humidity.

	Storage time (day)		
	0	4	8
Control	3.8 ± 0.1 aAB	3.6 ± 0.2 aB	4.1 ± 0.1 aA
Wet PL *30s	3.3 ± 0.1 bA	3.1 ± 0.5 aA	3.5 ± 0.2 aA
Wet PL *60s	3.0 ± 0.3 bA	3.5 ± 0.2 aA	3.5 ± 0.4 aA

Values represent the mean population (log CFU/g) of three replicates ± 1 standard

deviation.

Data in the same column followed by the same lowercase letter are not significantly

different (P > 0.05).

Data in the same row followed by the same uppercase letter are not significantly

different (P > 0.05).

4.4   Discussion

4.4.1   Effect of dry PL treatment on inactivation of *E. coli* O157:H7 and *Salmonella* on blueberries and resulting quality of blueberries.

The results in Table 4.2 show that the PL fluence level and inoculation site are

the two most important factors that affects the efficacy of dry PL treatment. Both

pathogens investigated were substantially reduced in a time-dependent manner by the

dry PL treatment. Our results are in general agreement with those reported by Bialka

and Demirci (2007) who obtained 1.3–4.9 log reductions of *E. coli* O157:H7 and 1–

3.8 log reductions of *Salmonella* on blueberry skin with dry PL at fluences of 1.1–32.4

J/cm^2. However, the impact of inoculation site on the efficacy of dry PL was not

determined in their study. The difference in efficacy of PL treatment against bacterial

cells inoculated on blueberry skin and calyx in our study was probably due to the

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different surface structures of those two sites. Compared with blueberry skin, the calyx has a much rougher surface structure, which potentially allows more shielding/shadowing of microorganisms inside surface details. It is known that PL has a very limited penetration depth (~2 μm) in nontransparent media (Wallen et al., 2001) and is only capable of targeting superficial microorganisms. Therefore, bacterial cells hiding in the sub-surface of the calyx were probably protected from PL. Similar findings were reported by other researchers. Kim and Hung (2012) observed a persistent higher population of *E. coli* O157:H7 recovered from the blueberry calyx than from the skin after UV treatment. Sapers et al. (2000) found a higher survival of *E. coli* in the calyx and stem areas of inoculated apples than the skin after a washing treatment. Woodling and Moraru (2005) studied the influence of surface topography of stainless steel coupon on the effectiveness of PL treatment and indicated a complex effect of various surface properties on inactivation. Han et al. (2000) reported that *E. coli* O157:H7 preferentially attached to coarse and porous intact surfaces and injured surfaces of peppers. Similar phenomena were also exhibited by raspberries and strawberries (Sy et al., 2005). Indeed, higher levels of bacteria were reported to be found in the calyx of naturally contaminated apples (Riordan et al., 2001). The surface structure of fresh produce is usually complex and bacterial cells may lodge in surface irregularities or crevices, *i.e.* calyx, therefore, reducing the efficacy of PL by preventing the highly directional, coherent PL beam from reaching its target (Lagunas-Solar et al., 2006). Hence, great care must be taken in selecting the representative inoculation site in a microbial challenge study.

Although PL treatment is considered "nonthermal", this usually only holds true for treatments of short durations. For a longer PL treatment, the temperature of a

sample could increase to a level high enough to cause thermal inactivation of microorganisms. In our study, a maximum temperature of 64.8 °C was observed during the 60-s dry PL treatment which caused elimination of *E. coli* O157:H7 (survivors undetectable by the enrichment method) on blueberry skin. It is very possible that both the thermal and nonthermal parts of PL contributed to the elimination of *E. coli* O157:H7 in the dry PL treatment. To investigate the level of thermal inactivation caused by sample heating, a simple experiment was conducted. Briefly, blueberries were inoculated with *E. coli* O157:H7 on the top of the berries (skin and calyx). The samples were then treated with dry PL or wet PL. For this particular experiment, water was not agitated during the wet PL treatments. After 60-s PL treatment, an extra 1.7-log reduction (P < 0.05) of *E. coli* O157:H7 were achieved for berries treated in the absence of water compared with berries that were immersed in un-agitated water during the PL treatment. However, similar reductions (P > 0.05) of *E. coli* O157:H7 were obtained for shorter treatment durations, i.e. 5, 15 and 30s. The results demonstrated that the high temperature (64.8 °C) reached during the 60-s dry PL treatment probably caused the extra 1.7-log reduction. Similar observation was also reported by Bialka and Demirci (2008) who indicated that the significant temperature increase of raspberries may result in a thermal inactivation of pathogens. Therefore, it can be concluded that heating effect plays an important role in long dry PL treatments, which should be taken into account when interpreting inactivation results.

Color is an important quality factor, which directly influences the fresh market value of berries. The color also determines the suitability of a processing method, which may remove or degrade the bloom/wax, therefore affecting Hunter *L* (lightness)

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values (Kim et al., 2011). A significant change in visual appearance of blueberries treated by dry PL was observed in this study (Figure 4.2), which was confirmed by the results of color measurements (Table 4.4). The discoloration of berries was also coupled with sample heating during the PL treatment. In fact, sample heating and browning were reported to be the most important limiting factor for the application of PL. Bialka and Demirci (2007) reported that PL-treated blueberries had a cooked appearance and lost structural integrity when samples were treated at a high fluence level. In a later study (Bialka and Demirci, 2008), they reported a maximum temperature of 80 °C on raspberry surface after dry PL treatment at a fluence level of 72 J/cm^2. Gomez et al. (2012) reported that cut-apple surface exposed to high PL fluences turned darker and less green due to browning reactions accelerated by temperature increase during PL treatments. Ramos-Villarroel et al. (2011) reported that the use of 30 pulses of PL (12 J/cm^2) affected the color and firmness of fresh-cut avocado, causing browning and softening. Fine and Gervais (2004) reported that PL treatment caused a rapid modification of color of black pepper and wheat flour. This color modification was thought to be attributed to overheating combined with oxidation.

4.4.2 Effect of wet PL treatment on the inactivation of *E. coli* O157:H7 and *Salmonella* on blueberries and resulting quality of blueberries.

It needs to be noted that direct comparison between the efficacy of wet and dry PL treatments based on fluence level may be misleading, although the measured fluence value in the wet PL setup was equivalent to that in the dry PL setup. The equivalence in fluence values was only a coincidence, which was caused by reflection and scattering effects of glassware and energy absorption by water. For dry PL

treatments, the inoculation site was facing the PL-strobe all the time and was the only surface to receive all the PL energy. For wet PL treatments, however, the surface being illuminated with PL was always changing as the berries were randomly moving and rotating in the agitated water. Therefore, the inoculation sites on the blueberries received less PL energy in the wet treatments than in the dry PL treatments.

Lagunas-Solar et al. (2006) reported that PL treatment could be interfered by shadowing effect and therefore modifications and/or improvements is necessary to generate random movement of fruits to provide uniform PL exposure of all fruit surfaces. In addition, temperature control of samples would have to be taken into consideration to make longer treatment possible without damaging the food quality. In this study, therefore, a wet PL system was designed to overcome the limitations associated with the dry PL system. In the wet PL system, blueberries were submerged in agitated water during PL treatment, which could provide three major benefits. First, the presence of water substantially reduced the sample heating thus preserving the sensory quality of blueberries as demonstrated in Figure 4.2 and Table 4.4. A lower sample temperature may also ensure better preservation of nutrition since some bioactive compounds such as anthocyanin and vitamins may be degraded during sample heating (Kong and Singh, 2012; Sablani et al., 2010). Second, the agitated water allowed blueberries to move and rotate freely so that uniform PL exposure of all the blueberry surfaces could be achieved. Third, the agitated water could physically remove bacterial cells from blueberries and these cells would be easily killed by PL once they were in the water. To our knowledge, this is the first study that used this wet system to enhance the efficacy of PL treatment and minimize the quality loss of blueberries caused by dry PL treatment.

Table 4.3 demonstrates that the inactivation of *E. coli* O157:H7 and *Salmonella* had a positive correlation with the treatment duration. It can be reasonably expected that higher PL fluence or longer treatment time would cause a higher bacterial inactivation rate. In the meantime, the longer treatment time probably allowed more bacterial cells to be washed off. As shown in Table 4.3, water washing alone for 60-s removed 0.9 and 2.8 log CFU/g of *E. coli* O157:H7 from blueberry calyx and skin, respectively. Once in water, the bacterial cells were expected to be easily killed by PL. To test this hypothesis, we inoculated 150 mL of DI water with the *E. coli* O157:H7 cocktail to a final level of 6.8 CFU/mL. The inoculated water was exposed to PL for 5 s and then analyzed for bacterial counts. After the 5-s PL treatment, the *E. coli* O157:H7 counts in the water were below the 1-log detection limit of the plating method (> 5.8-log reduction). Indeed, these results are somewhat predictable since water is known to be relatively transparent to a range of wavelengths including UV and visible light. In addition, PL has been shown to be highly effective against a range of microorganisms in water and clear fluids (Huffman et al., 2000; Sauer and Moraru, 2009).

Chlorine is commonly used by the food industry to wash different types of fresh produce to reduce levels of microorganisms and to prevent cross-contamination. It is also used to wash blueberries before they are further processed into frozen berries, puree and other products. However, concerns have been raised in recent years regarding the efficacy and safety of chlorine. It is known that chlorine loses its effectiveness quickly in the presence of soil, dirt and organic materials and forms various carcinogenic disinfection by-products, including trihalomethanes (Richardson et al., 1998). Chlorine solutions and fumes are irritating to the skin and the respiratory

tract. In addition, use of chlorine is associated with the production of high amounts of wastewater with very high levels of biological oxygen demand (BOD) (Oelmez and Kretzschmar, 2009). Due to the environmental and health risks posed by chlorine, there is a trend in reducing or eliminating the use of chlorine from the disinfection process. Our results in Table 4.3 demonstrated that the 60-s wet PL treatment is consistently more effective in inactivating *E. coli* O157:H7 and *Salmonella* on both inoculation sites of blueberries compared to the 60-s chlorine washing. As a physical method, PL leaves no chemical residues on the produce being treated. In addition, the wet PL treatment is more environmentally friendly since it generates no chemical residues in the water during the application and does not create chemical disposal issues. Therefore, the wet PL treatment could be a potential non-chemical alternative (residue free) to chlorine washing with greater efficacy and less environmental concern.

For industry application, a simple PL system similar to the laboratory-scale wet PL system used in this study is probably adequate. In this conceptual system, PL lamps are placed above an open flume containing shallow water. Blueberries would flow in single layer through the flume. Rotation and random movement of blueberries can be achieved through mechanical agitation of the water or through the use of water jets. The flow speed and/or the length of the flume could be controlled or designed to obtain target treatment time. To enhance the decontamination efficacy, multiple PL lamps could also be installed above, below and on both sides of the flume. In this case, the flume should be made of quartz to allow PL to pass through. Since water is sanitized during the PL processing, it can be re-used thus saving the water usage. Periodically, water needs to be filtered to remove suspended solids. Before entering

the PL system, blueberries might need a pre-treatment step using overhead water sprayers or an air blower to remove dirt, soil and plant debris since they could potentially reduce the efficacy of PL treatment. Although only blueberries were tested in this study, the PL system could probably be used to decontaminate other fresh produce with smooth surface such as tomatoes. Further study needs to be conducted to validate the feasibility.

In blueberries, natural microorganisms such as yeasts and molds are common contaminants, which cause spoilage of fruits. Thus, it is necessary to investigate the effect of PL treatments on fungal contaminants and their survival during subsequent storage. The results in Table 4.5 showed that the two wet PL treatments had very limited effect on reducing the natural yeasts and molds on blueberries. The reason for this result is not clear; however, it is possible that these natural yeasts and mold are strongly attached to the blueberries and protected by biofilms and surface details. In the study of Kim et al. (2011), a well-established biofilm of yeast with or without budding on the surface of fresh blueberry was observed under scanning electron microscope. It is known that biofilm could protect microorganisms from environmental changes and microorganisms in biofilms are much more resistant to many antimicrobial treatments (Simoes et al., 2010). The low efficacy of wet PL treatments may also due to a higher resistance of some natural fungal contaminants compared with *E. coli* O157:H7 and *Salmonella*. It was reported in two studies (Anderson et al., 2000; Rowan et al., 1999) that fungal spores were generally more resistant to PL; however, Gómez-López et al. (2005) did not observe any sensitivity pattern among different groups of microorganisms.

Acknowledgement

REFERENCES

Anderson, J., Rowan, N., MacGregor, S., Fouracre, R., Farish, O. 2000. Inactivation of food-borne enteropathogenic bacteria and spoilage fungi using pulsed-light. *IEEE Trans. Plasma Sci. 28*(1), 83-88.

Bialka, K.L., Demirci, A. 2007. Decontamination of *Escherichia coli* O157: H7 and *Salmonella enterica* on blueberries using ozone and pulsed UV-Light. J. Food Sci. 72:M391-M396. doi: 10.1111/j.1750-3841.2007.00517.x.

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. J. Food Sci. 73:M201-M207. doi: 10.1111/j.1750-3841.2008.00743.x.

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. Epidemiol. Infect. 131:745-751.

Centers for Diseases Control and Prevention. 2013. Foodborne Outbreak Online Database. Available at: http://wwwn.cdc.gov/foodborneoutbreaks/Default.aspx. Accessed 20 May 2013.

Economic Research Service, US Department of Agriculture. 2012. Fresh blueberries: Supply and utilization in the United States, 1980-2011. Available at: http://usda01.library.cornell.edu/usda/ers/blueberry/table04.xls. Accessed 28 November 2012.

Elmnasser, N., Guillou, S., Leroi, F., Orange, N., Bakhrouf, A., Federighi, M. 2007. Pulsed-light system as a novel food decontamination technology: a review. Can. J. Microbiol. 53:813-821. doi:10.1139/W07-042

Fine, F., Gervais, P. 2004. Efficiency of pulsed UV light for microbial decontamination of food powders. J. Food Prot. 67:787-792.

Food and Agriculture Organization of the United Nations. 2012. Food and Agricultural commodities production. Available at: http://faostat.fao.org/site/339/default.aspx. Accessed 27 September 2012.

Food and Drug Administration. 1996. Pulsed light for the treatment of food. 21CFR179.4.

Food and Drug Administration. 2001. FDA Survey of Imported Fresh Produce FY 1999 Field Assignment. Available at: http://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatory Information/ProducePlantProducts/ucm118891.htm. Accessed 29 March 2012.

Gomez, P. L., Salvatori, D. M., Garcia-Loredo, A., Alzamora, S. M. 2012. Pulsed light treatment of cut apple: dose effect on color, structure, and microbiological stability. Food Bioprocess Technol. 5:2311-2322.

Gómez-López, V. M., Devlieghere, F., Bonduelle, V., Debevere, J. 2005. Factors affecting the inactivation of micro-organisms by intense light pulses. J. Appl. Microbiol. 99:460-470.

Gomez-Lopez, V. M., Ragaert, P., Debevere, J., Devlieghere, F. 2007. Pulsed light for food decontamination: a review. Trends Food Sci. Technol. 18:464-473.

Han, Y., Linton, R., Nielsen, S., Nelson, P. 2000. Inactivation of *Escherichia coli* O157: H7 on surface-uninjured and -injured green pepper (*Capsicum annuum L.*) by chlorine dioxide gases demonstrated by confocal laser scanning microscopy. Food Microbiol. 17:643-655.

Hannum, S. 2004. Potential impact of strawberries on human health: A review of the science. Crit. Rev. Food Sci. Nutr. 44:1-17.

Hsu, L., Moraru, C. I. 2011. Quantifying and mapping the spatial distribution of fluence inside a pulsed light treatment chamber and various liquid substrates. J. Food Eng. 103:84-91.

Huang, Y., Ye, M., Chen, H. 2013. Inactivation of *Escherichia coli* O157:H7 and *Salmonella* spp. in strawberry puree by high hydrostatic pressure with/without subsequent frozen storage. Int. J. Food Microbiol. 160:337-343.

Huffman, D., Slifko, T., Salisbury, K., Rose, J. 2000. Inactivation of bacteria, virus and Cryptosporidium by a point-of-use device using pulsed broad spectrum white light. Water Res. 34:2491-2498.

Kim, C., Hung, Y. 2012. Inactivation of *E. coli* O157:H7 on blueberries by electrolyzed water, ultraviolet light, and ozone. J. Food Sci. 77:M206-M211. doi: 10.1111/j.1750-3841.2011.02595.x.

Kim, T. J., Corbitt, M. P., Silva, J. L., Wang, D. S., Jung, Y., Spencer, B. 2011. Optimization of hot water treatment for removing microbial colonies on fresh blueberry surface. J. Food Sci. 76:M353-M360. doi: 10.1111/j.1750-3841.2011.02209.x.

Kong, F, Singh, R.P. 2012. Effect of Processing on Nutrients in Foods, p. 193. *In* Ghosh D, Das S, Bagchi D, Smarta RB (eds.), Innovation in Healthy and Functional Foods. CRC Press.

Lagunas-Solar, M., Bolkan, L., MacDonald, J. D., Pina, C. 2006. Development of pulsed UV light processes for surface fungal disinfection of fresh fruits. J. Food Prot. 69:376-384.

Mitcham, E. J., Crisosto, C. H., Kader, A. A. 2013. Bushberries: blackberry, blueberry, cranberry, raspberry: recommendations for maintaining postharvest quality. Available at: http://postharvest.ucdavis.edu/PFfruits/Bushberries. Accessed 20 May 2013.

Oelmez, H., Kretzschmar, U. 2009. Potential alternative disinfection methods for organic fresh-cut industry for minimizing water consumption and environmental impact. LWT-Food Sci. Technol. 42: 686-693.

Oms-Oliu, G., Martin-Belloso, O., Soliva-Fortuny, R. 2010. Pulsed Light Treatments for Food Preservation. A Review. Food Bioprocess Technol. 3:13-23

Oregon Health Authority. 2011. Lab tests confirm source of *E. coli* O157 from deer droppings in strawberry fields in NW Oregon. Available at: http://www.oregon.gov/OHA/news/2011/2011-0817a.pdf?ga=t. Accessed 29 Mach 2012.

Ramos-Villarroel, A. Y., Martin-Belloso, O., Soliva-Fortuny, R. 2011. Bacterial inactivation and quality changes in fresh-cut avocado treated with intense light pulses. Eur. Food Res. Technol. 233:395-402.

Ramos-Villarroel, A. Y., Aron-Maftei, N., Martin-Belloso, O., Soliva-Fortuny, R. 2012. The role of pulsed light spectral distribution in the inactivation of *Escherichia coli* and *Listeria innocua* on fresh-cut mushrooms. Food Control. 24:206-213.

Richardson, S. D., Thruston, A. D., Caughran, T. V., Collette, T. W., Patterson, K. S., Lykins, B. W. 1998. Chemical by-products of chlorine and alternative disinfectants. Food Technol. 52:58-61.

Riordan, D., Sapers, G., Hankinson, T., MaGee, M., Mattrazzo, A., Annous, B. 2001. A study of US orchards to identify potential sources of *Escherichia coli* O157: H7. J. Food Prot. 64:1320-1327.

Roberts, P., Hope, A. 2003. Virus inactivation by high intensity broad spectrum pulsed light. J. Virol. Methods. 110:61-65.

Rowan, N., MacGregor, S., Anderson, J., Fouracre, R., McIlvaney, L., Farish, O. 1999. Pulsed-light inactivation of food-related microorganisms. Appl. Environ. Microbiol. 65:1312-1315.

Sablani, S. S., Andrews, P. K., Davies, N. M., Walters, T., Saez, H., Syamaladevi, R. M., Mohekar, P. R. 2010. Effect of thermal treatments on phytochemicals in conventionally and organically grown berries. J. Sci. Food Agric. 90:769-778.

Sapers, G. M., Miller, R. L., Jantschke, M., Mattrazzo, A. M. 2000. Factors limiting the efficacy of hydrogen peroxide washes for decontamination of apples containing *Escherichia coli*. J. Food Sci. 65:529-532.

Sauer, A., Moraru, C. I. 2009. Inactivation of *Escherichia coli* ATCC 25922 and *Escherichia coli* O157:H7 in apple juice and apple cider, using pulsed light treatment. J. Food Prot. 72:937-944.

Simoes, M., Simoes, L. C., Vieira, M. J. 2010. A review of current and emergent biofilm control strategies. LWT-Food Sci. Technol. 43:573-583.

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. J. Food Prot. 68:1165-1175.

Szajdek, A., Borowska, E. J. 2008. Bioactive Compounds and Health-Promoting Properties of Berry Fruits: A Review. Plant Foods Hum Nutr. 63:147-156.

Wallen, R.D., May, R., Rieger, K., Holloway, J.M., Cover, W.H., 2001. Sterilization of a new medical device using broad-spectrum pulsed light. Biomed. Instrum. Technol. 35:323-330.

Woodling, S., Moraru, C. 2005. Influence of surface topography on the effectiveness of pulsed light treatment for the inactivation of *Listeria innocua* on stainless-steel surfaces. J. Food Sci. 70:M345-M351. doi: 10.1111/j.1365-2621.2005.tb11478.x.

Chapter 5

INACTIVATION OF *ESCHERICHIA COLI* O157:H7, *SALMONELLA* AND HUMAN NOROVIRUS SURROGATE ON ARTIFICIALLY CONTAMINATED STRAWBERRIES AND RASPBERRIES BY WATER-ASSISTED PULSED LIGHT TREATMENT

ABSTRACT

This study investigated the inactivation of *Escherichia coli* O157:H7, *Salmonella* and murine norovirus (MNV-1), a human norovirus surrogate, on strawberries and raspberries using a water-assisted pulsed light (WPL) treatment. The effects of combinations of WPL treatment with 1% hydrogen peroxide (H_2O_2) or 100 ppm sodium dodecyl sulfate (SDS) were also evaluated. Strawberries and raspberries were inoculated with *E. coli* O157:H7 and treated by WPL for 5 – 60 s. *E. coli* O157:H7 on both strawberries and raspberries was significantly reduced in a time-dependent manner with 60-s WPL treatments reducing *E. coli* O157:H7 by 2.4 and 4.5 log CFU/g, respectively. Significantly higher reductions of *E. coli* O157:H7 were obtained using 60-s WPL treatment than washing with 10 ppm chlorine. Compared with washing with chlorine, SDS and H_2O_2, the combination of WPL treatment with 1% H_2O_2 for 60 s reduced *E. coli* O157:H7 on strawberries and raspberries by 3.3- and 5.3-log units, respectively. Similarly, *Salmonella* on strawberries and raspberries was inactivated by 2.8- and 4.9-log units after 60-s WPL-H_2O_2 treatments. For decontamination of MNV-1, a 60-s WPL treatment reduced the viral titers on strawberries and raspberries by 1.8- and 3.6-log units, respectively; however, the combination of WPL and H_2O_2 did not significantly ($P>0.05$) enhance the treatment

efficiency. These results demonstrated that the WPL treatment can be a promising chemical-free alternative to chlorine washing for decontamination of berries destined for fresh-cut and frozen berry products. WPL-H_2O_2 treatment was the most effective treatment for decontamination of bacterial pathogens on berries, providing an enhanced degree of microbiological safety for berries.

5.1   Introduction

Foodborne pathogens such as human norovirus, *Salmonella* and *Escherichia coli* O157:H7 have been frequently associated with outbreaks of fresh produce. During 1990-2005, human norovirus was ranked as the top causative agent for fresh produce outbreaks, accounting for 40% of all the outbreaks, followed by *Salmonella* spp. (18%), *Escherichia coli* (8%), *Clostridium* spp. (6%) and hepatitis A virus (4%) (Doyle & Erickson, 2008). According to a recent report by the US Department of Agriculture Economic Research Service, foodborne illnesses cost more than $15.6 billion annually in the US (USDA-ERS, 2014a), in which annual economic losses due to human norovirus, *Salmonella* and *E. coli* O157:H7 were estimated to be 2.6, 3.7 and 0.27 billion US dollars, respectively. Leafy vegetables and fruits/nuts are the 2nd and 4th leading food vehicles for foodborne illnesses, responsible for 13% and 11% of all the foodborne outbreaks in the US (Gould et al., 2013).

Fresh and frozen berries are popular fruit commodities for their unique flavors, textures and health benefits. Therefore, the consumption of berries has increased considerably in the last decade. From 2000 to 2010, the consumption of fresh strawberries increased from 1.4 billion lb. to 2.3 billion lb. and a five- and four-fold increase was observed for the consumption fresh blueberry and raspberry, respectively

(USDA-ERS, 2014b). However, concerns regarding the microbial safety of berries and berry products have been rising in recent years due to the implication of these small fruits in multiple foodborne outbreaks. Strawberries have been implicated in three outbreaks of hepatitis A (CDC, 1997a; Hutin et al., 1999; Niu et al., 1992). In 2011, fresh strawberries from a farm in Oregon were associated with an *E. coli* O157:H7 outbreak, which caused at least 15 people sick including two deaths (Laidler et al., 2013). In 2012, norovirus contaminated frozen strawberries caused the largest foodborne outbreak in Germany, which affected about 11,000 people (Mäde et al., 2013). Raspberries have been implicated in a series of outbreaks of *Cyclospora cayetanensis* (CDC, 1997b; Herwaldt & Beach, 1999; Herwaldt & Ackers, 1997). Frozen raspberries also have been reported to have caused multiple large scale human norovirus outbreaks in 2005 and 2009 (Friedman et al., 2005; Sarvikivi et al., 2012). Unfortunately, current production practices are not designed to reduce potential pathogen contamination; therefore, new food processing technologies that is able to inactivate both bacterial and viral pathogens from berries is urgently needed.

Pulsed light (PL) is an emerging nonthermal 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 (200 – 1100 nm) rich in UV light (Oms-Oliu, Martín-Belloso, & Soliva-Fortuny, 2008). This technology was approved by the U.S. FDA for food processing in 1996 (21 CFR 179.41). A number of studies has been published on the application of PL for inactivation of bacteria, fungi and viruses *in vitro* (Oms-Oliu et al., 2008). Efforts have been made to successfully apply PL for decontamination on both solid and liquid foods, food contact materials and food packaging materials (Bialka & Demirci, 2008;

McDonald et al., 2000; Sauer & Moraru, 2009). However, many studies also reported a significant temperature increase during the PL treatments of fresh produce, which caused undesirable sensorial changes in these fresh produce (Bialka & Demirci, 2008; Fine & Gervais, 2004; Huang & Chen, 2014; Ramos-Villarroel, Martín-Belloso, & Soliva-Fortuny, 2011). In addition, shadowing effect limits the PL efficacy on solid foods (Elmnasser et al., 2007); therefore, a 360° exposure of PL must be ensured for efficient PL decontamination. In our previous study, a water-assisted PL (WPL) system was developed and successfully addressing those issues (Huang & Chen, 2014). By treating blueberries with PL while they are being agitated in water, efficient rotation of blueberries were achieved during washing, allowing more uniform PL exposure on all the blueberry surfaces. Meanwhile, the agitated water acted as a cooling medium to prevent sample heating. Our results showed that the WPL could be a promising non-chemical alternative to chlorine washing since it is more effective and environmentally friendly.

In this study, we further investigated the inactivation of *E. coli* O157:H7, *Salmonella* and murine norovirus (MNV-1), as a surrogate for human norovirus, on both strawberries and raspberries using the WPL system we developed. In addition, the combination of PL with hydrogen peroxide (H_2O_2) or sodium dodecyl sulfate (SDS) was evaluated as a hurdle technology for decontamination of berries.

5.2 Materials and Methods

5.2.1 Bacterial strains and inoculum preparation

Five *E. coli* O157:H7 strains, kindly provided by Dr. Joerger and Dr. Kniel, University of Delaware, and four *Salmonella enterica* serotypes, kindly provided by

Dr. Gurtler, U.S. Department of Agriculture, were used in this study. The *E. coli* O157:H7 strains included H1730 (lettuce outbreak isolate), cider (cider outbreak isolate), 250 (sprout outbreak isolate), 251 (lettuce outbreak isolate) and J58 (lettuce isolate). The *Salmonella* strains included *S.* St. Paul 02-517-1 (Cantaloupe outbreak isolate), *S.* Newport H1275 (Sprout outbreak isolate), *S.* Montevideo G4639 (raw tomato outbreak isolate) and *S.* Stanley HO588 (Sprout outbreak isolate). Nalidixic acid-resistant mutants of the strains were generated as described by Huang et al. (2013). The bacterial cultures were maintained on tryptic soy agar (Difco Laboratories, Sparks, MD) supplemented with 0.6% yeast extract (Difco) and 50 µg/mL of nalidixic acid (Fisher Scientific, Hampton, NH) (TSAYE-N) at 10 °C for no more than 1 month. To prepare bacterial culture, 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 were transferred twice at 24-h interval. One mL of each culture was mixed to form a five-strain cocktail of *E. coli* O157:H7 or a four-strain cocktail of *Salmonella*. Bacterial cells were harvested by centrifugation at 2450 × *g* for 10 min (Centra CL2, Centrifuge, Thermo Scientific). The supernatant was discarded and the pellet was resuspended in 1 mL of sterile 0.1% peptone water (Fisher Scientific) to yield a final concentration of ~10^9 CFU/mL.

5.2.2 Virus and cell line

Strain MNV-1 and murine macrophage cell line RAW 264.7 were kindly provided by Dr. Li, the Ohio State University. RAW 264.7 cells were propagated in Dulbecco's Modified Eagle Medium (DMEM) (Gibco, Life Technologies Corporation) supplemented with 10% fetal bovine serum (FBS) (Gibco) at 37 °C under a 5% CO_2 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-h incubation at 37 °C under a 5% CO_2 atmosphere, 25ml of DMEM supplemented with 2% FBS was added. MNV-1 was harvested 2 days post inoculation by three freezing-thawing cycles and centrifugation. The virus stock was stored at −80 °C until use.

5.2.3   Sample inoculation

Fresh blueberries were purchased from local grocery stores the day before each experiment and stored at 4 °C until use. All the berries were illuminated with UV-C (254 nm) in a biosafety hood for 10 min to reduce the impact of background microflora before each experiment. Raspberries (~5 g /sample) were spot-inoculated with 30 μl of the *E. coli* O157:H7 or *Salmonella* cocktails and strawberries (~20 g/sample) with 100 μl of each cocktail. The final inoculation levels of *E. coli* O157:H7 and *Salmonella* on berries were ~6.8 log CFU/g. For viral inoculation, raspberries and strawberry were spot-inoculated with 100 and 400 μl of the MNV-1 stock, respectively, to a level of ~4.5 log PFU/g. After inoculation, the samples were air-dried in a biosafety hood at 22 ± 2 °C for 2 h to facilitate bacterial attachment.

5.2.4   PL treatment

A bench-top PL system (Steripulse-XL RS-3000, Xenon Corp., Wilmington, MA) that included a controller module, a treatment chamber and an air cooling module was used in this study. The Xenon flash lamp emitted PL in the wavelength of 200–1100 nm, with 40% of the energy being in the UV region (Hsu & Moraru, 2011). The pulses were generated at a rate of 3 pulses/s with a pulse width of 360 μs. The distance between the lamp and the quartz window in the PL unit was 5.8 cm and the

distance between the bottom of the treatment chamber and the quartz window was ~16 cm.

For PL treatments, the water-assisted PL (WPL) setup was as described by Huang & Chen (2014). Raspberry and strawberry samples were immersed in 300 mL and 500 mL tap water, respectively, in a 1-L glass beaker containing a 2.5-cm stir bar. The corresponding depths of water in raspberry and strawberry treatments were 4.0 cm and 6.3 cm, respectively. An ultra-thin magnetic stirrer (Lab Disc, Fisher Scientific) was placed under the PL chamber to agitate the water in the beaker through the stir bar. The berries were therefore continuously tumbled to achieve a 360° exposure to the PL source. The treatment durations were 5, 15, 30 and 60 s. In addition, the effect of adding 1% H_2O_2 or 100 ppm SDS to the wash water during WPL treatments was investigated. Washing treatments using tap water, 1% H_2O_2, chlorinated water (CW) containing 10 ppm free chlorine, and 100 ppm SDS were also evaluated using the same washing setup without the PL treatment. Solutions of 1% H_2O_2 and 100 ppm SDS were prepared by adding proper amounts of H_2O_2 (3%, Onpoint, Clifton, NJ) and SDS (Amresco Inc., OH, USA) into de-ionized (DI) water. CW were prepared by diluting household bleach (8.25% sodium hypochlorite, Clorox, Oakland, CA) in DI water with pH adjusted to 6.5 with citric acid. The free chlorine level in the CW was determined by a portable free/total chlorine meter (Hanna HI 93414, Hanna Instruments, USA).

5.2.5 PL fluence measurements

The broadband energy of each pulse, expressed in J/cm^2, was quantified using a Vega laser power meter (Ophir Optronics Inc., Wilmington, MA) equipped with a pyroelectric energy sensor (PE-50C, Ophir Optronics). Since the berry samples were

always moving up and down in the water, the PL fluence at heights equal to half of water depth during each treatment were measured to represent an average fluence. To measure the fluence received by raspberry samples during WPL treatments, a 1-L glass beaker with its bottom cut was placed on the pyroelectric sensor centered at the bottom of the treatment chamber. It needs to be noted that the thickness of the sensor is ~2 cm. Since the sensor could not be immersed in water, a 500-mL quartz beaker (GM Associates, Inc., Oakland, CA) containing water to a depth of 2 cm was placed inside the glass beaker and sit on the sensor. To measure the fluence received by strawberries, a similar approach was used except that a 1-cm spacer was placed under the sensor and the quartz beaker on the sensor was filled with water to a depth of 3.2cm. Precautions were taken to allow about 30-s pauses between each measurement to prevent sensor from overheating. All fluence measurements were performed in triplicate.

5.2.6 Color and temperature measurements

As a quality indicator, the color of the berries was measured and analyzed immediately after treatments. Un-inoculated strawberries and raspberries were treated with WPL for 60 s as described in section 5.2.4. A chroma meter (Minolta CR-400, Minolta, Osaka, Japan) was used to measure the color of untreated and treated berries. Three spots on the berry skin were randomly selected for color measurements after WPL treatments. Color parameters were quantified in the Hunter *L*, *a*, *b* color space where *L* refers to lightness, ranging from 0 (blackness) to 100 (whiteness), positive *a* means red and negative *a* green, and positive *b* means yellow and negative *b* blue.

The temperature profiles on berry surfaces during a 60-s WPL treatment was monitored at intervals of 5 s using a K-type thermocouple connected to a thermometer

(H84, Omega enineering, Stamford, CT). To measure the temperature profiles on berry surfaces, the thermocouple was placed under the skin of berries. Since it is impossible to secure the thermocouple to a moving berry in agitated water, the temperature profile of berries immersed in un-agitated water were measured instead. In comparison, the berries were also PL-treated directly without water and the temperature profiles were recorded.

5.2.7 Bacterial recovery and analysis

After treatments, each sample was transferred into a sterile filter bag (Whirl-Pak, Nasco, USA) containing 45 or 80 mL of Dey-Engley (D/E) neutralizing broth (Difco) for raspberries and strawberries, respectively, and pummeled in a laboratory 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 – 72 h. Presumptive colonies of *E. coli* O157:H7 or *Salmonella* on the plates were counted.

5.2.8 Extraction of MNV-1 from berries and viral plaque assay

MNV-1 was extracted from the berries as described by Dubois et al. (2002) and Kingsley et al. (2005) with some modifications. Individual berry samples were transferred into a sterile filter bag (Nasco). Ten mL and 40 mL of vegetable extraction buffer [100 mM Tris (Thermo Fisher Scientific Inc.), 50 mM glycine (Promega Corporation), 3% (m/v) beef extract (Becton Dickinson Company), 50 mM $MgCl_2$ (Thermo Fisher Scientific Inc.), pH 9.5] was added to the bags for raspberries and strawberries, respectively. The samples were immersed in buffer, incubated at 37 °C for 30 min and stomached at 260 rpm for 1 min. The sample homogenate was

centrifuged at $3000 \times g$ for 10 min at 4 °C. The supernatant was serially diluted in DMEM and used for plaque assays. The viral titer was quantified using plaque assays as described previously (Huang et al., 2014). Briefly, RAW264.7 monolayers were infected with 400 µl of MNV-1 series dilutions and incubated for 1 h at 37 °C and 5% CO_2 with gentle rocking every 10 min. The inoculum was then aspirated and cells were overlaid with 2.5 mL of Eagle Minimum Essential Medium (MEM) supplemented with 5% (v/v) FBS, 1% (w/v) sodium bicarbonate, 10 mM HEPES (pH 7.7), 100 U/mL penicillin, 100 µg/mL streptomycin, 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_2 for 48 h, fixed in 3.7% formaldehyde for 60 min and plaques were then visualized by staining with 0.05% (w/v in 10% ethanol) crystal violet.

5.2.9   Statistical analysis

At least three independent trials were conducted for all the experiments. Colony counts and viral counts were converted to log CFU/g and log PFU/g, respectively, with means and standard deviations calculated. Statistical analyses were conducted using JMP (SAS Cary, NC, USA). One-way analysis of variance and Tukey's one-way multiple comparisons were used to determine significant differences among treatments ($P < 0.05$).

5.3   Results

5.3.1   WPL treatments of strawberries and raspberries

Strawberries and raspberries inoculated with *E. coli* O157:H7 at a final concentration of ~6.8 log CFU/g were treated for 5 – 60 s using the WPL system. As

shown in Table 5.1, *E. coli* O157:H7 on strawberries and raspberries was significantly reduced in a time-dependent pattern by WPL treatments. On strawberries, *E. coli* O157:H7 was significantly reduced by 1.4 – 2.4 log units after 5 – 60 s WPL treatments ($P < 0.05$) with PL fluence ranging from 5.6 to 63.2 J/cm^2. Washing with tap water or CW only resulted in 1.4 – 1.5 log reductions of *E. coli* O157:H7 on strawberries, which was similar ($P > 0.05$) to a 5-s WPL treatment while a significantly higher reduction of *E. coli* O157:H7 was achieved by 60-s WPL treatment than washing with tap water or CW ($P < 0.05$). Raspberries consistently had higher log reductions of *E. coli* O157:H7 than strawberries. WPL treatment for 5 – 60 s, with fluence ranging from 4.8 to 53.9 J/cm^2, resulted in 1.6 – 4.5 log reduction of *E. coli* O157:H7 population on raspberries. Washing with tap water and CW caused 1.9- and 2.5-log reduction of *E. coli* O157:H7, which were not significantly different ($P > 0.05$) from 5 – 30 s WPL treatments. In contrast, a significantly higher reduction ($P < 0.05$) was observed for 60-s WPL treatment, which reduced the population of *E. coli* O157:H7 on raspberries by 4.5 log CFU/g.

Table 5.1 Effect of single treatments on inactivating *E. coli* O157:H7 on strawberries and raspberries. Berries spot-inoculated with *E. coli* O157:H7 were washed with tap water or 10 ppm free chlorine (CW) for 60 s or treated with PL for 5 – 60 s while being immersed in agitated water. Data represent mean of at least three replicates ± one standard deviation.

Treatment	Fluence (J/cm^2)		Log reduction of *E. coli* O157:H7 (log CFU/g)	
	Strawberries	Raspberries	Strawberries	Raspberries
WPL for 5 s	5.6	4.8	1.4 ± 0.4bA	1.6 ± 0.5bA
WPL for 15 s	16.1	13.7	1.6 ± 0.1abA	2.6 ± 0.9bA
WPL for 30 s	31.8	27.1	1.9 ± 0.6abA	3.0 ± 0.3abB
WPL for 60 s	63.2	53.9	2.4 ± 0.2aA	4.5 ± 0.4aB
Water for 60 s	NA	NA	1.4 ± 0.3bA	1.9 ± 0.5bB
CW for 60 s	NA	NA	1.5 ± 0.3bA	2.5 ± 1.0bB

The initial populations of *E. coli* O157:H7 on strawberries and raspberries were ~6.8 CFU/g.

Data in the same column followed by the same lowercase letter are not significantly different ($P > 0.05$).

Data in the same row followed by the same uppercase letter are not significantly different ($P > 0.05$).

5.3.2 Effect of H_2O_2 and SDS on WPL inactivation of *E. coli* O157:H7 on strawberries and raspberries

Strawberries and raspberries inoculated with *E. coli* O157:H7 were either washed with 1% H_2O_2, 100 ppm SDS or tap water alone or in combination with PL exposure for 60 s. As shown in Table 5.2, washing with 1% H_2O_2, 100 ppm SDS or tap water for 60 s reduced the population of *E. coli* O157:H7 on raspberries by 2.5, 2.5 and 2.0 log CFU/g, respectively. However, significantly higher ($P < 0.05$) reductions of *E. coli* O157:H7 on raspberries were achieved by WPL treatment alone or in combination with 1% H_2O_2 or 100 ppm SDS. WPL treatment for 60 s induced a 4.4-

log reduction of *E. coli* O157:H7 on raspberries. Treatments using WPL in combination with 1% H_2O_2 (WPL-H_2O_2) or 100 ppm SDS (WPL-SDS) reduced the population of *E. coli* O157:H7 by 5.3 and 5.1 log CFU/g, respectively. A similar pattern in decontamination effect was observed for strawberries, although lower inactivation efficacy was observed for strawberries than for raspberries. None of the washing treatments without PL was able to cause > 2-log reductions of *E. coli* O157:H7 on strawberry surface. However, combination of WPL and 1% H_2O_2 significantly enhanced the inactivation efficacy, achieving 3.3-log reductions of *E. coli* O157:H7 on strawberries.

Table 5.2 Effect of H_2O_2 and SDS on PL inactivating *E. coli* O157:H7 on strawberries and raspberries. Berries spot-inoculated with *E. coli* O157:H7 were washed with tap water, 1% H_2O_2 or 100 ppm SDS for 1 min or treated with PL for 1 min while being immersed in agitated tape water, 1% H_2O_2 or 100 ppm SDS solution. Data represent mean of at least three replicates ± one standard deviation.

Treatment	Log reduction of *E. coli* O157:H7 (log CFU/g)	
	Strawberry	Raspberry
Tap water	1.6 ± 0.1bA	2.0 ± 0.6bA
1% H_2O_2	1.9 ± 0.3bA	2.5 ± 0.2bB
100 ppm SDS	1.6 ± 0.5bA	2.5 ± 0.5bB
WPL	2.2 ± 0.2bA	4.4 ± 0.5aB
WPL-H_2O_2	3.3 ± 0.0aA	5.3 ± 0.4aB
WPL-SDS	2.3 ± 0.2bA	5.1 ± 0.6aB

The initial populations of *E. coli* O157:H7 were ~6.8 CFU/g.

Data in the same column followed by the same lowercase letter are not significantly different (P > 0.05).

Data in the same row followed by the same uppercase letter are not significantly different (P > 0.05).

5.3.3 Inactivation of *Salmonella* on strawberries and raspberries by WPL treatments

Based on results described in section 5.2.3, WPL-H_2O_2 treatment was selected for its highest decontamination efficiency among all the treatments and WPL was selected as a non-chemical alternative to chlorine for the decontamination of *Salmonella* on berries. As shown in Table 5.3, washing berries with tap water was not effective in reducing *Salmonella* population on berries. For strawberries and raspberries, only 1.3- and 1.4-log reduction of *Salmonella* were observed after 1-min water washing, while CW washing showed higher ($P > 0.05$) antimicrobial efficacy, reducing *Salmonella* by 2.1 log CFU/g on both berry types. For raspberries, WPL-H_2O_2 was the most effective treatment in inactivating *Salmonella* followed by WPL. For strawberries, both WPL-H_2O_2 and WPL achieved insignificantly higher log reductions of *Salmonella* than CW washing ($P > 0.05$).

Table 5.3 Effect of single and combined WPL treatments on inactivating *Salmonella* on strawberries and raspberries. Berries spot-inoculated with *Salmonella* were washed with tap water or 10 ppm free chlorine for 1 min or treated with PL for 1 min while being immersed in agitated tap water or 1% H_2O_2 solution. Data represent mean of at least three replicates ± one standard deviation.

Treatment	Log reduction of *Salmonella* (log CFU/g)	
	Strawberry	Raspberry
WPL	2.4 ± 0.4aA	3.2 ± 0.4bB
WPL-H_2O_2	2.8 ± 0.5aA	4.9 ± 0.7aB
Tap water	1.3 ± 0.6bA	1.4 ± 0.3cA
10 ppm Chlorine	2.1 ± 0.4abA	2.1 ± 0.4cA

The initial populations of *E. coli* O157:H7 were ~6.8 CFU/g.

Data in the same column followed by the same lowercase letter are not significantly different ($P > 0.05$).

Data in the same row followed by the same uppercase letter are not significantly different ($P > 0.05$).

5.3.4 Inactivation of MNV-1 on strawberries and raspberries by WPL treatments

Table 5.4 shows the effectiveness of WPL treatments in reducing MNV-1, as a human norovirus surrogate, on strawberries and raspberries. MNV-1 titers on strawberries and raspberries were decreased by 1.3 and 2.2 log PFU/g, respectively, after 1-min washing with CW. Higher reductions of MNV-1 were obtained by treatment of WPL and WPL-H_2O_2. For strawberries, WPL-H_2O_2 treatment achieved a significantly higher ($P < 0.05$) reduction of MNV-1 than CW washing, by reducing 2.2 log PFU/g of MNV-1. WPL treatment achieved a 3.6-log reduction of MNV-1 on raspberries which was significantly higher ($P < 0.05$) than CW washing which reduced

MNV-1 by 2.2-log units. However, H_2O_2 slightly decreased the decontamination

efficacy of WPL in raspberries, although not in a significant way (P > 0.05).

Table 5.4 Effect of single and combined WPL treatments on inactivating MNV-1 on strawberries and raspberries. Berries spot-inoculated with MNV-1 were washed with 10 ppm free chlorine for 1 min or treated with PL for 1 min while being immersed in agitated tap water or 1% H_2O_2 solution. Data represent mean of at least three replicates ± one standard deviation.

Treatment	Log reduction of MNV-1 (log PFU/g)	
	Strawberry	Raspberry
WPL	1.8 ± 0.3abA	3.6 ± 1.1aB
WPL-H_2O_2	2.2 ± 0.2aA	2.5 ± 1.1abA
10 ppm Chlorine	1.3 ± 0.1bA	2.2 ± 0.9bA

Data in the same column followed by the same lowercase letter are not significantly different (P > 0.05).

Data in the same row followed by the same uppercase letter are not significantly different (P > 0.05).

5.3.5   Color and temperature measurements

The color of berries treated by 1-min WPL was immediately measured to determine if WPL treatment had any negative effects on the color of berries as a direct quality indicator. For strawberries, treated berries had *L*, *a*, and *b* values of 33.5, 34.2 and 12.0, compared to the control samples, which had values of 33.4, 33.7, and 13.3, respectively (Table 5.5). For raspberries, the *L*, *a*, and *b* values were 29.6, 22.6, and 6.3 for treated berries and 29.3, 2, and 6.2 for untreated samples, respectively. No significant difference was found between treated and untreated berry samples for both berry types (P > 0.05).

During PL exposure, berries absorbed light energy resulting in an increase in surface temperature. The temperature profiles of berry surface during PL treatments are shown in Figure 5.1. When berries were treated with PL in the absence of water, the highest temperatures of 59.9 and 53.5 °C were recorded on raspberries and strawberries, respectively, after 60-s PL treatment. In contrast, the surface temperatures of both strawberries and raspberries were significantly lower during the WPL treatment, with the highest temperatures of 33.3 and 31.5 °C, respectively, by the end of the 60-s treatments.

Table 5.5 Hunter L, a and b color readings of berry samples before and after 60-s WPL treatment.

Color	Treatment	Strawberry	Raspberry
L value	Control	33.4 ± 0.5a	29.3 ± 0.5a
	WPL	33.5 ± 1.3a	29.6 ± 0.4a
a value	Control	33.7 ± 3.6a	22.0 ± 2.0a
	WPL	34.2 ± 1.9a	22.6 ± 2.1a
b value	Control	13.3 ± 2.4a	6.3 ± 0.6a
	WPL	12.9 ± 1.1a	6.2 ± 0.7a

Data represent mean color values of three replicates ± 1 standard deviation.

L refers to lightness, positive a means red and negative a green, and positive b means yellow and negative b blue.

For the same color parameter, values in the same column followed by the same lower case letter are not significantly different ($P > 0.05$).
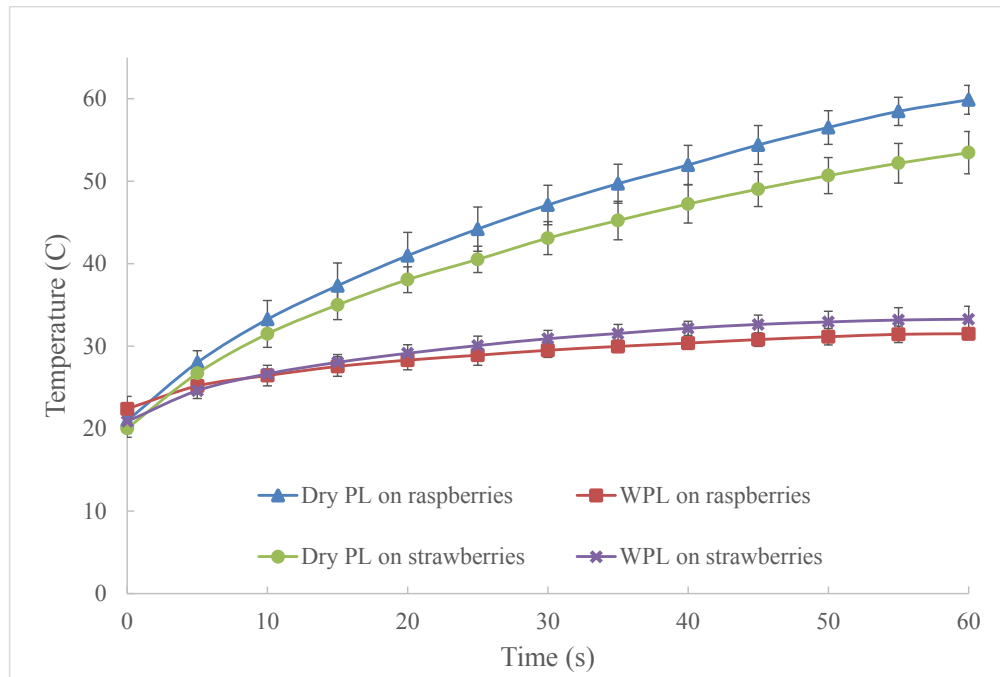
Figure 5.1 Increase in surface temperatures of strawberries and raspberries during 60-s PL treatments. Strawberries and raspberries were illuminated with PL either directly on a petridish (dry PL) or under water (WPL).

5.4 Discussion

Fresh produce is a high risk food due to possible bacterial and viral contamination. Unfortunately, no effective processing method has been established to ensure the safety of these foods. PL is a promising nonthermal technology for its established efficacy in inactivating various microorganisms. PL inactivation of bacteria, fungi and viruses in clear suspensions and some food contact surfaces has been well documented (Elmnasser et al., 2007; Oms-Oliu et al., 2008); however, few studies have focused on the improvement of the applicability and efficacy of this technology in foods, such as fresh produce. Indeed, the application of PL in fresh produce has been a challenge since sample heating is one of the factors limiting commercial application. In our previous study, we reported a significant change in

visual appearance of blueberries due to sample heating after the samples were treated with PL for 60 s (56.1 J/cm^2) (Huang & Chen, 2014). Gómez et al. (2012) reported that cut-apple surface exposed to high PL fluences turned darker due to browning reactions accelerated by temperature increases. Fine and Gervais (2004) reported that PL treatment caused a rapid modification of color of black pepper and wheat flour due to overheating combined with oxidation. In this study, we measured the surface temperatures of strawberries and raspberries that were PL-treated on a petridish without water. The maximum temperatures of both type of berries exceeded 50 °C after 60-s treatments. Shadowing effect is another limiting factor. Limited or reduced PL efficacy due to shadowing effect has been reported in foods matrices compared with clear liquid. Belliot et al. (2013) compared the PL inactivation of bacteriophage MS2 in PBS, glass bead and some food matrices. While two pulses of PL (1.88 J/cm^2) were adequate to result in >5 log reduction of MS2 in PBS, only marginal (<1 log unit) reduction of MS2 was achieved after 30 pulses when viruses were absorbed at the food surface. Lagunas-Solar et al. (2006) also reported the interference by shadowing effects during PL treatment of fruits and suggested modifications and/or improvements in the PL system to provide uniform PL exposure of all fruit surfaces.

In this study, a new strategy using the combination of washing process and PL illumination for decontamination of strawberries and raspberries was evaluated. During the WPL treatments, berry samples were immersed in agitated water while being illuminated by PL. The agitated water not only acted as a cooling medium to prevent sample heating but at the same time facilitated the berries to move and rotate randomly, therefore allowing a more uniform PL exposure of all the berry surfaces. The cooling efficacy of this design is demonstrated in Figure 5.1 as the surface

temperature of berry samples remained below 34 °C during the 1-min treatment. As shown in Table 5, WPL treatment for 1 min did not cause any significant change in the color readings of strawberries and raspberries. These results are in good agreement with those from our previous study (Huang & Chen, 2014), in which we found that WPL treatment did not cause any visible damage to blueberries as well.

A 60-s WPL treatment demonstrated significantly higher ($P < 0.05$) antimicrobial efficacy in inactivating *E. coli* O157:H7 on both strawberries and raspberries compared with 60-s washing treatment with tap water and CW (Table 5.1). In addition, we found that the levels of bacterial reduction due to WPL treatment varied with different topographies of berry surface (Tables 5.1 – 5.4). The presence of achenes of strawberries and the drupelets of raspberries can potentially shield microorganisms from the PL beams, leading to only partial decontamination. A similar phenomenon has also been observed in many other studies (Belliot et al., 2013; Bialka & Demirci, 2008; Fino & Kniel, 2008).

In order to further enhance the effectiveness of WPL treatments, two generally recognized as safe (GRAS) chemicals was used in combination with WPL treatments. Sodium dodecyl sulfate (SDS) is a surfactant that can potentially facilitate the release of microorganisms from fresh produce. Although not approved for use as a produce rinse, SDS is commonly used in a variety of foods, including marshmallows, egg whites, vegetable oils and fruit juices as food additives (21 CFR 172.822) and also widely used in household products such as toothpastes, shampoos, shaving foams and bubble baths. Surfactants can interact with proteins and influence protein folding, denaturation, and aggregation, therefore causing damages on cell membranes and viral capsids (Predmore & Li, 2011; Zhao, Zhao, & Doyle, 2009); however, our results

(Table 5.2) showed that the use of SDS did not enhance the removal of *E. coli* O157:H7 from berries and no additional bacterial reduction was observed when 100 ppm SDS was used in combination with WPL. Raiden et al. (2003) tested the efficacy of several detergents in removing bacterial cells from fresh produce at different temperatures and concluded that detergents were no more effective in removing organisms from produce than water was.

Hydrogen peroxide has GRAS status for some food application as an antimicrobial or bleaching agent in the range of 0.04 – 1.25% (21CFR184.1366). Since 2002, the use of H_2O_2 for raw agricultural commodities is exempt from the requirements of a tolerance by the U.S. Environmental Protection Agency (EPA) if the concentration used is 1% or less (40 CFR 180.1197). One of the main advantages of using H_2O_2 as a disinfecting agent is that it produces no residue as it is decomposed into water and oxygen by catalase, an enzyme naturally found in plants (Ölmez & Kretzschmar, 2009). The antimicrobial effectiveness of H_2O_2 against different foodborne pathogens and spoilage microorganisms are well documented for its use on various fresh commodities (Crowe et al., 2007; Huang & Chen, 2011; Sapers, 2001). Yu et al. (2001) reported a 1.2 – 2.2 log reduction in *E. coli* O157:H7 on strawberries by H_2O_2 washing. Lukasik et al. (2003) reported that washing with 0.5% H_2O_2 caused 1.6- and 2.7-log reduction of *E. coli* O157:H7 and *Salmonella*, respectively, on strawberries. Our results (Table 5.3) are in general agreement with those earlier studies and showed that 1%H_2O_2 washing reduced the population of *E. coli* O157:H7 on strawberries and raspberries by 1.9 and 2.5 log CFU/g, respectively.

In this study, we found that WPL-H_2O_2 was the most effective treatment for decontamination of strawberries and raspberries (Tables 5.2 – 5.4). The combination

of UV and H_2O_2 is actually a process based on advanced oxidation processes (AOPs), which has been mainly utilized to degrade organic pollutant during waste water treatment (Rosenfeldt et al. , 2006; Suty, De Traversay, & Cost, 2004) or to sterilize food packaging materials (Reidmiller et al.,, 2003). The interaction of H_2O_2 with UV produces highly reactive, but short-lived, hydroxyl radicals which is effective against various microorganisms (Rosenfeldt et al., 2006). In our study, only 1.9- and 2.5-log reduction of *E. coli* O157:H7 on strawberries and raspberries was achieved by washing with 1% H_2O_2; however, WPL-H_2O_2 treatment reduced the population of *E. coli* 157:H7 by 3.3- and 5.3-log units, respectively (Table 5.2). Bayliss and Waites (1979) reported a synergistic effect by using 1% H_2O_2 solution in combination with UV-C at 254 nm to inactivate *Bacillus subtilis* endospores. The researchers also showed that the optimal concentration of H_2O_2 was about 1% for spores of *Bacillus subtilis*, and killing was reduced at higher concentrations. Hadjok et al. (2008) reported a 4.1-log reduction of *Salmonella* on lettuce by using a combination of 1.5% H_2O_2 spray at 50 °C and UV-C (254 nm).

Viral contamination of fresh produce is a food safety concern as human norovirus has been recognized as the top causative agent in foodborne diseases (Scallan et al., 2011). Since there is no established cell culture system available for human norovirus, MNV-1, a common human norovirus surrogate, was used in this study. MNV-1 is a small (28 to 35 nm in diameter), icosahedral, non-enveloped, single-stranded (+) RNA virus that share many biochemical and genetic features with human noroviruses (Wobus, Thackray, & Virgin, 2006). Park et al. (2011) examined the UV inactivation of several human norovirus surrogates and concluded that MNV-1 and echoviruses 12 are more conservative surrogates than feline calicivirus for human

noroviruses. Compared with Tulane virus, another human noroviruses surrogate, MNV was reported to be a better surrogate as it is more stable over a wider range of pH, and persist longer at 2 ppm of chlorine and 4 °C storage (Hirneisen & Kniel, 2013). The inactivation of MNV-1 on strawberries and raspberries using the WPL treatments is displayed in Table 5.4. Despite of varied efficacy of WPL treatment in different types of berries, WPL treatments demonstrated a higher effectiveness against MNV-1 on both types of berries than CW washing. However, no significant increase ($P > 0.05$) in the efficacy of WPL treatment was observed when H_2O_2 was used in combination with PL. A similar phenomenon was observed by Xie et al. (2008), who reported a 3-log reduction of MS2 F(+) coliphage on lettuce treated by H_2O_2 (2%) spray at 50 °C and found no significant increase in the viral inactivation when H_2O_2 and UV light were applied simultaneously. In contrast, Li et al. (2011) reported that vaporized H_2O_2 (2.52%) only induced a marginal reduction (<1 log unit) of MNV-1 on stainless steel discs, but the combination of UV and vaporized H_2O_2 reduced the viral titer by 4-log units. The effectiveness of H_2O_2 on viral inactivation is not conclusive as varied results have been reported. In a recent study, Kingsley et al. (2014) reported that 4% H_2O_2 treatment of up to 60 min resulted in only ~ 0.1-log reduction in the ability of human noroviruses to bind to porcine gastric mucin binding magnetic bead, which was used as an indirect indicator of viral infectivity. Lukasik et al. (2003) reported that washing with 0.5% H_2O_2 caused 1.5- to 2.4-log reduction of bacteriophages and Poliovirus 1 on strawberries. Tuladhar et al. (2012) reported that vaporized H_2O_2 treatment (127 ppm for 1 h) reduced > 4-log units of MNV-1, poliovirus, rotavirus and adenovirus on stainless steel. It is plausible that the form of H_2O_2 (liquid and gas) and its concentration as well as the type of virus and treatment

time all contributed to the difference in the efficacy of the H_2O_2/H_2O_2-UV treatments (Finnegan et al., 2010). Therefore, more research is needed to determine whether H_2O_2 and combination of PL and H_2O_2 are effective for inactivation of human noroviruses and its surrogates.

Our results also showed a consistently lower reduction of virus on berries than of bacteria after WPL treatments (Table 5.1 – 5.4). These results are congruent with a previous report showing that viruses are generally more resistant to UV treatment than vegetative bacteria and protozoa (Hijnen, Beerendonk, & Medema, 2006). On the other hand, the complexity of food matrices might make it more challenging for the highly directional, coherent PL beam from reaching its target. As MNV-1 is much smaller than bacterial cells, they can hide in smaller crevices and surface details of berry surface where even bacterial cells were not able to fit, therefore, reducing the efficacy of PL.

In conclusion, the present study demonstrated that WPL treatment was an effective intervention method to inactivate both bacterial and viral pathogens on strawberries and raspberries. The WPL treatment represents an alternative to CW washing currently applied in fresh produce industry. WPL-H_2O_2 treatment was the most effective treatment for the bacterial pathogens, *Salmonella* and *E. coli* O157:H7, compared with washing with CW, SDS and H_2O_2 could provide an enhanced degree of microbiological safety for fresh berries.

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REFERENCES

Bayliss, C. E., & Waites, W. M. (1979). The synergistic killing of spores of *Bacillus subtilis* by hydrogen peroxide and ultra-violet light irradiation. *FEME Microbiology Letters*, *5*, 331–333.

Belliot, G., Loutreul, J., Estienney, M., Cazeaux, C., Nicorescu, I., Aho, S., Gervais, P., Orange, N., Pothier, P., & Morin, T. (2013). Potential of pulsed light to inactivate Bacteriophage MS2 in simple liquid medium and on complex foodstuffs. *Food and Environmental Virology*, *5*(3), 176–179. doi:10.1007/s12560-013-9110-8

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), M201–M207. doi:10.1111/j.1750-3841.2008.00743.x

CDC. (1997a). *Hepatitis A Associated with Consumption of Frozen Strawberries -- Michigan, March 1997* (pp. 288–295). Retrieved 12/12/14 from http://www.cdc.gov/mmwr/preview/mmwrhtml/00047129.htm

CDC. (1997b). *Outbreak of Cyclosporiasis -- Northern Virginia-Washington, D.C.-Baltimore, Maryland, Metropolitan Area, 1997* (pp. 689–691). Retrieved 12/12/14 from http://www.cdc.gov/mmwr/preview/mmwrhtml/00048551.htm

CFR - Code of Federal Regulations. 21 CFR 172.822. Retrieved 12/12/14 from http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=172.822

CFR - Code of Federal Regulations. 21 CFR 179.41. FDA. Retrieved 12/12/14 from http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=179.41

CFR - Code of Federal Regulations. 21 CFR 184.1366. Retrieved 12/12/14 from http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=184.1366

CFR - Code of Federal Regulations. 40 CFR 180.1197. Retrieved 12/12/14 from http://www.gpo.gov/fdsys/pkg/CFR-2005-title40-vol23/pdf/CFR-2005-title40-vol23-sec180-1204.pdf

Crowe, K. M., Bushway, A. a, Bushway, R. J., Davis-Dentici, K., & Hazen, R. a. (2007). A comparison of single oxidants versus advanced oxidation processes as chlorine-alternatives for wild blueberry processing (*Vaccinium angustifolium*). *International Journal of Food Microbiology*, *116*(1), 25–31. doi:10.1016/j.ijfoodmicro.2006.12.027

Doyle, M. P., & Erickson, M. C. (2008). Summer meeting 2007 - the problems with fresh produce: an overview. *Journal of Applied Microbiology*, *105*(2), 317–30. doi:10.1111/j.1365-2672.2008.03746.x

Dubois, E., Agier, C., Traoré, O., Hennechart, C., Merle, G., Crucière, C., & Laveran, H. (2002). Modified concentration method for the detection of enteric viruses on fruits and vegetables by reverse transcriptase-polymerase chain reaction or cell culture. *Journal of Food Protection*, *65*(12), 1962–1969.

Elmnasser, N., Guillou, S., Leroi, F., Orange, N., Bakhrouf, a, & Federighi, M. (2007). Pulsed-light system as a novel food decontamination technology: a review. *Canadian Journal of Microbiology*, *53*(7), 813–21. doi:10.1139/W07-042

Fine, F., & Gervais, P. (2004). Eficiency of pulsed UV light for microbial decontamination of food powders. *Journal of Food Protection*, *67*(4), 787–792.

Finnegan, M., Linley, E., Denyer, S. P., McDonnell, G., Simons, C., & Maillard, J. Y. (2010). Mode of action of hydrogen peroxide and other oxidizing agents: differences between liquid and gas forms. *The Journal of Antimicrobial Chemotherapy*, *65*(10), 2108–15. doi:10.1093/jac/dkq308

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), 908–913.

Friedman, D. S., Heisey-Grove, D., Argyros, F., Berl, E., Nsubuga, J., Stiles, T., Fontana, J., Beard, R., Monroe, S., McGrath, M., Sutherby, H., Dicker, R., DeMaria, A., & Matyas, B. (2005). An outbreak of norovirus gastroenteritis associated with wedding cakes. *Epidemiology and Infection*, *133*(6), 1057–63. doi:10.1017/S0950268805004760

Gómez, P. L., Salvatori, D. M., García-Loredo, A., & Alzamora, S. M. (2012). Pulsed light treatment of cut apple: dose effect on color, structure, and microbiological stability. *Food and Bioprocess Technology*, *5*(6), 2311–2322. doi:10.1007/s11947-011-0598-3

Gould, L. H., Walsh, K. A., Vieira, A. R., Herman, K., Williams, I. T., Hall, A. J., & Cole, D. (2013). Surveillance for Foodborne Disease Outbreaks - United States, 1998-2008. *MMWR SURVEILLANCE SUMMARIES*, *62*(2), 1–34.

Hadjok, C., Mittal, G. S., & Warriner, K. (2008). Inactivation of human pathogens and spoilage bacteria on the surface and internalized within fresh produce by using a combination of ultraviolet light and hydrogen peroxide. *Journal of Applied Microbiology*, *104*(4), 1014–24. doi:10.1111/j.1365-2672.2007.03624.x

Herwaldt, B., & Beach, M. (1999). The return of Cyclospora in 1997: Another outbreak of cyclosporiasis in North America associated with imported raspberries. *Annals of Internal Medicine*, *130*(3), 210–20.

Herwaldt, B. L., & Ackers, M. L. (1997). An outbreak in 1996 of cyclosporiasis associated with imported raspberries. The Cyclospora Working Group. *The New England Journal of Medicine*, *336*(22), 1548–56. doi:10.1056/NEJM199705293362202

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*(1), 3–22. doi:10.1016/j.watres.2005.10.030

Hirneisen, K. A., & Kniel, K. E. (2013). Comparing human norovirus surrogates: murine norovirus and Tulane virus. *Journal of Food Protection*, *76*(1), 139–43. doi:10.4315/0362-028X.JFP-12-216

Hsu, L., & Moraru, C. I. (2011). Quantifying and mapping the spatial distribution of fluence inside a pulsed light treatment chamber and various liquid substrates. *Journal of Food Engineering*, *103*(1), 84–91. doi:10.1016/j.jfoodeng.2010.10.002

Huang, R., Li, X., Huang, Y., & Chen, H. (2014). Strategies to enhance high pressure inactivation of murine norovirus in strawberry puree and on strawberries. *International Journal of Food Microbiology*, *185*, 1–6. doi:10.1016/j.ijfoodmicro.2014.05.007

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*(8), 1178–1183.

Huang, Y., & Chen, H. (2014). A novel water-assisted pulsed light processing for decontamination of blueberries. *Food Microbiology*, *40*, 1–8.

Huang, Y., Ye, M., & Chen, H. (2013). Inactivation of *Escherichia coli* O157:H7 and *Salmonella* spp. in strawberry puree by high hydrostatic pressure with/without subsequent frozen storage. *International Journal of Food Microbiology*, *160*(3), 337–43. doi:10.1016/j.ijfoodmicro.2012.11.008

Hutin, Y. J., Pool, V., Cramer, E. H., Nainan, 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*(8), 595–602. doi:10.1056/NEJM199902253400802

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), 1748–1751.

Kingsley, D. H., Vincent, E. M., Meade, G. K., Watson, C. L., & Fan, X. (2014). Inactivation of human norovirus using chemical sanitizers. *International Journal of Food Microbiology*, *171*, 94–9. doi:10.1016/j.ijfoodmicro.2013.11.018

Lagunas-Solar, M. C., Piña, C., MacDonald, J. D., & Bolkan, L. (2006). Development of pulsed UV light processes for surface fungal disinfection of fresh fruits. *Journal of Food Protection*, *69*(2), 376–384.

Laidler, M. R., Tourdjman, M., Buser, G. L., Hostetler, T., Repp, K. K., Leman, R., Samadpour, M., Keene, W. E. (2013). *Escherichia* coli O157:H7 infections associated with consumption of locally grown strawberries contaminated by deer. *Clinical Infectious Diseases : An Official Publication of the Infectious Diseases Society of America*, *57*(8), 1129–34. doi:10.1093/cid/cit468

Li, D., Baert, L., De Jonghe, M., Van Coillie, E., Ryckeboer, J., Devlieghere, F., & Uyttendaele, M. (2011). Inactivation of murine norovirus 1, coliphage phiX174, and Bacillus fragilis phage B40-8 on surfaces and fresh-cut iceberg lettuce by hydrogen peroxide and UV light. *Applied and Environmental Microbiology*, *77*(4), 1399–404. doi:10.1128/AEM.02131-10

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. doi:10.1007/s12560-013-9118-0

McDonald, K.F., Curry, R.D., Clevenger, T.E., Brazos, B.J., Unklesbay, K., Eisenstark, A., Baker, S., Golden, J., & Morgan, R. (2000). The development of photosensitized pulsed and continuous ultraviolet decontamination techniques for surfaces and solutions. *IEEE Transactions on Plasma Science*, *28*(1), 89–96.

Niu, M.T., Polish, L.B., Robertson, B.H., Khanna, B.K., Bradley, A., Shapiro, C.N., Miller, M.A., Smith, J.D., Gedrose, J.K., Miriam, J., Margolis, H.S., Woodruff, B.A., & Alter, M.J., (1992). Multistate outbreak of hepatitis a associated with frozen strawberries. *The Journal of Infectious Diseases*, *166*(3), 518–24.

Ölmez, H., & Kretzschmar, U. (2009). Potential alternative disinfection methods for organic fresh-cut industry for minimizing water consumption and environmental impact. *LWT - Food Science and Technology*, *42*(3), 686–693. doi:10.1016/j.lwt.2008.08.001

Oms-Oliu, G., Martín-Belloso, O., & Soliva-Fortuny, R. (2008). Pulsed light treatments for food preservation. A review. *Food and Bioprocess Technology*, *3*(1), 13–23. doi:10.1007/s11947-008-0147-x

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*(2), 162–7. doi:10.1111/j.1472-765X.2010.02982.x

Predmore, A., & Li, J. (2011). Enhanced removal of a human norovirus surrogate from fresh vegetables and fruits by a combination of surfactants and sanitizers. *Applied and Environmental Microbiology*, *77*(14), 4829–38. doi:10.1128/AEM.00174-11

Raiden, R. M., Sumner, S. S., Eifert, J. D., & Pierson, M. D. (2003). Efficacy of detergents in removing *Salmonella* and *Shigella* spp. from the surface of fresh produce. *Journal of Food Protection*, *66*(12), 2210–2215.

Ramos-Villarroel, a. Y., Martín-Belloso, O., & Soliva-Fortuny, R. (2011). Bacterial inactivation and quality changes in fresh-cut avocado treated with intense light pulses. *European Food Research and Technology*, *233*(3), 395–402. doi:10.1007/s00217-011-1533-6

Reidmiller, J. S., Baldeck, J. D., Rutherford, G. C., & Marquis, R. E. (2003). Characterization of UV-peroxide killing of bacterial spores. *Journal of Food Protection*, *66*(7), 1233–1240.

Rosenfeldt, E. J., Linden, K. G., Canonica, S., & von Gunten, U. (2006). Comparison of the efficiency of *OH radical formation during ozonation and the advanced oxidation processes O3/H2O2 and UV/H2O2. *Water Research*, *40*(20), 3695–704. doi:10.1016/j.watres.2006.09.008

Sapers, G. M. (2001). Efficacy of washing and sanitizing methods for disinfection of fresh fruit and vegetable products, *39*(4), 305–311.

Sarvikivi, E., Roivainen, M., Maunula, L., Niskanen, T., Korhonen, T., Lappalainen, M., & Kuusi, M. (2012). Multiple norovirus outbreaks linked to imported frozen raspberries. *Epidemiology and Infection*, *140*(2), 260–7. doi:10.1017/S0950268811000379

Sauer, A., & Moraru, C. I. (2009). Inactivation of *Escherichia coli* ATCC 25922 and *Escherichia coli* O157: H7 in apple juice and apple cider, using pulsed light treatment. *Journal of Food Protection*, *72*(5), 937–944.

Scallan, E., Hoekstra, R.M., Angulo, F.J., Tauxe, R. V, Widdowson, M.-A., Roy, S.L., Jones, J.L., & Griffin, P.M. (2011). Foodborne illness acquired in the United States--major pathogens. *Emerging Infectious Diseases*, *17*(1), 7–15. doi:10.3201/eid1701.091101p1

Suty, H., De Traversay, C., & Cost, M. (2004). Applications of advanced oxidation processes: present and future. *Water Science and Technology*, *49*(4), 227–233.

Tuladhar, E., Terpstra, P., Koopmans, M., & Duizer, E. (2012). Virucidal efficacy of hydrogen peroxide vapour disinfection. *The Journal of Hospital Infection*, *80*(2), 110–5. doi:10.1016/j.jhin.2011.10.012

USDA-ERS. (2014a). USDA Economic Research Service - Cost Estimates of Foodborne Illnesses. Retrieved 12/12/2014 from http://www.ers.usda.gov/data-products/cost-estimates-of-foodborne-illnesses.aspx#.VDW27r4mUfy

USDA-ERS. (2014b). *USDA Economic Research Service - Yearbook Tables*. Retrieved 12/12/2014 from http://www.ers.usda.gov/data-products/fruit-and-tree-nut-data/yearbook-tables.aspx

Wobus, C. E., Thackray, L. B., & Virgin, H. W. (2006). Murine norovirus: a model system to study norovirus biology and pathogenesis. *Journal of Virology*, *80*(11), 5104–12. doi:10.1128/JVI.02346-05

Xie, Y., Hajdok, C., Mittal, G. S., & Warriner, K. (2008). Inactivation of MS2 F (+) coliphage on lettuce by a combination of UV light and hydrogen peroxide. *Journal of Food Protection*, *71*(5), 903–907.

Yu, K., Newman, M. C., Archbold, D. D., & Hamilton-kemp, T. R. (2001). Survival of *Escherichia coli* O157 : H7 on strawberry fruit and reduction of the pathogen population by Chemical Agents. *Journal of Food Protection*, *64*(9), 1334–1340.

Zhao, T., Zhao, P., & Doyle, M. 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*(5), 928–936.

Chapter 6

APPLICATION OF WATER-ASSISTED PULSED LIGHT TREATMENT TO DECONTAMINATE RASPBERRIES AND BLUEBERRIES FROM *SALMONELLA*

ABSTRACT

We developed and evaluated a small scaled-up water-assisted pulsed light (WPL) system, in which berries were washed in a flume washer while being treated by pulsed light (PL). Hydrogen peroxide (H_2O_2) was used in combination with PL, as an advanced oxidation process. The effects of organic load, water turbidity, berry type and PL energy output on the inactivation of *Salmonella* were thoroughly investigated. The combination of WPL and 1% H_2O_2 (WPL-H_2O_2) was the most effective treatment as it reduced *Salmonella* on raspberries and blueberries by 4.0 and > 5.6 log CFU/g, respectively, in clear water. When high organic load and SiO_2, as a soil simulator, were added into the wash water, the free chlorine level in chlorinated water decreased significantly ($P < 0.05$); however, no significant difference ($P > 0.05$) was observed for the decontamination efficacy of a 1-min WPL-H_2O_2 treatment. Even in the presence of high organic load and water turbidity, no viable bacterial cells were recovered from the wash water, thus WPL-H_2O_2 effectively prevented the risk of cross-contamination during treatment. Lowering the PL fluence by half did not significantly influence the efficacy of WPL-H_2O_2 treatment even under high turbidity conditions. Taken together, WPL treatment without H_2O_2 could provide a chemical-free alternative to chlorine washing with higher bactericidal efficacy. Compared with chlorine washing, the combination of WPL and H_2O_2 resulted in significantly higher

(P < 0.05) reduction of *Salmonella* on berries, providing a novel intervention for processing of small berries intended for fresh-cut and frozen berry products.

6.1   Introduction

Small fruits such as raspberries and blueberries are high-value commodities due to their well-reported health benefits. Unfortunately, they are susceptible to contamination caused by pathogenic bacteria and viruses since they are usually grown in open fields and are constantly exposed to potential pre-harvest contamination from soil, insects, birds, irrigation water as well as human contacts during harvesting. In recent years, an increasing number of foodborne illness outbreaks has been associated with small fruits such as raspberries and blueberries (Calder et al., 2003; Luna and Griffin, 2010; Miller et al., 2013; Sarvikivi et al., 2012). At least two outbreaks of *Salmonella* were reported in the U.S. due to the consumption of blueberries in 2009-2010 according to the CDC-Foodborne Outbreak Online Database (http://wwwn.cdc.gov/foodborneoutbreaks/). During September-October 2012, a norovirus gastroenteritis outbreak affected about 11,000 people in Germany due to contaminated frozen strawberries (Mäde et al., 2013). Although implementation of Good Agriculture Practices (GAPs), Good Manufacturing Practices (GMPs) and HACCP plans can minimize the chance of contamination, effective post-harvest interventions that can effectively reduce the microbial load are also very critical for ensuring the safety of these foods.

Chlorine is the most widely used sanitizer in the fresh produce industry. Although many studies have demonstrated that washing with chlorine cannot completely eliminate pathogens on fresh produce, it is still one of the most important

processing steps in fresh produce production to remove soil, plant debris, and microorganisms from fresh produce and to prevent cross-contamination. However, chlorine is known to react with organic matter, which can significantly diminish its antimicrobial efficacy (Luo, 2007). Although it is a common practice in the fresh produce industry to periodically monitor and replenish chlorine in the processing line, repeated addition of chlorine in to wash water containing high organic load results in formation of noxious chlorine by-products such as trihalomethanes and chlorine off-gassing problems (Luo et al., 2012). Therefore, there is a sustained effort to find chlorine-alternatives and new decontamination technologies (Bialka and Demirci, 2008; Huang and Chen, 2011; Sy et al., 2005; Udompijitkul et al., 2007).

Pulsed light (PL) is an emerging nonthermal technology that utilizes short, intense pulses of broad spectrum light ranging from UV to near-infrared (200 – 1100 nm) to inactivate microorganisms (Gómez-López et al., 2007). This technology was approved by the U.S. FDA for food processing in 1996 (21CFR179.41). Numerous studies have been published on the high efficacy of PL for inactivating bacteria, fungi and viruses (Oms-Oliu et al., 2008). Many efforts have also been made to enhance the safety and/or shelf-life of fresh produce by using PL treatment (Bialka and Demirci, 2008; Bialka et al., 2008; Huang and Chen, 2014; Lagunas-Solar et al., 2006; Sauer and Moraru, 2009). Some promising results have been reported when microorganisms were inoculated on the surface of fresh produce and directly exposed to PL. However, to become a feasible option for the fresh produce industry, some practical issues with the application of PL must be addressed. Many studies have reported sample heating during PL treatment, which could significantly alter the sensorial quality of fresh produce (Bialka and Demirci, 2008; Fine and Gervais, 2004; Huang and Chen, 2014;

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Ramos-Villarroel et al., 2011). In addition, microorganisms on an opaque food surface must directly face the PL lamp to be inactivated due to the shallow penetration depth of PL. To address these two issues, in our previous study, we developed a prototype water-assisted PL (WPL) system, in which three blueberries were treated by PL while being immersed in clear tap water agitated by a stir bar (Huang and Chen, 2014). The agitated water acted as a cooling medium to prevent sample heating and at the same time caused the blueberries to move and rotate randomly, thus allowing a more uniform PL exposure for all the blueberry surfaces. In this study, we designed and fabricated a small scaled-up WPL system and the bactericidal efficacy of the WPL system was evaluated on blueberries and raspberries. In addition, the impact of organic load and turbidity in the wash water, as well as the PL energy output, on the efficacies of WPL treatments was thoroughly investigated.

Processes based on advanced oxidation processes (AOPs) have been mainly applied in waste water treatment to degrade peptides and organic pollutant (Rosenfeldt et al., 2006; Suty et al., 2004). Another major application has been seen in sterilization of packaging materials (Reidmiller et al., 2003). One form of the AOPs includes the interaction of H_2O_2 with UV to produce highly reactive, but short-lived, hydroxyl radicals which could kill microorganisms (Rosenfeldt et al., 2006). The application AOPs for inactivating foodborne pathogens is very limited (Crowe et al., 2007; Hadjok et al., 2008). To our knowledge, no study has been published on the use of AOPs to inactivate foodborne pathogens such as *Salmonella* and *E. coli* O157:H7 on berries so far.

The objectives of this study were 1) to assess the bactericidal efficacy of the scaled-up WPL system; 2) to evaluate the effect of H_2O_2 in combination with PL for

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inactivating *Salmonella* on blueberries and raspberries; and 3) to determine the effects of PL fluence, organic load, and water turbidity on the bactericidal efficacy of WPL treatments.

6.2 Materials and Methods

6.2.1 Bacterial strains and inoculum preparation

Four nalidixic-acid resistant strains of *Salmonella enterica* (serotypes: St. Paul, Newport, Montevideo, and Stanley) were used in this study. Detailed strain information can be found in our previous study (Huang and Chen, 2014). The working cultures were maintained on tryptic soy agar (Difco Laboratories, Sparks, MD) supplemented with 0.6% yeast extract (Difco) and 50 µg/mL of nalidixic acid (Fisher Scientific, Hampton, NH) (TSAYE-N) at 10 °C. Prior to experiments, 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 a new tube of TSBYE-N for another 24-h incubation at 35 °C. One mL of each culture was mixed to form a 4-strain cocktail of *Salmonella*. Bacterial cells were harvested by centrifugation at $3000 \times g$ for 15 min (Sorvall ST16 R, Thermo Scientific). Then, the supernatant was discarded and the pellet was resuspended in 0.8 mL of sterile 0.1% peptone water (Fisher Scientific) to yield a final concentration of ~10^9 CFU/mL.

6.2.2 Sample inoculation

Fresh blueberries and raspberries were purchased from local grocery stores and used within 24 h. All the berries were illuminated with UV-C (254 nm) in a biosafety hood for at least 10 min to lower the impact of natural background microflora. Each sample consisted of ~ 50 g of blueberries or raspberries. For sample inoculation, 150

µl of the *Salmonella* cocktail was deposited on the skin of berries in multiple small droplets. Samples were air-dried in a biosafety hood for 2 h at 22 ± 2 °C to facilitate bacterial attachment. The final inoculation level of *Salmonella* on berry samples was ~ 6.2 log CFU/g.

6.2.3   Water-assisted pulsed light treatment

The PL treatments were performed using a laboratory scale PL unit (Steripulse-XL RS-3000, Xenon Corp.,Wilmington, MA), which included a controller module, a treatment chamber and an air cooling module. The pulses were generated at a rate of 3 pulses/s with a pulse width of 360 µs. The distance between the lamp and the quartz window in the PL unit was 5.8 cm and the distance between the bottom of the treatment chamber and the quartz window was ~16 cm.

A small scaled-up WPL system was built by incorporating a miniature circulating flume washer in the PL treatment chamber. The miniature circulating flume washer was fabricated at our lab using a stainless steel hotel pan (26.7 cm (L) × 16.5 cm (W) × 6.4 cm (H)) and an inline aquarium pump (Hydor 320 Universal Pump, 320 gph, Hydor, CA) as shown in Figure 6.1. Two water jets were fixed on the opposite side of the circulating flume to create turbulent flow. The water jets were connected to the pump with tygon tubings (PVC, ID = 3/8 inch, Fisher) and an electrical switch was added to control the water pump. The volumetric flow rate of the flume washer system was 3.64 L/min.

Figure 6.1 Water-assisted pulsed light treatment setup. (a) Water-assisted pulsed light experimental setup; (b) circulating flume washer.

For WPL treatments, 1.1 L of tap water was loaded into the WPL treatment system. Inoculated blueberries or raspberries (50 g) were introduced into the circulating flume washer where samples were washed. Random rotation of samples

was achieved during the washing process, which could potentially facilitate a 360°
exposure of berries to the PL source. At the same time, the PL lamp above the flume
washer was turned on for 30 – 60 s at either high or low energy output. The energy
output of the PL lamp was adjusted by changing the system's charge voltage. To
evaluate the potential synergy between H_2O_2 and PL due to AOP, 1% H_2O_2 was added
to the flume washer during WPL treatments (WPL-H_2O_2). Washing treatments using
1% H_2O_2 and chlorinated water (CW) with 100 ppm free chlorine was also evaluated
for comparison. Solutions of 1% H_2O_2 was prepared by adding H_2O_2 (3%, Onpoint,
Clifton, NJ) into de-ionized (DI) water. CW were prepared by diluting a proper
amount of commercial bleach (8.25% sodium hypochlorite, Clorox, Oakland, CA) in
DI water with pH adjusted to 6.5 with citric acid. The free chlorine level in the CW
was determined by a portable free/total chlorine meter (Hanna HI 93414, Hanna
Instruments, USA).

6.2.4 Effect of water quality on inactivation of *Salmonella*

Berry extracts (BE) were prepared from fresh blueberries and raspberries using
a household juice extractor (CJ14, Hamilton Beach, VA, USA). Extracted berry juice
was centrifuged at $3000 \times g$ for 10 min at 4 °C to remove coarse particles and berry
debris. The supernatant was stored at -20 °C until use. Blueberry and raspberry
extracts were used to simulate organic load during washing treatments. Silicon dioxide
(SiO_2) was used to simulate the effect of soil and clay to artificially increase the
turbidity of the wash water. SiO_2 suspension was made by suspending 1.5 g of SiO_2
(0.5-10 μm, with approx. 80% between 1-5 μm, Sigma-Aldrich) in 40 mL DI water
and 1 mL of SiO_2 suspension was added to 1 L of water to obtain water turbidity of
63.7 NTU. During the treatment, berry extracts and/or SiO_2 suspension were added to

the wash water at targeted concentrations and mixed for 30 s before introduction of berry samples.

6.2.5   Water quality measurement

Chemical oxygen demand (COD) of the washing solutions was determined using a COD colorimeter (YSI 250910Y COD Colorimeter), a thermoreactor (YSI 1P22-2Y Thermoreactor) and a COD test kit (YSI 251992Y COD Test Kit), which were all purchased from Cole Parmer. The free chlorine level and turbidity of water samples were determined with a portable turbidity and free/total chlorine meter (Hanna HI 93414, Hanna Instruments, USA).

6.2.6   *PL fluence measurements and fluence distribution*

The broadband energy of each pulse, expressed in J/cm^2, was quantified using a Vega laser power meter (Ophir Optronics Inc., Wilmington, MA) coupled with a pyroelectric energy sensor (PE-50C, Ophir Optronics) as described previously (Huang and Chen, 2014). To measure the PL fluence at both high and low PL output, the pyroelectric sensor was placed at different locations in the flume washer as indicated in Figure 6.2. All fluence measurements were performed in triplicate and precautions were taken to allow about 30-s pauses between measurements to prevent the sensor from overheating. Ideally, the pyroelectric sensor should be placed under the water to measure the fluence received by the samples treated in water; however, no water was added to the system during the measurement since the pyroelectric sensor could not be immersed in water. This may introduce some error into the measured fluence values as the presence of water may absorb some PL energy, causing an overestimation of the real fluence received by samples. However, it needs to be noted that the depth of water

was only ~3 cm when the flume washer was running and the thickness of the pyroelctric sensor was ~2 cm. The actual average fluence on samples should be very close to the measured values, considering that the PL has very good penetration ability in shallow clear water and the berries were always floating up and down in the flume.
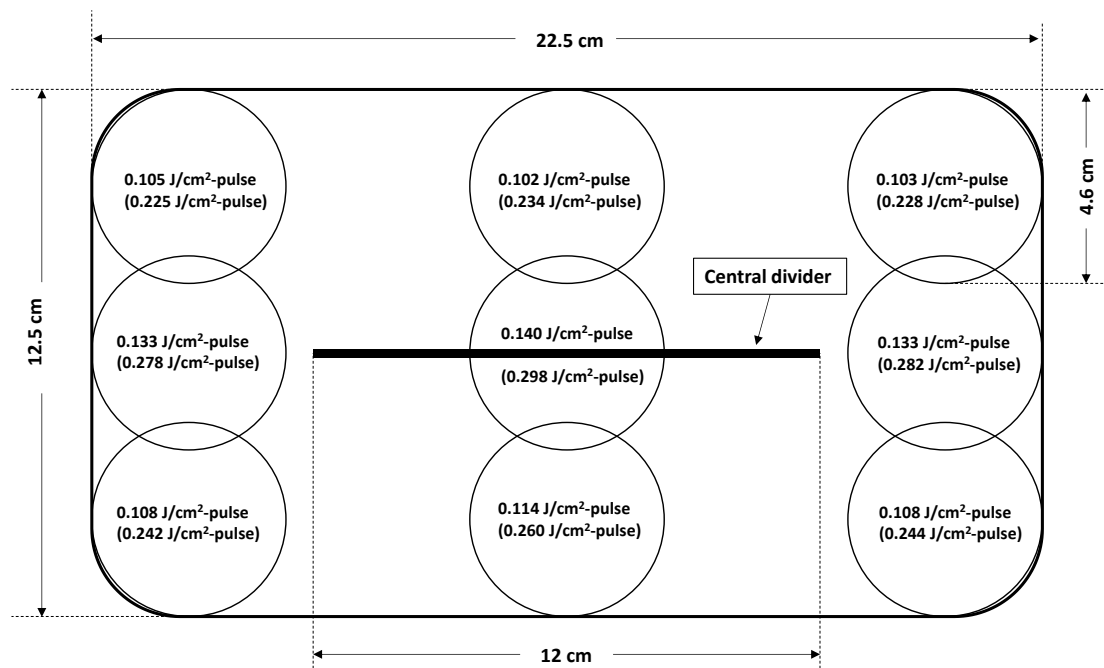
Figure 6.2 An aerial view of the miniature flume washer with the locations where PL fluence values were measured. At each location, data on the top and data in the parentheses showed PL fluence measured at low and high PL output, respectively.

6.2.7 Microbiological analysis

After PL treatments, each sample (~50 g) was transferred into a sterile filter bag (400 mL, Fisher Scientific, USA) containing 200 mL of Dey-Engley (D/E) neutralizing broth (Difco) and pummeled in a laboratory stomacher (Seward 400, Seward, London, U.K.) at 260 rpm for 2 min. 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 – 72 h. Presumptive colonies of *Salmonella* on the plates were counted. Homogenates of berry samples (25 mL) were also enriched in 25 mL of $2 \times$ TSBYE-N and incubated for 48 h at 35 °C to allow resuscitation of sub-lethally injured bacterial cells. Enriched samples were streaked onto Xylose Lysine Desoxycholate agar (Difco) supplemented with 50 µg/mL of nalidixic acid. After 24-h incubation, the presence of growth exhibiting typical morphology of *Salmonella* was determined by visual inspection. Water samples (1 mL) were collected from the flume washer and mixed with 1 mL of 5% sodium thiosulfate (Sigma-Aldrich, USA) to neutralize the residual chlorine or H_2O_2. Then, neutralized water samples were surface-plated on TSAYE-N and the survival of *Salmonella* was determined after the plates were incubated at 35 °C for 48 – 72 h.

6.2.8 Statistical analysis

At least three independent trials were conducted for all the treatments. Colony counts were converted to log CFU/g and means and standard deviations were calculated. Statistical analyses were conducted using JMP (SAS Cary, NC, USA). One-way analysis of variance and Tukey's one-way multiple comparisons were used to determine significant differences among treatments (P < 0.05).

6.3 Results

6.3.1 Effect of WPL treatments and organic load on inactivation of *Salmonella* on raspberries.

Table 6.1 shows the decontamination of *Salmonella* on raspberries using WPL treatments and the effect of organic load on the efficacy of WPL treatments. When no

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berry extract was added into the flume washer, CW washing reduced *Salmonella* on raspberries by 2.2-log units. In comparison, 0.5- and 1-min WPL treatments achieved 2.6- and 3.0-log reduction of *Salmonella*, respectively. Washing with 1% H_2O_2 only resulted in 2.3-log reduction of *Salmonella* on raspberries, which was not significantly different from CW washing (P > 0.05). However, a synergistic effect was observed when 1% H_2O_2 was used in combination with PL. A 4.0-log reduction of *Salmonella* was observed using WPL-H_2O_2, which resulted in significantly higher reduction of *Salmonella* than all the other treatments.

Table 6.1 Inactivation of *Salmonella* on raspberries by water-assisted pulsed light (WPL) treatments at high PL energy output under different organic load conditions. Raspberries inoculated with *Salmonella* were washed with 1% H_2O_2 or 100 ppm chlorine (CW) or treated with WPL treatments with or without 1% or 2% of berry extract (BE). Data represent mean of at least three replicates ± one standard deviation. For the water sample, the fraction numbers in parentheses represent the number of samples showing positive plating results out of the number of total trials.

Treatments	Log reduction of *Salmonella* on berries (log CFU/g)			*Salmonella* survival in wash water (CFU/g)		
	0% BE	1% BE	2% BE	0% BE	1% BE	2% BE
0.5-min WPL	2.6 ± 0.3Ba	2.5 ± 0.4Ba	2.0 ± 0.1BCa	0.7 ± 1.2 (1/3)	1.0 ± 1.2 (2/4)	19.0 ± 31.7 (2/4)
1-min WPL	3.0 ± 0.1Ba	2.6 ± 0.3Ba	2.8 ± 0.7Ba	ND	0.5 ± 1 (1/4)	1.8 ± 2.4 (2/4)
1-min WPL+H_2O_2	4.0 ± 0.8Aa	3.4 ± 0.5Aa	4.2 ± 0.6Aa	ND	ND	ND
1-min H_2O_2	2.3 ± 0.2Ba	1.5 ± 0.2Cb	1.7 ± 0.3Cb	ND	ND	9.0 ± 18.0 (1/4)
1-min CW	2.2 ± 0.2Ba	1.8 ± 0.4BCa	1.7 ± 0.3Ca	ND	0.5 ± 1 (1/4)	34.5 ± 45.8 (3/4)

ND: Not detectable by direct plating (2 CFU/mL).

The detection limit of the plating method for berry samples was 0.7 log CFU/g.

Data in the same column followed by the same uppercase letter are not significantly different (P > 0.05).

Data in the same row followed by the same lowercase letter are not significantly different (P > 0.05).

When organic load in the form of 1 and 2% raspberry extract was added into wash water, WPL-H_2O_2 was the most effective treatment, as it showed significantly higher (P < 0.05) efficacy against *Salmonella* by inactivating 3.4 – 4.2 log CFU/g compared with all the other treatments on raspberries. WPL treatment for 1 min reduced the population of *Salmonella* by 2.6 – 2.8 log CFU/g. No significant difference was observed in the efficacy between WPL treatments with or without addition of berry extract. In contrast, the efficacy of 1% H_2O_2 washing was significantly lowered (P < 0.05) when organic load was introduced into the wash solution. Figure 6.3 shows the linear relationship between the organic load level and the remaining free chlorine in CW. With an initial free chlorine concentration of 100 ppm in CW, the free chlorine concentration dropped to 37 ppm when 2% raspberry extract was added. However, the decontamination efficacy of CW was not significantly affected by the addition of berry extracts.
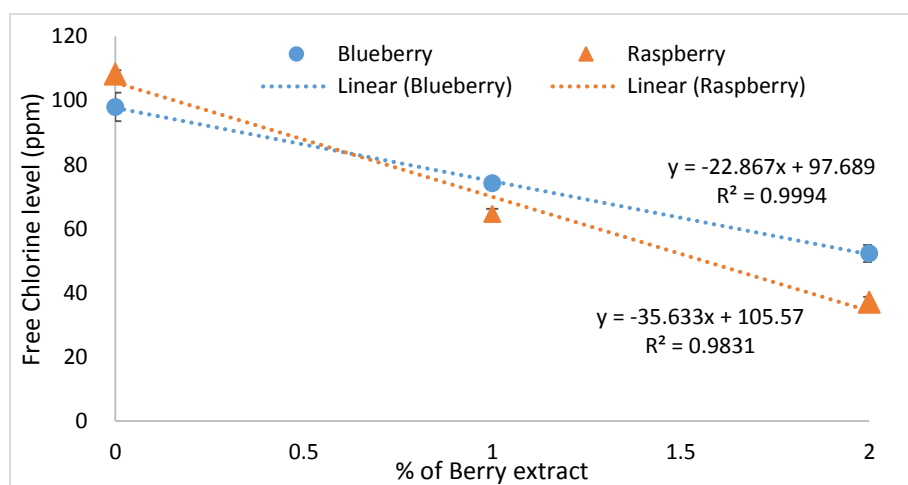

Figure 6.3 Effect of organic load on the free chlorine concentration in wash water. Blueberry extract and raspberry extract were added to CW containing 100 ppm free chlorine and the residual free chlorine levels were measured.

To evaluate whether the WPL treatments and CW could prevent the risk of cross-contamination under different organic load conditions, water samples were immediately taken after treatments were finished. In the absence of organic load in the wash water, all the water samples except one from 0.5-min WPL treatment were negative for *Salmonella* (Table 6.1). When 1% berry extract was added, no survival of *Salmonella* was found for all the water samples from 1% H_2O_2 washing and WPL+H_2O_2 treatments. Although some of the water samples from 0.5-min WPL, 1 min WPL and CW washing showed survival of *Salmonella*, the counts were all relatively low in these sporadic incidences. When 2% raspberry extract was added, water samples from 0.5- and 1-min WPL, 1% H_2O_2 washing and CW showed 1.8 − 34.5 CFU/mL of *Salmonella*, indicating that high loads of raspberry exudates might impact the effectiveness of these treatments. In contrast, WPL-H_2O_2 was not affected by the high organic load, showing negative results for the presence of *Salmonella* in all of the water samples.

6.3.2 Effect of WPL treatments and organic load on inactivation of *Salmonella* on blueberries.

The decontamination of *Salmonella* on blueberries using WPL treatments and the effect of organic load on the efficacy of WPL treatments is shown in Table 6.2. In the absence of blueberry extract, washing with 100 ppm CW for 1 min reduced *Salmonella* on blueberries by 3.9-log units. Significantly higher reductions ($P < 0.05$) of *Salmonella* were achieved by WPL treatments which resulted in $\geqslant$ 5.3-log reduction of *Salmonella*. Treatments of WPL (1 min) and WPL-H_2O_2 were able to reduce the population of *Salmonella* by > 5.6 log units, which was below the detection

limit of plating (0.7 log CFU/g). No survival of *Salmonella* was detected from water samples in all the treatments.

Addition of 2% blueberry extract did not significantly reduce the decontamination efficacy of the treatments, except for 0.5-min WPL. WPL (1 min) and WPL-H_2O_2 were the two most effective treatments achieving 5.0-log reductions of *Salmonella*, while CW washing reduced the population of *Salmonella* by 4.6-log units. Washing with 1% H_2O_2 only reduced *Salmonella* by 2.6 log units, which was significantly lower than all the other treatments. An average of 14.5 CFU/ml of *Salmonella* was detected in post-treatment water samples from 1% H_2O_2 washing with all the other treatments showing negative result for the presence of *Salmonella* in corresponding water samples.

Table 6.2 Inactivation of *Salmonella* on blueberries by WPL treatments at high PL energy output under different organic load conditions. Blueberries inoculated with *Salmonella* were washed with 1% H_2O_2 or 100 ppm chlorine (CW) or treated with WPL treatments with or without 2% of berry extract (BE). Data represent mean of at least three replicates ± one standard deviation. For berry samples, the fraction numbers in parentheses represent the number of samples shown positive after enrichment out of the number of total trials. For water samples, the fraction numbers in parentheses represent the number of samples showing positive plating results out of the number of total trials.

Treatments	Log reduction of *Salmonella* on berries (log CFU/g)		*Salmonella* survival in wash water (CFU/mL)	
	0% BE	2% BE	0% BE	2% BE
0.5-min WPL	5.3 ± 0.3ABa	4.4 ± 0.5Ab	ND	ND
1-min WPL	>5.6 (2/3)Aa	5.0 ± 0.7Aa	ND	ND
1-min WPL+H_2O_2	>5.6 (1/3)Aa	5.0 ± 0.7Aa	ND	ND
1-min H_2O_2 washing	2.7 ± 0.0Ca	2.6 ± 0.4Ba	ND	14.5±11.2 (3/4)
1-min CW washing	3.9 ± 0.4BCa	4.6 ± 0.8Aa	ND	ND

ND: Not detectable by direct plating (2 CFU/mL).

The detection limit of the plating method for berry samples was 0.7 log CFU/g.

Data in the same column followed by the same uppercase letter are not significantly different (P > 0.05).

Data in the same row followed by the same lowercase letter are not significantly different (P > 0.05).

6.3.3 Effect of PL energy output on the efficacy of WPL treatments

In order to study the effect of PL energy output on the inactivation of *Salmonella*, the charge voltage of the PL unit was lowered from 3800 V to 2670 V, which reduced the PL energy output by roughly half. The actual PL fluence level and distribution in the flume washer were quantified and are shown in Figure 6.2. Our results showed that the PL fluence at different locations in the flume washer ranged from 0.102 to 0.140 J/cm^2-pulse for low PL output, which was roughly half of the PL fluence at high PL output (0.225–0.298 J/cm^2-pulse).

Table 6.3 shows the efficacy of WPL treatment in the presence of 2% berry extract when the PL output was lowered by half. For both types of berries, WPL-H_2O_2 was still the most effective treatment, reducing *Salmonella* by 3.4 and 5.0 log CFU/g on raspberries and blueberries, respectively. Although no significant difference (P > 0.05) was found between 0.5- and 1-min WPL treatments, consistently higher reductions were observed for 1-min WPL treatment in both berries. Moreover, lowering the PL energy output by half did not significantly reduce the efficacies of all the WPL treatments (P > 0.05), compared to the WPL treatments at high PL output (Tables 6.1 and 6.2).

The microbiological quality of post-treatment water was also tested for each WPL treatment at low PL output. *Salmonella* was recovered in water samples from 0.5- and 1-min WPL treatments although the counts were relatively low (Table 6.3). In contrast, no survival of *Salmonella* was detected in all the water samples from treatment of WPL-H_2O_2.

Table 6.3. Inactivation of *Salmonella* by WPL treatments at low PL energy output. Berries inoculated with *Salmonella* were treated with WPL treatments in the presence of 2% of berry extract (BE). Data represent mean of at least three replicates ± one standard deviation. For water samples, the fraction numbers in parentheses represent the number of samples showing positive plating results out of the number of total trials.

Treatments	Log reduction of *Salmonella* on berries (log CFU/g)		*Salmonella* survival in wash water (CFU/mL)	
	Raspberry	Blueberry	Raspberry	Blueberry
0.5-min WPL	1.8 ± 0.2Ba	3.9 ± 0.5Bb	10.0 ± 3.5 (3/3)	3.0 ± 2.0 (4/4)
1-min WPL	2.1 ± 0.4Ba	4.5 ± 0.5ABb	1.3 ± 1.2 (2/3)	1.5 ± 1.9 (2/4)
1-min WPL+H_2O_2	3.4 ± 0.5Aa	5.0 ± 0.6Ab	ND	ND

ND: Not detectable by direct plating (2 CFU/mL).

The detection limit of the plating method for berry samples was 0.7 log CFU/g.

Data in the same column followed by the same uppercase letter are not significantly different (P > 0.05).

Data in the same row followed by the same lowercase letter are not significantly different (P > 0.05).

6.3.4 Effect of water quality on the efficacy of WPL treatments

As shown in Table 6.4, the water turbidity increased to 27.6 and 4.3 NTU, respectively when 2% berry extract of raspberry and blueberry was added to the wash water. The resulting COD level was 2006.7 mg/L and 2116.7 mg/L for raspberry and blueberry, respectively (Table 6.4). SiO_2 was selected as a simulator of soil to increase the turbidity of wash water. Adding 1 mL of SiO_2 suspension in 1 L of water increase the water turbidity to 63.7 NTU (Table 6.4). When both 2% berry extract and 1 mL of SiO_2 suspension was added to the wash water, the turbidity increased to 98.3 and 61.3 NTU for raspberry and blueberry, respectively. The visual appearance of water sample added with berry extract and SiO_2 is shown in Figure 6.4.

Table 6.4 Turbidity and COD of wash water

Water containing	Turbidity (NTU)		COD (mg/L)	
	Raspberry	Blueberry	Raspberry	Blueberry
1% BE	13.3 ± 0.1	2.4 ± 0.1	1100.0 ± 55.7	1043.3 ± 85.0
2% BE	27.6 ± 0.1	4.3 ± 0.1	2006.7 ± 141.9	2116.7 ± 80.2
2% BE+SiO_2	98.3 ± 0.5	61.3 ± 0.1	Not Applicable	
SiO_2	63.7 ± 0.2		Not Applicable	

Table 6.5 Effect of water turbidity on the efficacy of WPL treatments at low PL energy output. Berries inoculated with *Salmonella* were treated with WPL treatments in the presence of 2% of berry extract (BE) and SiO_2. Data represent mean of at least three replicates ± one standard deviation. For the water sample, the fraction numbers in parentheses represent the number of samples showing positive plating results out of the number of total trials.

Treatments	Log reduction of *Salmonella* on berries (log CFU/g)		*Salmonella* survival in wash water (CFU/mL)	
	Raspberry	Blueberry	Raspberry	Blueberry
1-min WPL	2.3 ± 0.4Ba	3.2 ± 0.5Ba	8.7 ± 9.0 (2/3)	1.3 ± 1.2 (2/3)
1-min WPL+H_2O_2	3.4 ± 0.8Aa	5.3 ± 0.3Ab	ND	ND
1-min CW washing	2.0 ± 0.2Ba	4.2 ± 0.7ABb	ND	ND

ND: Not detectable by direct plating (2 CFU/mL).

The detection limit of the plating method for berry samples was 0.7 log CFU/g.

Data in the same column followed by the same uppercase letter are not significantly different (P > 0.05).

Data in the same row followed by the same lowercase letter are not significantly different (P > 0.05).
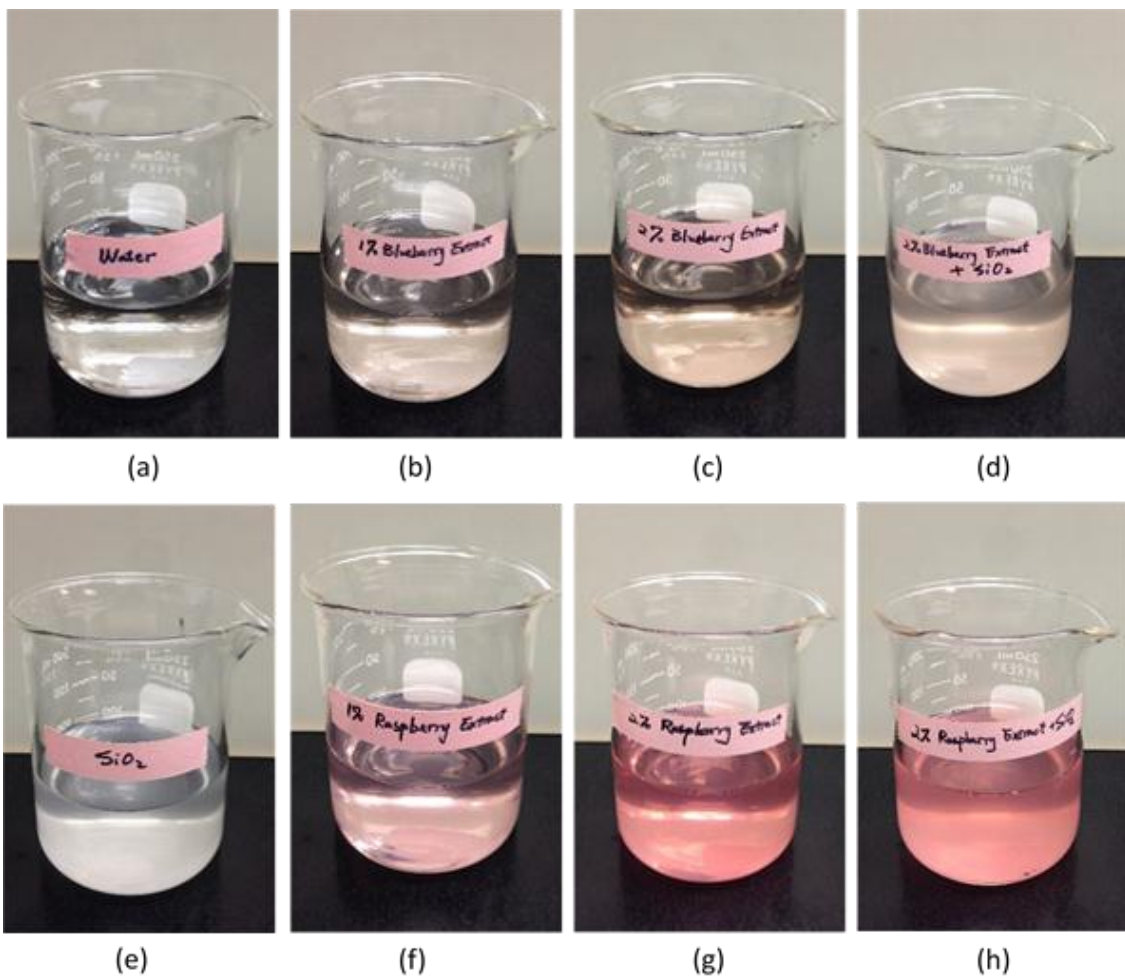
Figure 6.4 Visual appearance of water added with berry extract and SiO_2. (a) tap
water; (b) 1% blueberry extract; (c) 2% blueberry extract; (d) 2%
blueberry extract+SiO_2; (e) SiO_2; (f) 1% raspberry extract; (g) 2%
raspberry extract; (h) 2% raspberry extract+SiO_2.

As seen in Table 6.5, WPL-H_2O_2 was the most effective treatment as it reduced *Salmonella* on raspberries by 3.4-log units, followed by 1-min WPL treatment with a 2.3-log reduction of *Salmonella*. The population of *Salmonella* on raspberries was reduced by 2 log CFU/g after CW washing, which is significantly lower (P < 0.05) than the reduction with WPL-H_2O_2. For blueberries, significantly higher (P < 0.05) reductions of *Salmonella* were observed for WPL-H_2O_2 and CW washing compared to the same treatments in raspberries. WPL-H_2O_2 was the most effective treatment achieving a 5.3-log reduction of *Salmonella* on blueberry, followed by CW washing and 1-min WPL treatment. No significant decrease (P > 0.05) of free chlorine was noticed when SiO_2 was added into CW but adding berry extract in CW reduced the free chlorine level immediately. For CW washing, adding SiO_2 and 2% berry extract did not significantly alter the washing efficacy (P > 0.05) as compared with results in Tables 6.1 and 6.2.

No significant difference in the efficacy of WPL-H_2O_2 was observed regardless of PL energy output and turbidity level. Although a significantly lower (P < 0.05) reduction of *Salmonella* (3.2-log reduction) on blueberries was found for 1-min WPL treatment when water turbidity was increased to 61.3 NTU, no significant difference (P > 0.05) was observed for 1-min WPL treatment of raspberry at high and low water turbidity and PL output (Tables 6.1 – 6.3 & 6.5). No survival of *Salmonella* was detected in water samples from WPL-H_2O_2 and CW washing, but sporadic incidence of *Salmonella* survival, in the range from 2 to 18 CFU/mL, was observed in WPL treatment without H_2O_2.

6.4 Discussion

One of the main disadvantages of using chlorine washing is its sensitivity to organic matters in wash water. During washing, considerable amounts of organic materials including exudate from cut surfaces of fresh produce, soil and plant debris are released into the washing solution, which cause rapid deterioration of water quality manifested by increase in COD and turbidity and decrease in free chlorine level in wash water (Luo et al., 2012). This could create opportunities for pathogens to survive and cross-contaminate produce in the same batch or even subsequent batches (Allende et al., 2008). In our study, berry extracts were used to simulate berry exudate to artificially increase the organic load in wash solutions. Although the decontamination efficacy on raspberries and blueberries by CW washing was not significantly affected by organic load (Tables 6.1 and 6.2), *Salmonella* survivors were detected in the wash water with 1% or 2% of raspberry extracts. This is probably due to the significant decrease of free chlorine level in CW after the addition of berry extract (Figure 6.3). It should be pointed out that the free chlorine level used in the fresh-cut produce industry is usually much lower than 100 ppm, which could potentially cause cross contamination. In a pilot plant study, Luo et al. (2012) reported that the consumption of free chlorine by organic materials in wash water accelerates exponentially as organic load increases. In order to cope with deteriorating wash water, food processors need to repeatedly replenish chlorine into wash water in a timely manner, which could be a laborious technical challenge.

WPL-H_2O_2 was the most effective treatment, showing higher log reductions of *Salmonella* on both types of berries than all the other treatments and in most cases these differences were statistically significant ($P < 0.05$) (Tables 6.1 – 6.3 & 6.5). No *Salmonella* survivors were detected in all the water samples from the WPL-H_2O_2

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treatment. In addition, the effectiveness of WPL-H_2O_2 treatment was not significantly affected (P > 0.05) by the presence of organic load (Tables 6.1 – 6.2). The combination of UV and H_2O_2, as an AOP, has been demonstrated to be effective against various microorganisms. Bayliss and Waites (1979) reported a synergistic effect by using a 1% H_2O_2 solution in combination with UV-C at 254 nm to inactivate *Bacillus* subtilis endospores. The researchers also showed that the optimal concentration of H_2O_2 was about 1% for spores of *Bacillus subtilis*, and killing was reduced at higher concentrations. McDonald et al.(2000) treated *Bacillus subtilis* spores with 1% H_2O_2 in combination with either pulsed or continuous UV and noticed synergism in both combinations. Xie et al. (2008) sprayed H_2O_2 onto lettuce samples for 10 s and illuminated lettuce with UV-C light (254 nm) for 20 s. An enhanced efficacy (4.12 log reduction of MS2 F(+) coliphage) was reported compared to a 1.67-log reduction achieved by 200 ppm chlorine washing. Hadjok et al. (2008) later used the same setup to decontaminate different fresh produce and reported a 4.12-log reduction of *Salmonella* on lettuce by using a combination of 1.5% H_2O_2 spray at 50 °C and UV-C (254 nm).

The effectiveness of PL treatment depends on many factors such as treatment fluence, light peak power, and distance between light source and samples (Gómez-López et al., 2007). Unlike light from a point source, whose intensity follows the inverse square law, light intensity from non-point source lamps cannot be calculated easily and usually needs to be measured. The distribution of PL fluence is a result of distance, light absorption, scattering and reflection (Hsu and Moraru, 2011). The fluence value was highest at the center of the flume washer, which was also centered in the treatment chamber. The fluence value decreased at locations either parallel or

perpendicularly away from the washer center. These results are in general agreement with the results reported by Hsu and Moraru (2011), who measured the spatial distribution of fluence inside the same model of PL treatment chamber and described the distribution of PL fluence using a Gaussian model.

PL inactivation of microorganisms was reported to follow a fluence-dependent manner in clear liquid and food surfaces (Huang and Chen, 2014; Krishnamurthy et al., 2004; Uesugi et al., 2007). However, no significant difference in log reductions of *Salmonella* on berries was found between treatments of high and low PL fluence in our study (Tables 6.1 – 6.3). It is very likely that some *Salmonella* cells might have lodged in surface or subsurface structures inaccessible to PL; therefore, increasing PL fluence did not result in further reduction of *Salmonella* from the berry surface.

Higher reductions of *Salmonella* were achieved on blueberries than on raspberries for all the PL treatments (Tables 6.1 – 6.3). The effect of surface property probably contributed to these differences. Compared to the smooth skin of blueberries, the gaps between the drupelets of a raspberry might allow better shielding of microorganisms from PL exposure. Tissue exudate from broken pulp may provide additional protection for pathogens. Many studies have shown a complex effect of various surface properties on the inactivation of microorganisms by PL. In our previous studies, we found a consistently higher reduction of *E. coli* O157:H7 and *Salmonella* on blueberry skin than on the blueberry calyx (Huang and Chen, 2014). In another study, we found that *E. coli* O157:H7 inoculated on the stem part of green onions was more resistant to WPL treatment than on leaf portions (Xu et al., 2013). Sapers et al. (2000) found a higher survival of *E. coli* in the calyx and stem areas of inoculated apples than the skin after a washing treatment. Sy et al. (2005) also

reported that *Salmonella* inoculated on raspberries showed a higher resistance to gaseous ClO_2 than on blueberries.

Soil carried by fresh produce is thought to impact the wash water quality during washing process, causing decrease of free chlorine and increase in turbidity and COD. In our study, SiO_2, with its inert chemical property and abundance in soil, was selected as a simulator of soil to increase the turbidity of wash water. Compared with the direct use of soil or sand collected from fields, where soil composition varies significantly among different areas, the SiO_2 used in our study has a defined content and particle size, which provides an easy and reproducible way to study the effectiveness of WPL and CW washing under various water turbidity conditions.

The efficacy of PL has a strong dependence on liquid turbidity as high turbidity will diminish the PL intensity, which would successively reduce the bactericidal efficacy of PL. Therefore, 2% berry extract and SiO_2 suspension were added into wash water to artificially increase the organic load as well as turbidity in our study. Our results showed that no significant difference ($P > 0.05$) in the efficacy of WPL-H_2O_2 treatment was observed regardless of PL energy output and turbidity level (Table 6.5). Additionally, WPL-H_2O_2 treatment showed significantly higher efficacy in reducing *Salmonella* on both berry types while WPL and CW washing were not significantly different from each other.

The COD of water increased to > 2000 mg/L after 2% berry extracts were added and turbidities increased to 98.3 and 61.3 NTU, respectively, after both 2% berry extract and SiO_2 were added (Table 6.4). In a recent study by Nou et al. (2011), spent water was obtained from a fresh-cut lettuce processing plant. Water turbidity and COD level in the post-wash water samples were reported to be 59.8 NTU and 1857.8

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mg/L, respectively. In another study by Van Haute et al. (2013), the COD values of spent water samples from two lettuce processing plants were 465 and 1405 mg/L. Therefore, the water quality used in our study was comparable or even worse than the industrial water sample in these studies. Moreover, lettuce generally carries much more soil and plant debris into the washing process than blueberries and raspberries. Therefore, supplemented with both berry extract and SiO_2, the water quality used in our study mimicked a worst-case scenario.

In the fresh produce industry, one of the main purposes of using CW is to prevent cross-contamination. Therefore, the microbiological quality of wash water becomes a good indicator of the integrity of the washing process since when free chlorine in wash water is depleted, washing process might become an ideal medium for pathogen cross-contamination. To evaluate WPL treatment as a good alternative to chlorine, water samples were taken for microbiological analyses after each treatment. In general, WPL treatment and CW washing showed similar ability in effectively preventing cross-contamination, especially in clear water. However, sporadic incidence of *Salmonella* survival in water samples from WPL treatment were observed, although no survival of *Salmonella* were detected after WPL-H_2O_2 treatment, even in water containing high organic load and water turbidity.

It is known that PL can efficiently inactivate microorganisms in a clear suspension. Uesugi et al. (2007) reported a 7-log reduction of *Listeria innocua* in clear liquid broth after 9 J/cm^2 PL exposure. Krishnamurthy et al. (2004) reported that a 7.5-log reduction of *Staphylococcus aureus* in buffer solution was achieved after a 5.6 J/cm^2 PL exposure. In our previous study (Huang and Chen, 2014), we reported that a 5-s PL treatment at high PL output (5 J/cm^2) reduced the population of *E. coli*

O157:H7 in clear water by > 6.8 log units. However, the addition of 2% berry extract and SiO_2 complicated the situation by changing the water property and diminishing the PL influence. It should be pointed out that although a portion of *Salmonella* cells was either killed by PL directly or washed off into water and subsequently killed by PL, there were still live cells remaining on the berry surface after the PL treatments. There was a time gap between the time PL was turned off and the water samples were taken for microbial analysis. During this time period, it was highly possible that some live cells just dislodged from the berry surface into the wash water, which could have subsequently ended up in the water samples taken for microbial analysis. In addition, water was continuously circulating in the system. Cells could only be inactivated when they were exposed to PL in the flume washer, but the water pump and tubing, which were located outside the PL chamber, could temporarily protect these cells from PL exposure. On the other hand, the use of PL and H_2O_2 generated highly reactive hydroxyl radicals, which provided a longer antimicrobial effect by effectively inactivating any bacterial cells in wash water, even after the PL was turned off.

Taken together, the WPL treatment could be a promising chlorine alternative. In the current small scaled-up system, WPL treatment without H_2O_2 provided a chemical free alternative to chlorine with higher bactericidal efficacy than 100 ppm CW at different water conditions. Additional decontamination capability was obtained by using combination of WPL and H_2O_2, which provided significantly higher bactericidal efficacy than both CW and WPL treatments regardless of PL energy output, turbidity level and organic load in water.

Acknowledgements

REFERENCES

Allende, A., Selma, M. V., López-Gálvez, F., Villaescusa, R., Gil, M.I., 2008. Impact of wash water quality on sensory and microbial quality, including *Escherichia coli* cross-contamination, of fresh-cut escarole. Journal of Food Protection 71, 2514–2518.

Bayliss, C., Waites, W., 1979. The synergistic killing of spores of *Bacillus subtilis* by hydrogen peroxide and ultra-violet light irradiation. FEME Microbiology Letters 5, 331–333.

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, M201–M207. doi:10.1111/j.1750-3841.2008.00743.x

Bialka, K.L., Demirci, A., Puri, V.M., 2008. Modeling the inactivation of *Escherichia coli* O157:H7 and *Salmonella enterica* on raspberries and strawberries resulting from exposure to ozone or pulsed UV-light. Journal of Food Engineering 85, 444–449. doi:10.1016/j.jfoodeng.2007.08.007

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 and infection 131, 745–751.

CFR - Code of Federal Regulations 21CFR179.41, FDA.

Crowe, K.M., Bushway, A.A., Bushway, R.J., Davis-Dentici, K., Hazen, R.A, 2007. A comparison of single oxidants versus advanced oxidation processes as chlorine-alternatives for wild blueberry processing (*Vaccinium angustifolium*). International Journal of Food Microbiolpgy. 116, 25–31. doi:10.1016/j.ijfoodmicro.2006.12.027

Fine, F., Gervais, P., 2004. Eficiency of pulsed UV light for microbial decontamination of food powders. Journal of Food Protection 67, 787–792.

Gómez-López, V.M., Ragaert, P., Debevere, J., Devlieghere, F., 2007. Pulsed light for food decontamination: a review. Trends in Food Science & Technology 18, 464–473. doi:10.1016/j.tifs.2007.03.010

Hadjok, C., Mittal, G.S., Warriner, K., 2008. Inactivation of human pathogens and spoilage bacteria on the surface and internalized within fresh produce by using a combination of ultraviolet light and hydrogen peroxide. Journal of Applied Microbiology 104, 1014–24. doi:10.1111/j.1365-2672.2007.03624.x

Hsu, L., Moraru, C.I., 2011. Quantifying and mapping the spatial distribution of fluence inside a pulsed light treatment chamber and various liquid substrates. Journal of Food Engineering 103, 84–91. doi:10.1016/j.jfoodeng.2010.10.002

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.

Huang, Y., Chen, H., 2014. A novel water-assisted pulsed light processing for decontamination of blueberries. Food Microbiology 40, 1–8.

Krishnamurthy, K., Demirci, A., Irudayaraj, J., 2004. Inactivation of *Staphylococcus aureus* by pulsed UV-light sterilization 67, 1027–1030.

Lagunas-Solar, M.C., Piña, C., MacDonald, J.D., Bolkan, L., 2006. Development of pulsed UV light processes for surface fungal disinfection of fresh fruits. Journal of Food Protection 69, 376–384.

Luna, R.E., Griffin, P.M., 2010. Memorandum: Non-O157 Shiga toxin-producing *E. coli* (STEC) outbreaks, United States. http://blogs.cdc.gov/publichealthmatters/files/2010/05/nono157stec_obs_0521 10.pdf. accessed on 12/01/14

Luo, Y., 2007. Fresh-cut produce wash water reuse affects water quality and packaged product quality and microbial growth in romaine lettuce. HortScience 42, 1413–1419.

Luo, Y., Nou, X., Millner, P., Zhou, B., Shen, C., Yang, Y., Wu, Y., Wang, Q., Feng, H., Shelton, D., 2012. A pilot plant scale evaluation of a new process aid for enhancing chlorine efficacy against pathogen survival and cross-contamination during produce wash. International Journal of Food Microbiology 158, 133–9. doi:10.1016/j.ijfoodmicro.2012.07.008

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. doi:10.1007/s12560-013-9118-0

McDonald, K.F., Curry, R.D., Clevenger, T.E., Brazos, B.J., Unklesbay, K., Eisenstark, A., Baker, S., Golden, J., Morgan, R., 2000. The development of photosensitized pulsed and continuous ultraviolet decontamination techniques for surfaces and solutions. IEEE Transactions on Plasma Science 28, 89–96.

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, 762–9. doi:10.4315/0362-028X.JFP-12-407

Nou, X., Luo, Y., Hollar, L., Yang, Y., Feng, H., Millner, P., Shelton, D., 2011. Chlorine stabilizer T-128 enhances efficacy of chlorine against cross-contamination by *E. coli* O157:H7 and *Salmonella* in fresh-cut lettuce processing. Journal of Food Science 76.

Oms-Oliu, G., Martín-Belloso, O., Soliva-Fortuny, R., 2008. Pulsed light treatments for food preservation. A review. Food and Bioprocess Technology 3, 13–23. doi:10.1007/s11947-008-0147-x

Ramos-Villarroel, a. Y., Martín-Belloso, O., Soliva-Fortuny, R., 2011. Bacterial inactivation and quality changes in fresh-cut avocado treated with intense light pulses. European Food Research and Technology 233, 395–402. doi:10.1007/s00217-011-1533-6

Reidmiller, J.S., Baldeck, J.D., Rutherford, G.C., Marquis, R.E., 2003. Characterization of UV-peroxide killing of bacterial spores. Journal of Food Protection 66, 1233–1240.

Rosenfeldt, E.J., Linden, K.G., Canonica, S., von Gunten, U., 2006. Comparison of the efficiency of *OH radical formation during ozonation and the advanced oxidation processes O_3/H_2O_2 and UV/H_2O_2. Water Research 40, 3695–704. doi:10.1016/j.watres.2006.09.008

Sapers, G., Miller, R., Jantschke, M., Mattrazzo, A.M., 2000. Factors limiting the efficacy of hydrogen peroxide washes for decontamination of apples containing *Escherichia coli*. Journal of Food Microbiology and Safety. 65, 529–532.

Sarvikivi, E., Roivainen, M., Maunula, L., Niskanen, T., Korhonen, T., Lappalainen, M., Kuusi, M., 2012. Multiple norovirus outbreaks linked to imported frozen raspberries. Epidemiology and Infection 140, 260–7. doi:10.1017/S0950268811000379

Sauer, A., Moraru, C.I., 2009. Inactivation of *Escherichia coli* ATCC 25922 and *Escherichia coli* O157: H7 in apple juice and apple cider, using pulsed light treatment. Journal of Food Protection 72, 937–944.

Suty, H., De Traversay, C., Cost, M., 2004. Applications of advanced oxidation processes: present and future. Water Science and Technology 49, 227–233.

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, 1165–1175.

Udompijitkul, P., Daeschel, M.A., Zhao, Y., 2007. Antimicrobial effect of electrolyzed oxidizing water against *Escherichia coli* O157:H7 and *Listeria monocytogenes* on fresh strawberries (*Fragaria x ananassa*). Journal of Food Science 72, M397–M406. doi:10.1111/j.1750-3841.2007.00531.x

Uesugi, A.R., Woodling, S.E., Moraru, C.I., 2007. Inactivation kinetics and factors of variability in the pulsed light treatment of *Listeria innocua* cells. Journal of Food Protection 70, 2518–2525.

Van Haute, S., Uyttendaele, M., Sampers, I., 2013. Organic acid based sanitizers and free chlorine to improve the microbial quality and shelf-life of sugar snaps. International Journal of Food Microbiology 167, 161–9. doi:10.1016/j.ijfoodmicro.2013.09.007

Xie, Y., Hajdok, C., Mittal, G.S., Warriner, K., 2008. Inactivation of MS2 F (+) coliphage on lettuce by a combination of UV light and hydrogen Peroxide. Journal of Food Protection 71, 903–907.

Xu, W., Chen, H., Huang, Y., Wu, C., 2013. Decontamination of Escherichia coli O157:H7 on green onions using pulsed light (PL) and PL-surfactant-sanitizer combinations. International Journal of Food Microbiology 166, 102–8. doi:10.1016/j.ijfoodmicro.2013.06.027

Chapter 7

FUTURE RESEARCH

Fresh and frozen berry products are minimally processed foods that are susceptible to contamination of pathogenic bacteria and viruses during pre- and post-harvest stages. The findings from the present dissertation and previous studies on the application of high pressure processing (HPP) and pulsed light (PL) show that these intervention technologies have the potential to deliver a higher safety margin to the berry products than traditional chlorine washing with minimal impact on the quality of these foods. With proper design, modification and combination with other hurdles, the cost and efficiency of those intervention methods could be further improved.

In our study, we found that HPP could efficiently eliminate *Escherichia coli* O157:H7, *Salmonella*, and natural yeasts and molds in strawberry puree. The use of HPP treatment followed by a short period of frozen storage could significantly reduce the pressure level needed to eliminate the target microorganisms. More research can be carried out using HPP at different temperatures, pH values of berry puree, type of berry puree, etc. The storage temperature after HPP treatment could also have a profound impact on the survival of injured cells by HPP. More studies can be done using refrigerated temperatures and room temperature storage after pressure treatment. Moreover, the shelf life and nutrition qualities of berry puree processed with different processing strategies can be determined.

The novel water-assisted PL (WPL) treatment developed in our study provided a new strategy to use PL as a practical chlorine alternative in the fresh produce industry. The combination of WPL treatment with hydrogen peroxide as an advance oxidation process enhanced the efficacy of WPL treatment against bacterial pathogens,

but a similar pattern was not observed in murine norovirus (MNV). More hurdles such as low temperature, ultrasonication, surfactant, different forms of antimicrobials, and photosensitizers can be incorporated with PL treatment to potentially enhance the inactivation of viruses as well as bacteria. In addition, FDA only allows the use of PL with a pulse width not exceeding 2 ms and a cumulative fluence less than 12 J/cm^2; therefore, future research is needed to evaluate the efficacy of WPL at a lower fluence level by customizing the current PL system. Although a small scaled-up WPL system was built and tested for its efficacy in our study, the robustness of the WPL treatment needs to be further validated at a larger scale, through pilot plant to full production scale before recommendations can be made to the industry. Further efforts are needed to design and build a larger laboratory PL system with refinements to the parameters such as fluence level, flow rate, treatment time, treatment temperature, sample to water ratio, etc. Finally, a predictive model estimating the inactivation of bacterial and viral pathogens on different type of berries as a function of the above parameters would be beneficial to readily evaluate the efficacy of the WPL treatment system.

More research is needed on the inactivation of viruses using PL and WPL treatments. While surrogates are helpful tools to gain an estimate of the effectiveness of PL treatment on human norovirus, there are structural differences and their behavior may not be indicative of human norovirus. Therefore, further validation using human norovirus is needed. However, human norovirus study is hampered by lack of suitable cell culture system and the inactivation mechanism of PL treatment is not well delineated yet. In order to assess the infectivity of human norovirus, molecular methods such as RT-PCR are usually used but genome integrity does not indicate viral infectivity. Recently, a porcine gastric mucin-magnetic bead (PGM-MB) binding

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assay has been developed to offer quantitative information about the inactivation of non-cultivable human norovirus. This assay is based on the premise that the inactivation mechanism of the treatment is targeting the capsid by damaging the viral binding ability to its receptor-like molecules. Therefore, the correspondence between the viral binding ability and infectivity needs to be determined case by case for different treatments. In our preliminary study, suspensions of human norovirus and MNV were treated by PL and the viral infectivity was assessed by both plaque assay and PGM-MB binding assay. However, the MNV infectivity results obtained using those two methods did not correspond well with each other. Therefore, further study using human norovirus and the PGM-MB binding assay was not conducted. Until a feasible cell culture system for human norovirus is established, surrogates will still be a valuable tool for studies of food safety intervention technologies.

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